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Research Article

Cover caption
This special issue commemorates the International Year of Plant Health 2020 and will serve as an excellent source of the latest research, reviews and commentaries from leading plant pathologists and entomologists in South Africa. It will inform not only plant pathologists and food growers, but also policymakers and the public of the importance of plant health. As Mirko Montuori (UN Food and Agriculture Organization) states in his Commentary, '…our own health depends on plants while the health of plants depends on us'.

Celebrating the International Year of Plant Health 2020 (IYPH 2020)

The COVID-19 pandemic clearly illustrates the impact of a microbial disease outbreak on the human population. Throughout the course of human history, microbial plant diseases and insect pests have had a similar impact, resulting in loss of life due to food shortages. Notable examples include the Irish potato famine in the 1840s caused by *Phytophthora infestans* and the more recent locust swarms in East Africa. Conservative estimates predict that up to 5 million people may be at risk of starvation due to this locust invasion. Yet, despite years of extensive research, we still lack a comprehensive understanding of, for example, host–pathogen interactions and resistance mechanisms deployed by plants.

In this special issue, we highlight research on plant health by pathologists and entomologists in South Africa. The Commentary by Mirko Montuori from the Food and Agricultural Organization (FAO) provides a clear synthesis of contemporary plant health research. He discusses the concept of how protecting plants directly protects human health. This topic is explored further by Ida Wilson who specifically discusses the importance of plant health for sustainable crop production in South Africa. Her suggestion is that a holist approach should be implemented when considering a management strategy against pests and pathogens.

Microbe and insect collections may be considered national biodiversity resources. These collections, which detail the historical records of disease and pest outbreaks, are important for decision-makers as they contain critical information for phytosanitary regulations. In her insightful Commentary, Adriaana Jacobs outlines the importance of these natural science collections, one of which has a long and sustained history in South Africa; for instance, the national fungal culture collection was established in 1905.

The Southern African Society of Plant Pathology and Microbiology was formed in 1962. Initially the presentations at the annual congresses focused on plant pathogens, but this changed in the 1970s with microbiological topics dominating proceedings. In 1980, the disciplines split into the South African Society of Microbiology and the Southern African Society of Plant Pathology. In our Commentary, Mike Wingfield and I describe the history of the Southern African Society of Plant Pathology, which has developed into a highly successful professional society.

One of the primary objectives of plant pathologists is to manage diseases to prevent significant economic losses. In 1926, Paul van der Bijl, the head of the Department of Plant Pathology and Mycology at Stellenbosch University, wrote a seminal paper in the *South African Journal of Science* on 'Landmarks in the development of the science of plant pathology and of disease control'. In his Commentary on this article, Bernard Slippers compares key factors that influence plant disease development and management today compared with what was known almost 100 years ago. One of the most renowned epidemiologists in the world during the 20th century was James E. Vanderplank, and Mike Wingfield, Andre Drenth and I present the achievements of this most remarkable scientist in our Commentary. In the contribution by Trudy Paap and colleagues, the epidemiology in South Africa of an invasive exotic pest, the shot hole borer, is discussed. Although initially only a problem in the urban environment, it is now a tremendous threat to agriculture and forestry.

Victoria Pastor from Jaume I University in Spain reviewed two volumes of the book *Plant Health Under Biotic Stress*', edited by Dr Rizwan Ali Ansari and Dr Irshad Mahmood. Volume 1 focuses on organic strategies while Volume 2 examines microbial interactions. She entitled her review 'Green actions for a better plant health', thus highlighting the trend towards using biological alternatives as opposed to pesticides to manage plant pests and diseases.

This year (2020) the National Science and Technology Forum (NSTF) included a special category to celebrate the IYPH 2020. Several distinguished plant pathologists and entomologists were nominated for this award and Mike Wingfield, a highly accomplished researcher in the field of forest protection, received the award. Salmina Mokgehle, a *South African Journal of Science* Associate Editor mentee, who has been integrally involved in the compilation of this special issue, interviewed Mike. Her Profile of Mike's accomplishments over the course of his 40-year career is included in this issue.

This issue also includes six review articles focusing on viruses and other pathogens that have caused, and remain the cause of, significant losses to the agricultural and forestry sectors in South Africa. The first, written by Chrissie Rey, is similar to a detective story as it records the early history of the discovery of plant viruses in the country. The two reviews by Jacquie van der Waals and Kerstin Krüger cover the diseases and the vectors of the most important pathogens affecting potato production areas in South Africa. Mike Wingfield and co-authors reflect on the history of forest protection in South Africa while Zakkie Pretorius discusses the accomplishments of his group in wheat rust research over the past 40 years. Kwasi Yobo and co-authors highlight the importance of Fusarium head blight on wheat and emphasise the significance of multiple strategies to manage the disease.

The six research articles focus almost entirely on plant diseases. Although attempts were made to encourage submissions from applied entomologists, none was received. Some of the most devastating plant diseases in South Africa are thus the core of these articles. Our collection includes research undertaken by Maryke Craven and co-authors on northern corn leaf blight as a predisposing factor of ear rot incidence and severity; the development and improvement of an epidemiological model for citrus black spot by Providence Moyo and colleagues; and the first report of tropical race 4 (TR4) of the pathogen responsible for Fusarium wilt of banana in Mozambique by Altus Viljoen and his collaborators. The occurrence of TR4 in Mozambique is considered a major threat to banana production in the rest of Africa. Mapotso Kena and her colleagues describe the use of plant extracts (from *Monsonia burkeana* and *Moringa oleifera*) and *Trichoderma harzianum* as a biocontrol option to manage damping-off of tomato seedlings caused by *Rhizoctonia solani*. Research by Kwasi Yobo and co-authors on the entomopathogenic fungus and endophyte, *Beauvaria bassiana*, as a control measure against the rice stem borer is also included. Finally, Dave Berger and his team determined that northern corn leaf blight is the most serious disease of maize in smallholder farms in South Africa and that it can be successfully managed by the deployment of resistant cultivars.

We hope that you enjoy reading this special issue in honour of the IYPH 2020.

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INTERNATIONAL YEAR OF PLANT HEALTH 2020

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Recognising the importance of plant health in today's world

Protecting plants is protecting life

Plants are the source of the oxygen we breathe and most of the food we eat, as almost all our food is directly or indirectly plant-based. We need plants for medical reasons and the medicinal components of many plant species are either directly used for human medication or serve as a blueprint for producing pharmaceutical drugs. Plants provide building materials for shelter and supply us with energy for heat and transportation.[1]

Nevertheless, plant health is often overlooked by policymakers and the public. Between 2000 and 2016, international agricultural trade increased more than three-fold in value.[2] With increasing trade, opportunities for plant pests and diseases to move around the world have grown exponentially. International travel and the movement of people have also fundamentally increased over the past decades, bringing fresh pathways for pest and disease introduction and spread. New pests and diseases appear now in territories where they had never before been encountered, with negative consequences on local ecosystems, agriculture and food security. Pests and diseases are responsible for losses of between 20% and 40% of global food production and for trade losses in agricultural products exceeding USD220 billion every year. What is more, once established in a new area, plant pests are often impossible to eradicate.

This situation is worsened by climate change, the impact of which on plant health is incontrovertible and affects plant pests' epidemiology, distribution and impact. Due to global warming, it is observed that more pests are not only appearing earlier in the season, but rising temperatures also create new pathways for pests and diseases to thrive and spread. Climate change influences the movement of pests and threatens both the quality and quantity of crops. Balancing the impact of climate change on pest and disease occurrences and food production is an unprecedented global challenge for the scientific community because we need to produce more food with fewer resources and soils that are being degraded in order to feed the nine billion people on earth by 2050.

Urgent action is needed to protect plants

Protecting plants from pests and diseases and keeping them healthy is more important than ever to secure food for a growing world population of humans and domestic stock. By protecting plants, we thus also protect the key components of our diets and those of the animals we eat.

Much still needs to be done to secure plant health worldwide. Plant pest and disease outbreaks and their increasing incidences pose new challenges for the global phytosanitary community. Being aware of the risks and taking responsible actions when travelling and purchasing plants and plant products online could help to secure plant health worldwide. Driving innovation in the plant health domain, private sector businesses also have a key role to play as they can contribute to the development of global plant health standards and help to implement them.

Currently, plant pests and diseases such as *Spodoptera frugiperda*, *Schistocerca gregaria*, *Rhynchophorus ferrugineus* and *Xylella fastidiosa* are just some of the many that raise serious challenges across borders and, indeed, across regions and continents.

The role of the International Plant Protection Convention

Going by the ancient adage 'prevention is better than cure', by protecting plants from the spread and introduction of pests and diseases, governments, farmers, and other actors can help preserve natural biodiversity at local and national levels, contribute to the saving of billions of dollars and ensure access to sufficient and safe food for all. The application of solid, science-based normative instruments, such as the International Standards for Phytosanitary Measures (ISPMs) of the International Plant Protection Convention (IPPC), helps to achieve sustainable and fair trade globally, thus facilitating market access, particularly for developing countries. Farmers, especially smallholders, and small businesses may acquire better opportunities when such normative tools are implemented in a harmonised and coordinated manner. The mission of the IPPC is to protect the world's plant resources from pests and diseases while facilitating the safe trade of plants and plant products.

Plant health and One Health

Health debates have dominated since COVID-19 became a pandemic, emphasising the importance of preventive health measures. In fact, human health has always been, rightly, a priority on the international agenda. Given the absolute necessity of saving as many human lives as possible, international organisations and their members also have the duty to preserve and continue to improve life in the widest possible sense: from everyday life to the wider natural environment.

In fact, when considering the matter of 'health', what comes to mind is usually the health of humans or, at most, the health of animals. It is seldom that one thinks about the health of plants or that of the environment except in their relation to human health. Plants may appear healthy to untrained eyes, yet plants also get sick. While applying sanitary precautions, procedures, and treatments to tackle the threat of COVID-19 are paramount, we should nevertheless remember that our own health depends on plants while the health of plants depends on us. Therefore, we need to lower the risk of transmitting plant pests and diseases through our actions.

Plant pests and diseases are introduced through similar mechanisms that we are observing with the tragic spread of COVID-19. This is why the prevention advocated by international regulations, such as the ISPMs of the IPPC, approved through participatory methods and based on scientific evidence, becomes even more essential.

The International Year of Plant Health

Given the increasing threats to plant health and the impact on food security, the environment, and safe trade, for more than 5 years, the government of Finland, in collaboration with the Secretariat of the IPPC, has engaged to bring this issue to the notice of the general public. The proclamation of the International Year of Plant Health (IYPH) by the UN General Assembly is the result of this essential work.[3] The year 2020, therefore, is the opportunity for the global phytosanitary community to present its work to an audience broader than only disciplinary experts or institutional offices.

When first conceived, the plan for the IYPH anticipated many global, regional, and national events being held face-to-face. With the current global pandemic, the programme for the International Year is adapting the global series of initiatives that began with the launch of the IYPH at the FAO headquarters in Rome on 2 December 2019. The launch demonstrated the promise of global participation. However, the current global health crisis prompted the IPPC Secretariat and FAO to postpone other key global events, including the Fifteenth Session of the Commission on Phytosanitary Measures and the First International Plant Health Conference. Many other virtual initiatives have been, and will be organised, at the national level, in which all operators in the agricultural sector and consumers are called upon to participate. Among these, FAO with the Secretariat of the IPPC and *National Geographic* magazine launched a photographic contest to depict healthy plants and diseased plants, an art and drawing competition, and a call for national plant health champions.

The implementation and adherence to IPPC standards represent other important successes. By implementing phytosanitary standards, countries can contribute substantially to a zero-hunger world. ISPMs ensure that pest risks are effectively managed, pests and diseases can be prevented from spreading worldwide and we can ensure that the trade in, and transportation of, plants, seeds, and other agricultural products are safe.

What can we do?

1. Think twice before bringing plants along for the ride

It may be tempting to bring home that exotic flower from your summer holiday, but make sure you are not getting more than you bargained for – foreign plants can carry unseen pests and diseases, therefore make sure that you bring back only certified plant products. Every year, up to 40% of global food crops are lost to plant pests and diseases. As with human health, prevention is key.

The mobility of plants and plant products is subject to ISPMs, so when travelling to and from other countries, refrain from taking plants and plant products with you to ensure that foreign pests and diseases are not accidentally introduced. It would also help if you were careful when ordering plants and plant products online or through postal services because packages can easily bypass the regular phytosanitary controls that are vital for keeping our agricultural industries safe from external threats.

2. Trade safely and according to international standards

Many countries depend on trading plants and plant products to sustain their economies. In fact, the annual trade value of agricultural produce is now USD1.7 trillion! However, trade can quickly spread plant pests and diseases and seriously damage native plants and biodiversity. Government representatives and policymakers should ensure that they have implemented the International Plant Protection Convention (IPPC) standards. The IPPC is a global treaty signed by 184 contracting parties that provides an international framework for protecting plant resources from pests and diseases and promotes safe trade among all countries.

Anyone in the transportation and trade sectors can practise safe trading too. Ensure that your business enforces existing phytosanitary legislation and investigate adopting innovative technologies, such as electronic phytosanitary certification (ePhyto), when importing or exporting plants and plant products. Ensure that your clients are aware of the risks associated with transporting plants and plant products across borders and are knowledgeable about the best practices to follow.

3. Promote and adopt more sustainable environmentally friendly pest and disease control solutions

Plant pests and diseases are among the main reasons for biodiversity loss and poor plant health. However, we need to deal with pests and diseases in environmentally friendly ways and minimise the use of hazardous pesticides. Pesticides can kill pollinators and beneficial insects which are natural pest enemies and crucial for a healthy environment. Policymakers, as well as farmers, need to take heed of important information on pesticide use and the use of appropriate alternatives where these are available.

More sustainable solutions lie at the heart of Integrated Pest Management, an ecosystem-based approach that focuses on long-term prevention of plant ill-health through a combination of techniques such as biological control, biopesticides, intercropping, physical barriers such as tree cover, insect nets and good airflow, and the use of pest/disease-resistant plant varieties. Other environmentally friendly ways of controlling plant diseases include using pest- and disease-free planting materials, avoiding monoculture systems, and employing crop rotation methods that suppress the accumulation of pests and pathogens and favour biodiversity. Citizens can also support plant health through daily actions such as buying produce from farms that use ecological approaches for pest control, biological fertilisers and biopesticides.

Implementing scientifically based phytosanitary measures is also essential to sustain plant and seed health. Countries should invest more in research and technologies related to plant and seed health and provide incentives for commercial and smallholder farmers to adopt innovative practices and sustainable solutions. Building capacity in a cohort of phytosanitary experts is crucial, as is improving the infrastructural development for seed testing and certification at global and regional levels.

In conclusion, the proclamation of the IYPH 2020 itself marks a key global achievement. It has finally brought to light how protecting plant health can help achieve food security and solve other global challenges. The IYPH is not only a once-in-a-lifetime opportunity to raise global awareness on how protecting plant health can end hunger, reduce poverty, protect the environment, and boost economic development, but also to demonstrate that everyone has a specific role to play in this global action. The private sector, governments, farmers and agribusiness, the scientific community, travellers and citizens can all contribute to this important global goal by taking specific actions. Collaborative global efforts to proclaim an International Day of Plant Health (IDPH) are currently being championed by the government of Zambia as a lasting legacy to espouse the vision and mission of the IYPH.[4,5]

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INTERNATIONAL YEAR OF PLANT HEALTH 2020

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The importance of plant health as a key to sustainable crop production in South Africa

The South African population has grown from 40.5 million in 1996[1] to 56.6 million in 2020[2] and it is estimated to reach 73 million by 2050[3]. This unprecedented population growth escalates the demand for human food and animal feed and impels increased crop productivity in future.[4] Concurrently, crop production contributes to biodiversity loss, deforestation, desertification, soil degradation and climate change. As the population grows, and agricultural production intensifies, the question is whether it is possible to boost crop production while conserving natural resources and the environment.[3]

Crop productivity is directly related to crop health; thus, it stands to reason that improved plant health will contribute to greater crop productivity, but exactly what is a healthy crop? Healthy crops are those for which the biotic and abiotic factors that cause injury to plants are minimised, and which receive sufficient water, nutrition and light, as needed for photosynthesis and healthy growth. Such plants are less susceptible to pests and diseases and can reach their full genetic yield potential.[5]

For several reasons there is a gap between the current crop health attained in our agricultural systems and the potential for cultivating healthy crops. The growth and productivity of a crop are reliant on the health of the *whole production system*, yet the thought processes around the present inputs into crop cultivation systems remain *segmented*. Inputs on soil health, nutrition, irrigation and crop protection are given to producers by different service providers, who are often also salespersons for products or technologies. The reality, however, is that the crop production system is an *integrated system* in which all the variables related to the crop interact and essentially contribute either to a healthy or to an unhealthy crop.

So, if the production system is viewed as a holistic system, how could we achieve absolute optimal plant health? Take soil for example: when plants are exposed to prolonged wet soil conditions, they are more prone to infection by soil-borne diseases.[5] The management of such a disease therefore cannot rely on the application of a crop protection agent alone, and the cause of the problem, which is long-lasting exposure to wet soil, should also be addressed.[6] Crop health is therefore the result of the best and timely interventions, and these often need to take place concurrently and comprise a variety of components in the production system. There is an ongoing dynamic among soil, water, nutrition and protection of the crop (against pests and diseases). If any of these are neglected, then the health of the crop could be compromised.

So how do we support better plant health in commercial crop production in South Africa? We need to strengthen capacity in *detection, accurate identification* and *quantification* of plant health problems. Saline water, for instance, causes the yellowing of leaves in crops, but so do nutritional deficiencies and certain microbial infections. The application of products (nutritional or agrochemical) in this instance will not help the crop to recover. It is therefore important to understand – and address – the underlying causes in crop diseases. Moreover, if biological agents are found to be responsible for poor plant health, agrochemical intervention is only needed if the pest or disease incidence is above a certain economic threshold. The concept of a 'threshold' refers to the magnitude of the pest or disease population. If the population is smaller than the threshold value, the application of crop protection agents is not economically justifiable[5] as the input costs of the control measures will exceed the monetary benefit likely to arise from the given control measure.

In commercial agriculture in South Africa, there is a strong focus on *preventative measures* for controlling plant diseases by agrochemical spray applications in the absence of the pest or disease, but in anticipation of its presence or arrival. In these systems, strengthening the detection, identification and quantification of specific risks may allow for tailor-made intervention which could reduce reliance on agrochemicals.

In some instances, the climate may not be suitable for the occurrence of a specific organism, or the cultivated variety of the crop may have an inherent resistance to a given organism. So, it remains important to know to what extent a certain pest or disease creates a risk in the production unit and how crucial the management of that specific risk is.

With this knowledge at hand, producers could save money, effort and time, and reduce chemical inputs that are harmful to the environment. In South Africa, the plant protection community needs to be strengthened to support producers with reliable information about the plant health risks that crops face. The prospect of precision detection and identification technology, such as electronic noses, will allow for accurately informed crop protection intervention.[7] Such precision diagnoses will also allow meticulous spray applications on segments of the production unit where the target organism was identified at perfectly timed intervals.[8]

Furthermore, there are various drivers that will determine which pests and diseases may come to dominate certain geographical areas, including:

1. biological shifts, as the displacement of one organism by agrochemical products makes way for another[9],
2. the changing climate's effect on pathogens and pests[10] and the crop host[11],
3. the genetic composition of current and future cultivated varieties[12], and
4. the impacts of the continued evolution of cultivation practices, including the influence of the Internet of things on cultivation practices and crop production.[13]

Future solutions in crop health therefore cannot be seen to be a standard recipe that is applied year after year. The challenge of crop health remains a *dynamic challenge* best addressed with *scientific knowledge*.

Contemporary plant health management approaches also tend to focus on day-to-day operational efforts. Decisions are mainly made in terms of which chemical active ingredients to apply to the crop. The long-term strategic outlook on the environmental impact of the use of these chemicals on non-target organisms (such as insects useful for biological control) and the long-term impact on the environment and a sustainable planet for humans are not properly considered, despite substantial evidence that it is possible to use less pesticide and simultaneously increase crop yields.[14]

Finally, end-point monitoring and evaluation of the success of plant protection efforts are generally lacking, and in order to better manage the risks to crop production and crop health, the true measurement of the impact of the application of certain measures will go a long way in supporting the future use of a technology or product. There is currently a void in terms of the *measurement of the impact of given pests and diseases* on certain crops. Improved access to real-world knowledge could support more appropriate and targeted management of plant health risks in our systems and enhance crop production in future.

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The importance of natural science collections in South Africa

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Our ability to understand the natural world depends on the collection, preservation, and study of natural history specimens. These natural science collections are the physical record of earth's biological diversity. As a megadiverse country, it is essential that South Africa documents not only its aboveground diversity but also its below-ground richness.

Ecosystem functionality is equivalent to biodiversity. This is particularly evident in the soil environment, and Pimentel et al.[1] estimated the global economic benefits of soil diversity at USD1.5 trillion. Soils provide a high number of ecosystem services due to the complex communities of organisms living there. These biota contribute to nutrient cycling, waste decomposition, soil formation and water regime control[2], and the majority of these organisms are still unknown. These interactions were also highlighted by Louw et al.[3] in a South African context, and led to the establishment of the South African Soil Ecology Group in 2011, and a subsequent publication by Janion-Scheepers et al.[4] highlighting the gaps in knowledge regarding soil biota in South Africa.

It is thus essential to establish a platform that encompasses all biodiversity, from a micro- to macroscale, and not only a fragment of it.[5,6] Having a publicly accessible platform for different research sectors to submit and compare taxonomic data of specimens will go far in establishing this inclusivity. Natural history collections and herbaria provide such a platform in South Africa under the umbrella of the Natural Science Collections Facility (NSCF). With the establishment of the NSCF, over 30 million preserved plant, animal, fungal and fossil specimens from more than 40 institutes across the country have been integrated under a single coordinating hub.

A fundamental role of natural history collections and herbaria involves safeguarding type specimens. These are preserved specimens of the individuals that were used to describe and name a species. As well as these, every natural history specimen with good data provides a physical snapshot of a species or community at a particular point in time and space. It is this physical scientific record that makes collections so valuable. The specimens play a vital role in our understanding of biodiversity, evolution, population genetics and the environmental impacts of climate change and pesticide use. This is because historical collections provide baseline data against which modern observations can be compared and from which various mathematical models can be produced. These observations and models can inform the vast majority of our agricultural production, essential ecosystem services for rural communities and agro-ecology systems. In turn, these benefits will support agricultural productivity, improved conservation planning, management of global climate change and maintenance of ecosystem services as demonstrated internationally.[7] All this is only possible if there are records to consult on the pathogens, pest and beneficial species.

Furthermore, the baseline ecosystem data that are documented in collections support the enhancement of dryland agricultural resilience through the improved assessment of climate change scenarios, and identification of research gaps and information systems, amongst others.[8] For example, the first high-throughput sequencing study on soil diversity was published in 2006[9], while the impact of such technologies has led to the discovery and description of a much larger below-ground diversity than was originally expected. The majority of soil organisms is still unknown and it has been estimated that the currently described fauna of Nematoda and Acari represents only 5% of the total number of species that actually exist.[10] Thus the need for collections in documenting and preserving the known and newly discovered biodiversity is vital.

Collections also provide a public platform for decision-making in fields such as quarantine and diagnostic services. The identifications based on specimens catalogued in these collections, and the taxonomic skills associated with collections that scientists provide, are clearly evident from the hundreds of specimen identifications done by NSCF partner institutions over the past 3 years. These identifications are either based on morphological characteristics or Sanger sequence data. This service also supports biosecurity agencies in making decisions about imports and exports, such as whether to authorise, or to request, quarantine, or recommend another treatment of horticultural products, which impacts on food security and the economy. A study by Van den Hoogen et al.[11] includes data based on specimen holdings in the National Collection of Nematodes and has contributed to high-resolution models serving as first steps towards representing soil ecological processes in global biogeochemical models, thus supporting the prediction of elemental cycling under current and future climate scenarios.

Furthermore, the information presented in the national collections assists in identifying taxa that have the potential to be commercially adapted under the regulations of the *National Environmental Management: Biodiversity Act, 2004*. There is already a successful drive by the National Collection of Fungi to support the development of biocontrol agents based on its specimen holdings, with one commercial product already on the market both locally and internationally. The specimens and associated data in the collections can also reduce the monetary losses incurred by South Africa due to unscrupulous bioprospecting and the development of products without proper permits and intellectual property protection.

Data and specimens in national natural history collections and herbaria contribute to spatial planning and decision-making for development. Examples of these include the plan developed with the South African National Biodiversity Institute's Threatened Species Programme for assessing the threat status of biota in areas with ecologically or economically important biodiversity. Other assessments included the National Biodiversity Assessment, and the Department of Environmental Affairs' land-use decision-making tool. These are used by conservation authorities and the national Departments to make decisions on development applications (e.g. infrastructure such as mining, housing, roads). Promotion of specimen data sets amongst national and provincial conservation

authorities, municipalities and consultants for use in impact assessments and decision-making in development applications is another product of data sets based on catalogued specimens in natural history collections and herbaria.

In conclusion, it should also be emphasised that collections of objects often serve us in ways that could not have been imagined at the time at which they were created. Sometimes these unanticipated uses can help solve today's most pressing scientific problems. Likewise, in years, or even decades from now, new analytical techniques will allow researchers to use the same specimens to answer new questions. There are countless examples of 'new' specimens being 'discovered' in collections and recognised as scientifically important long after their original acquisition.

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INTERNATIONAL YEAR OF PLANT HEALTH 2020

The Southern African Society for Plant Pathology: 1962–2020

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Scientific societies play an enormously valuable role in promoting important disciplines. This role is perhaps even greater in countries that are relatively isolated from the rest of the world, such as those in the southern hemisphere, with no long histories of scientific endeavour nor well-established networks of communication. In this regard, scientific societies in South Africa are crucially important; in our view, their value is often under-appreciated. This might also be true for the Southern African Society for Plant Pathology (SASPP), which has drawn together and coordinated the activities of plant pathologists for almost 60 years. In so doing, the SASPP has contributed substantially to the health of plants that sustain agriculture and forestry and food security, and those that constitute one of the most biodiverse and fascinating flora on earth, the Cape Floristic Region.

Reflecting on the history of the SASPP in 2020, the United Nations International Year of Plant Health, is timely and relevant. The Society, then named the South African Society of Plant Pathology and Microbiology (SASPPM), was initially established by a small group of five plant pathologists and microbiologists in 1962. At that time, the fields of plant pathology and microbiology were deeply integrated. This integration stems from the fact that plant diseases are primarily, but not exclusively, caused by microbes such as fungi, bacteria and viruses. Consequently, microbiologists working on plant diseases or microbes that enhance plant growth, operated at the confluence of classical microbiology (dominated by bacteriologists and virologists with interests in animal health and industrial microbiology) and that more closely focused on the biology of plant pathogens and the control of plant diseases. The fact that most plant diseases are caused by fungi and that relatively few microbiologists worked on these organisms, must even in the early days, have warned of potential conflicts of interest amongst the first members of the Society.

Despite complications relating to the boundaries of plant pathology and microbiology, the SASPPM was effectively established and held successful annual meetings (symposia, later termed congresses) commencing in Pretoria in the latter half of 1962. These annual congresses moved between the then four provinces of South Africa, although on one occasion, in 1973, the meeting was held in Salisbury (now Harare), Zimbabwe.

As time passed, the boundaries between microbiology (at least in the classical sense) and plant pathology became increasingly problematic for the SASPPM. This is not surprising given the fact that the two disciplines were growing rapidly in the country and there was a clear difference in the interests between microbiologists working on animal and human diseases, water purity, and industrial processes involving microbes, and those focused specifically on the health of plants. This is illustrated by the fact that from 1962 to 1969, most presentations concerned plant pathogens, whereas from 1970 onwards, presentations on microbiological topics began to dominate congresses. From 1975, presentations at congresses were separated and devoted either to microbiology or plant pathology. This is known to have been difficult for those microbiologists with dual interests and loyalties spanning the two distinct but overlapping disciplines. Arguments ensued as to whether the words 'plant pathology' were fairly positioned in front of 'microbiology' in the name of the society, which was dominated by microbiologists with little or no interest in plant diseases.

As tensions rose between members of the SASPPM, it became increasingly clear that the focus of the Society required substantial change. Thus, at the 1980 meeting held in Bloemfontein, a decision was made by the classical microbiologists to separate from the plant pathologists. They would then establish a new society to become known as the South African Society of Microbiology (SASM). Microbiology was removed from the name of the plant pathology society, thereafter known as the South African Society for Plant Pathology (SASPP).

The split was formally reported to have been congenial. But some current (now older) members of the SASPP remember being shocked by the high level of animosity and anger amongst some senior plant pathologists, who were clearly upset by the changes. The fact that many university departments incorporated both microbiology and plant pathology resulted, at least in some cases, in rifts in relationships between academic colleagues. It is interesting to reflect that, even today, complications relating to the intersections between plant pathology and microbiology persist in some domains.

The SASPP, now separated from SASM, was a much smaller group than had been the case prior to 1981. Some plant pathologists despaired of the split and even felt that there were insufficient members to maintain a viable society. This sentiment was rapidly shown not to be relevant as the SASPP grew in membership, in relevance and in vibrance. Successful congresses were held annually and a decision was made in 1994 to expand the geographical scope of the society to include countries in southern Africa. This led to changing the name of the Society to the Southern African Society for Plant Pathology, still the SASPP.

Congresses and other meetings have always played an important role in the history of SASPP. In a large country where plant pathologists are commonly separated by great distances, these gatherings provided opportunities for colleagues to meet, to exchange knowledge and to establish important collaborations. As is true for all scientific societies, they have also provided the platform for students to meet, to draw mentorship and support from those more experienced than themselves, as well as to establish their own career paths. Consequently, the proposal in 2006 and the final decision the following year for the Society to meet formally only every second year, was deeply debated and not necessarily accepted enthusiastically by all members.

The most relevant argument to hold SASPP congresses only every second year, was that it would afford members the opportunity to attend congresses of other societies relevant to them in the intervening years. In retrospect, this

change to less frequent meetings has not affected society membership or attendance at the now biennial congresses. Looking back, the first congress of the SASPP in 1981 – after the split from the microbiologists – was attended by approximately 100 members. At the time of the last meeting of the Society held at Club Mykonos in the Western Cape in 2019, there were just over 300 members, not substantially different to the prior congress in 2017. Clearly, and contrary to the predictions of some members including one of the authors of this narrative, holding meetings every second year has not had a negative impact on the SASPP. This is possibly due, at least in part, to the fact that tools for communication linked to the Internet and social networking have negated the need for more regular in-person meetings.

All scientific societies and related organisations have the important role of recognising the accomplishments of their members. The SASPP appreciated this fact in 1978 when it established its highest honour, the Christiaan Hendrik Persoon Gold Medal. The medal was named for Christiaan Hendrik Persoon (1761–1836), one of the "fathers' of mycology (the study of fungi) who was born in Cape Town. The globally recognised epidemiologist, JE Vanderplank, was the first recipient of the Medal in 1979. Many years were to pass before another South African plant pathologist, Professor WFO Marasas, was considered worthy of receiving this high honour in 1987. Professor Marasas was at that time employed by the Medical Research Council, and later in his career held extraordinary professorships at both the University of Pretoria and Stellenbosch University. The Medal has now been awarded six times over a period of 33 years and in 2015 was awarded for the first time to a female plant pathologist, Professor Brenda Wingfield (University of Pretoria). In addition to the Christiaan Hendrik Persoon Gold Medal, the SASPP has established many awards and honours that play an important role in promoting excellence and activities across the broad range of areas that constitute the field of plant pathology.

While the fact that plants suffer from diseases is well known, the field of plant pathology as a discipline is poorly known or understood amongst the general public. This is a situation that requires active attention, especially given the importance of plant health and consequently the tremendous threat of pathogens to food and fibre security, clean water availability and the quality of the air that we breath. New and novel plant pathogens that threaten humankind are arising regularly and this trend is set to continue. The currently experienced global health pandemic caused by the novel SARS-CoV-2 virus vividly illustrates the terrible impact that pathogens can have on humanity. Plant pathogens are equally threatening and deserve far more attention than they currently attract. The SASPP, with its long and proud history, has an important role to play in addressing this important goal. And the International Year of Plant Health is providing many opportunities to promote the importance of plant pathology in southern Africa, as well as globally.

INTERNATIONAL YEAR OF PLANT HEALTH 2020

The Plant Disease Pyramid: The relevance of the original vision of plant pathology in 2020

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In 1926, Professor Paul A. van der Bijl made an address to the South African Association for the Advancement of Science in his capacity as President of Section C of the Association, entitled 'Landmarks in the development of the science of plant pathology and of disease control'.[1] The talk itself marks a key moment in the development of plant pathology as a discipline in South Africa, as Van der Bijl had been appointed 5 years earlier as the first Professor of Plant Pathology and Mycology in South Africa, at Stellenbosch University. The field was just being established, not only in South Africa, but in many parts of the world, and its distinction from other disciplines was a matter of pride.

In this Commentary I use the Van der Bijl address as a 'lens of history' to reflect on the state and role of plant pathology today, and what might lie ahead as we approach the centenary of his presentation. In doing so I do not cover all elements of relevance to plant pathology currently, but focus on key issues he raised that influence plant disease development and management, the interdisciplinary and interconnected nature of the discipline, and the role it has in society. Despite the immense progress in knowledge and the power of the tools of the discipline since 1926, it is also necessary to reflect on the reasons why we need an International Year of Plant Health in 2020 (IYPH 2020) – not to celebrate success, but to highlight a growing crisis globally facing food security and environmental health due to the increasing pressure on plant health.

Despite the revolutions in plant pathology since 1926, pathogens and disease continue to outpace our efforts to manage them. The United Nations, in its communication about the IYPH 2020, claims that as much as 40% of global food production is lost due to pathogens, pests and weeds. In hindsight it is perhaps not unexpected news, as we understand clearly today that pathogens will evolve, and will evolve faster, under the strong selection pressure we place on them. In fact, the very measures we have developed over the past century to feed a growing world population, and that have delivered the Green Revolution, have also created an ideal scenario that speeds up the evolution and spread of virulent and resistant pathogens.[2] Clearly there are no silver bullets, irrespective of how extensive our new knowledge may be. There is a need to urgently reassess how we use what we have learned since 1926 and to apply it in a different manner if plant pathology is to optimally contribute to the health and well-being of our society, and our environment.

When reading the address by Van der Bijl in 1926 one is shocked to be reminded of how far our understanding of the biological cause of plant disease has developed since then. For example, at that time it was thought that most plant disease was caused by bacteria and fungi, except for the 'so-called mosaic diseases' for which no infective organism could be defined by the 'highest power of the microscope'. The term 'virus' was used for a potential 'ultra-microscopic' infective agent, but its nature remained obscure (as it did for human diseases such as the Spanish flu). In contrast, today the sequence of every nucleic acid building block of a pathogen can be determined as a matter of routine, we can consider the relevance of the 'pangenome' of a pathogen species, and accurately measure the changes in networks of molecular interactions at subcellular levels across minute time scales.[3,4]

Van der Bijl describes at some length the importance of the interaction between the host, pathogen and its environment in the development of disease – what has since become known as the 'Disease Triangle'. This concept has become deeply embedded in ecological and epidemiological concepts of disease development and has advanced to sophisticated mapping of the changes in these factors over time and space in order to direct management. An even more holistic picture is now emerging, sometimes called a (triangular) disease pyramid, that recognises the role of symbioses (in particular the extended genotype and phenotype of the host through its associated microbiome) in both animal and human disease development.[5,6] I would argue, however, that this picture is still incomplete, and that a fifth dimension – a square pyramid – that considers the influence of human social systems is also needed. This dimension is increasingly recognised for its importance in ecological systems that influence sustainability[7], and cannot be ignored in the management of plant health, because political, economic and cultural factors all directly interact or influence each of the other four factors that are accepted as drivers of plant disease development (Figure 1). Nowhere is this more evident than in two of the major drivers of global disease emergence, namely climate change and the rate of spread of invasive pathogens.

Van der Bijl notes that 'practically all countries have laws and regulations aimed at protecting them against the introduction of serious diseases from elsewhere, as well as against the spread of serious diseases…in the same country'. Unfortunately, these measures have proven woefully inadequate, with multiple waves of invasive pathogens causing devastation to crops and native ecosystems alike since then. In fact, nearly 100 years later, the emergence and spread of invasive pests and pathogens are increasing at a faster rate than ever before.[8] Ecological 'neighborhoods', referring to connected ecosystems, are nearly global for some pathogens. Rapid and repeated spread of pathogens or resistance across continents are now commonplace and create pools of genetic diversity and evolutionary potential in pathogens (a global evolutionary experiment) that is unprecedented. To make matters worse, climate change is increasingly placing unknown levels of stress on plant communities and opening new areas for infection for some plant pathogens (while possibly restricting others). While scientific advice to counter these negative trends is plentiful, their outcome is almost wholly determined by political and economic decisions. These social factors cannot be excluded from disease development, modelling and associated management decisions. What is certain is that we need to prepare to deal with a continued onslaught of emerging pathogens in agricultural and native systems for decades to come (even under the best scenarios).

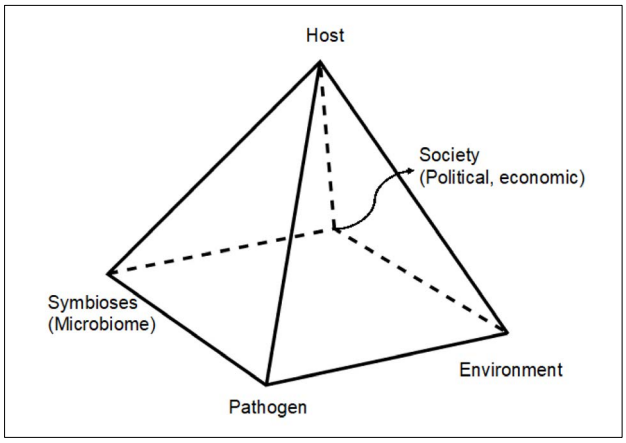

Figure 1: The Plant Disease Pyramid. Factors that influence disease development have been described in the classical 'Disease Triangle' concept. Recent work suggests that a fourth dimension, that of symbioses such as the microbiome are also critical to consider.[5] Here I argue for a fifth dimension, namely the societal factors (cultural, political and economic) that have profound impact on disease development and outcomes on scales from the local to the global. Social-ecological interdependencies are increasingly well defined for sustainable development[7] and would provide a useful starting point to integrate these concepts into disease development models and management forecasts.

Capacity is required to face the enormity of the current global plant health crisis and to implement an integrated systems approach to plant health. One must thus be alarmed by a common reflection in recent years in South Africa in the agricultural sciences, including plant pathology, over the concern for the 'health' of the discipline, mergers and the disappearance of historical departments or faculties, an 'aging' cohort of leading researchers, lower student numbers and reduced financial support. In preparing this article, it was interesting to read the expression of almost identical concerns in reflections from the early 1900s, 1950s, 1970s and more recently, from across the world. Yet breakthroughs in the field now, as in the past, continue to come from universities and research institutes without formalised 'Plant Pathology' units. The problem thus does not seem external, i.e. how university structures or student choices influence the discipline, but how those who understand its importance, and carry the current responsibility and knowledge in the discipline, are able to provide leadership and innovation in developing structures that respond to the current realities. From that perspective I am optimistic when I see many exceptional and innovative young leaders in the field today. As Van der Bijl had to do, those in leadership positions must focus on creating opportunities for the generations to come, as opposed to only for themselves.

The challenge of capacity was even harder in Van der Bijl's time, as he was the only formal plant pathologist appointed at professorial level. The challenge was undoubtedly made worse by the fact that the teaching of plant pathology in 1926 was only available for white 'men ... to be better farmers …, government agents …, teachers … and research workers' (which is a small fraction of the population). There is fortunately a much broader base of capacity in South Africa today, and plant pathology is no longer only seen as merely a 'phase of botany', as Van der Bijl described it. The country has a well-established South African Society for Plant Pathology, and the topic is taught in many universities in South Africa, as it is globally. Yet, in industry and in government, one still often hears of frustration about capacity constraints. Unfortunately, some consequences of a sexist and racist history are also not yet completely eradicated, as in many other parts of the world, leaving much of the true potential talent in the country untapped for the field. As we look to the future, we need to use all the insight and courage we have to tackle remaining hurdles in this regard. Targeted efforts are needed to recruit South Africa's brightest stars, from the youngest ages to undergraduate courses, by exposure to the critical role and exciting options that the field offers. The interdisciplinary nature of plant pathology offers opportunity to target students from a very broad background of original training to enter the field and work as part of interdisciplinary teams.

Throughout his address Van der Bijl refers to the farming community, research community and government as an integrated network dealing with plant diseases – demonstrating that a transdisciplinary, team- and system-based approach is part of the foundation of plant pathology. It is a pity that this team-based foundation of the discipline has given way to a competition driven, individualistic development of the 'PI-lead Lab' approach, and that we have often (as in many fields of science) celebrated the lone figure (often referred to as the 'father') of certain fields or breakthroughs, as opposed to the teams inevitably behind them. Van der Bijl refers to the first university in the world to establish a Department of Plant Pathology, the University of California, Berkeley, where Ralph E. Smith at the time fostered an approach of the 'department as a family working together towards a common end, solving problems that baffle and discourage people…'.[9] A team-based approach not only gives the opportunity to develop a more interdisciplinary scope, but also one that can address complex, real-world problems in a more effective manner, aim for higher quality outputs and impact, and attract a broader range of talent. It is my view that we should be celebrating the success and impact of teams, both as an accurate reflection of actual input and for the sake of the health of the attractiveness of the discipline. It is something to aim for in the years ahead.

The control measures that Van der Bijl was excited about reflect very crude, and sometimes blind, application of chemicals such as the Bordeaux mixture (lime and copper sulfate mixture) at the time. While an increasing understanding of the epidemiology of diseases was starting to direct more sensible use of chemicals, even in 1926, we unfortunately look back on a history since then of very injudicious use of chemicals, with a focus on immediate increases in productivity that ignore longer-term impacts on sustainability. There have been very substantial negative impacts on the environment, and the emergence of resistance to many chemicals in pathogens that limit choices for future use. There is a very real possibility today to apply these chemistries with precision in time and space, and in combinations with various others tools, in ways that minimise waste, optimise impact and consider plant health as part of a holistic system. In addition, the diversification of crops (for both nutritional and disease management reasons), unlocking of genetic sources of resistance in wild populations through genetic engineering, and the increasing use of biologicals for management, amongst other options, hold potential to contribute even more to disease control in future. We need to learn from the past that none of these will be a silver bullet or provide permanent solutions. Rather, a continuously adaptive and resilient system that can buffer against shocks such as pandemics, and that can continue to evolve as various elements of the 'five dimensions' of disease development and management change (Figure 1), should be the aim of plant pathologists, in collaboration with other fields and sectors of society.

One of the most neglected areas of plant pathology in South Africa is the systematic capture of information about disease outbreaks and impact. Van der Bijl expresses the hope that the newly established 'divisions of Agricultural Economics and Agricultural Extension' will gather more systematic knowledge on the impact of plant disease. Sadly, this has not happened and data on the impact of disease on yield, its geographic variation, changes over time and other vital information are not available for most plant diseases in a systematic manner in South Africa today. Information that is available internationally through bodies such as the FAO and CABI is often lacking in detail, especially from Africa. International efforts have recently been launched to attempt to address some of these knowledge gaps and it is important that plant pathologists in South Africa and Africa participate in these to unlock hidden and collect missing information (see for example the project on the Global Burden of Crop Loss; www.croploss.org). It is even more urgent than it was in 1926, given increasing pressure and resource constraints, that South Africa develops strong national pest and disease information systems for its local planning and forecast.

While the description of a squared disease pyramid (Figure 1) as a fundamental basis for plant health management might be relatively new as a concept, the importance of every one of the five dimensions I mentioned was described and discussed in Van der Bijl's address in 1926. We have clearly come a long way since then in our ability to characterise plant disease at the finest scales, and have enormously powerful tools to target and manage these diseases. The same can be said for human disease. Yet, this article is being written in a time when the world is facing the pandemic caused by the SARS-CoV-2 virus. More knowledge and more powerful tools do not necessarily translate into better management over time. It is essential in the years that lie ahead that plant pathologists consider how to deploy the tools at their disposal in ways that reduce the opportunity for evolution and spread of pathogens. As with human disease we have a long way to go to increase connection, representation, reach and impact of the powerful tools at our disposal to secure the nutrition and fibre the world needs, as well as our planetary health. A well-functioning and structured network of collaboration amongst government departments, research institutions, universities and industries is even more necessary today than it was in 1926 to manage national plant health, and should be the primary concern for plant pathologists today. Knowledge only becomes powerful when it is used.

The collision of the COVID-19 pandemic and the IYPH 2020 has wreaked havoc on a year of meetings and workshops aimed at dealing with the global crisis facing plant health management. This collision, however, also offers an opportunity to capitalise on the greater societal understanding of the threat of globally spreading diseases, and the need to be prepared and to invest in the resilience of health management sciences. It demonstrates that the capacity to respond to such a crisis is not a tap you can turn on, but rather a reservoir one needs to build to feed the tap when the crisis hits. It would be a mistake, however, to think that the message will come across automatically. As an example, there have been at least four global flu pandemics since the Spanish flu of 1918, and yet society and its political machinery seemed to quickly forget their warnings. Keeping society informed, and policies implemented, fighting for resources for training and research to reduce the devastation that invasive plant pathogens can cause to our livelihoods, our food safety and our environment, will require an ongoing effort and dedicated leadership.

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James E. Vanderplank: South African and globally recognised plant pathologist

James Edward Vanderplank, best known to plant pathologists globally simply as 'Vanderplank', is widely regarded as one of the world's most influential plant scientists. This recognition stems from his reputation as the founding father of modern quantitative plant disease epidemiology.[1] Professional plant pathologists and students in the discipline are familiar with Vanderplank's work as it provides a theoretical framework to study disease epidemics and breeding for disease resistance. The United Nations declaration of 2020 as the international Year of Plant Health provides an apt opportunity to reflect on the contributions Vanderplank has made to both plant pathology and plant breeding.

Vanderplank was born in 1908 in Eshowe (KwaZulu-Natal, South Africa). He was the youngest of four children. His father, Walter, was a solicitor and his mother, Agnes, a nurse. Although his name might suggest otherwise, he came from an English background[2], with his surname having been anglicised in the 18th century to Vanderplank. His British ancestors were of Flemish–Belgium descent.[2] His grandfather, John Vanderplank, emigrated to South Africa in 1838 and was responsible for bringing *Acacia mearnsii* (black wattle) seed into South Africa from Australia in 1864, to be used for the production of tannins needed for the leather industry.[3] Vanderplank attended Eshowe Primary School and completed his secondary education at Durban High School for Boys. It is intriguing to know that, prior to his retirement in 1973[1], he used van der Plank, the Dutch form of his surname, but in his subsequent publications, he chose to present his name as Vanderplank. This has led to some confusion in the literature with scientists unsure whether these two names refer to the same person.

After his primary and secondary education, Vanderplank obtained a BSc from the University of Natal in 1927. He completed a MSc in Botany (1928) under the supervision of Prof. J.W. Bews at Natal University College (now the University of KwaZulu-Natal) who taught him plant ecology. Known to be a great admirer of Bews, Vanderplank commented that the knowledge he acquired from Bew's teachings was used in a chapter of his 1975 book *Principles of Plant Infection*.[2]

In 1928, Vanderplank was appointed as a mycologist in the South African Department of Agriculture. He continued his studies and obtained a second MSc in Chemistry (1932) from Rhodes University College where he was taught by Prof. JLB Smith, later to become the famous ichthyologist. Vanderplank suggested jokingly in his autobiographical preface in the *Annual Review of Phytopathology*[2] that Smith probably left chemistry to study fish after the traumatic effect of teaching him. The following year he was awarded an 1851 Exhibition Scholarship to undertake a doctorate in Graz, Austria. However, because Hitler had just assumed power in Germany and Nazi terrorism had begun to destabilise Austria, Vanderplank entered a PhD programme in botany specialising in physiology at Imperial College London. His PhD, awarded in 1935, was supervised by the renowned Prof. Vernon Blackman FRS and focused on plant photosynthesis and the biosynthesis of sugars.

After completing his PhD, he returned to South Africa and to his employment in the Department and met and married Elsa Niemeyer, a botanical artist. He was then transferred to the Low Temperature Laboratories at the Cape Town docks as a biochemist to work on the preservation of fruit for export. While there, he developed a method of bleaching oranges to remove rust spots and this formed the basis of his second doctoral thesis in chemistry, conferred by the University of South Africa in 1944. Vanderplank returned to Pretoria in 1941 and began his research career in plant pathology. In 1958, he became Chief of the Division of Plant Protection. He was appointed director of the newly established Plant Protection Research Institute in 1962 and remained in that position until 1973[1] when he retired.

Much of Vanderplank's career was spent doing what he termed 'down-to-earth' potato breeding.[2] During World War II, seed potatoes were in short supply as they were imported from Scotland. Vanderplank persuaded the South African government to allow him to establish a potato breeding programme. This research was undertaken in greenhouses at Vredehuis, close to the Union Buildings in Pretoria, as well as at the Vaalharts Irrigation Scheme. While in Pretoria he was known to meet his visitors in dirty overalls after planting potatoes. They often mistook him for a labourer and he appeared to delight in this anonymity. One author (MJW) remembers as a young and inexperienced plant pathologist being introduced to Vanderplank during a Vredehuis morning tea break and expressing his delight in having the honour of meeting the famous man. Vanderplank simply answered with the single word 'baloney'. This illustrates his understated nature and his down-to-earth humility.

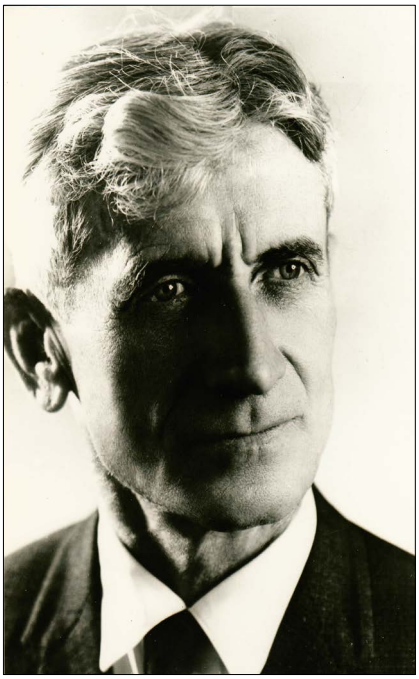
Vanderplank was particularly proud that one of his potato cultivars was named after him. He had predicted that, in the future, 60% of all potatoes grown in South Africa would come from his breeding programme.[2] Although true at the time, owing to substantial subsequent advances in potato breeding, this is no longer the case. Yet the cultivar 'Van Der Plank', known for its excellent eating and processing qualities, remains the most popular early maturing potato variety in South Africa.

While plant breeders and potato growers in South Africa know of Vanderplank for his potato breeding, his global fame rests on introducing a theoretical framework to our understanding of plant disease epidemics and disease resistance. Vanderplank was often heard to say that he had never attended a single course in plant pathology, genetics or plant breeding. To his many followers, this was truly remarkable given his later reputation as one of the world's most highly recognised scientists in all three of these fields. His interest emanated from the work he conducted after formal office hours and during his holidays, analysing the published research work of others. He regarded himself as a 're-viewer of evidence'[2].

Vanderplank's interrogation of previous studies led him to interpret the published data such that he often arrived at conclusions different from those of the original authors. Based on these views, and his alternative hypotheses, his first, and arguably his most famous book, *Plant Diseases: Epidemics and Control*[4], was published in 1963. This book has been reprinted several times and has been cited at least 2800 times. More importantly, it was often used as a textbook, which over the years has introduced scores of plant pathologists and plant breeders to the world of plant disease epidemiology.

Vanderplank's particular contribution in 1963 was that he was the first person to propose a 'unifying' theory on plant disease epidemiology.[3] Essentially, he suggested that epidemiology represents the science of diseases and pathogens in populations. These ideas were not only new and applicable to plant pathology but also to the broader field of epidemiology and across many fields of biology. Importantly, Vanderplank used mathematical models, such as the monomolecular and logistic equations and infection rates, to quantify the relationship between the amount of inoculum and disease progress. Although his descriptions, including the simple and compound interest analogy, were an oversimplification of monocyclic and polycylic development of a disease over time and space, they undoubtedly inspired research on disease dynamics.[5]

Vanderplank established the terms 'vertical resistance' and 'horizontal resistance' in his 1963 book and extended this concept in 1968 in *Disease Resistance in Plants*.[6] These remain fundamental principles of plant pathology and terms crucial to the field of disease resistance breeding. Vertical resistance refers to when a plant variety/cultivar is bred to have complete resistance to particular races or strains of a pathogen which is controlled by a single gene in the host. By contrast, horizontal resistance refers to when a plant variety/cultivar is bred to have a general level of resistance, or incomplete resistance, to many races or strains of a pathogen, which is controlled by multiple host genes. These concepts of horizontal and vertical resistance were debated by many researchers in the fields of plant pathology and plant breeding.



James E. Vanderplank [photo courtesy of Elspeth van Duuren]

Vanderplank's concepts and ideas not only fuelled debate but more importantly forced many researchers to conduct detailed experiments and to consider their data differently. An appropriate example can be found in the landmark paper by Parlevliet and Zadoks[7] who convincingly showed that horizontal and vertical resistance do not represent different forms of resistance but are a continuum. Using more detailed analyses, they showed that horizontal resistance can involve specific multiple additive interactions, but in some cases can also be conferred by a single major gene. They also suggested that the value r (rate of infection), as used by Vanderplank, is an inaccurate measure for quantitative resistance and certainly of no value for breeders because its assessment is quite laborious.[7] A more commonly used method today is to measure the area under the disease progress curve. These examples illustrate how notable plant pathologists utilised and tested Vanderplank's theoretical framework when dealing with host plant resistance, thus substantially advancing the field of plant disease epidemiology.

While Vanderplank's theories and concepts may not be applicable to all pathogen systems, he clearly incited plant pathologists to question his views. However, the challenger needed to be prepared with rigorously analysed data or face Vanderplank's often-used response 'please go and do the experiment and prove your point'.

Vanderplank's 1963 book introduced the 'vertifolia effect' in which horizontal resistance is eroded during breeding for vertical resistance. The story behind this demonstrates his penchant for reading. He is known to have conceived the 'vertifolia effect' during his visits to the library in Wageningen (the Netherlands) during an international potato conference held in that city. Publication of his 1968 book led to intense and heated debates amongst the scientific community, with many researchers intent on proving or disproving his theories regarding plant resistance to disease. He was later shown to be fundamentally incorrect regarding horizontal resistance because it cannot be proven experimentally.[8] The terms 'vertical resistance' and 'horizontal resistance' are used less frequently today but race specificity and race non-specificity remain central concepts in the quest for durable resistance in crops.

By the late 1960s, Vanderplank was globally recognised and widely sought after as guest speaker at international congresses. He preferred not to travel and was known to have declined many opportunities and invitations that could have added to his stature. In addition, the peak of his fame came at the same time as the height of South Africa's apartheid era. The academic boycott led to his being prevented, on at least one occasion, from presenting an invited lecture in the Netherlands. Knowing of his country's pariah status might, in part, have influenced his reticence to speak at international meetings or to receive awards when these were suggested to him.

After 1968, Vanderplank published three additional books: *Principles of Plant Infection* in 1975[9], *Genetics and Molecular Basis for Pathogenicity* in 1978[10] and *Host-pathogen Interactions in Plant Disease* in 1982[11]. In the last of these, he extended and elaborated on some of his earlier basic ideas and re-argued the basis of resistance and the development of epidemics. There were several objections to his ideas and innovative theories. It has been suggested that his later books were less worthy and detracted from the impact of his first two and most important contributions. This seemed not to bother Vanderplank and it certainly did not diminish the 'greatness'[12] of this South African scientist with a remarkable mind.

In 1966, Vanderplank spent 6 months in the Department of Plant Pathology at Pennsylvania State University (USA) working with Prof. C. Wernham. At that time, the Department had just initiated a programme in plant disease epidemiology. He was that University's first distinguished visiting professor, and he found that he had both admirers and adversaries of his theories. He also undertook study leave at Wageningen Agricultural University in the Netherlands, with Prof. J.C. Zadoks in 1968, and with Prof. J. Kranz at the Justus Liebig University, Giessen, Germany in 1979. Zadoks and Kranz were great admirers of Vanderplank's theories on plant disease epidemiology. Zadoks and Schein[1] expressed their admiration for him by stating that 'he changed the faces of two sciences, plant pathology and plant breeding'.

Owing to the general acceptance of his theories on quantitative plant disease epidemiology, Vanderplank received numerous awards, both in South Africa and abroad.[11] Amongst others, they included the Junior and Senior Captain Scott medals (1928 and 1948, respectively) from the South African Biological Society. In 1979, he was awarded the

Christiaan Hendrik Persoon Gold Medal from the South African Society of Plant Pathology and Microbiology. This was the first time that this award, the most prestigious of the Society, was conferred. Given his huge accomplishments, 8 years were to pass before another member of the now Southern African Society of Plant Pathology was considered deserving of this honour! Vanderplank also received the Ruth Allen Award from the American Phytopathological Society in 1978 and was awarded honorary doctorates by the University of Natal (now the University of KwaZulu-Natal) and the Justus Liebig University, Giessen, Germany. To celebrate Vanderplank's 80th birthday in 1988, a special issue of the *international Journal of Plant Diseases and Protection* (Vol. 93), edited by Jűrgen Kranz, was published to honour him.

Vanderplank (middle) at the ceremony in Pretoria where he received the prestigious Stakman Award with Prof. Mike Wingfield (left) and Prof. Mike Martin (right) of the University of KwaZulu-Natal.

Vanderplank was regarded as a 'superb lecturer'[3] even though he tended to come across to his students and staff as shy and claimed not to relish the public spotlight. This was illustrated when one of us (MJW) was tasked with arranging an appropriate ceremony for Vanderplank to receive the prestigious Stakman Award, conferred by the Department of Plant Pathology of the University of Minnesota. Vanderplank had reluctantly agreed to attend the ceremony in Pretoria (not in Minnesota), but he had made it clear that guests on the occasion, which included an emissary (Prof. Chet Mirocha) from the University of Minnesota, should be informed that he would not speak, but merely say 'thank you'. It therefore came as a surprise to everyone present when he spoke at some length, even regaling the audience with anecdotes from his career. One of these included his explaining that as a chemist with the Department of Agriculture during World War II, he was instructed to produce chocolate with a high melting temperature so that it could be shipped to the South African troops fighting in the hot Egyptian desert. He suggested in his address that this might have been his greatest scientific accomplishment, and then joked that it could even have been the origin of chocolate chip cookies!

Zadoks and Schein[1] describe Vanderplank's personality as modest and courteous. He was well aware of his status as a globally recognised scientist, and was considerate of opposing points of view, even when he disagreed with them. James Vanderplank passed away on 2 June 1997 after sustaining fractures after a fall at his home in Pretoria where he had lived all his working life with his wife Elsa and their two children, Elspeth and Adrian. He had a brilliant mind, and his name endures in the plant pathology halls of fame with other great leaders in this field. It is also a fitting tribute to remember him in the International Year of Plant Health.

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Lessons from a major pest invasion: The polyphagous shot hole borer in South Africa

The arrival and establishment of invasive forest pests can cause devastating environmental damage and great economic impact. For example, the cost over the past decade of dealing with the arrival of a single invasive beetle in the USA, the emerald ash borer (*Agrilus planipennis*), is estimated at more than USD10 billion.[1] Originating from Asia, this beetle has killed hundreds of millions of native ash trees since it became established in the USA. However, this beetle is but one of hundreds of invasive insect pests that impact forests in the USA, and that contribute to a global tree health crisis caused by invasive insects and pathogenic microorganisms.[2-4]

South Africa is no different from other countries and is experiencing an increasing rate of introductions of damaging forest pests.[5,6] These invasions are largely unintentional and are a by-product of globalisation and increasing global trade.[7,8] The movement of living plants and plant products, including untreated wood packaging materials (i.e. pallets, dunnage and crating), is known to be a major pathway for these pests.[9,10] For clarification, in this commentary we use the terms 'insect' and 'pathogen' to distinguish between the two types of organisms, although we also use the general term 'pest' to refer to both groups. The term 'invasive pest' is used for introduced species that, in addition to maintaining a self-sustaining population, show evidence of spread and impact.

Millions of years of co-evolution between plants and their pests has led to close ecological dependencies. Damaging outbreaks of native pests in their natural environments are rare; instead, pests play a vital role in shaping the dynamics and diversity of natural ecosystems.[11] For this reason, forest pests are often not known to be problematic, or not even known to science, prior to their arrival and establishment in a non-native environment. However, on their arrival, alien pests may encounter suitable native hosts that lack co-evolved resistance, with the potential for devastating consequences. Well-known examples in addition to that of the emerald ash borer in the USA, include chestnut blight (caused by *Cryphonectria parasitica*) in the USA and Europe, and *Phytophthora cinnamomi* in southwest Australia.[1,12,13] These pests and others have fundamentally altered ecosystems, with entire tree species being practically eliminated from invaded landscapes. In addition to these dramatic changes in forest canopy composition, wide-ranging ecological impacts may result following these invasions. These impacts include significantly altered species richness and abundance, and the loss of important ecosystem services.[14]

The International Plant Protection Convention provides for the protection against invasive pests through the International Standards for Phytosanitary Measures. These measures are acknowledged by the Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organisation.[15] Under current protocols, Pest Risk Analysis is the mechanism by which an organism can be recognised as a potential threat that requires regulation. An important issue arises: in order for an organism to be subject to regulation, it must be named and known to be harmful. In addition, phytosanitary inspections (by both exporting and importing countries) are typically visual, and therefore inadequate for detecting asymptomatic infections or infestations.[16] Consequently, these pests have the potential to avoid interception at check points.[17]

Given the shortfalls in current biosecurity regulations, there has been a move to use sentinel trees (trees planted outside of their natural range) to identify new and emerging pest risks.[18] As hubs of human movement and traded goods, urban areas are frequently the first point of contact for alien pests.[19,20] Therefore, when located in urban environments, monitoring of sentinel trees also provides an opportunity for early detection of recently arrived pests.[17]

A sentinel project supported by the South African National Biodiversity Institute was initiated in 2016. In 2017, this project led to the discovery of the polyphagous shot hole borer (PSHB, *Euwallacea fornicatus*) in a national botanical garden in the country.[21] A tiny ambrosia beetle, 2 mm in length and native to Southeast Asia[22], the PSHB has a symbiotic relationship with three species of fungi, including the tree pathogen *Fusarium euwallaceae*. This fungus provides a food source for the beetle and its larvae, but, in susceptible trees, it kills the vascular tissue, causing branch die-back and tree death.

Since its initial discovery, the PSHB has spread rapidly. The beetle is now well established in South Africa, with its presence currently confirmed in all provinces except Limpopo. Worryingly, its host list also continues to grow. Over 100 tree species have been attacked in South Africa.[23] Not all of these are threatened with rapid death. Usually only trees in which the beetle is able to breed – so-called 'reproductive hosts' – are in immediate danger. To date, 36 host species (including exotic and indigenous trees) have been found to support PSHB reproduction in South Africa. This number will continue to grow as the beetle spreads to new areas and encounters new hosts. Importantly, some 'non-reproductive' hosts may become reproductive under certain environmental conditions. This makes compiling host lists problematic and the outcome of infestation difficult to predict.

The greatest impact of the PSHB invasion observed to date has been in urban environments. These, for example, include the outbreaks in Johannesburg, George and Knysna. A similar situation has been observed in two other countries invaded by the PSHB – the USA (California) and Israel[24,25] – where the PSHB went on to emerge as a damaging pest to the avocado industry and trees in natural ecosystems. For this reason, there is concern regarding the possible impacts that the PSHB will have on economically important tree crops including avocados and plantation acacias in South Africa. But the threat to natural ecosystems is the most worrying. There are already indications that indigenous tree species at the fringes of infested urbanised areas are susceptible to PSHB infestation.

Shot hole borer beetles can be seen in the gallery of this tree.

Nearly three years have passed since the detection and reporting of what is arguably the most damaging tree pest to ever arrive in South Africa. An invasion of this magnitude should have elicited a rapid response and the development of a strategic action plan. However, South Africa has never before had to deal with a tree-killing pest of this importance. Moreover, with limited resources available and confusion regarding which government department should take responsibility, a coordinated response has failed to emerge.

In South Africa, the management of agricultural pests falls under the Department of Agriculture, Land Reform and Rural Development (DALRRD), specifically Plant Health Early Warning Systems. Where a pest is deemed an 'emergency plant pest' (EPP), the South African EPP Response Plan provides for a rapid response to prevent establishment and spread, and coordination of communication between government agencies, academia and plant industry professionals.[26] A second piece of legislation, the *South African National Environmental Management: Biodiversity Act* (*NEMBA, Act 10 of 2004*) Alien and Invasive Species Regulations, provides for the listing of invasive alien species that threaten biodiversity.[27] This Act is overseen by the Department of Environment, Forestry and Fisheries (DEFF), and contains explicit guidelines for the development of monitoring, control and eradication plans.

The PSHB presents a unique challenge in that it crosses boundaries between agriculture, commercial forestry, natural forests and urban trees.[28] It has been detected on backyard avocado trees and roadside weedy acacias, but not yet in commercial avocado orchards or plantation forests. The only commercial crop on which PSHB has been detected to date is pecan, although early indications suggest the impact to this tree species may not be high. The PSHB has not yet been declared an agricultural EPP and no formal response has been triggered. This may be a consequence of the PSHB not yet appearing as a pest in commercial settings.

Similar challenges have been encountered in regard to listing PSHB under NEMBA. Despite submission (in November 2018) of a detailed pest risk analysis, the process by which listing is facilitated, its addition to the NEMBA list is yet to be finalised. Perhaps the lack of empirical evidence for the impact of the PSHB in natural ecosystems resulted in a reluctance on the part of the then Department of Environment Affairs to take full responsibility for the management of this pest. The recent relocation of the Forestry portfolio from the previous Department of Agriculture, Forestry and Fisheries to the now combined Department of Environment, Forestry and Fisheries may resolve this matter, as two portfolios which are threatened by this pest, now reside in the one department.

The major impact of the PSHB has been in urban environments, which leads to the question of where the responsibility for management of urban forests lies. In terms of government policy, urban forestry is mentioned briefly in the Forestry White Paper, prepared in 1996 by the then Department of Water Affairs and Forestry.[29] The National Forestry Action Programme[30] also provides for some recognition of urban forestry. Despite the existence of these policies, it seems urban forestry is poorly represented in South Africa, and a dedicated research and advocacy focus is lacking.[31] Consequently, in the absence of a national strategy and with no clear structure in place to guide their response, most municipalities have understandably struggled to adequately manage this threat to the urban forest.

There is also the complication of understanding the extent to which local governments are responsible for urban forests within their municipalities. For example, a Public Road and Miscellaneous By-law gazetted by the City of Johannesburg Metropolitan Municipality forbids any damage to trees on any public road within the municipality (no lop, top, trim, cut down or removal of such trees) without prior written permission of the Council.[32] Realistically, however, urban forestry is likely a low priority for local government in urban areas, where issues such as the provision of housing, potable water, sanitation and other services remain priorities.[31] Given the limited financial resources available to local government to manage urban forest issues, there is a clear need for leadership and support from national government.

Dealing with the arrival of damaging invasive forest pests is challenging anywhere in the world. Australia, a country associated with a strong stance on biosecurity, is still grappling to provide a coordinated government response to the myrtle rust incursion, 7 years later.[33] It is perhaps not surprising, given the challenging nature of dealing with such incursions, that the listing of the PSHB and the development of a national strategy for response to this pest has not yet occurred. But this delay has not been without consequence.

In the absence of a nationally coordinated strategy, and without strong stakeholder and public engagement, a vacuum has been allowed to develop. This vacuum has provided a space in which opportunists could appear, ready to tout expensive and unproven treatments as the solution. It has also resulted in the public being exposed to conflicting messages, leading to confusion as to the most appropriate action to take in response to infestations. In the meantime, the beetle has continued to multiply and spread unchecked across the country.

Experiences from California, Israel, and South Africa indicate that the management of the PSHB is particularly challenging. This is largely due to its inbreeding mating system, wide host range, and ability to survive in felled wood for many months. Heavily infested reproductive hosts become 'reservoirs' of beetles, threatening the health of adjacent trees. Therefore, current best practice recommends removal of heavily infested trees in which beetles are breeding. Infested wood should be disposed of appropriately at designated dumping sites. Applying sanitation treatments such as chipping, composting, solarising or kiln-drying infested wood will greatly reduce the risk of further spread of the PSHB to new environments.[34]

Treatment trials conducted in California suggest chemical control may have an application in protecting individual high-value trees. But this should not be seen as a 'silver bullet' for the problem. Ambrosia beetles (such as the PSHB) have cryptic habits and are notoriously difficult to control using pesticides. They spend little time on the tree surface and only ingest small amounts of wood, limiting their contact with pesticides.[35] In addition, research from California suggests treatments may only be effective when applied either as a preventative measure or during the very early stages of infestation.[35,36] The duration of therapeutic effects following pesticide application are finite, with repeated applications required over time. Therefore, cultural practices such as removing dead and dying trees and the sanitation of infested wood remain the most important management tools.

The development of a consolidated national management strategy and action plan for the PSHB is crucially important. This strategy should be prepared in conjunction with strong stakeholder engagement, and intergovernmental coordination between the relevant government departments (DALRRD and DEFF) must be ensured. A well-coordinated public awareness campaign informing local government, residents and stakeholder groups about the beetle and its impact must be an essential component of this strategy. Municipalities should be encouraged and supported to remove and destroy heavily infested reproductive host trees. Staff from affected sectors (including private, municipal, provincial and national parks and gardens, and landscaping, nursery, tree felling, farming and forestry industries) need to be trained to identify and appropriately handle PSHB-infested material. There should be a strong emphasis on the dangers of moving untreated infested wood, and best

management practices for disposal of infested wood must be developed and promoted.

In the USA, campaigns such as 'Buy it where you burn it' have been strongly promoted to educate the public about the risk of movement of invasive forest pests by long distance transport of wood. The campaign promotes the local buying and burning of wood to limit the movement of firewood. While this campaign has been effective to some degree in the USA, managing the movement of wood in South Africa may prove more challenging. Within South Africa, the informal urban firewood trade is widespread and provides an important energy source and income security for many poorer urban residents.[37] The movement of infested wood through informal trade will be an important pathway of internal spread of the PSHB. However, managing this pathway has the potential to impact traders directly (through loss of access to harvestable wood), as well as to impact those who rely on this wood for cooking and heating. Careful consideration will need to be given to this issue.

The Forestry and Agricultural Biotechnology Institute of the University of Pretoria, where the PSHB invasion was first recognised, has initiated numerous baseline studies on the pest. But these have largely been restricted to surveillance and monitoring. Importantly, a multidisciplinary and multi-institutional Polyphagous Shot Hole Borer Research Network has recently been established to extend work on the pest. The network will coordinate research efforts undertaken by researchers from various institutions across the country. Current projects are investigating invasions in urban, agriculture and natural areas, and the search for a biological control agent will soon be initiated. Key to this network is a cross-sector collaborative approach. Now with funding made available by DEFF, further studies can be conducted under the framework of this network to underpin science and knowledge-based advice and policy processes.

While we hope that South Africa will never again have to see the arrival of a pest as damaging as the PSHB, the reality is that there seems to be no end to the accumulation of alien species worldwide.[38,39] Of note is that we find ourselves in this, the International Year of Plant Health, facing a global threat to human health due to the COVID-19 pandemic. Many parallels can be drawn between the emergence of novel diseases of humans and invasions by forest pests. Not least of these is that they are both largely a consequence of human activites. There is strong evidence linking the role of anthropogenic disturbance as a major driver of emerging infectious diseases of both humans and forests. Moreover, the unprecedented level of global connectedness via trade and travel networks, facilitates the rapid spread of pathogens responsible for human and tree disease pandemics more than ever before.[40,41] There is a very strong economic case for investing in strategies to reduce the threat posed by future pandemics to both human and forest health. It is clear that we require a greater capacity to predict, prevent and respond to emerging infectious diseases and forest pest invasions, and cross-disciplinary and global collaboration will enhance our ability to achieve this goal.[40-42]

We would do well to seriously reflect on the shortcomings of how we as a country have responded to the arrival of the PSHB and learn from them. Importantly, South Africa needs to be able to deal with new invasions more effectively in the future.

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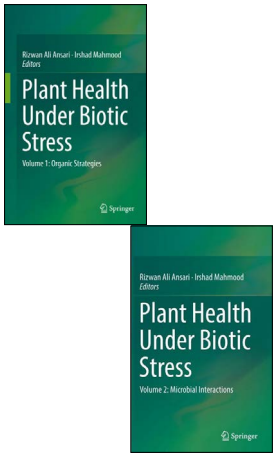

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Green actions for better plant health

Plant health is a planetary issue with major implications for global welfare. Beyond the anthropocentric vision we tend to profess, plants are essential for our lives: we need them to breathe and to eat. Healthy plants are essential for providing these extremely basic human needs. Thus, we need to keep our plants safe from diseases that can impact on humanity's food security and safety.

In the two volumes of *Plant Health Under Biotic Stress,* edited by Dr Rizwan Ali Ansari and Dr Irshad Mahmood, important aspects relative to plant health are presented. Overall, these two books make a compendium of articles focused mostly on promoting plant health ranging from soil amelioration and care to the application of older natural actions and biotechnological approaches. They invite us to consider that a good substrate and knowledge of plant physiology will provide better fundamental health in important plants and cultivars. Along with the information in these chapters, we are given several recommendations to provide sustainable plant health. Moreover, these books provide interesting information through specific examples and synopses that can orientate state-of-the-art knowledge to minimise biotic stress. Three main actions are suggested: the use of genetic techniques and biotechnological systems; the application of beneficial microorganisms; and following agronomic practices that might lower the impact of harmful chemicals.

Organic strategies

The first volume presents distinct approaches, homing in on the different organic and natural applications of distinct organic material and focusing on particular cases of cultivars. Organic strategies are conceived for ameliorating soil to enhance the production of a cultivar that would reduce the use of agrochemicals, as well as for other ecofriendly practices with the same objective. Organic amendments are considered to be animal manure, municipal biosolids and septage, green manure, food residues and wastes, and compost. The application of these organics impacts differently on the physical, chemical and biological properties of the soil, promotes a healthy environment for plants and fosters plant health through plant biomass promotion and plant disease management. Nevertheless, these materials can be costly to prepare before they can be used in agriculture; microorganisms that might bear non-desirable effects for food safety must be removed, and the material must be in a suitable form to avoid negative consequences on the physical and chemical properties of the soil. This volume also presents other agrofriendly management ideas on practices that mimic the action of agrochemicals. For instance, the grafting process has a similar effect on the plant's defensive response to applying agrochemicals in inducing oxidative enzymes. The application of botanicals and antagonistic fungus is a green option to avoid the harmful biocides to combat biotic stress, especially in the tropics where the high temperatures and humidity may favour the proliferation of fungi that may be pathogenic. In general, there is growing interest in integrated pest management that allows for equilibrium in the ecosystem, but landholders also need to earn their living and obtain a profit for their work. The example of the action against rice blast caused by *Magnaporthe oryzae* explains how one might go about this by integrating several approaches like the integration of biocontrol agents (ex. *Trichoderma viride* or *Pseudomonas fluorescens*), botanicals (for example the application of an extract of *Atalantia monophylla*), nutrition, water and the controlled use of chemicals. Kumar and Ashraf (Chapter 5) also support the use of biotechnological approaches and the search for resistant cultivars. Taking all these 'green' considerations into account, it is easy to outline the urgency for increasing research for food safety and security. Each plant–pathogen interaction has a different treatment, even between the same species, while the selection of resistant varieties is also an option for implementing plant health. Moreover, another chapter emphasises the relevance of this 'green' health in improving quality traits for the final product that reaches the consumer. These include organoleptic and nutritional characteristics, and hygienic/sanitary and commercial aspects. At this point, the authors do not neglect the combination of different stresses and their consequences on plant health. Plants try to live in equilibrium with their environment and must respond to, or to be immune to, potential pathogenic pests. Forecasting may introduce a disequilibrium in the ecosystem and plants must respond to an excessive inoculum or population produced by the changes in humidity and temperature, or weakness of the plant due to a hostile environment (salinity, drought) may be a good trait for phytopathogenic agents to colonise plants.

Microbial interactions

Plants are not alone in their environments. They are surrounded by different microorganisms living in the rhizosphere and the rhizoplane sites that are in continuous dialogue. The second volume presents different approaches for biocontrol tools based on the use of endophytic bacteria, biofilmed biofertiliser, and beneficial fungi like the genus *Trichoderma*. Along with the chapters, the reader will find a review of the different mechanisms used by all these biological tools in ameliorating the impact of biotic stress. Despite being a beneficial known interaction there is still a long way to go. The efforts for sustainably handling biotic problems are described in these chapters. Concepts such as phytoremediation, mycorestoration and rhizoengineering are explained. The primary focus is on the application of promoting growth-promoting rhizobacteria (PGPR) and their role as a biostimulant, in phytoremediation, and in biocontrol, with a long list of already known and registered bacteria for agricultural use. The study of mechanisms by which the endophytes can promote health in plants is of great interest because it allows for the introduction of policies for improving food production and environmental health. When scrutinising the table of mechanisms proposed by Tewari et al. (Chapter 10), the complexity of these biocontrol actions is made clear. These findings point to a need to prioritise future studies and to contemplate more complex situations than two-way interactions, like plant–pathogens or plant–endophytes. The scenario is becoming increasingly challenging as the players in this drama are not acting alone, but within a net of interactions together with abiotic factors that will determine the final result in overcoming the biotic threat.

INTERNATIONAL YEAR OF PLANT HEALTH 2020

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NSTF-South32 award for Plant Health: Honouring excellence and celebrating Professor Mike Wingfield

Professor Michael (Mike) Wingfield received the 2019/2020 NSTF-South32 Special Annual Theme Award: Plant Health on 30 July 2020 for his commitment to fostering a collaborative and innovative environment in this field of science. I had the privilege of interviewing him shortly after the award ceremony, and he was gracious and friendly in answering my questions.

Wingfield was responsible for establishing the first integrated forest pathology programme in South Africa, providing laboratory facilities and a working environment for many postgraduate candidates. In his 20 years as Director of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, he conducted research that led to the identification and diagnosis of many organisms causing diseases of plants. Wingfield has studied their pathways of movement to understand their global impact and the management of plant health problems caused by harmful organisms. He has partnered with industries, both locally and worldwide, to resolve plant disease problems, and has fostered collaborations with distinguished scientists internationally, particularly championing the development of new technologies. Together with students and colleagues, his work has led to the successful identification of pathogens and pests, leading to improved quarantine strategies and the development of disease and pest-tolerant planting stock. From these research activities, he has published over 1000 scientific articles in collaboration with other researchers, locally and globally, and is listed as one of South Africa's most highly cited researchers. He has an A1 rating from the National Research Foundation.

Wingfield has held many distinguished positions and has received many awards. In 2014, the tree health team at FABI was recognised by the Department of Science and Technology, now the Department of Science and Innovation (DSI), as one of the first government-supported Centres of Excellence. This became known as the DSI/NRF Centre of Excellence in Tree Health Biotechnology (CTHB) at FABI and contributed substantially to the expansion of the research team. Establishment of the CTHB alongside the South African Forestry Industry supported Tree Protection Co-operative Programme – which Wingfield established in 1990 and which was already successful and highly recognised – led to a fundamental expansion of the tree health research being conducted at FABI. Wingfield was awarded honorary doctorates by the University of British Columbia (Vancouver, Canada) and North Carolina State University (USA). He is the Immediate Past President of the International Union of Forest Research Organizations, having served a 5-year term as President from 2014 to 2019 – a tremendous accolade for a South African scientist.

He is a Fellow of several scientific societies, including the Academy of Science of South Africa, the Royal Society of South Africa, the Southern African Society for Plant Pathology, and the American Phytopathological Society, and is one of few honorary members of the Mycological Society of America. He received the prestigious African Union Kwame Nkrumah Scientific Award in the Life and Earth Sciences category in 2013, and the Distinguished Leadership Award for International Scientists for 2016 from the University of Minnesota, his alma mater. In 2017, he received the esteemed Royal Society of South Africa John FW Herschel medal – this medal is the highest honour that can be bestowed on a Fellow and is awarded to an outstanding scientist whose work straddles a number of disciplines.

Professor Mike Wingfield with his trophy for the 2019/2020 NSTF-South32 Special Annual Theme Award: Plant Health

In his general approach to life and his research, Wingfield is committed not only to delivering quality research, but also to enhancing people's lives. As well as being a specialist laboratory scientist, he has a holistic philosophy that emphasises the role that research can play in improving ecosystem function that provides a better quality of life for humans and the beneficial organisms with which we interact. The *South African Journal of Science*, to which Wingfield and his team have contributed over the years, congratulates Prof. Wingfield on his award and is sincerely proud of his outstanding leadership in his field of science.

INTERNATIONAL YEAR OF PLANT HEALTH 2020

The hunt for plant viruses in South Africa: The early days

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Plant viruses cause widespread disease in agriculturally important crops, resulting in a reduction in both quality and quantity of produce. The introduction of intensive crop monoculture has resulted in an exponential increase in viral diseases which can cross over from wild indigenous plants. Viral pathogens also can occur in mixed infections, and rapid, sensitive and reliable diagnostic methods are required to identify and characterise the viruses responsible for the field diseases. In comparison to bacterial and fungal diseases, viral diseases are more difficult to diagnose. This review covers a period (1985–2011) in the history of virus discovery in South Africa during which several plant viruses from commercial and small-scale farms were identified and characterised. Interestingly, novel viruses were discovered in three crops, namely guar and cassava grown by small-scale farmers in Mpumalanga, and in commercial tobacco. The implication of these plant diseases is potential yield loss to farmers which can affect their livelihoods, and result in severe economic loss for the food and agriculture industries. Accurate identification of the causal viral agents of these viral diseases is a prerequisite for development of effective management strategies.

Significance:

- This review provides a historical account of the discovery and characterisation of several viral pathogens of important agricultural crops grown by small-scale and commercial farmers in South Africa.
- Three novel plant viruses were isolated for the first time during the period (1985–2011) of this review.

Introduction

Origin, evolution and diversity of plant viruses

The history of virology interestingly began with the discovery of a novel infectious agent (tobacco mosaic virus), not in animals or humans, but in a tobacco plant.[1] Viruses represent the most diverse, ubiquitous and numerous microorganisms defined to date. It has been speculated that viruses contributed to the origin of cellular life.[2] Although the origin of viruses is not known due to lack of 'molecular fossil' information, extant evidence indicates a polyphyletic origin. As with human and animal viruses, plant viruses arose multiple times as hosts evolved and diverged in defined geographical regions. Plant viruses have a longstanding tight co-evolutionary history with their plant hosts, and while many of these do not cause disease, pathogenic viruses appear to dominate in economically important agricultural crops. Virus emergence is generally associated with ecological change or domestication of crops leading to intensive mono-agronomical practices. Complex ecological factors play a major role in plant virus emergence, host range expansion/diversification and plant–virus interactions.[3] In nature, virus infections occur in multivirus–multihost communities, whereas in monoculture, although mixed infections do occur frequently, a single dominant virus species is usually associated with the disease. These viruses are transmitted by undiagnosed infected plant material or seeds introduced into geographical regions; or often these viruses transgress from wild plants in natural ecosystems into agri-systems, although from domestic crops into wild hosts can also occur.[4] Virus populations are continuously evolving and adapting to new environments, vectors and hosts.[5] Genetic diversity is achieved by several molecular mechanisms such as horizontal gene transfer, mutations (nucleotide substitutions), virus genome re-assortment or recombination. Virus populations behave as mutant spectra (quasispecies) composed of heterogeneous genetic variants around a master dominant sequence.[6] Genetic diversity of a quasispecies at any given time is a result of natural selection and genetic drift, enabling viral emergence and altered pathogenesis. Factors determining the origin, emergence and diversification of virus populations in any specific geographical region/country are highly complex.

Diagnosis

Many viruses can remain undetected in the field, in particular those in wild plant hosts. Due to previous limitations of methods for virus detection and identification, many viruses remained 'hidden'. Early studies in the first six decades of plant virology (~1900–1960) were mainly focused on insect and mechanical transmission, centrifugation, serological assays and electron microscopy. The rise of molecular virology, including nucleic acid and protein technologies, has allowed for more rapid and accurate viral genome identification. Next-generation sequencing and metagenomics applied to plant virology in the last decade has provided rapid, efficient and high-throughput sequencing of DNA and RNA virus and viroid genomes.[7] Metagenomic studies have also revealed a large diversity of viruses in wild plants.[8] Next-generation sequencing combined with bioinformatics is also a powerful tool for *de novo* virus discovery and virus genome diversity studies. The potential contribution of minor genetic variants in a quasi-species to disease aetiology in the field is not yet known. Future next-generation sequencing studies on both temporal and spatial regulation of viral quasi-species in plant hosts merits further attention.[9]

A personal journey in plant virus hunting in southern Africa

To our knowledge, the first virus disease symptoms reported in South Africa was streak disease in maize.[10] Maize streak disease was shown to be caused by a virus that is transmitted by a leafhopper vector.[11] More recent studies have identified several ssRNA viruses occurring singly or in mixed infection in sweet potato in KwaZulu-Natal[12],

Limpopo[13], and Eastern and Western Cape Provinces[14]. Several geminiviruses have also been detected in South Africa and southern African neighbouring counties causing serious yield reductions in cassava, maize, tomatoes, beans and sweet potatoes.[15] Two monopartite begomovirus isolates, occurring either alone or in mixed infection in sweet potato (*Ipomoea batatas*) plants, were identified for the first time in South Africa in 2011 from samples near Louis Trichardt in the Limpopo Province.[16] The complete genome sequence of one of the isolates corresponded to *Sweet potato mosaic-associated virus* (SPMaV; SPMaV-[ZA:WP:2011]), with which it shared 98.5% nucleotide identity. The second genome isolate sequence corresponded to a new variant of *Sweet potato leaf curl Sao Paulo virus* (SPLCSPV; SPLCSPV-[ZA:WP:2011]), with which it shared 91.4 % nucleotide identity.

This review reports a number of new viruses or virus isolates that were identified in several provinces in South Africa between the years 1985 to 2011. These viruses are depicted in a geographical map (Figure 1).

A new green-sterile viral disease of guar in Mpumalanga

Guar (*Cyamopsis tetragonoloba* L.) is native to tropical Africa and Asia, and is grown in the USA, Pakistan, India and several countries in Africa as a livestock feedstuff.[17] Guar meal has more recently also been used in poultry, and guar gum obtained from an annual pod is used as an emulsifier in baking mixes, cheeses, fats, oils, sauces and jams. The guar market in North America, Europe, Asia-Pacific, South America and Africa is projected to grow at a rate of 3.0% during the forecast period 2015–2024. Major importers of guar gum are Italy, South Africa, Russia, Australia, Netherlands, Japan, Brazil, Belgium and Canada. Guar was introduced into South Africa in the late 1940s and was grown by rural farmers in Mpumalanga and Northern Province.[18] Guar was often used in intercropping with maize, cassava and groundnuts in Mpumalanga.

A number of potyviruses have been shown to infect guar, including *peanut mottle potyvirus* (PeMoV), *Bean common mosaic virus* (BCMV), and a symptomless seed-transmitted potyvirus from Indian, African and North American guar.[19]

While researching cassava mosaic disease in the Mpumalanga region, disease symptoms of reduced leaf size and number, and fewer, often sterile, inflorescences along the stem were observed on guar plants in the fields in KaNgwane (now incorporated into Mpumalanga). Often the stems remained green long after plant senescence, and 50% of the seeds were discoloured and distorted.[18] The disease was named guar green sterile disease. It was suspected that this disease may be due to a potyvirus (ssRNA flexuous particles) and was named *Guar green sterile virus* (GGSV). Host range study in bean cultivars, and serological tests with antisera to *Bean common mosaic virus* (BCMV-SA) and *Bean common mosaic necrosis virus* (BCMNV), indicated that GGSV was serologically related. Further characterisation of guar green sterile disease was undertaken in order to elucidate the transmission, biological properties, and immunological relatedness to several other potyviruses. Mechanical inoculations on several indicator hosts[18(Table 1)] resulted in symptoms of red vein necrosis, chlorosis and mosaic, while non-persistent aphid transmission on guar was not observed. Symptoms of leaf malformation and mosaic were observed on soybean (*Glycine max*) and several bean (*Phaeseolus vulgaris* L.) cultivars. Inability to obtain green sterile symptoms in guar was explained by the slow spread of the virus, low virus concentrations, and lack of symptoms in young guar plants. In the few cases in which local lesions were obtained, plants had a positive reaction to homologous antiserum raised against GGSV isolated from guar with green sterile symptoms. Seeds showing disease symptoms were positive when tested with BCMV antiserum, and antiserum raised to purified potyviruses extracted from guar leaves. Serological tests also demonstrated virus presence in seed coat and

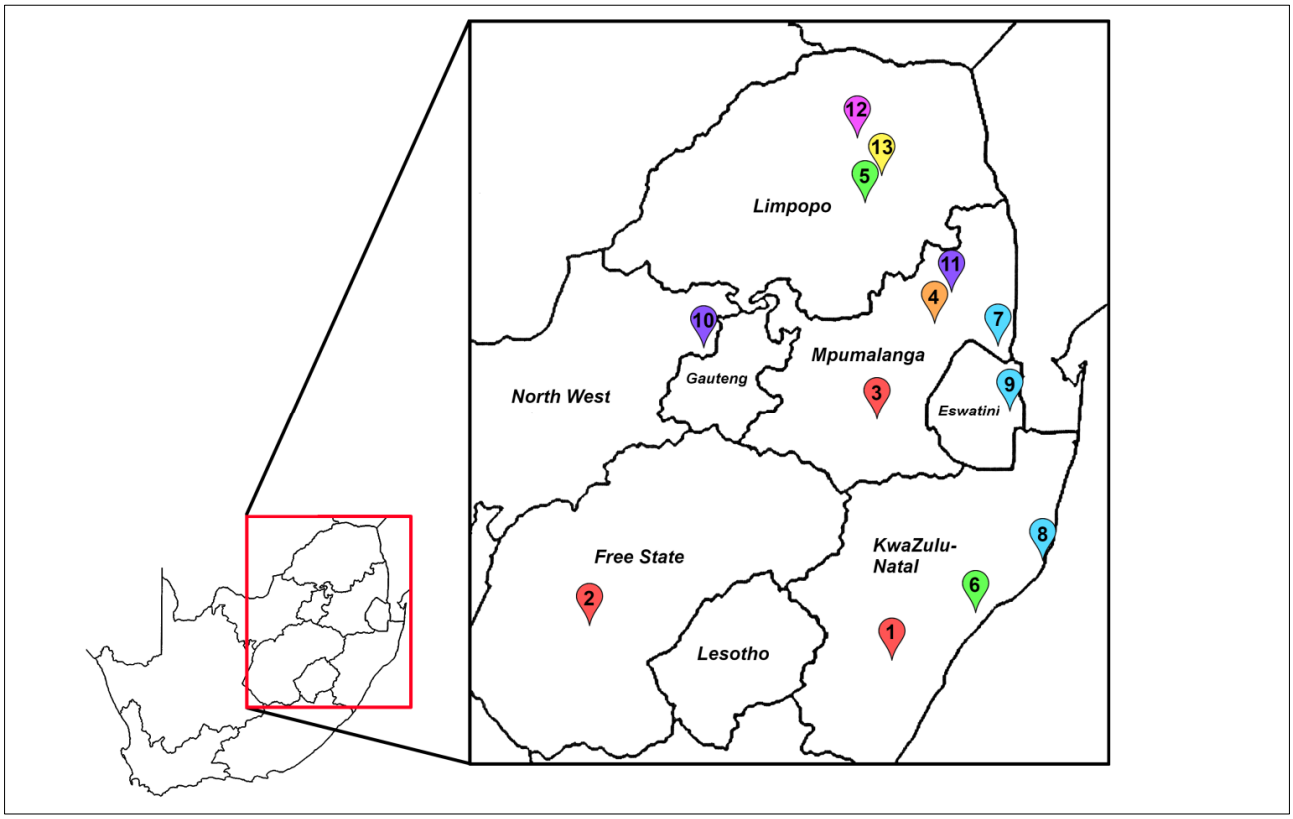


Figure 1: Map of plant viruses identified between 1985 and 2012: *Ryegrass mosaic virus* in KwaZulu-Natal (1), Free State (2) and Mpumalanga (3); *Guar green-sterile virus* in KaNgwane (now eastern Mpumalanga near White River) (4); *Avocado sunblotch viroid* near to Magoebaskloof, Limpopo Province (5) and near Eshowe (KwaZulu-Natal) (6); *African cassava mosaic virus* and *East African mosaic virus* in Mpumalanga (7) and in St. Lucia in KwaZulu-Natal (8); *South African cassava mosaic virus* in Mpumalanga (7) and Swaziland (9); *Tobacco leaf enation virus* in Brits region (North West Province) (10) and near Hazyview in Mpumalanga (11); genetic variants of *Sweet potato mosaic-associated virus* and *Sweet potato leaf curl Sao Paulo virus* near Louis Trichardt, Limpopo Province (12); and *Pepino mosaic virus* near Mooketsi, Limpopo Province (13).

embryo tissue. Virus purification was only successful from fresh guar material collected from the field. Long flexuous particles averaging 750 X 15 nm were observed by transmission electron microscopy in leaf-dip preparations. Guar cv.TX-79-2741 was also strongly positive for *Bean yellow mosaic virus* (BYMV) antiserum. This study described a new disease symptom in guar, and indicated the putative involvement of a seed-transmitted legume potyvirus. A ssRNA shown to be 9.4 kb was isolated from purified GGSV virions, and Western blots showed the coat protein to be ~34 kD.[20] The nucleotide sequence of the 3'-terminal region (1359 nt) of GGSV and translated amino acid was determined and compared with the latent seedborne guar symptomless potyvirus in guar imported into the USA from India.[21] The presence of the amino acid triplet DAG in both GGSV and guar-US was consistent with aphid transmissibility. The coat protein amino acid sequence was 96% similar between GGSV and BCMV-NL4. GGSV and guar-US formed a tight cluster that was most closely related to the BCMV sub-group of potyviruses based on the coat protein and 3' UTR, strongly suggesting that GSV-US and GGSV are strains of BCMV-NL4.

Potyviruses represent one-quarter of known plant RNA viruses, and exhibit high nucleotide variation.[22] This is an indication that potyviruses have an exceptional capacity to adapt to new hosts, vectors and environments. Further surveys of guar may demonstrate other potyvirus infections, and this could potentially have a negative impact on the use of this valuable crop, not only in the food and livestock industries but also in pharmacotherapy, where guar gum has more recently been used in the treatment of gut disorders.

The challenge of routine avocado sunblotch viroid detection in avocado trees

The avocado is one of the most important fruits in South African and global subtropical industries, and the local industry has increased rapidly in South Africa over the years.[23] Avocado production in South Africa has traditionally been concentrated in the warm subtropical areas of the Limpopo and Mpumalanga Provinces in the northeast of the country between latitudes 22°S and 25°S. However, due to growing global demand and to produce year round, production is expanding in KwaZulu-Natal and the Eastern and Western Cape Provinces (up to 33°S).[24] Avocado sunblotch is a serious disease of avocado (*Persea americana* Miller) worldwide. The disease affects both the yield and quality of the fruit. Fruit yield due to avocado sunblotch disease was reported to be reduced by 27.3% in cultivar Fuerte in the early 1980s in South Africa.[25] The causal agent of sunblotch disease was first identified as a viroid (ASBVd)[26], and is a low molecular mass circular ssRNA with a compact secondary structure comprising 247 nucleotides.

Sunblotch disease can be detected in avocado trees by identifying the typical symptoms in fruits; however, this approach is not applicable to infected asymptomatic trees.[27] Diagnosis based on symptoms is not reliable and other sensitive diagnostic techniques are necessary to determine the health status of an avocado tree. Despite rapid diagnostic methods developed for viroid detection in the early 1980s, such as polyacrylamide gel electrophoresis (PAGE) and use of ^{32}P-labelled complementary DNA probes[28], these techniques were not reliable due to variability in viroid levels within branches and leaves. Problems with PAGE indexing for ASBVd were also reported in South Africa.[29] Furthermore, contaminating polyphenols and polysaccharides in plant RNA extracts posed problems in RNA purification and gel separation. In order to improve screening for ASBVd, we developed a modified method to separate the viroid from other contaminating RNA species and contaminating plant compounds.[30] Furthermore, we compared sensitivity of a cDNA versus a synthetic oligonucleotide probe for detection of ASBVd in plant extracts. From our study we showed that DNase treatment, and removal of polysaccharides, polyphenols, 4S and 5S ribosomal RNA by a modified method of CF-11 cellulose chromatography, improved hybridisation efficiency by 100-fold. The use of cDNA probes appeared to be a more reliable method for ASBVd-RNA detection compared to PAGE; however, the costs at the time of this study were high, and diagnosis laboratories were not equipped to handle cDNA techniques. The procedure was also very time intensive.

Other molecular techniques have been developed more recently; for example, a highly sensitive novel SYBR green-based method based on real-time reverse transcription (RT)-PCR was reported.[31] The RT-qPCR is 100 times more sensitive to ASBVd than conventional RT-PCR. Infected asymptomatic trees play an important role in the epidemiology of this disease, and avocado nurseries need to be certified to ensure they provide pathogen-free avocado material. Although there is no cure for infected trees, early detection and sanitation practices may have a significant impact on avoiding the spread of this pathogen.

First report, characterisation and phylogenetic justification of *Ryegrass mosaic virus–South African* isolate

Annual ryegrass (*Lolium multiflorum*) is an important forage crop in many parts of the world including South Africa, where it is grown as a cool season pasture species under irrigation by commercial farmers for intensive dairy, lamb and beef production.[32] It is mainly cultivated in Gauteng, North West, Free State and Kwa-Zulu-Natal Provinces. *Ryegrass mosaic virus* (RGMV) is reported to infect only members of the *Poaceae* family, including ryegrass (*Lolium perenne* L. and *L. multiflorum* Lam.), bromegrass (*Bromus*) species and oats (*Avena sativa* L.).[33] *Ryegrass mosaic virus* belongs to the Potyviridae family, and is a flexuous filamentous particle, approximately 703 nm in length and 15 nm in diameter, and is transmitted by the mite vector *Abaracus hystrix*.[33] Symptoms of infection usually range from yellow to light green mosaic or streaking.[34]

While symptoms in ryegrass had been reported in South Africa, there was no information on the distribution, biology or strain of the virus in South Africa prior to 1989, and both the vector and causal agent of ryegrass mosaic disease had not been confirmed. From 1989 to 1990, a broad survey of viruses in pasture grasses was carried out in several provinces, and mechanical and vector transmission was performed.[34] Transmission results showed that RGMV-SA was present at only three of the sites (Cedara, Nooitgedacht and Glen). Dot-blot and leaf press immunobinding assays, using anti-RMV-SA antiserum raised to purified RMV particles in rabbits, and electron microscopy confirmed the presence of RGMV.[35(Fig.1)] Vector identification (by Dr E Ueckermann at the Plant Protection and Research Institute, Pretoria) also confirmed the mite to be *A. hystrix* belonging to the Eriophyidae family. A further host range study was performed on several indicator hosts using both mechanical and vector transmission. Of the 15 plant species screened, RMV-SA was shown to be transmissible to all the tested cultivars of *L. multiflorum* as well as *L. perenne*, *A. sativa*, *A. fatua*, *Dactylus glomerata*, *Bromis mollis* and *Festiuca arundinaceae*. RGMV-SA was found to be a severe isolate as it also induced severe tissue necrosis in several hosts, including *L. multiflorum*, a similar result as shown in *L. multiflorum* cv.s22 by RGMV isolates from Wales.[33] Transmission of RGMV-SA by *A. hystrix* was found to be in a semi-persistent manner. In order to study the relationship with other virus members of the *Rymovirus* and *Potyvirus* genera, antisera for various potyviruses were used in dot-blot immunobinding assays and indirect ELISA. Immunoblots probed with antisera raised to RGMV-SA, RGMV-W and RGMV-Ca from South Africa, Wales and Canada, respectively, were positive; however, antisera to potato virusY (potyvirus) and other rymoviruses [Hordeum mosaic virus (HorMV); Agropyron mosaic virus (AgMV); wheat streak mosaic virus] were negative. The coat protein from purified virus particle preparations was determined to be 32.1±0.52 kDa, and the size of RGMV-SA RNA extracted from purified virions was estimated to be 2.8 x 10^6 on 1% agarose gels.[35] Further, molecular cloning and nucleotide sequencing of the 3'terminal end (2094 nt) of the first strain of a RGMV isolate was accomplished.[36] Two putative polyprotein cleavage sites, Q/L and E/A, were found, both of which are novel amongst potyviruses.

Alignment of the amino acid sequence of RGMA-SA with other *Rymovirus* genus members showed limited identity with *Potyvirus*. These results indicated that RGMV-SA was a distinct virus genus *Rymovirus* within the Potyviridae family.[37] Additionally, phylogenetic analyses of the rymovirus sequences revealed a distinct group of two clusters: RGMV, HorMV and AgMV in one group and *Wheat streak mosaic virus* (WSMV) and

Brome streak mosaic virus (BrSMV) in the second group. Rymoviruses also clustered separately from *Ipomovirus* and *Bymovirus* genus members.[37(Fig.1)]

While no extensive studies on RGMV-SA have ensued in the years following its discovery and characterisation, breeding of this important forage crop is ongoing. Yield data from the *L. multiflorum* breeding programme at ARC-Cedara in KwaZulu-Natal Province provides substantial evidence of the benefits of breeding to improve yield.[38] Because ryegrass mosaic disease can affect yields, it is important that ryegrass growers are aware of a potential problem and remain vigilant.

Unravelling the disease aetiology of tobacco leaf curl disease in southern Africa: *Tobacco leaf enation virus*, a novel field phytoreovirus in tobacco reported for the first time

Tobacco leaf curl disease occurs mainly in tropical and sub-tropical regions, but is also reported in temperate regions such as Japan and parts of Europe and USA. Tobacco leaf curl disease was first reported in the Netherlands East Indies in 1912; however, it is thought that a disease fitting the description of leaf curl was present in South Africa as early as 1902.[39] Leaf curl in tobacco was once reported as the most destructive disease in East Africa, Zimbabwe and the North West Province of South Africa.[39] Although the disease syndrome was attributed to a virus, named *Tobacco leaf curl virus* (TLCV)[40], which was shown to be transmitted by a species of whitefly, only one attempt was successful in isolating geminivirus particles.[41] Symptom variability in tobacco leaf curl disease, however, has been reported by several researchers worldwide, including in South Africa[42], and while suspected to be caused by different geminivirus strains, this suspicion was never confirmed experimentally. Furthermore, repeated attempts to isolate a geminivirus from tobacco in South Africa had failed.[43] Tobacco plants exhibiting leaf curl symptoms were collected between 1992 and 1995 from Zimbabwe and from the North West and Mpumalanga Provinces in South Africa. Three different leaf symptoms, based on type and severity, were distinguished and placed into three classes (I, II and III).[44] Class I tobacco plants from South Africa predominantly exhibited stunting and stems appeared bent and irregular and the thickened leaves curled and twisted with thickened veins on the ventral surface and frilly enations along the midrib. Class II leaf curl symptoms were considered non-viral, and attributed to a physiological disorder. Transmission electron microscopy, dot-immunobinding assays and polyacrylamide gel electrophoresis (PAGE) failed to detect geminivirus particles or coat protein from symptomatic class I plants.[45] DNA hybridisation with eight geminivirus-specific probes and PCR with three sets of geminivirus-specific degenerate primers failed to detect DNA. Interestingly, dsRNA extraction revealed 12 bands ranging from 4350 bp to 810 bp, which resembled plant reoviruses that are transmitted by leafhopper or planthopper vectors. Plant reovirus-like symptoms include enations, vein swellings and dwarfing.[46] *Wound tumour phytoreovirus* (WTV) was at the time the only plant reovirus known to infect dicotyledonous plants.[47] As the dsRNA pattern and sizes and symptoms of class I leaf curl tobacco strongly suggested a phytoreovirus, further studies were performed to confirm this diagnosis. Reovirus extractions successfully isolated icosahedral particles with an outer core 60–65 nm in diameter and an inner core 40–45 nm in diameter. Twelve distinct non-polyadenylated dsRNAs were isolated from purified virions, and the total molecular masses of the dsRNAs ranged from 17.86 to 18.40 × 106 Da in polyacrylamide and agarose gels, respectively.[48] Comparisons of the tobacco phytoreovirus from South Africa with other known phytoreoviruses (*Maize rough dwarf fijivirus* (MRDV), *Garlic dwarf reovirus* (GDV), R*ice ragged stunt reovirus* (RRSV), *Rice black-streaked dwarf fijivirus* (RBSDV), WTV and *Rice dwarf phytoreovirus* (RDV)) revealed a unique dsRNA banding pattern that was distinct but most similar to WTV, the type species of the genus *Phytoreovirus*. Hybridisations of WTV-cloned DNA probes (segments S4 and S6 to S9) and dsRNAs from infected tobacco indicated no significant sequence similarity, whereas an indirect ELISA with a polyclonal antiserum to WTV showed strong positive cross-reactivity to tobacco virions. Western blot analysis of structural viral proteins (apparent molecular weights of 93 kDa, 58 kDa, 48 kDa, 39 kDa and 36 kDa) associated with the dsRNAs isolated from infected tobacco in South Africa, suggested that these proteins may correspond to structural WTV-like proteins. The virus was named 'tobacco leaf enation virus' (TLEV).

To further establish the nature of TLEV-associated disease phenotype in tobacco, molecular characterisation of six dsRNA components was undertaken.[49] Results of this study revealed the conserved terminal sequence: 5'GG(U/C)...UGAU 3' of segments S6–S12, while adjacent to these conserved terminal sequences are imperfect inverted repeats (7–15 bp in length), both features being common to reoviruses. The complete nucleotide sequences were determined for segments S5 (2610 bp), S7 (1740 bp), S8 (1439 bp), S10 (1252 bp), S11 (1187 bp) and S12 (836 bp). Comparison of full-length nucleotide sequences with corresponding segments of other phytoreoviruses, *Rice gall dwarf virus* (RGDV), *Rice dwarf virus* (RDV) and WTV has shown nucleotide and predicted amino acid identities within the range of 30–60%. TLEV consistently showed a higher identity to WTV than to other phytoreovirus species for which sequence data were available. Each segment had a single predicted open reading frame encoding proteins with calculated molecular weights of 90.6 kDa (S5), 58.1 kDa (S7), 47.7 kDa (S8), 39.8 kDa (S10); 35 kDa (S11) and 19.5 kDa (S12). The relatively low nucleotide and amino acid identity to other members of the genus confirmed that TLEV is a novel phytoreovirus, distinct from the only other reported dicotyledonous-infecting WTV and was the first report of a phytoreovirus in tobacco, and the first discovered reovirus on the African continent.

Discovery of *Tobacco leaf curl Zimbabwe virus* – a new distinct monopartite begomovirus associated with subgenomic defective DNA molecules

Class III leaf curl plants from Zimbabwe tested positive in PCR using two sets of geminivirus-specific degenerate primers that amplify the core region of the coat protein of DNA A or the bottom half of DNA A of most whitefly transmitted geminiviruses. Dot-blot hybridisation and triple antibody sandwich ELISAs for geminiviruses also were positive.[50] The Zimbabwe virus isolates were named *Tobacco leaf curl Zimbabwe virus* (TbLCZWV). The complete DNA A sequence of TbLCZWV comprises 2767 nucleotides with six major open reading frames encoding proteins with molecular masses greater than 9 kDa.[50] Agro-inoculation with a full-length TbLCZWV DNA A infectious clone resulted in systemic infection of tobacco and tomato. Symptoms of field-infected tobacco leaves (*Nicotiana tabacum* cv. HG) collected from Zimbabwe exhibited distinct downward leaf margin curling, vein thickening, wavy midribs and significantly distinct cup-shaped enations on the ventral surface. Symptoms observed in *Nicotiana benthamiana*, following TbLCZWV agro-inoculation, were stunting, severe upward leaf curling, leaf distortion, ventral vein thickening and bending of petioles (Figure 2a). In *N. tabacum* cv. Samsun, extensive puckering of dorsal leaf surfaces (Figure 2b) and upward leaf margin curling in newly developing leaves occurred (Figure 2c). *N. tabacum* cv. Samsun ventral leaf surfaces of infected plants exhibited vein thickenings on smaller veins of the infected plants that were absent in the healthy plants (Figure 2d).

Efforts to identify a DNA B component were unsuccessful. These findings suggested that TbLCZWV was a novel member of the monopartite group of begomoviruses, with its closest relative being *Chayote mosaic virus* (Figure 3). Abutting primer PCR amplified two ~1300-bp subgenomic defective DNA molecules originating from TbLCZWV DNA A. Many monopartite begomoviruses are associated with co-dependent betasatellites for successful infection.[51] While a betasatellite was not found in *N. tabacum* cv. HG, it would prove interesting to revisit tobacco leaf curl disease in Zimbabwe. Since this discovery, a TbLCZWV isolate was reported from the Comoros archipelago.[52] Further, a complex tobacco leaf curl begomovirus exhibiting differential disease phenotypes in the Comoros archipelago has recently been observed.[53] Extensive genetic material exchange through recombination, molecular diversity and evolution of these begomovirus complexes warrants further research.

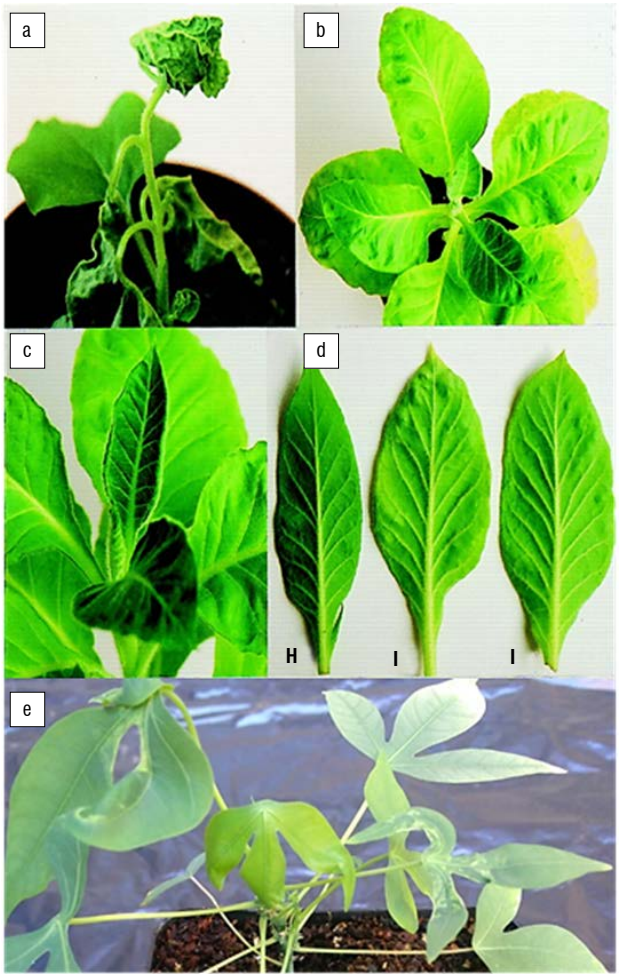


Figure 2: Symptoms seen on tobacco following agro-inoculation with monopartite *Tobacco leaf curl Zimbabwe virus* DNA A. (a) *Nicotiana benthamiana* (100% infection 2 weeks post-inoculation) exhibiting stunting, severe upward leaf curling, leaf distortion, ventral vein thickening and bending of petioles. (b) *N. tabacum* cv. Samsun (100% infection 4 weeks post-inoculation), showing extensive puckering of dorsal leaf surfaces. (c) *N. tabacum* cv. Samsun showing leaf margins of newly developing leaves curling upwards. (d) *N. tabacum* cv. Samsun ventral leaf surfaces of healthy (H) and infected (I) plants, showing vein thickenings present on smaller veins of the infected plants that are absent in the healthy plants (source: Paximadis and Rey[50]). (e) Symptoms of leaf curl and mosaic on cassava cv.60444 agro-inoculated with bipartite *South African cassava mosaic virus* DNA A and B.

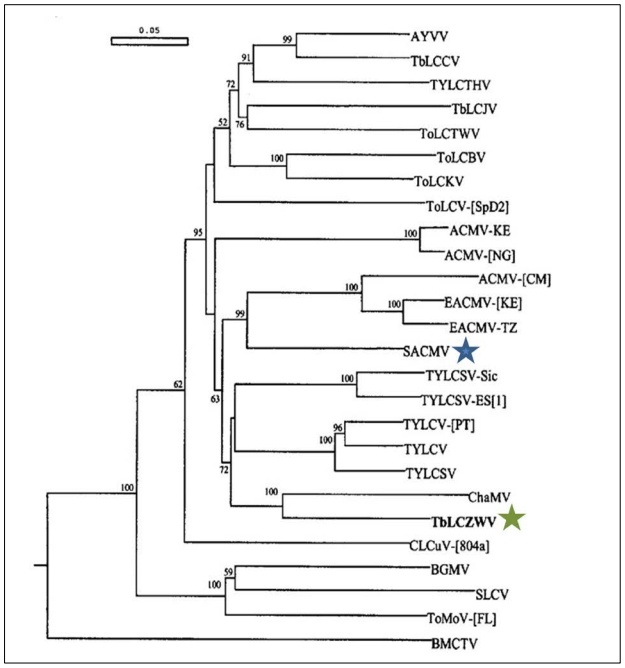
The elusive identification of *South African cassava mosaic virus*: A novel geminivirus provides further evidence for recombination

Cassava (*Manihot esculenta* Crantz) is one of the most important staple root crops whose starchy roots are a major source of dietary energy for more than 500 million people in tropical and sub-tropical regions and thus occupies a uniquely important position as a food security crop for smallholder farmers. In low-income areas of these countries, the crop is a staple food consumed by over 700 million people. Over the period 1980 to 2013, global cassava production has increased from 124 million tons to an estimated 263 million tons, and global fresh root production reached an estimate of 275.7 million tons in 2018.[54] Cassava mosaic disease is considered to be the first virus disease reported in Africa[55], where symptoms of leaf curl and mosaic were reported (Figure 2e). Cassava (*Manihot esculenta* Crantz) is thought to have been introduced, in two independent events, from the Americas onto the African continent in the 16th and 18th centuries[56] and cultivation spread throughout sub-Saharan Africa. The introduction of this exotic crop into a new ecosystem provided opportunities for infection of cassava by geminiviruses from native plant species. The geminiviruses (family Geminiviridae) constitute the largest family of plant viruses, with over 450 distinct species that occur across all world regions with favourable climates for their insect vectors.[57] Among the geminiviruses, begomoviruses (genus *Begomovirus*) are responsible for a large number of emergent crop diseases over the past 50 years throughout the tropical and subtropical regions worldwide.[58] Begomoviruses are composed of circular ssDNA genomes, and can be monopartite (DNA A) or bipartite (DNA A and B). *African cassava mosaic virus* (ACMV) was the first reported begomovirus associated with cassava mosaic disease.[59] Cassava mosaic viruses frequently occur in mixed synergistic infections, and readily exchange their genomes through recombination or re-assortment which leads to high genetic variability within species.[60]

It is speculated that cassava was introduced into South Africa from Zimbabwe, Mozambique and Mauritius, and was spread across the northern and eastern regions of Natal and Mpumalanga by Tsonga tribesman in the 1830s.[61] Cassava is mainly cultivated by small, resource-poor farmers. A study in the early 1980s reported cassava mosaic disease symptoms in South Africa and Swaziland.[61] While it is not known how cassava mosaic disease emerged in South Africa, it is speculated to have been introduced through infected cassava vegetative material via other neighbouring countries. An initial transmission electron microscope study to identify the causal agent of cassava mosaic disease in cassava in South Africa, and in mechanically inoculated indicator host *N. benthamiana*, was unable to detect typical geminivirus particles or inclusion bodies in the nucleus.[62] However, using antiserum raised against ACMV-Kenya, immunogold labelling located geminiviral antigen in infected cassava leaf tissue. On the basis of a partial DNA A sequence, and comparative serological results with three monoclonal antibodies against EACMV, ACMV and *Indian cassava mosaic virus* (ICMV), a novel geminivirus was identified, *South African cassava mosaic virus*.[63,64] The individual common region and coat protein nucleotide sequences of SACMV clustered with the monopartite *Tomato yellow leaf curl virus–Sardinia* (TYLCV-SR), followed by *East African cassava mosaic virus* [Malawi-8N-2007] and East African cassava mosaic virus–Tanzania [Tanzania].[64] Southern blots with three DIG-labelled ACMV-Kenya, ICMV and *Tomato golden mosaic virus* (TGMV) DNA B probes, and nucleotide sequencing of a 687-bp DNA B component, proved that SACMV was a bipartite begomovirus that clustered with the Old World sub-group.

Earlier serological surveys in west, central and east African countries suggested that cassava mosaic begomoviruses, ACMV, EACMV and the ACMV-EACMV recombinant (EACMV-Uganda) had separate non-overlapping distributions[65]; however, *East African cassava mosaic virus-Cameroon* [Cameroon-1998] was reported more west in Cameroon.[66] A later PCR study using core coat protein and EACMV-Uganda primers in southern Africa (Angola, Zambia, Swaziland, Mozambique and Zimbabwe) demonstrated that the three viruses occurred in most of these countries, but EACMV-Uganda only occurred in mixed infections.[67] All three viruses were present in South Africa, and further heteroduplex mobility assay profiling was able to distinguish four different virus species and 11 different strains/isolates.[68] In addition to EACMV, ACMV, SACMV, EACMV-Uganda and *East African cassava mosaic virus–Malawi* were also detected. Full-length DNA A sequence comparisons with other begomoviruses available first demonstrated that SACMV was most closely related to EACMV-Tanzania and Malawi isolates (Figure 3). Later, full-length DNA A and B genomes were shown also to be closely related to EACMV-Malawi and EACMV-Uganda mild and severe isolates, respectively.[69] One significant recombination event spanning the entire AC4 open reading frame on DNA A was identified, and interestingly, this open reading frame clustered with a group consisting of ICMV, ACMV, *Tomato leaf curl virus–Australia* (TLCV-AU) and *Tomato yellow leaf curl virus* (TYLCV) isolates from Sardinia and Sicily.[69] Following the discovery of SACMV in South Africa, Swaziland, Mozambique and Zimbabwe, it was later reported from Madagascar.[70] Collectively, studies confirm that SACMV is most closely related to a clade that contains *East African cassava mosaic*

virus, *East African cassava mosaic virus–Tanzania, East African cassava mosaic virus–Cameroon, East African cassava mosaic virus–Uganda, East African cassava mosaic virus–Zanzibar, East African cassava virus–Malawi,* and *East African cassava mosaic virus–Kenya*, which occur in east Africa. Evidence supports recombination events amongst African, Mediterranean and southwest Indian Ocean islands begomoviruses.[71] More recently, three additional cassava begomovirus species, *Cassava mosaic virus–Madagascar*, *East African cassava mosaic virus–Kenya* and *East African cassava mosaic virus–Zanzibar* – have been discovered in cassava in sub-Saharan Africa. Two distinct species, *Indian cassava mosaic virus* and *Sri Lankan cassava mosaic virus,* are described from the Asian sub-continent.[58,72]



Source: Paximadis and Rey[50].

Figure 3: Relationship dendrogram of the DNA A nucleotide sequences of *Tobacco leaf curl Zimbabwe virus* (TbLCZWV) and other geminiviruses. Bootstrap scores >50% were placed at major nodes, and nodes lacking a score are considered dubious. Geminiviruses and their respective GenBank accession numbers are as follows: ACMV-[CM] (*African cassava mosaic virus–Cameroon*: NC 000859); ACMV-[KE] (*African cassava mosaic virus–Kenya*: J02057); ACMV-[NG] (*African cassava mosaic virus–Nigeria*: X17095); AYVV (*Ageratum yellow vein virus*: X74516); BMCTV (*Beet mild curly top virus*: U56975); BGMV (*Bean golden mosaic virus*: M88686); ChaMV (*Chayote mosaic virus*: AJ223191); CLCuV-[804a] (*Cotton leaf curl Lahore virus*: AJ002455); EACMV-[KE] (*East African cassava mosaic virus–Kenya*: AJ006458); EACMV-TZ (*East African cassava mosaic virus–Tanzania*: Z83256); SACMV (*South African cassava mosaic virus*: AF155806); SLCV (*Squash leaf curl virus*: M38183); TbLCCV (*Tobacco leaf curl China virus*: AF240674); TbLCJV (*Tobacco leaf curl Japan virus*: AB028604); TbLCZWV (*Tobacco leaf curl Zimbabwe virus*: AF350330); ToLCV-[SpD2] (*Tomato leaf curl virus–Australia*: AF084007); ToLCBV (*Tomato leaf curl Bangalore virus*: Z48182); ToLCKV (*Tomato leaf curl Karnataka virus*: U38239); ToLCTWV (*Tomato leaf curl Taiwan virus*: U88692); ToMoV-[FL] (*Tomato mottle virus–Florida*: L14460); TYLCV (*Tomato yellow leaf curl virus*: X15656); TYLCV-[PT] (*Tomato yellow leaf curl virus–Portugal*: AF105975); TYLCSV (*Tomato yellow leaf curl Sardinia virus*: X61153); TYLCSV-Sic (*Tomato yellow leaf curl Sardinia virus–Sicily*: Z28390); TYLCSV-ES[1] (*Tomato yellow leaf curl Sardinia virus–Spain*: Z25751); TYLCTHV (*Tomato yellow leaf curl Thailand virus*: X63015).

With growing human populations and drought associated with climate change predicted for the future, cassava can provide one solution for food security in South Africa, and in other countries in the SADC region. Cassava starch has other potential diversified industrial uses such as food additives, biofuels, biodegradable packaging and animal feedstock.[73] Introduction of high-yielding varieties, improved pest and disease control and better processing methods could increase cassava production in Africa. Because begomoviruses are a major threat, genetic engineering for virus resistance through RNA interference and gene editing can provide critical solutions for virus disease management.

First report of *Pepino mosaic virus* infecting tomato in South Africa

Pepino mosaic virus (genus *Potexvirus*) is a highly infectious virus that is responsible for significant losses in yield of tomato fruit (*Solanum lycopersicum*) across Europe, Asia and the Americas. *Pepino mosaic virus* (PepMV) rapidly evolved from an emerging to an endemic pathogen in tomato crops worldwide.[74] During the winter growing season of 2008, symptoms of uneven discolouration of tomato fruit and mosaic symptoms on leaves were detected in tomato farms in Limpopo Province. Double antibody-sandwich (DAS)-ELISA using polyclonal antibodies against PepMV (Agdia, Elkhart, IN, USA) confirmed PepMV in leaf and fruit samples. Mechanical inoculation of susceptible *S. lycopersicum* cv. Rooikhaki seedlings with infected sap was achieved, and all inoculated plants developed characteristic PepMV symptoms including leaf bubbling, distortion and curled leaves. A 986-bp region, that included the coat protein of the PepMV genome, was RT-PCR amplified and sequenced. Phylogenetic analysis clustered the South African sequence with EU (European), LP (Peruvian), US1 (United States)/CH1 (Chilean) and US2/CH2 isolates. This confirmed the first report of PepMV in South Africa.[75]

Concluding remarks

A number of diverse RNA and DNA viruses from many agriculturally important crops have been uncovered in South Africa over the past 11 decades. More recently, virus metagenomic surveys have been extended from agricultural systems to natural ecosystems. For example, a survey in 2010 in the fynbos in South Africa revealed a highly divergent geminivirus from wild spurge (*Euphorbia caputmedusae*), which is symptomless in this natural host but causes severe symptoms in *N. benthamiana* and tomato.[76] Metagenomic studies will continue to reveal novel plant viruses in both wild plants and agricultural crops, and characterising the impact of viruses in natural ecosystems may lead to informed agricultural practices or alternative solutions to controlling these pathogens. One of the future challenges will be to understand the equilibrium between plant viruses and their hosts, and how the long term co-evolutionary balance between natural hosts and plant viruses can be broken by large-scale monoculture.[9] While many useful molecular interaction studies have been performed in plant virus–model host systems, future studies of interactions in specific virus–host combinations in more complex ecosystems are warranted. Furthermore, next-generation sequencing and new sample preparation techniques will also allow researchers to sequence ancient viral genomes from archaic plant material, and shed more light on virus evolution.

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Competing interests

I declare that there are no competing interests.

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INTERNATIONAL YEAR OF PLANT HEALTH 2020

Emerging potato pathogens affecting food security in southern Africa: Recent research

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Potato is a staple crop that contributes to food security and poverty alleviation in developing nations. Despite this, yields in developing nations are often unsustainably low, due to various biotic and abiotic factors that negatively affect production. Some of the most important biotic constraints are pathogens, many of which are disseminated by seed tubers. The lack of functional or formal seed certification systems in many southern African countries results in a continual increase in pathogen pressure. Short rotation cycles, poor plant nutrition and inefficient control measures exacerbate the crop production challenges faced by resource poor growers. In this review, we discuss five of the most important diseases on potatoes in southern Africa, namely late blight, bacterial wilt, soft rot / blackleg, powdery scab and zebra chip. Management options for small-scale growers are provided.

Significance:

- Potato production in southern Africa is threatened by tuber-borne pathogens.
- Establishment and implementation of seed certification systems in southern African countries will increase potato yields and subsequently contribute to food security.
- Late blight, bacterial wilt, soft rot / blackleg and powdery scab are important emerging diseases on potatoes in southern Africa.
- Improved understanding of the biology of pathogens and the epidemiology of diseases will contribute to the management thereof.

Introduction

Potato is regarded as one of the most important crops in addressing the challenge of food security, particularly among smallholder farmers.[1,2] Potato production has drastically increased since the 1960s in the developing world, in comparison to that in the developed world.[3] However, the sustainability of potato production globally, and particularly in developing countries, is threatened by adverse abiotic conditions, pests and pathogens. Pest and pathogen control is difficult for subsistence potato growers in southern Africa – a region fraught with challenges, not least of which is the need to increase agricultural productivity in the face of climate change and a rapidly growing population.

Many of the important diseases affecting potato can be defined as emerging infectious diseases.[4] Emerging infectious diseases are caused by pathogens that have '(i) increased in incidence, geographical or host range; (ii) have changed pathogenesis; (iii) have newly evolved or (iv) have been discovered or newly described'[4]. Similarly, Secor and Rivera-Varas[5] classified important potato diseases as caused by emerging, changing or surviving pathogens. The primary reasons for the occurrence of emerging infectious diseases in potatoes and other crops are related to increased trade and travel, intensified and expanded land use, changes in agricultural practices, planting of new varieties, and extreme weather events linked to climate change.[4-7]

Using the classification proposed by Secor and Rivera-Varas[5], important diseases on potatoes in the last decade (2010–2020) in southern Africa include late blight, bacterial wilt, soft rot and blackleg, and powdery scab. Zebra chip disease, although not recorded in southern Africa, has been included as a threat because of its severe impact on potato production. In this review, we investigate the drivers behind the increase in these diseases, mitigation measures and routes to prevent additional emerging infectious diseases from appearing in the southern African potato industry.

Late blight

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is considered the most devastating potato disease globally, and causes severe yield losses when uncontrolled.[4,7] The disease begins as small, light to dark green, circular to irregular-shaped water-soaked lesions on leaves. In cool, moist conditions the lesions expand to form dark brown to black lesions (Figure 1a), often surrounded by chlorotic halos. As the disease progresses, symptoms extend to petioles and haulms, eventually killing the plant (Figure 1b).[8]

In some countries in sub-Saharan Africa, including southern Africa, where the majority of potato crops are produced by small-scale or subsistence growers, losses due to late blight are estimated to be as high as 40–70% in susceptible varieties.[9,10] In the South African commercial potato production industry, however, late blight is not considered the most important yield limiting disease. Climatic conditions during potato growing seasons in most of the 16 growing regions in South Africa are sub-optimal for the development of late blight.[11] Given the climate change forecast for the next few decades, it is also unlikely that late blight will increase in severity – in fact, the contrary has been predicted.[11] However, the SimCastMeta model simulation by Sparks et al.[12] for five different potato growing regions globally, has indicated that blight units in some other southern African countries may increase slightly in the period 1975–2050. These possible increases in disease could, however, be mitigated by shifting planting dates to avoid conditions favourable for the development of late blight.[12]

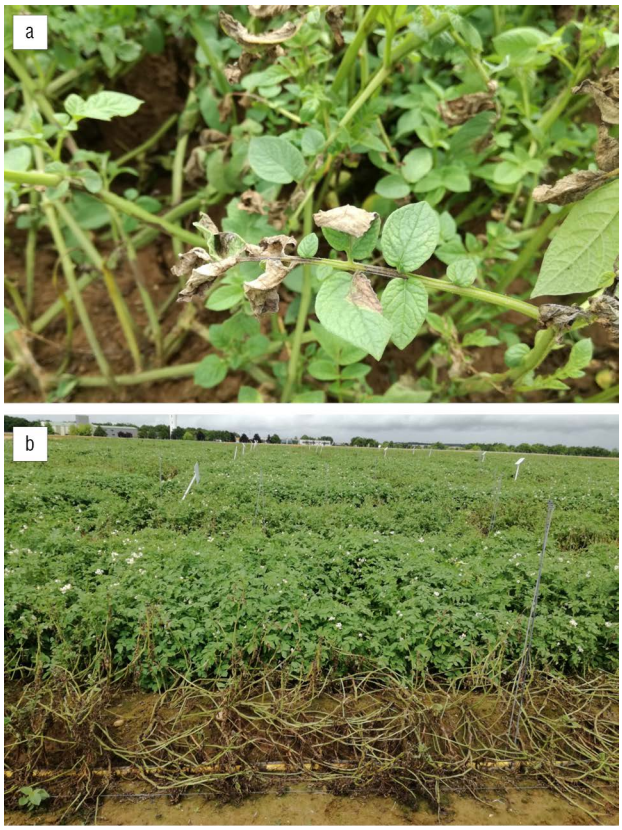

Figure 1: (a) Initial late blight symptoms on potato foliage. (b) Destruction of a susceptible potato cultivar (foreground) in a field.

A study by Pule et al.[13] showed that the US-1 clonal lineage predominates in potato production areas of southern Africa. However, the recent first report of the EU_33_A2 clonal lineage of *P. infestans* causing late blight in Nigeria is concerning, as this lineage is suspected to be more widely spread than previously thought.[14] The introduction of a new pathogen population into an area is often followed by an increase in disease severity and subsequent negative socio-economic impacts.[15] This might be the case in southern Africa should EU_33_A2 spread south on the continent.

Late blight control in developed nations is achieved through seed certification, integrated management, intensive fungicide spray programmes, early planting dates, elimination of inoculum and planting resistant cultivars.[10,15] However, in developing nations, where systemic fungicides are not easily accessible or are unaffordable, growers often use large amounts of low-cost dithiocarbamate-type contact fungicides, in particular mancozeb, which presents significant health hazards to farmworkers and their families.[16] It has been suggested that late blight in developed countries can be managed by phosphonate applications, as a safer alternative to the hazardous dithiocarbamate derivatives currently used.[16] Additional research is, however, needed to determine optimal timing, application rates, specific host reactions, environmental effects and interactions of phosphonates with other chemical compounds.[16] The same level of control of the pathogen as that achieved by intensive spray programmes that incorporate both contact and systemic fungicides will not be attained by phosphonate application alone.

Host resistance may be the most effective way for resource poor growers to manage late blight.[16,17] However, the adoption of resistant cultivars in developing countries is often slow, because of the scarcity of functional seed certification systems[18] and because *P. infestans* rapidly overcomes cultivar resistance, and there is therefore a lack of truly resistant varieties[19].

Bacterial wilt

Bacterial wilt (or brown rot) is another serious disease of potatoes that can result in substantial yield losses. It is caused by members of the *Ralstonia solanacearum* species complex (RSSC). The first symptoms of the disease are wilting of the young leaves of plants, which is most commonly observed at the hottest time of day. As the disease progresses, plants become stunted, display general wilting and chlorosis, and will eventually die.[20] The most obvious symptoms are noted in tubers, as an initial brown discolouration of the vascular tissue, followed by rotting thereof (Figure 2a and 2b). Members of the RSSC globally were previously assigned to four phylotypes, with phylotype classification based on phylogenetic analysis of the 16S-23S ITS region.[21] However, Safni et al.[22] elevated these phylotypes to species level. The phylotypes generally correlate with the geographical origin of the strains: phylotype II originating from the Americas was assigned to *R. solanacearum,* strains in phylotype IV from Indonesia were assigned to *R. syzygii*, and strains in phylotype I and III originating from Asia and Africa were assigned to *R. pseudosolanacearum.*[23] The most threatening potato pathogens, causing bacterial wilt and brown rot globally, fall into phylotype IIB sequevar 1 (IIB1), historically known as race 3, biovar 2, i.e. *R. solanacearum.*[21,23] Strains of both *R. pseudosolanacearum* and *R. solanacearum* can cause wilt in potato, but IIB1 strains are most persistent, can cause latent infections in tubers, are highly destructive and are cold tolerant.[21]

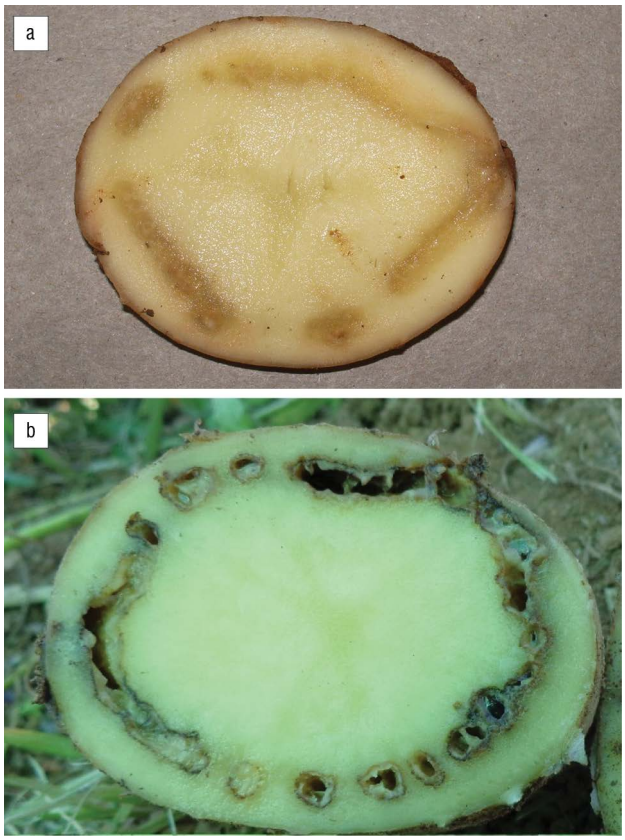

Figure 2: (a) Initial and (b) advanced symptoms of brown rot in potato tubers (images: Fienie Niederwieser).

RSSC is considered to be one of the most important phytopathogenic bacteria globally, because of the destruction it can cause, its wide geographical distribution and host range, and its ability to survive for extended periods in soil, water, on plant debris and in asymptomatic hosts.[24] In southern Africa, bacterial wilt has been reported in Angola, Malawi, Mozambique, South Africa, Swaziland, Zambia and Zimbabwe.[25-27] A report by Nortje[28] indicated that, historically, the most important phylotypes in Africa are I and II, including the virulent IIB1 strains. There is, however, a dearth of recent documentation on the species causing bacterial wilt of potatoes in southern African countries, but it is assumed that both *R. pseudosolanacearum* and *R. solanacearum* are present.

Management strategies for bacterial wilt include planting pathogen-free seed tubers, avoiding cutting or dipping seed tubers, good

management of root-knot nematodes, planting of resistant varieties, soil fumigation, crop rotation with non-hosts, treatment of irrigation water, and disinfection of implements, machines and equipment.[29] Messiha et al.[30] demonstrated the potential of biological soil disinfestation to reduce soil populations of *R. solanacearum* through the incorporation of fresh organic matter into soil and then preventing the re-supply of oxygen into soils by covering with plastic. This practice results in shifts in the composition of bacterial communities, allowing biocontrol organisms to establish themselves in the soil.[30] This practice may be a practical and affordable way for subsistence farmers in southern Africa to manage this devastating disease.

With the exception of South Africa, most southern African countries do not have formal or functional seed potato certification schemes. Growers thus rely on seed that is visually selected and saved from harvested tubers for the following seasons' planting.[29] This practice has resulted in the spread of *R. solanacearum,* as growers usually select smaller tubers for seed, which are often latently contaminated with the pathogen.[29] Numerous studies have shown that latently infected farm-saved seed is the primary source of inoculum and the pathway of dissemination of *Ralstonia solanacearum* IIB1 in many countries in sub-Saharan and southern Africa, thus contributing substantially to the severity of bacterial wilt in these countries.[26,29,31] The importance of seed inoculum is emphasised by the success of the South African Potato Certification Service in drastically reducing the incidence of bacterial wilt in both registered seed potato and table potato plantings since the inception of the scheme.[28] This decrease in the disease in the commercial potato production sector of South Africa can be ascribed to strict implementation of a formal seed certification scheme, and good disease management practices.

Despite the many methods by which bacterial wilt can be managed, it still remains a yield-limiting disease in many countries in southern Africa[32,33], particularly for small-scale or subsistence growers. The wide host range and ability of the pathogen to survive for extended periods in soil and irrigation water renders many of the current potato practices useless in terms of reducing *R. solanacearum* soil inoculum. The wide genetic diversity of *R. solanacearum* hampers the success of cultivars bred for resistance against the pathogen, and makes detection and correct identification of the pathogen difficult, which in turn affects management decisions.[28,33,34] An integrated pest management strategy – which combines numerous cultural, biological and chemical control measures – remains the most effective approach to reduce losses caused by *R. solanacearum.* It is vital that potato growers in southern Africa implement as many of these practices as possible to avoid serious economic losses.

Soft rot / blackleg disease complex

The soft rot / blackleg disease complex caused by *Pectobacterium* and *Dickeya* species is a potential threat to potato production worldwide.[35-38] The soft rotting Pectobacteriacea (SRP) cause systemic and vascular infections in potatoes, which result in the development of various symptoms on the stem and tubers.[35-38] SRP, like *R. solanacearum,* are considered to be among the top 10 most important bacterial phytopathogens globally, and cause significant losses in crop yield and quality.[24] SRP are primarily tuber-borne, but environmental sources and contamination through wind currents, insects and irrigation water are established secondary routes.[39-43]

Soft rot (Figure 3a) can occur in tubers at any stage (in the field, during harvest, post-harvest or in-store) and the disease will spread if a source of contamination is present and conditions are favourable for the development of the disease. The disease is most likely to occur in wet conditions with high temperatures.[35] Blackleg (Figure 3b) develops when the bacteria in contaminated seed potatoes spread upwards in the stems.[35,37] Aerial stem rot is a secondary soft rot of stems and leaves that develops after plants are wounded and subsequently infected by SRP.[35,37]

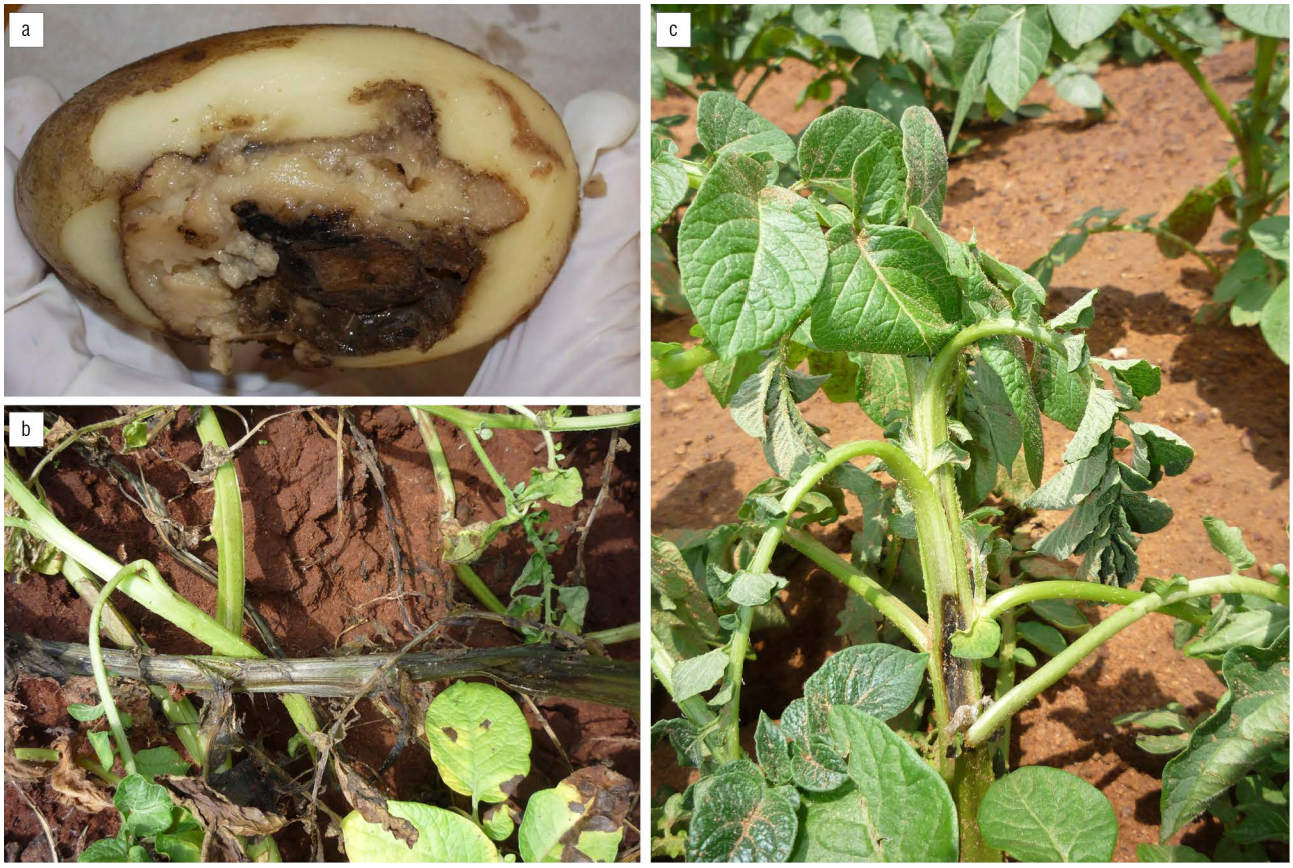

Figure 3: (a) Soft rot of potato tuber. (b) Blackleg of potato plant in field. (c) Early symptoms of aerial stem rot on a potato plant.

The disease complex is a major concern to the potato industry worldwide and a significant contributing factor to yield losses in southern Africa.[44,45] This disease has increased in both severity and distribution, likely due to an increase in planting of susceptible varieties, and the effect of climate change on the composition of the pathogen population causing soft rot and blackleg in southern Africa.[11] The majority of research on the SRP in southern Africa has been done in South Africa, and to a lesser extent in Zimbabwe.[44-46] The most prevalent pathogen causing soft rot and blackleg in these countries is *Pectobacterium brasiliense*.[44,45] *Pectobacterium brasiliense* has been shown to grow at temperatures between 20 °C and 38 °C[47], which are the prevailing temperatures during the main potato growing seasons in southern Africa.

The primary means of management of SRP is planting disease-free seed potatoes[37] and implementation of various cultural management practices, such as roguing of infected plants, increasing calcium fertilisation, avoidance of wounding at harvest and during grading, and storage in well-ventilated, low-temperature conditions. These practices have variable success rates due to latent infections of tubers, rapid reproduction of bacteria and subsequent disease development under favourable environmental conditions.[37] Nevertheless, as with *R. solanacearum*, an effective seed certification system can help to reduce disease incidence.[37,38]

Powdery scab

Spongospora subterranea f. sp. *subterranea* (Sss), the causal agent of root galls (Figure 4a) and powdery scab (Figure 4b) on potatoes, has been described as an emerging pathogen on potatoes.[5] For many years, powdery scab was considered a minor disease on potatoes; however, it is rapidly becoming an increasing threat to potato production globally.[48-51] The global increase in powdery scab intensity can be attributed to increased potato production, planting of susceptible cultivars, more frequent use of irrigation, and discontinuation of mercury-based soil and seed treatments.[48] Only a few published records on the distribution of Sss in southern Africa are available. According to Manditsvara[52], powdery scab is a significant disease of potatoes in Zimbabwe. Likewise, research on powdery scab in South Africa has shown that this disease causes major economic losses to the potato industry in the country.[53,54]

Control of Sss is difficult, but a reduction in disease incidence and severity can be achieved through integration of a number of management measures. These measures include informed selection of pathogen-free fields, cultivar choice, seed and soil treatment, optimal plant nutrition, crop rotation with non-hosts or trapping crops, planting of disease- and pathogen-free tubers, and post-harvest hygiene.[55] As with bacterial wilt and late blight, the implementation of many of these control methods by smallholder or subsistence growers in southern Africa is, however, often problematic or impractical. A compounding factor in the management of powdery scab is the correct identification of symptoms. The symptoms of common scab of potatoes, caused by *Streptomyces* spp., and powdery scab, are difficult to distinguish; although favourable conditions for disease development and control measures for the two diseases are different.[56] Accurate identification of symptoms is thus critically important for the management and containment of the disease. Many African countries, however, do not have active and accurate pest and pathogen diagnostic services for low-value staple crops, in particular, due to lack of expertise and the costs involved in establishing such services.[7,57]

Most of the research on Sss in southern Africa has focused on management of powdery scab. Wright et al.[53] demonstrated the importance of good hygiene and regular testing of growing media in the production of Sss-free mini-tubers in tunnels. Manditsvara[52] and Simango and van der Waals[54] established the potential of biofumigation and various soil treatments in suppressing root galling and powdery scab. These results may provide small-scale, subsistence and commercial growers with sustainable options for suppression of the pathogen in the soil.

The choice of crops in a rotation programme with potatoes is important. Growers should familiarise themselves with the host range of the pathogen[58-60], and plant non-hosts or trapping crops in rotation with potatoes to prevent increase of the soil inoculum. No single management measure will control powdery scab; therefore, growers should use as many of the available options as possible to manage the disease.

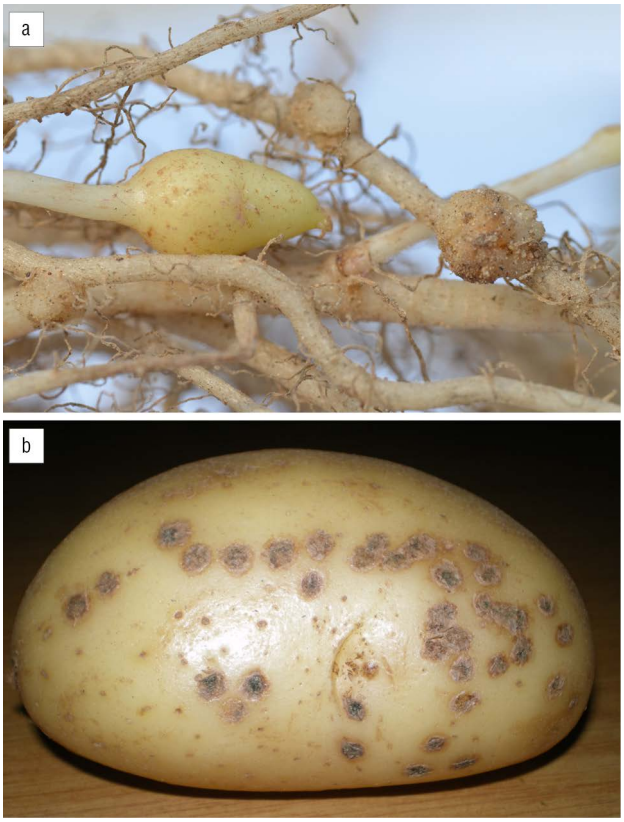

Figure 4: (a) Root galls on potato roots. (b) Powdery scab on a potato tuber.

Zebra chip disease

Zebra chip is an emerging potato disease that poses a serious threat to potato production worldwide, including in southern Africa. Symptoms in potato include leaf curling, yellowing or purpling of leaves or shoots, leaf chlorosis, shortened internodes with aerial tubers, and early necrosis.[61] Potato tubers have necrotic flecking of the vascular tissue and streaks along the medullary rays.[61] The name of the disease originates from potato slices that have brown blotches, stripes and streaks when fried. The disease is associated with the phloem-limited bacterium '*Candidatus* Liberibacter solanacearum'. The pathogen is transmitted in the field by the potato psyllid *Bactericera cockerelli* (Hemiptera, Triozidae), to potato and other solanaceous crops. Other psyllid species have been identified as vectors of 'Ca. L. solanacearum' to plants within the Apiaceae. Another route of infection is planting of infected plant material.[62] The insect vector is native to North and Central America.[62] The disease has been introduced into Europe and New Zealand, with the cause of spread not yet clear.[63] Pathways of introduction of the pathogen into southern Africa include importation of infected plant material or the infected insect vector.[63] An integrated pest management approach is recommended, which includes the planting of certified disease-free material, conservation of natural enemies of the psyllid vector, application of insecticides and use of barriers for smaller areas to prevent the insect vector from reaching plants.[63] The latter two strategies could be practically applied in southern Africa.

Discussion

Due to the vegetative propagation of potatoes by seed tubers, most of the important yield limiting pathogens are seed-borne and thus result in seed degeneration. Thomas-Sharma et al.[64] define seed tuber degeneration as 'an increase in pest and/or pathogen incidence or severity, associated with reduction in yield or quality of seed tubers over successive cycles of vegetative propagation' and thus an indication

of decreasing seed health. Most farmers in developing nations, such as the majority of those in southern Africa, use farm-saved or poor-quality seed, which is often infected with various pathogens, or is physiologically inferior.[20,65-67] This, along with the use of low-yielding varieties with little or no resistance to important diseases, poor disease management, and nutritionally depleted soils, results in low yields in these countries. The Agricultural Research Council of South Africa has a dedicated potato breeding programme, the aim of which is to release varieties that are adapted to local growing conditions in Africa.[68] If sufficiently supported, the varieties developed through this breeding programme could contribute positively to sustainable potato production in other southern African countries.

The introduction of specialised seed production systems, implementation of seed certification schemes[69], reduction in the cost of good-quality seeds, and alleviation of the bottleneck in the seed supply in developing African countries will improve potato production and sustainability[64-69]. The production of seed tubers through a combination of tissue culture and aeroponics may also provide a viable alternative to conventional production of tubers for growers in developing countries.[64,70-72] Aeroponics systems reduce the time and cost of production of seed, improve the growth and survival rate of plantlets, and are environmentally friendly. This approach will ensure the production of vast quantities of pathogen-free seed tubers for growers, and subsequently improve profitability of the farming operations.[64,70-72]

As the demand for potatoes in southern Africa increases, growers often repeatedly crop potatoes in succession on the same fields, which leads to increases in pathogen inoculum in the soil and subsequent yield losses.[6,34,67] Numerous studies have shown the importance of crop diversification and rotation in the suppression of pathogens, the optimisation of nutrient use efficiency, crop productivity and, when legumes are included in the cycle, minimisation of the dependence on external nitrogen applications.[73,74]

Grower training plays a critical role in improving crop production.[75] Demonstration plots and workshops run by regional extension officers should explain the importance of clean seed, crop rotation, hygiene, plant nutrition, and pest and pathogen management in potato production to growers. The success of such training efforts has been shown in Kenya and Swaziland inter alia.[45,75]

One of the challenges encountered in gathering the information for this review was the lack of published data on disease occurrence and epidemiology, type of pathogen strains present, prevalence of pathogens or effect of diseases on potato production in southern Africa. Smith et al.[57] made similar observations on the reporting of plant pests and pathogens in Africa, with the number of reports of new pest introductions in Africa having dropped over the last century compared to Europe. These discrepancies could be attributed to either the actual rate of introductions or the lack of plant protection expertise and capacity to report introductions in many African countries.[6,76] Potato yield losses due to major pathogens in (central) Africa may be more than 50%, compared with 24% in northern Europe, indicating clear differences in crop protection intensity between these two regions.[77] These observations imply that Africa is ill-prepared for pest or pathogen introductions, which could threaten production of staple crops, such as potatoes.[6] Improved border control, plant quarantine or monitoring of the movement of pathogens is imperative to prevent incursions of pathogens into new areas. Border porosity and lack of enforcement of plant quarantine of monitoring services in Africa results in unhindered movement of pests and pathogens across both regional and national borders on the continent[27], and should receive urgent attention to ensure food security in southern Africa.

Competing interests

We declare that there are no competing interests.

Authors' contributions

J.E.v.d.W.: Conceptualisation; data curation; writing – initial draft. K.K.: Conceptualisation; writing – revision.

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INTERNATIONAL YEAR OF PLANT HEALTH 2020

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Potato virus Y and *Potato leafroll virus* management under climate change in sub-Saharan Africa

Potato has increased in importance as a staple food in sub-Saharan Africa, where its production is faced with a multitude of challenges, including plant disease development and spread under changing climatic conditions. The economically most important plant viruses affecting potatoes globally are *Potato virus Y* (PVY) and *Potato leafroll virus* (PLRV). Disease management relies mostly on the use of insecticides, cultural control and seed certification schemes. A major obstacle in many sub-Saharan Africa countries is the availability of disease-free quality seed potatoes. Establishment and implementation of quality control through specialised seed production systems and certification schemes is critical to improve seed potato quality and reduce PVY and PLRV sources. Seed could be further improved by breeding virus-resistant varieties adapted to different environmental conditions combined with management measures tailored for smallholder or commercial farmers to specific agricultural requirements. Innovative technologies – including more sensitive testing, remote sensing, machine learning and predictive models – provide new tools for the management of PVY and PLRV, but require support for adoption and implementation in sub-Saharan Africa.

Significance:

- *Potato virus Y* (PVY) and *Potato leafroll virus* (PLRV) are the two major potato viruses threatening profitable seed potato production.
- High-quality seed shortage in many sub-Saharan Africa countries has been identified as a constraint to increasing yield.
- Specialised seed grower or seed certification programmes should be implemented to prevent virus transmission from seed to daughter tubers.
- Sustainable PVY and PLRV management in seed potatoes requires specific regional approaches to growth, farming and climatic conditions.
- Future research should include predictive models and new innovative technologies such as more sensitive testing, machine learning and remote sensing.

Introduction

Potato (*Solanum tuberosum* L.; Solanaceae) is a high yielding cash crop in sub-Saharan Africa.[1] Its production has experienced one of the largest increases in comparison with other staple food crops in the region.[1] The potato production area more than doubled in sub-Saharan Africa between 1998 (655 447 ha) and 2018 (1.47 million ha), including in regions with high poverty rates, but yields vary greatly across the region (Figure 1).[2] However, food security in sub-Saharan Africa remains a chronic problem.[3] Although there is sufficient food to satisfy global average food consumption in lower-income countries, several countries in sub-Saharan Africa suffer from food insecurity due to low production with limited access to food produced in other countries.[4] Food security is likely to worsen with the impact of climate change and a growing population.[4] The effects of climate change are expected to be most severe in sub-Saharan Africa because of the high dependence on agriculture and its vulnerability to extreme weather events.[3,5]

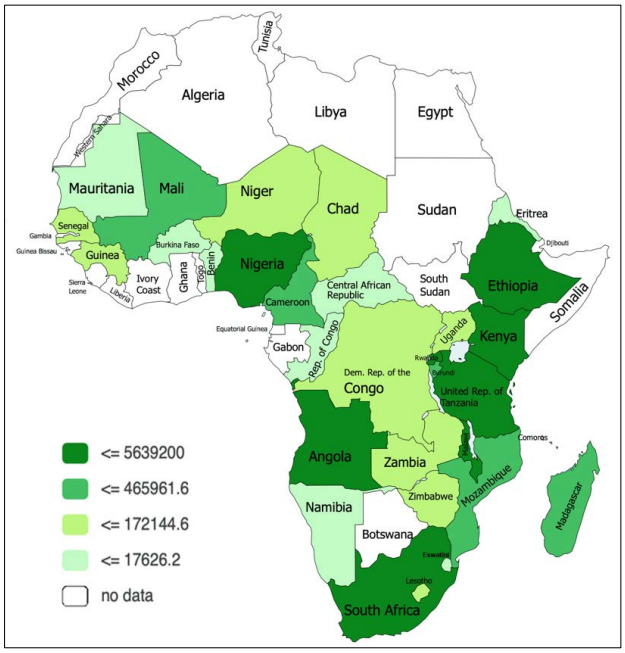
Predicted climate change impact in sub-Saharan Africa is highly complex and region specific. Droughts and heat waves, unseasonal rainfall, and an increase in the frequency of extreme weather events are likely in future.[6,7] The climate in sub-Saharan Africa has already changed with mean increases in temperature from a 1951–1980 baseline to 2019 of 1.5 °C, ranging from 1.0 °C in South Sudan and Eritrea to 2.3 °C in Namibia.[2] Simultaneously, rainfall patterns have become even more variable with regional decreases in southern Africa and increases in eastern Africa.[7] This variability is exacerbated by erratic severe drought episodes.[8,9] Regional adaptations to maintain at least current yields in potato production in the increased area planted[10] are therefore crucial as most potatoes are grown under dry-land conditions during specific rainy seasons[11].

The impact of climate change on potatoes globally has been reviewed by Hijmans[12], Haverkort and Verhagen[13], Raymundo et al.[14] and George et al.[15] Little detailed information is available for sub-Saharan Africa. Simulation models for agro-ecosystems with continental and Mediterranean climates in South Africa suggest that increased CO_2 levels will impact positively on water use by potato plants and thus yield, compensating for negative effects of increased temperature and reduced water availability, provided crops are grown at suitable times.[16,17] However, potatoes grown under heat stress are likely to have a lower water use efficiency and reduced yields, even under increased CO_2 levels.[16,17] Changing climatic conditions could therefore reduce potato production in the lowlands in sub-Saharan Africa by up to 50% by 2050.[14] In eastern Africa, where potatoes are grown mainly in the highlands, heat and water stress have been predicted to reduce yield, with the exception of Rwanda.[18] Potato production regions in the Ethiopian Highlands, for example, are faced with a potential increase of 0.7 °C from 1975 to 2050.[19]

One of the major biotic limitations to potato production are plant diseases. Their impact may be even more severe in warmer regions where seed potato tubers are propagated over several generations, and year-round plantings are

already under continuous pressure from insect-transmitted pathogens.[20] Furthermore, an increase in temperature or milder winters may have a negative effect on seed potato systems that rely on cooler growing regions or cool winters for reducing plant virus inoculum and low insect vector pressure.[20]

More than 50 plant viruses that infect potatoes have been recorded[20], of which two – *Potato virus Y* (PVY; genus *Potyvirus*, family Potyviridae) and *Potato leafroll virus* (PLRV; genus *Polerovirus*, family Luteoviridae) – affect profitable potato production globally[20,21]. PVY has overtaken PLRV as economically the most important of the potato viruses.[20] PVY has been a challenge worldwide during the past 20 years due to the emergence of recombinant PVY variants.[22,23] Both viruses result in dramatic yield and quality losses.[21,24] Infection levels are increased by planting infected tubers, leading to increased infection levels over successive generations. Thus, PVY or PLRV infection can lead to downgrading or rejection of seed lots if tolerance levels set by seed potato certification schemes are exceeded.[25-28] Tubers from informal seed systems, previous crops or tubers that are unmarketable are often used for planting[29]; this becomes a severe problem, especially for smallholder farmers in sub-Saharan Africa.



Source: ©FAO[2]

Figure 1: Potato production (in tonnes) in sub-Saharan Africa (mean 2014–2018).

Both PVY and PLRV are transmitted to the new potato crop through aphid (Hemiptera: Aphididae) vectors (horizontal transmission; primary infection) or via infected seed tubers to daughter tubers (vertical transmission; secondary infection). The severity of infection depends on host plant tolerance, time of infection (with early infection leading to higher yield loss), environmental factors and virus strains involved.[30,31] PVY and PLRV management relies largely on cultural preventative measures that limit virus inoculum or curative measures using insecticides to suppress aphid vector species.

Climate variability and climate change as well as agricultural changes (including introduction of new genotypes, germplasm movement and cultural intensification) add to the intricacy of the already complex virus–insect vector–plant–environment interactions.[32,33] The impact of climate change on plant viruses has been recently reviewed by Islam et al.[33], Jones[34,35], Jones and Naidu[36], and Trebicki[37].

The impact of climate change on aphid vector species and abundance is complex, difficult to predict and region specific[38-41] and depends on plant species, variety and age, and duration and severity of climate stressors and aphid species[42,43]

This review focuses on strategies to manage the spread of PVY and PLRV in potato crops in sub-Saharan Africa under changing climatic conditions. Research findings are reviewed and evaluated with specific reference to potato production in the sub-Saharan African context. Areas requiring further research to manage PVY and PLRV in sub-Saharan Africa are identified.

Potato virus Y

Potato virus Y (PVY) is a single-stranded RNA virus. Several strains have been identified – PVY^{O} (common strain), PVY^{N} (tobacco veinal necrosis strain), and PVY^{C} (stipple-streak strain, including potato virus C), and during the past two decades recombinant variants derived from PVY^{O} and PVY^{N} have become prevalent worldwide, e.g. $PVY^{N:O}$, PVY^{NTN} (N-tuber necrotic), and PVY^{N-Wi} (N-Wilga).[22,23,44] PVY infection increases the number of undersized tubers. Foliar symptoms include mosaic (chlorotic patches; Figure 2a) that may be severe or mild and hardly detectable.[45] Mild symptoms cause problems for virus management in potato seed production because symptomless plants remain unidentified and therefore are not eliminated and serve as virus inoculum.[30] Some recombinant variants cause potato tuber necrotic ringspot disease (Figure 2b).[45]

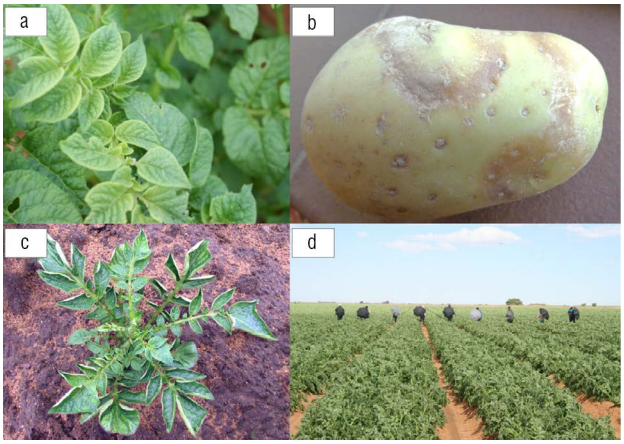


Figure 2: (a) Potato leaflets with mosaic (chlorotic patches) symptoms of *Potato virus Y* (PVY). (b) Potato tuber with necrotic lesions caused by PVY^{NTN} infection (photo: Potato Certification Service, South Africa). (c) Foliar symptoms of *Potato leafroll virus* (photo: Diedrich Visser, Agricultural Research Council – Vegetable and Ornamental Plants). (d) Roguing of plants expressing PVY symptoms in South Africa. Farm employees use umbrellas to facilitate the recognition of leaflets with mosaic symptoms.

PVY is primarily transmitted through aphid vectors, but can also be transmitted mechanically or by grafting but not through true seed.[31] PVY is transmitted non-persistently. The virus does not replicate in the vector and there is no latent period (time between virus acquisition and when a vector becomes infective). Aphid vector species acquire and transmit PVY to an uninfected plant during short feeding probes of seconds to minutes of epidermal cells. Aphids tend to lose the ability to transmit PVY after probing one or two uninfected plants or virus non-host plants. They can become infected again when feeding on an infected plant. PVY can be transmitted by 65 aphid species or species groups that either breed on potato (colonising species; e.g. *Myzus persicae,* the most efficient vector) or are transient species that land and probe or feed but do not breed on potato (non-colonising species; e.g. *Rhopalosiphum padi*).[30] Transient species, although less efficient in PVY transmission[30], are important because they may occur in high numbers. Therefore, both colonising and transient species are of importance when devising management strategies.[30]

PVY transmission and infection of plants is temperature dependent. High temperatures of 25–30 °C negatively influence virus transmission and virus titre at these temperatures decreases over time.[46] Whereas virus establishment in *Nicotiana benthamiana* (Solanaceae) was highest between 20 °C and 30 °C, the virus titre decreased over time in plants at 25 °C and 30 °C.[46]

Potato leafroll virus

Potato leafroll virus (PLRV) is a single-stranded RNA virus. Primary infection symptoms include upward rolling of the leaflets (Figure 2c), while secondary infection symptoms include stunting of shoots and upward rolling of leaflets.[21] Yield losses through stunting of plants and reduced tuber size and number are highest when planting infected tubers and through early infection of young virus-free plants by viruliferous aphids.[31] Apart from reducing yield, PLRV causes internal net necrosis of tubers of some cultivars, rendering them unsuitable for seed, marketing and processing.[20] Mature plants are less affected than young seedlings.[31]

PLRV is most commonly transmitted by aphids, and through grafting, but not mechanically nor through true seed.[31] The virus is transmitted through aphid vector species in a persistent, circulative, non-propagative manner, i.e. individuals remain infected for life (the virus is passed on through moults), but PLRV does not replicate in the vector and the virus is not passed on to offspring.[31] Vectors of PLRV must feed on the phloem sap of potato plants to acquire and transmit the virus. PLRV can be both acquired from infected plants and transmitted to healthy plants by aphid vectors with feeding times of 10–15 min but maximum virus acquisition occurs after feeding of approximately 12 h. Aphid vectors become infective after a latent period of 8–72 h.[31] To date, 13 potato-colonising aphid species or species groups have been identified as vectors.[47]

The highest titre of PLRV was recorded between 20 °C and 30 °C in *Physalis floridana* (Solanaceae).[46] The PLRV growth rate decreased from 25 °C and ceased at 35–40 °C, possibly due to gene silencing.[46,48]

PVY and PLRV management

Potato management, including disease management, requires an integrated approach and regional cooperation among growers.[10,31] Potato virus control worldwide is currently largely achieved through seed quality and certification, vector control with insecticides and cultural control methods.[28,31] Management of PVY poses an even greater challenge than that of PLRV, because it is transmitted non-persistently within seconds and insecticides may have a limited effect.[49] Effective PVY management relies therefore on prevention.[28] Depending on the prevalence of PVY or PLRV in a region and local conditions, a combination of various management strategies may be required for both viruses.

Climate change might affect commercial and smallholder farmers in different ways because of different production systems. In general, farmers may have to reassess current disease management practices. Apart from maximising soil and water conservation, integration of a variety of existing and innovative emerging strategies is likely to be required to cope with increasing uncertainty, and variable rainfall and temperatures.[10,50] General reviews of plant virus management strategies can be found in Jones and Naidu[36] and Kreuze et al.[20] Radcliffe and Ragsdale[31] and Dupuis et al.[28] reviewed management strategies for PVY and Radcliffe and Ragsdale[31] for PLRV. The following provides a brief overview of management options with emphasis on sub-Saharan Africa.

Seed quality and seed certification

The risk of PVY and PLRV spread can be minimised by planting disease-free seed potatoes.[28] Even so, seed quality has been identified as a major limiting factor to successful potato production in a number of countries across sub-Saharan Africa[1,10,29,51] where lack of specialised growers, informal seed systems and the use of unmarketable ware potatoes often result in poor-quality tubers, which produce low yields and tubers with low market values[1,29].

Effective, usually government-regulated, seed potato certification programmes, together with virus testing regimes, have long been implemented in developed countries for the control of seed potato quality.[26-28] Certification thresholds or disease tolerances are set to limit secondary virus transmission from infected seed tubers to daughter tubers and to limit primary infection within a crop in the next season. However, the recent emergence of new strains, e.g. PVY^{NTN} and $PVY^{N:O}$, together with potato cultivars that are tolerant, i.e. do not exhibit symptoms, pose new challenges to seed certification programmes.[52] Furthermore, higher temperatures due to climate change may reduce levels of PVY and PLRV[46] below the detection limit of enzyme-linked immunosorbent assays (ELISAs) used for detection in certification schemes. This is especially problematic for seed potatoes produced in low-lying seed potato regions in sub-Saharan Africa where temperature increases are expected to be more severe than in high-lying production areas. New technologies for reliable and sensitive virus detection methods, such as real-time reverse transcriptase polymerase chain reaction (qPCR) assays, should be implemented in schemes for seed certification to overcome the problem.

Some countries in sub-Saharan Africa, e.g. Kenya and South Africa, have introduced seed certification schemes.[25,53] Both largely use ELISA for detection of PVY and PLRV. Due to underdetection, ELISA has been replaced with more sensitive molecular techniques in many European countries.[28] The lack of capacity, aggravated by the cost of certification schemes, and a low demand because farmers are unwilling to pay the high price for quality seed[1,29,54], has delayed the implementation of seed certification schemes in many countries in sub-Saharan Africa. In a survey in eastern Africa (Kenya, Uganda and Ethiopia), only 2% of seed potatoes were sourced from seed growers, whereas 65% originated from own fields and 31% from rural markets.[55] This lack of certified seed could be overcome through government agencies that support smallholder farmers and provide advice through extension services and testing through national agencies. Furthermore, alternative strategies are being developed.[1,29] These include the use of true seed of hybrid potato varieties, which is virus free and easier to transport, benefiting smallholder farmers in remote areas[56], or prolonged seed health in informal systems in sub-Saharan Africa through an 'integrated seed health strategy' where disease resistance is combined with on-farm management[29].

Disease-resistant potato varieties

Resistant varieties, in which the host limits pathogen multiplication, are thought to provide the most efficient way to manage viruses, but the majority of varieties grown currently in sub-Saharan Africa do not possess adequate resistance to PVY or PLRV.[28,57] Furthermore, mature plant resistance has often been overcome by recombinant strains.[58] Breeders have introduced genes for hypersensitive resistance and extreme resistance to PVY.[20,57] Hypersensitive resistance is mostly PVY-strain specific and the death of virus-infected cells leads to localised necrotic lesions. However, as hypersensitive resistance is temperature sensitive, the higher temperatures induced by climate change can be expected to neutralise its beneficial effect.[24] Extreme resistance is effective against a broad range of PVY strains and does not cause visible lesions.[20] Although various molecular techniques (e.g. RNA-mediated resistance, RNA interference and more recently CRISPR/Cas technologies) are available for introducing resistance to viruses, only a few transgenic resistant potato varieties are available and none in developing countries.[36,59] In many countries in sub-Saharan Africa, the introduction of transgenic crops remains a highly contested issue.

Breeding of potato varieties with glandular trichomes, that may reduce aphid settling and thus virus transmission, may be more successful for PLRV than PVY because of the difference in their mode of transmission (persistent versus non-persistent).[57] However, it has been met with limited success because of negative effects on the growth period, tuber size and yield.[57] For recent reviews on PVY-resistant varieties see Valkonen et al.[60] and Kreuze et al.[20], and for PLRV see Halterman et al.[26].

Apart from planting virus-resistant varieties and increasing yield and nutrient content in varieties to maximise the use of the limited area

available, more diversified disease-resistant varieties are needed that are at the same time more resource efficient, requiring less water, fertiliser and pesticides to provide greater resilience to extreme weather events.[50,61-63] Some sub-Saharan African countries have initiated breeding programmes, frequently involving the National Agricultural Research System supported by the International Potato Center, to provide varieties suited for local growing conditions.[3,64]

Preventative cultural control

Spatial and temporal isolation

Tubers that exceed virus infection levels set by certification schemes or infected retained tubers in informal seed systems are unsuitable as seed potatoes but are frequently planted as commercial crops, which subsequently serve as virus reservoirs.[47] Geographical isolation and temporal separation of seed from commercial potato plantings and other crops that are potential inoculum sources of PVY or PLRV have been listed amongst the most effective preventative management strategies[28,31,47], which could also be applied by smallholder farmers in sub-Saharan Africa, although effective isolation distances are region specific[31].

Roguing

Another strategy to reduce PVY and PLRV spread within a growing season is roguing – that is the visual inspection and removal of plants expressing symptoms within seed-potato crops (Figure 2d), especially when virus incidence is low.[20,31] For roguing to be effective, seed potato fields require multiple inspections throughout the growing season and it is therefore labour intensive.[31] Infected plants should be removed before aphid numbers increase.[31] In order to recognise infected plants, cultivars expressing symptoms should be grown[47], but this is in conflict with planting disease-resistant varieties that mask symptom expression. In this case, the only alternative to roguing for the elimination of infected plants is detection by laboratory testing, which therefore requires careful consideration.

Elimination of virus sources – volunteer potatoes and weed control

Volunteer potatoes, other susceptible crops and weed species that are hosts of PVY or PLRV are important virus reservoirs.[28] Volunteer potatoes, which are plants that grow from tubers remaining in the soil after harvest, pose a significant threat as a source of virus inoculum and consequently viruliferous aphids in the next growing season, especially if warmer winters enhance their survival, and should be removed before the emergence of new plantings.[28] Another aspect to reduce virus inoculum is weed management as the host ranges of PVY and PLRV span over several families.[65,66]

Crop mulching, intercropping, crop borders

Various options are available to reduce aphid landing in potato fields. The rationale is to create less contrast between the green plant canopy and the soil in order to lower aphid landing rates in seed potato fields.[28] Straw mulches and intercropping with cereals, for example, may reduce PVY spread but may be expensive strategies that are effective only before the canopy closes and may require removal.[28,47] Crop borders consisting of non-virus host plants rely on the same principle. Aphids tend to land in high numbers at field edges due to the contrast in light reflected from the soil and the plants.[49] Aphid landing in the main crop is reduced by displacing the edge of the main field with a non-virus host plant. Crop borders for the management of non-persistent viruses have been reviewed by Schröder et al.[49] Crop borders may be suitable for smallholder farmers in sub-Saharan Africa, whose fields tend to be smaller than those of commercial farmers, as the area needed for a border depends on field size. Crop borders consisting of non-virus crops that are already part of a farming system, e.g. maize or cereals, may provide additional support for smallholder farmers.

Aphid monitoring

Aphid vector species composition and abundance vary greatly with time of year and region.[30,67] Thus, aphid monitoring is a key aspect of managing virus spread. Regular aphid monitoring data enable growers strategically to target location and timing of control measures, to optimise the timing of planting, haulm destruction and harvest. Aphid vector species composition together with their virus transmission efficiency and abundance are used to calculate vector pressure indices for specific regions.[30]

Aphid flight activity is usually monitored with suction traps, often in combination with yellow water traps to provide rapid information on vector pressure (Figure 3). The height of suction traps and the variation in topography determine the area over which aphids are sampled. Suction traps commonly used are 1.8, 8 or 12.2 m high.[68] Traps above 10 m height provide random aerial samples of aphids. Suction traps with a height of 1.8 and 8 m usually monitor aphids at local level. However, depending on the landscape, trap catches of 8-m traps may be representative of aphid flight activity over a 30-km radius.[69] South Africa has an extensive suction-trap network and seed potato farmers receive weekly information on aphid numbers and vector pressure to alert growers to the risk of PVY and PLRV spread.[30] Establishment and maintenance of aphid monitoring programmes may be challenging, because of the cost involved and the difficulty in identifying aphid species, especially for smallholder farmers. However, government- or industry-supported aphid monitoring initiatives should be considered and are recommended as they can be hugely beneficial for controlling viral spread.

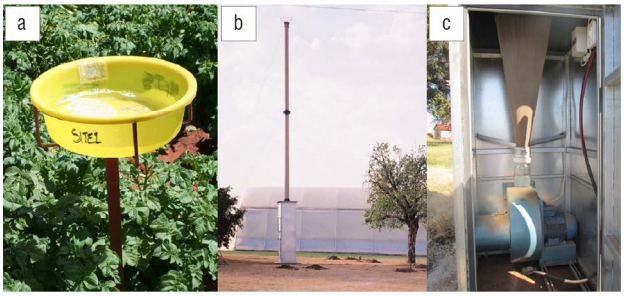


Figure 3: Traps used for aphid monitoring: (a) yellow bucket trap; (b) 12.2-m Rothamsted-type suction trap; and (c) inside of a suction trap showing the insect collection jar.

Planting and harvesting dates

The risk of virus transmission by an increase in aphid vector numbers can be reduced through adjustment of planting and harvesting dates to avoid peak vector activity[47,70], which is region specific[30,39,67]. However, this strategy is dependent on aphid monitoring systems being in place.

Chemical control

The longer transmission time of PLRV compared to PVY makes it easier to manage virus spread with chemical control because insecticides may affect aphid vectors before they can transmit the virus.[44] Synthetic insecticides may not be effective in preventing PVY transmission but may be effective in reducing aphid populations[49] and are one of the main methods for the management of PVY and PLRV. Chemical control using insecticides and mineral oils has been reviewed by Dupuis et al.[28], Lacomme et al.[30] and Yang et al.[71] Mineral oil may reduce PVY spread but its effectiveness depends on environmental conditions.[28,71] In African countries, farmers have been reluctant to use mineral oils because of concerns of phytotoxicity at high temperatures and potential yield loss.[72] Synthetic insecticides for smallholder farmers in sub-Saharan Africa are often not available or affordable.[59]

Predictive models

The complex interactions of different abiotic (e.g. climate change) and biotic stressors (e.g. plant pathogens and insect vectors) on plant growth make it challenging to predict the risk of plant disease development and spread. Climate modelling has progressed over the past years with models being able to guide farmers in planning climate change adaptation strategies.[73] However, adoption of forecasting models for

decision-making in sub-Saharan Africa by smallholder and commercial farmers alike has been limited due to lack of sufficient forecasting skills and perceived lack of relevance of forecasting models for specific farming decisions.[74]

New technologies

A variety of new and emerging technologies are being developed to improve virus disease management (e.g. through detection of diseased plants, insect infestations) or to alleviate climate change impact on virus outbreaks at various geographical levels.[36] To improve virus detection, management and prediction, new detection technologies should be implemented, e.g. qPCR. Innovations to provide decision support and curb virus spread include remote sensing, machine learning, aerial surveillance, and precision agriculture.[36,75-77] For example, Gómez et al.[78] developed a model to predict potato yield using satellite remote sensing and machine learning. Griffel et al.[79] proposed the use of support vector machine classification, based on machine learning and using different spectral profiles of PVY-infected and uninfected plants, for the detection of PVY infestations. Furthermore, technologies have been developed to identify areas of insect infestation, including aphids, based on plant stress.[80]

Conclusion

Current knowledge on the effect of climate change on plant disease management is limited. However, various measures may alleviate the predicted impact of climate change on the spread of PVY and PLRV and their aphid vectors. Future work in sub-Saharan Africa should concentrate on improving seed quality, currently a major constraint, especially for smallholder farmers, together with the development of region-specific management programmes that include cultural control methods. Another aspect is breeding for more disease-resistant varieties that (1) are more diversified to have a greater pool of varieties to choose from for different growth conditions, (2) provide greater resilience to extreme weather events, (3) have a higher nutrient content and yield to maximise the use of the area available, and (4) are more resource efficient. Further research should be directed at innovative technologies to improve disease detection, management and prediction. Considerable progress has been made in predictive modelling and its application in crop production, and research should be directed at improving the adoption and implementation thereof in sub-Saharan Africa. However, key to the implementation of any of these advances will be communication so that the benefits of these new technologies can be understood by stakeholders.

Competing interests

We declare that there are no competing interests.

Authors' contributions

K.K. and J.E.v.d.W. jointly conceived and wrote the article.

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INTERNATIONAL YEAR OF PLANT HEALTH 2020

Tree health in South Africa: Retrospect and prospect

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South Africa is a country with very limited natural forest cover. Consequently, the timber and fibre needs of the country cannot be provided for from indigenous forest. It is largely for this reason that South Africa initially developed a highly productive plantation forest industry, which today makes a substantial contribution to the local economy. These plantations are based on non-native species of *Eucalyptus*, *Pinus* and Australian *Acacia*. In the early years of establishment, South African plantations were relatively free of pest and pathogen problems. But, over time, an increasing number of insects, fungi and bacteria have emerged as serious threats to the sustainability of the forestry industry. Numerous native pests and pathogens, especially insects, have adapted to these introduced tree species to cause damage or disease. The problem is compounded by the accidental introduction of non-native pests and pathogens, and this has been at a rapidly increasing rate over the past three decades. Some of these introduced pests and pathogens also threaten the fitness and even the survival of many indigenous South African tree species. Fortunately, South Africa has developed an impressive knowledge base and range of integrated management options to deal with these problems. This development was first driven by government programmes, and in more recent years by public–private partnerships between industry, universities and government. It is clear from the pattern of emergence of pests and pathogens in recent years that South Africa will deal with an increasing number of these problems and a continuously changing tree health environment. This requires robust investment in both quarantine and mitigation mechanisms to protect the country's biodiversity as well as to ensure the sustainability of its wood and fibre industries.

Significance:

- This review about tree health in South Africa was in part inspired by the 2020 International Year of Plant Health. Plant health, and particularly tree health, is an important topic in regard to the sustainability of our forestry industry and conservation of our native forests. South Africa has been a leader in the field and this review highlights some of the achievements that researchers in the country, both past and present, have attained.

Introduction

South Africa is an arid country and consequently has very limited resources of natural forest in the western part of the country. This is the primary reason why South Africa was one of the first countries in the world to establish commercial plantation forestry based on non-native tree species. These plantations were able to accommodate local demand for wood products, especially for construction and fuelwood. In the process, this allowed small tracts of natural forest and woody ecosystems to be spared from destruction.[1]

The first commercial plantations established in South Africa were those of *Pinus pinaster* (around 1825), soon overtaken by *P. radiata* and much later by *P. patula*. Likewise, *Eucalyptus* was an early addition to the exotic tree resource with the first planting of *Eucalyptus globlus* in 1887. Subsequently, commercial forestry has grown considerably in South Africa based mainly on *Pinus* and *Eucalyptus*, but also including Australian *Acacia* species, mainly *Acacia mearnsii*. According to Forestry South Africa, as of February 2020, the current landholding representing commercial plantation forestry in South Africa is approximately 1.2 million hectares.

Many factors affect the health of trees. These factors include damage by insect and nematode pests and pathogens such as bacteria, fungi and viruses. Climatic factors such as rainfall, temperature and wind, as well as edaphic factors including soil structure and quality, are all important contributors to the overall health and vigour of trees. For the purpose of this review, we deal exclusively with pests and pathogens – thus broadly the fields of forest entomology and forest pathology as they relate to the health of forest trees in South Africa.

When considering forest tree health in South Africa, it is important to clearly distinguish between trees in natural woody ecosystems and those that are planted commercially. These are very different situations, both in terms of the impact and the management of insect pests and pathogens. Plantations, especially in the southern hemisphere, typically comprise non-native tree species often planted in high-density monocultures that are intensively managed. The choice of species, provenance or genotype is carefully controlled, and typically tailored to particular regions and sites based on numerous biotic and abiotic conditions as well as risk factors. Natural forest and woody ecosystems in South Africa are biodiverse and composed of large numbers of native trees and other plants in a complex matrix, and are typically protected from logging. In contrast, plantations are managed mainly by commercial enterprises and are of high value. Among other factors, this dichotomy leads to different approaches to managing pests and pathogens, although significant tree health challenges exist in both commercial and non-commercial woody ecosystems.

In plantations, serious damage due to pests and pathogens is usually very obvious and is considered important and worthy of intervention. In contrast, disease and pest problems in natural forests have been afforded very little attention in South Africa in the past. The value of understanding and managing health risks in natural forests has changed in recent years, due in part to the recognition of bidirectional transfer of pests and pathogens between native and non-native plantation systems, and the resulting substantial impacts on trees grown as non-natives in plantations.[2,3] Thus, the establishment of the South African Department of Science and Innovation (DSI) and

National Research Foundation (NRF) Centre of Excellence in Tree Health Biotechnology (CTHB) in 2004, which includes the health of trees and shrubs in natural woody ecosystems as well as commercial plantations, is particularly important.[4]

It is not the intention of this review to provide a detailed history of forest protection in South Africa. There are various previous reviews dealing with the history of forest pathology and forest entomology in the country, which treat this topic relatively comprehensively.[5-9] It is also not an aim to consider the finer details of the likely future concerning forest tree health in this country. That topic has also been treated in some detail in various recent reviews, particularly those relating to plantation forestry.[10,11] In contrast, we attempt to briefly capture some of the key elements of the history of forest protection in South Africa. Furthermore, we broadly consider the current situation and the likely requirements for this field in the future. Rather than focus on the specifics of numerous insect pests and diseases, for which detailed information can be found in the most recent edition of the South African Forestry Handbook, broad concepts with selected examples are provided.[12]

Pest and pathogens of native woody plants

There are extensive records of fungi and insects for South Africa[13-15], which are mainly housed in the National Collections and, in the case of insects, also in various museums. Many among these fungi and insects colonise the living tissue of trees. Yet, very little is known about most of these collections beyond some basic taxonomy. Generally, there has been little support for studies of insects or pathogens occurring in natural ecosystems. This is largely due to the fact that they are not considered to be of economic importance.

Where native trees or shrubs have exhibited signs of serious decline or damage, the causal agents are typically known or thought to be non-native. Perhaps the best example of a non-native organism with severe negative impacts on native plants is the root-feeding fungus-like heterokont, *Phytophthora cinnamomi*, which is particularly important on species of the Proteaceae in natural areas within the Cape fynbos.[16-18] Among the most susceptible native species is *Leucadendron argenteum* (the Cape silver tree), which has died in large numbers on the Cape Penisula.[19] Research on *P. cinnamomi* has shown that the pathogen was most likely introduced into South Africa.[20] The unusually large numbers of plants that have been killed, together with the rapid onset of this disease, support this view. Another contemporary and well-publicised example of an introduced pathogen that has the potential to damage native woody plants in South Africa is the root-feeding fungus, *Armillaria mellea*. This fungus was accidentally introduced into the country, most likely from Europe by early European settlers[21], and has gradually become established in the natural environment of the Cape Penisula[22,23], with devastating effects on *Protea* and *Leucodendron*.

In many cases in which trees or woody plants are diseased in natural ecosystems, the origin (i.e. native versus introduced) of associated pests or pathogens is difficult to determine.[2,24] Organisms that are new to science (commonly the case) are often erroneously designated as native owing to the lack of knowledge of their true worldwide distribution. This is important because geographic origin aids in predicting the spread and severity of a novel pest or pathogen problem and informs the search for potential biological control agents. The rapidly growing availability of population-based molecular genetic tools has, however, changed this situation. A growing number of studies show that pathogens found associated with tree diseases in natural ecosystems are in many cases likely to be of exotic origin.[20,24-27]

The importance of tree health in natural forests has recently been highlighted by the accidental introduction of the polyphagous shot hole borer, *Euwallacea fornicatus*, and its fungal symbiont *Fusarium euwallaceae*.[28] The beetle is native to Asia and was first detected in South Africa in 2016, as part of a programme to survey botanical gardens for new and emerging pest risks. Originally isolated from the non-native London plane tree (*Platanus* x *acerifolia*), it has subsequently been recorded on a large number of tree species in South Africa, including native forest species. The impact of this invasive beetle and pathogen on South Africa's natural forest is currently being investigated (De Beer ZW 2020, personal communication). Its introduction strongly emphasises the importance of surveillance programmes, such as those in botanical gardens and arboreta, amenity tree plantings as well as in natural and plantation forests, and ports of entry.

Pests and pathogens of plantation trees

Native insects and fungi

Not surprisingly, the history of recording and studying pests and pathogens of plantation-grown trees dates back to the beginning of the forestry industry in South Africa.[2,29] Some of the first records of pests and pathogens in plantations were those caused by native organisms that were able to feed on the non-native trees. Noticeable examples were of the pine emperor moth (*Nudaurelia cytherea*) recorded damaging *Pinus radiata* in 1885 and Armillaria root rot[30] (now known to be caused by the native *Armillaria fuscipes*)[31] recorded on *Pinus* species in various provinces of South Africa[32].

Many other native insect pests and pathogens are now known to cause serious damage to commercially propagated species of *Pinus*, *Eucalyptus* and *Acacia* in South Africa.[3] The analysis by Crous and co-authors[3] showed that native insect pests more commonly shift to and cause damage to these non-native plantation trees than do native pathogens. Examples include the wattle bagworm, *Kotochalia junodi*, that has been severely damaging to *Acacia mearnsii* virtually since the tree was first planted in this country.[6,9] Numerous other native insects, including defoliating and wood-boring Lepidoptera and Coleoptera, white grubs and sap-sucking insects are pests of *Eucalyptus*, *Pinus* spp. and *A. mearnsii*.[12,33] Recent outbreaks of the wattle semi-looper, *Achaea lineardi*, the pine brown tail moth, *Euproctis terminalis,* and *N. cytherea* (authors' personal observation) indicate the importance of these native insect pests and the need for research to acquire knowledge on their biology, population dynamics, diversity and other aspects that will inform management actions.

The relatively large number of native insects that have been able to feed on non-native plantation trees, at least in comparison to examples of pathogens, might relate to the fact that a subset of insects are highly polyphagus. They consequently have wide host ranges and thus easily adapt to feed on non-native trees.[3] There are nevertheless a number of examples of damaging native pathogens that have adapted to damage non-native plantations. This number is also increasing because a number of contemporary studies using DNA-based techniques have shown that fungal pathogens, which might originally have been thought of as introduced into South Africa, are actually native.

A recent and fascinating example of a native pathogen originally believed to be introduced into South Africa is found in the case of the eucalyptus canker pathogen *Chrysoporthe austroafricana.* When first discovered in South Africa, this fungus was thought to be the notorious *Cryphonectria cubensis* (Figure 1). But it was later shown to be a native fungus occurring naturally on South African Myrtaceae which had undergone a host range shift to infect introduced *Eucalyptus* spp.[34-36] Likewise, the canker stain and wilt pathogen of *A. mearnsii*, *Ceratocystis albifundus,* was originally thought to be the pathogen *C. fimbriata* and was later shown to be a common natural inhabitant on the wounds of many native South African woody plants.[37] Likewise, a relatively large number of Botryosphaeriaceae canker and dieback pathogens that occur in non-native plantations are likely native to the region.[38] The origin of many pathogens remains unclear and it is expected that further sampling and growth in the number of molecular population genetic and phylogeographic studies will reveal that other pathogens on non-native plants are native.

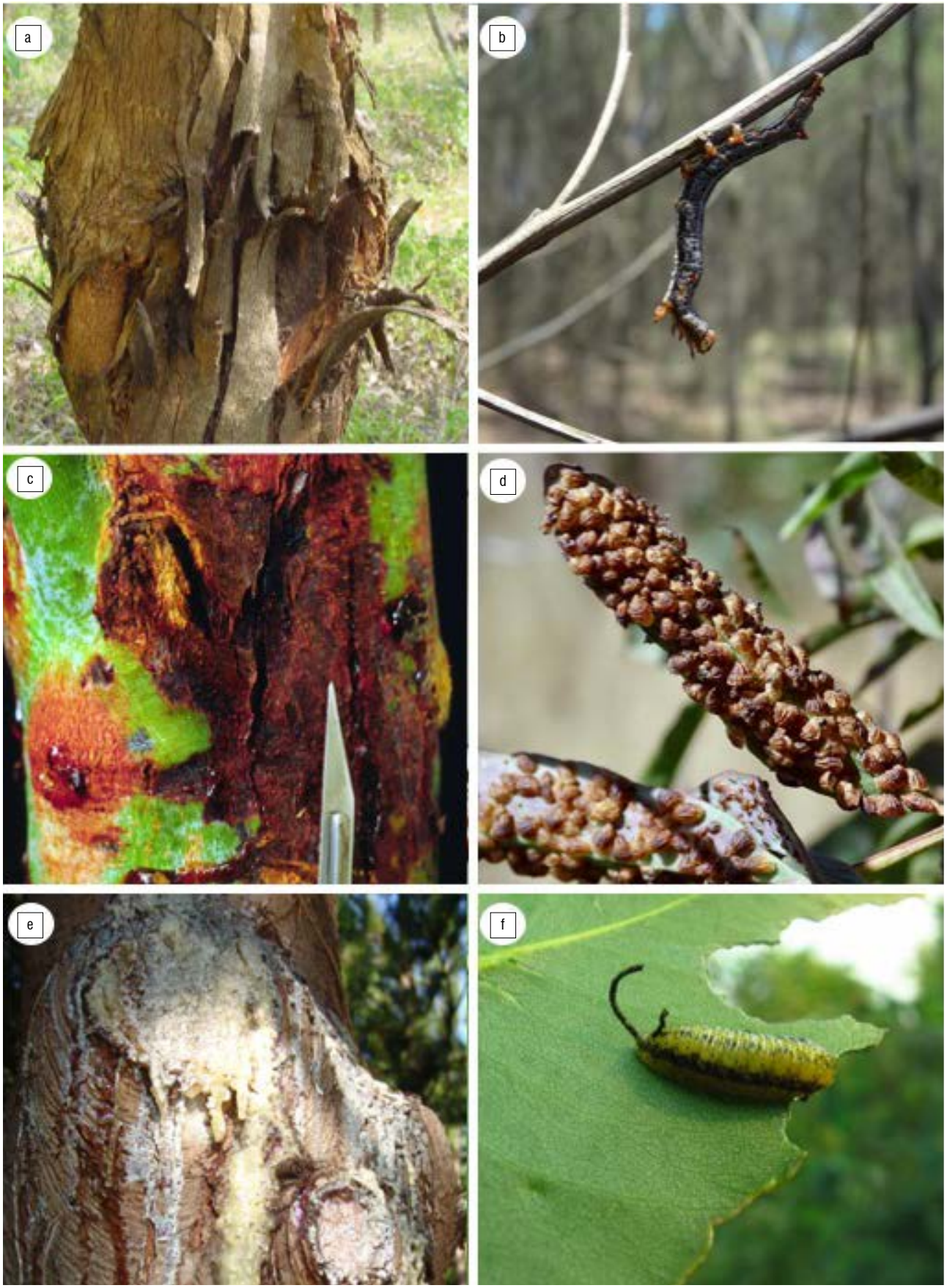

Figure 1: Pathogens and insect pests of plantation trees in South Africa: (a) *Cryphonectria* canker on *Eucalyptus* – one of the first serious diseases to emerge in South African clonal forestry; (b) the wattle semi-looper, *Achaea lineardi*, a native insect and sporadic pest of *Acacia mearnsii*; (c) symptoms of *Coniothyrium* canker caused by *Teratosphaeria zuluensis* on a susceptible *Eucalyptus* clone; (d) the shell lerp psyllid, *Spondyliaspis* c.f. *plicatuloides*, a recently introduced pest of *Eucalyptus*; (e) canker on pine stem with resin bleeding caused by the pitch canker fungus *Fusarium circinatum*; (f) larva of the eucalypt snout beetle, *Gonipterus* sp. n. 2, feeding on *Eucalyptus*.

Non-native insects and fungi

While native pests and pathogens can clearly cause very serious damage to non-native trees in plantations, it is generally recognised that the outstanding growth of these trees is largely due to the fact they have been separated from their natural enemies. In this respect, they behave much like weeds with their superior performance being attributed to 'enemy release'.[39] It is for this reason that the accumulation of insects and pathogens and the accelerating rate of introduction into the non-native plantation resource of South Africa represent a serious threat to the local industry.[8,9,11,29,40]

Host-specific insect pests and pathogens of species of *Pinus*, *Eucalyptus* and *Acacia* known to occur in the areas of origin of these trees have appeared in South African plantations with increasing frequency. When cumulative data for insects and pathogens of any of these trees are examined, there is a clear trend of an accelerating problem and it is one that is likely to continue in the foreseeable future.[9,29,40] Risk abatement and management strategies must clearly take this growing threat into serious consideration.

The eucalyptus snout beetle, *Gonipterus* sp. n. 2 (originally recorded as *G. scutellatus*)[41], recorded in South Africa in 1916 (Figure 1), was the first serious non-native pest to affect non-native plantations in the country[42,43]. Numerous other non-native insect pests, especially those on *Pinus* spp. and *Eucalyptus* spp., have subsequently entered the country, with varying impact. These include various guilds of insects, including sap-suckers, gall formers, bark and wood borers, and defoliators.[12,33] The more recent arrivals are the bronze bug (*Thaumastocoris peregrinus*), bluegum chalcid (*Leptocybe invasa*), red gum lerp psyllid (*Glycaspis brimblecombei*), shell lerp psyllid (*Spondyliaspis* c.f. *plicatuloides;* Figure 1), and the eucalypt gall wasp (*Ophelimus maskelli*) – all pests of *Eucalyptus* and recorded in 2003, 2007, 2012, 2014 and 2014, respectively.[44-46]

Non-native pathogens have had a very substantial impact on plantation forestry in South Africa. It can be reasonably argued that they have substantially influenced the choice and distribution of species planted. The pine shoot and dieback pathogen *Diplodia sapinea* was the first non-native pathogen recorded in South African plantations[47,48] and was rapidly recognised as leading to the death of large numbers of trees after hail damage[49,50]. Thus, susceptible species, such as *P. radiata* and *P. patula,* were specifically not planted on sites prone to hail storms. Likewise, leaf blotch caused by *Teratosphaeria nubilosa* (originally recorded as *Mycosphaerella molleriana*) is thought to have contributed to the failure of *E. globulus* as a plantation species in South Africa.[51] From the insect side, the eucalyptus snout beetle is at least in part responsible for the discontinued planting of *Eucalyptus viminalis* and *E. globulus* in the country.[40,42,43] And the recent introduction of the gall wasp *L. invasa* has already substantially influenced the *Eucalyptus* genotypes that can be planted in affected areas due to considerable differences in host resistance.[45,52]

Much as in the case of introduced insect pests, a large number of host-specific pathogens of *Pinus*, *Eucalyptus* and *Acacia* species have been recorded in South Africa.[2,3,12] Many of these are relatively weak pathogens that have not caused serious damage, while others are much more important. Certainly, the most important pathogen affecting commercial forestry in recent years has been the pine pitch canker pathogen, *Fusarium circinatum* (Figure 1). This pathogen was first found in a single nursery in 1991 and it has subsequently spread to all pine production nurseries in the country.[53,54] For many years, it was known only as a nursery problem, but in 2005 it was first recorded on mature *P. radiata* trees on the Cape Peninsula.[55] While the canker disease on established trees is of concern, particularly in coastal plantations, the most important impact of *F. circinatum* has been that it has rendered *P. patula* virtually impossible to establish cost effectively.[56] Essentially, the most important *Pinus* species planted in South Africa will most likely need to be replaced due to this pathogen.

Non-native pests and pathogens entering South Africa may arrive from the native range of plantation trees. As these agents of disease are increasingly being moved around the world, the probability of establishment increases non-linearly.[2,9,11,40,57] Available evidence suggests that once a pest or pathogen has become established in a new environment, it is more likely to move again – a trend referred to by Lombaert and co-authors[58] as 'a bridgehead effect'. The worldwide movement of the Sirex woodwasp *Sirex noctilio*, one of South Africa's most serious pine pests, is one of many insects and pathogens that illustrates this effect.[29,59-62] Trees in urban environments often serve as a convenient bridgehead between regions, before pests and pathogens spread into natural or plantation forests.[63] For this reason, urban environments, and botanical gardens in particular, offer important opportunities to study and monitor invasive or potentially invasive pathogens.[63-65]

Management of pests and pathogens

Efforts to reduce the impact of insect pests and pathogens in South African plantations date back to the time of the first records of these problems.[5,6] Broadly, the available options include chemical control, biological control (mainly for insects), avoidance through planting non-susceptible species and efforts to reduce the populations/inoculum loads of the pests/pathogens. While chemical control was quite widely used in the early period of South African forestry (see for example Tooke[66]), the negative environmental and health effects, and consequently rules set by, for example, the Forestry Stewardship Council, have rendered this approach increasingly difficult.

South Africa has a long and well-established history of using biological control to reduce the impact of forest pests (Figure 2). This use dates back to the introduction of the parasitoid wasp *Anaphes nitens* for the biological control of *Gonipterus* sp. 2 (then known as *G. scutellatus*)[42,43], which remains one of the classic examples of successful biological control. Other examples of classical biological control for non-native insect pests include *Pauesia* sp. for the control of *Cinara cronartii*, various biological control agents for the control of *Phoracantha* species, *Deladenus siricidicola* and *Ibalia leucospoides* for the control of *S. noctilio* (Figure 2), *Selitrichodes neseri* for the control of *L. invasa*, and *Cleruchoides noackae* for the control of *T. peregrinus*.[6,67-70] Biological control remains the most effective option currently available to manage the impact of damaging introduced forest insects.[40,71]

Various strategies have been used to reduce the impact of diseases in South African plantations.[10] Silvicultural methods such as thinning to reduce stress and the removal of dead and dying plant material from plantations are commonly applied for both insect and pathogen management. But the most commonly used approach is planting resistant species or clones in areas prone to infection by fungal pathogens. The most notable and long-standing example is found in the case of the shoot and dieback pathogen *Diplodia sapinea.* This fungus is opportunistic and infections typically occur on stressed trees, as mentioned above. The most commonly encountered of these stresses is that associated with hail damage.[49,50] Thus, highly susceptible species such as *P. radiata* and *P. patula* have been confined to areas where the risk of hail is minimal. Likewise, damage due to pruning produces wounds for infection and, at least for some time, stress on the trees, which often results in infection. Thus, recommendations for pruning at times of the year when *D. sapinea* is unlikely to infect[50] have been implemented.

By far the most commonly used and effective means to deal with disease in plantations is to establish trees that are highly tolerant or even resistant to infection.[10,72] As mentioned previously, this approach has been very effective in reducing the damage caused by various pathogens. Particularly for *Eucalyptus*, the emergence of vegetative propagation and, thus, clonal forestry has had a remarkable impact on the ability to manage disease problems. Here, the selection of clones of single species, and increasingly hybrids, has allowed forestry companies to avoid disease problems (Figure 2).

Opportunities to avoid disease problems by deploying *Eucalyptus* clones with low levels of susceptibility first emerged at the onset of the serious canker diseases caused by *Chrysoporthe austroafricana* and *Teratosphaeria zuluense* (=*Coniothyrium zuluense*). Over a 20-year period, the diseases caused by these serious pathogens have been reduced to a tolerable level.[10,34] This has necessitated extensive screening trials.[73-75] Planting resistant genotypes is also important for the control

of insect pests, where host resistance coupled with biological control is likely to be the main strategy for the management of pests such as *L. invasa*[45] and *G. brimblecombei*[45].

In the longer term, understanding the biology and global movement of insect pests and pathogens affecting plantation trees, including those in South Africa, will be facilitated by molecular genetic tools that are rapidly emerging for this purpose.[72,76] Sequencing of the genomes of trees such as *Eucalyptus*[77] as well as those of important *Eucalyptus* and *Pinus* pathogens[78-81], pests[61,82] and their biological control agents[83], is already providing important insights. Ultimately, DNA-based genetic markers will also be produced to detect traits such as susceptibility to a particular disease based on small tissue samples.[72]

Looking ahead

South Africa has had a long history of dealing with insect pests and diseases affecting plantation-grown trees. Initially, most work in this field was done by small groups of scientists working in research institutes (government and private) or at universities. Up until the early 1970s, the larger proportion of the forest plantation patrimony was in government hands and support for forest pathology and entomology came primarily from government. Later, as the private forestry industry began to grow, and together with growing numbers of emerging insect pest and disease problems, the need for a more unified forest protection resource has also grown. This need has been filled largely by the Tree Protection Co-operative Programme established in 1990 and representing a collaborative venture between university and private forest owners, together with financial support by various government funding agencies. In more recent years, private companies have also begun to support some field-level research and development 'in house' in order to increase their capacity to deal with the increasing threats due to pests and diseases. The Eucalyptus and Pine Pathogen Interactions Programme, together with the Forest Molecular Genetics Programme, is also increasingly supporting gene and genome based approaches to pest and pathogen management.

In the early 1960s, at a time when plantation forestry based on non-native species, particularly in the tropics and southern hemisphere, was growing rapidly, the pioneer South African forest researcher Dr J.A. Lückhoff made the point that South African forestry had been particularly fortunate in not having been severely affected by tree pests and pathogens.[84] Given the fact that there had been a number of serious disease and pest problems even at that time, Lückhoff's statement might better be interpreted as a recognition that the forest resource could easily have been much more seriously affected. The pool of potentially damaging species is vast, and only a fraction of possible invaders have established to date. If one considers the situation today with the growing numbers of new pests and pathogens that continue to appear, there is little doubt that these factors will challenge plantation forestry greatly in the future.

One of the reasons that plantation forestry has not been devastated by insects and diseases must be attributed to the fact that a wide variety of trees has been grown in South Africa over time, changing species and

Figure 2: Management strategies for pathogens and insect pests of plantation trees in South Africa: (a) a clone of *Eucalyptus grandis* seriously damaged by *Coniothyrium* canker caused by *Teratosphaeria zuluensis,* alongside a disease tolerant clone, illustrating the potential benefits of breeding for resistance; (b) inoculating the nematode *Deladenus siricidicola* into a pine tree infested with *Sirex noctilio*, as part of a successful biological control programme first implemented in South Africa in 1995; (c) releases of the parasitic wasp *Psyllaephagus bliteus* to control the red gum lerp psyllid, *Glycaspis brimblecombei*; (d) a lure-based trap used to monitor populations of *S. noctilio* and thus inform management strategies.

clones as disease and pest problems have arisen. This has provided a buffering effect and the absence of an undue reliance on any particular species over space and time. The available variability of planting stock to deal with changing pest and disease problems has come about, not so much as a result of careful planning to minimise risk, but rather due to the fortuitous fact that South Africa is a large country with hugely variable climatic and edaphic zones that are not suitable to any single species of *Pinus*, *Eucalyptus* or *Acacia*. Yet, in terms of risk, continuous attention must be paid to ensure the maintenance of a genetically variable, yet manageable planting stock. The heavy reliance on *P. patula* and the potential loss of this species due to the pitch canker pathogen provides a strong warning signal in this regard.

Intensive commercial forestry practices such as those employed in South Africa can, of themselves, elevate the threat of damage due to insect pests and pathogens. Large-scale planting of single species, and especially blocks of identical clones of trees, can allow populations of insects and pathogens to build up rapidly. Planting disease- or insect-tolerant clones can also produce genetic adaptation, resulting in new and potentially more damaging pest or pathogen strains in the environment. Likewise, short rotations of trees planted on the same sites can result in the build-up of populations of soil-borne insects and microbial pathogens.

While plantation forestry based on non-native species might be considered a relatively high-risk enterprise, there are also many options to combat pest and pathogen problems. New technologies continue to emerge that promise to improve our ability to deal with these problems.[72] The introduction of vegetative propagation and the ability to hybridise between species has thus provided many examples of solutions to pest and pathogen problems.[10] Molecular genetic techniques which have made it possible to 'fingerprint' clones and thus to select and more carefully deploy planting stock, have already had a significant positive effect on dealing with diseases and insect problems.[10,72] In the longer term, there seems little doubt that genetic modification will become an important tool for this purpose. In effect, the intensive propagation of fast-growing trees represents a conflict between pests, pathogens and the successful production of timber and timber products. Recognising the challenge is perhaps the most important part of overcoming the enemy and ensuring forest plantation sustainability.

Native forests and woody ecosystems are particularly vulnerable to invasive alien pests and pathogens. Once a serious invasive alien organism becomes established in these heterogeneous and sensitive environments, there is little chance of recovery. There are many examples, particularly in the boreal region, that illustrate this fact.[85,86] Although there are some very worrying examples, South Africa has been relatively fortunate in not having been severely affected by disease problems in natural woody ecosystems. Unfortunately, this situation is also likely to change, well illustrated by the recent report of the polyphagous shot hole borer *Euwallacea fornicatus*[28], a stem canker disease of *Rapanea melanophloeos*[87] and the recent arrival of the myrtle rust pathogen *Austropuccinia psidii*[88]. *Austropuccinia psidii* is likely to cause serious issues for *Eucalyptus* forestry in South Africa, but these problems can be resolved through breeding and selection. It will most likely also severely impact some native Myrtaceae to varying degrees, and may even drive highly susceptible species such as *Heteropyxis natalensis* to extinction. *Euwallacea fornicatus* might have even more serious consequences, and might also threaten certain species with extinction should a biological control management option not be found.

Every effort must be made to strengthen quarantine measures and to ensure that new and damaging insect pests and pathogens of trees are not accidentally introduced into South Africa. At the same time, the capacity to deal with pests and pathogens after their introduction should be strengthened. Yet, as history has shown, even the best quarantine does not provide complete protection. Given that the current quarantine systems are far from effective, South African forestry is likely to have to deal with many more serious pests and pathogens affecting forests and forestry in the future.

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Competing interests

We declare that there are no competing interests.

Authors' contributions

All authors contributed to the conceptualisation and writing of the article; M.J.W. produced the initial draft.

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INTERNATIONAL YEAR OF PLANT HEALTH 2020

Accomplishments in wheat rust research in South Africa

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Rust diseases, although seasonal, have been severe constraints in wheat production in South Africa for almost 300 years. Rust research gained momentum with the institution of annual surveys in the 1980s, followed by race identification, an understanding of rust epidemiology, and eventually a focused collaboration amongst pathologists, breeders and geneticists. Diversity in South African populations of *Puccinia triticina*, *P. graminis* f. sp. *tritici* and *P. striiformis* f. sp. *tritici* has been described and isolates are available to accurately phenotype wheat germplasm and study pathogen populations at national, regional and global levels. Sources of resistance have been, and still are, methodically analysed and molecular marker systems were developed to incorporate, stack and verify complex resistance gene combinations in breeding lines and cultivars. Vigilance, capacity, new technologies, collaboration and sustained funding are critical for maintaining and improving the current research impetus for future management of these important diseases.

Significance:

- Rust diseases threaten wheat crops worldwide, including in South Africa.
- Management of rusts includes regular surveillance, pathogen diversity studies, rigorous screening of wheat germplasm, and efficient breeding and selection for resistance.
- Collaboration among plant pathologists, geneticists and breeders has provided momentum in rust research and control in South Africa in recent years.

Background

The sowing of small grain cereals in South Africa occurred within 2 months after the United (Dutch) East India Company set foot on land in present-day Cape Town in 1652.[1,2] Crop failures, in particular due to damage caused by heavy rains, wind storms and unadapted cultivars, were common occurrences. Nonetheless, efforts to successfully grow wheat continued and systematically included new production areas, different sowing times, new cultivars – not only from Europe but also from India, and exports when grain supplies allowed. Varietal assessments during the early years provided evidence for the first selection of higher-yielding types in South Africa.[2] The pioneering wheat cultivars are not well documented, but reference is made of 'white' wheat in 1659, 'Sarut' from India in 1673, 'Roode' and 'Grijse Winter' in 1677, until names based on phenotype (e.g. 'Bloukoring', 'Kleinkoring', 'Baardkoring', 'Zwartbaard', 'Vroeëbaard'), origin (e.g. 'Ciciliaans', 'Bengaalsch'), or growers (e.g. 'Du Toits', 'Niewoudts', 'Tautes') became customary.[2]

No mention is made of rust during the foundational years of cereal production in South Africa but, according to Theal[3], a critical shortage of wheat in 1727 was ascribed in the previous year to rust – a disease known in South Africa only on rye at the time. The regular occurrence of rust led Neethling[2] to conclude: 'There is no doubt that rust, owing to the severe damage caused, is the most important factor which caused the extinction and origin of varieties in South Africa'. Nhemachena and Kirsten[4] gave a detailed account of wheat cultivar development in South Africa, Smit et al.[5] summarised wheat research between 1983 and 2008, and overviews of wheat rust research in South Africa were provided by De Jager[6], Lombard[7] and Pretorius et al.[8] Early milestones were interspecies crosses to transfer stem rust resistance genes to bread wheat (*Triticum aestivum* L.) in 1912 followed by pathotyping isolates of *Puccinia graminis* f. sp. *tritici* Erikss. & E. Henn. (*Pgt*) and *P. triticina* Erikss. (*Pt*) in the 1920s and 1930s.[8] The establishment of a centre for dedicated small grains research at Bethlehem in 1976, currently named Agricultural Research Council – Small Grain (ARC-SG), resulted in appropriate training in rust methodologies, surveillance, race analysis and germplasm evaluation. These initiatives were expanded with the formation of a rust laboratory at the University of the Free State in 1989.

In recent decades, notable events and initiatives in South African wheat rust research include annual surveys[8], *Sr24* virulence[9], the appearance of stripe rust (caused by *P. striiformis* Westend. f. sp. *tritici*, *Pst*)[10], Ug99 stem rust studies[11-15], the mapping of quantitative resistance loci[16,17], genetic characterisation of *Puccinia* isolates[18-20], comprehensive phenotyping of wheat germplasm, and establishment of a marker service laboratory with a particular focus on rust resistance genes (https://www.cengen.co.za).

The objective of this review is to provide a summary of recent accomplishments in wheat rust research in South Africa.

Rust surveillance and phenotypic analysis

Surveillance and race typing are routinely conducted by the ARC-SG to determine rust distribution, impact and pathogenicity in the major wheat-producing areas of South Africa. Recent reports of similarities in races between southern African countries have also emphasised the importance of regional sampling.[21-23]

Handling obligate rust fungi in controlled experiments such as race typing or host plant screening requires specific infrastructure. In addition to facilities for plant growth, inoculation and incubation, equipment for collection

and application of small amounts of urediniospores is essential. Because these specialised items are not commercially available, Pretorius et al.[24] developed an additive manufacturing process to assemble spore collectors and atomisers through 3D printing. Using these devices, traditional race analysis is done by infecting seedlings of a predetermined (differential) set of wheat host lines with a rust isolate. An appropriate experimental set-up and experience in achieving accurate seedling infection types are essential for reliable phenotyping. Examples of infection types are shown in Figure 1.

Figure 1: Seedling (top, left to right: wheat leaf rust, stem rust and stripe rust) and adult plant phenotypes commonly encountered for leaf rust (second from top), stem rust (third from top) and stripe rust (bottom).

Based on the pathogenicity of an isolate on entries in the differential set, a race (pathotype) name is allocated. Apart from an alpha-numerical code to name leaf and stem rust races in South Africa[8], the North American system of nomenclature[25,26] is used to place races in an international context. The standard South African differential set for determining seedling infection types to *Pt* isolates contains 20 entries.[27] Except for Thew (*Lr20*) and Agent (*Lr24*), all *Lr* genes occur in a Thatcher wheat background. New races are further characterised on an additional set containing 23 *Lr* genes.[27] Infection types on the lines RL6011 (*Lr12*), CT263 (*Lr13*), RL6044 (*Lr22a*), RL6058 (*Lr34*), RL6082 (*Lr35*) and Thatcher control (*Lr22b*) are determined on flag leaves of adult plants.

No new *Pt* races were detected between 1988 and 2008 in South Africa.[8,28] This situation changed with the report of race 3SA145 (CCPS North American race code) in 2009, followed by races 3SA146 (MCDS, 2010), 3SA147 (FBPT, 2010), 3SA115 (CBPS, 2012), 3SA10 (CFPS, 2016), 3SA38 (CDPS, 2016) and 3SA248 (CFPS, 2016).[27-30] The frequency of *Pt* races with virulence to *Lr3*, *Lr12*, *Lr13*, *Lr15*, *Lr26* and *Lr37* is high and varied between 79% and 98% during recent surveys.[27] The *Pt* population was dominated for many years by race 3SA133 (PDRS) which initially was common on winter wheat in the Free State. This changed significantly with the appearance of races 3SA145, 3SA146 and 3SA115 which accounted for >80% of isolates typed during the 2012–2016 surveys.[27] The more recently described races 3SA38 and 3SA10 are increasing in prevalence and comprised more than 50% of the isolates typed from the 2018 growing season.[31] *Pt* race MCDS was common in Zimbabwe and Zambia with FBPT and SCDS detected in Zimbabwe and Malawi.[23]

Twenty differential wheat lines are used for stem rust pathotyping. Although the resistance genes are similar to the proposal of Jin et al.[26], Acme (*Sr9g*), Renown (*Sr17*) and Trident (*Sr38*) have replaced CnSr9g, Combination VII and VPM1, respectively. Additional differentials include Barleta Benvenuto (*Sr8b*), the triticales Coorong (*Sr27*), Kiewiet (*SrKw*) and Satu (*SrSatu*), and either LcSrWst-2Wst (*Sr9h*) or Matlabas (*Sr9h*).[32] New races are further characterised on an extended set of tester lines.[33] Although differential lines grown in the field can provide an indication of prevailing *Pgt* races, Boshoff et al.[34] showed that certain resistance genes are not well expressed in adult plants whereas other lines contain resistance in addition to that observed in seedling assays.

The most significant change in the *Pgt* population since 2005 was the regular appearance of new races in the Ug99 lineage. African race Ug99, named after the country of first detection (Uganda) and year of description (1999)[11], was the first race with virulence for the widely used *Sr31* resistance gene. Its broad virulence and subsequent specialisation in 13 pathotypes have raised serious concerns about sustained wheat production in many regions of the world.[15] Stem rust race 2SA106 (TTKSP North American race code) detected in 2007, 2SA107 (PTKST, 2009), 2SA88+ (TTKSF+, 2010) and 2SA42 (PTKSK, 2017) all show phenotypic similarities to race 2SA88 (TTKSF, 2000), which was the first stem rust race in the Ug99 lineage detected in South Africa.[12,13,18,21,32,35] These races are phenotypically characterised by differences in virulence for *Sr9h*, *Sr21*, *Sr24* and *Sr31*.[13,32] *Sr24* and *Sr31* have been reported to occur in South African wheat germplasm[36] and virulence was not unexpected. Likewise, the virulence adaptation of TTKSF+ was recently confirmed by the endorsement of *Sr9h* in the wheat cultivar Matlabas.[37] Despite being less virulent compared to the more recently detected Ug99 races, TTKSF remains the dominant variant.[8,31,32,38] Stem rust races TTKSF (2009), TTKSF+ (2010) and PTKST (2010) were also identified in samples collected in Zimbabwe and PTKST was confirmed in Mozambique.[15,21]

Seedling infection types produced on the World and European differential sets[39,40], followed by an A+ or A- suffix to describe virulence or avirulence for the *YrA* gene in Avocet R[41], are used for *Pst* race designations in South Africa. Near-isogenic lines with Avocet S as the recurrent parent are used as additional tester lines for race characterisation and in field plots.[42] Following the detection of *Pst* race 6E16A- in 1996[10], proposed to be a foreign introduction from Central or Western Asia either by wind or human intervention[43,44], there is strong evidence that adaptation to the host genes *Yr25* (race 6E22A-, cultivar Hugenoot, 1998) and *YrA* (6E22A+, PAN 3195, 2005) resulted from selection pressure[10,19]. The *Pst* population has remained relatively stable since the detection of race 6E22A+ on winter wheat in the eastern Free State in 2005[19] with 6E22A+ persisting as the most dominant race, comprising 58% of isolates in 2018[31].

The outbreak of stripe rust on irrigated spring wheat in 2018 represented the first report of the disease in Zimbabwe.[45] Showing virulence to *Yr3a*, *Yr4a*, *Yr9* and *Yr27*, race 30E142A+ was distinctly more virulent on South African wheat cultivars than 6E22A+ and poses a potential threat to the local industry. However, it is not yet known if *Pst* will successfully establish in Zimbabwe and, as anticipated, migrate to South Africa.

It has been suggested that wheat cultivated at a higher elevation in Lesotho during summer serves as a source of *Pst* inoculum for winter-grown crops in South Africa.[8] Although not customary, some hectares may also be sown to wheat in the Free State during summer. The impact of these formal off-season productions on the epidemiology of the rusts has, however, not been studied in detail. Volunteer wheat has generally been assumed to provide a green bridge for the survival of these biotrophic pathogens between seasons.

Genetic analysis of *Puccinia* isolates

Analysis of *Pt*, *Pgt* and *Pst* with microsatellite markers has contributed to explaining genetic diversity within the three populations. In the absence of functional alternate hosts for wheat rust pathogens in South Africa, wind dispersal, human activities, mutation and possibly somatic recombination are considered as drivers of variation. The South African *Pgt* population consists of two highly diverse genetic lineages.[18] In the absence of viable historical samples in South Africa, the close genetic similarity of members of the non-Ug99 genetic lineage with Australian standard races 21-0 collected in 1954, and 326 and 194 collected in 1969, respectively, suggested that this lineage represents the original South African population.[46] Included in this lineage are races that are specific for both wheat and triticale. The acquisition of virulence within this group appears to be the result of step-wise mutations.[32,33] On a global scale, this lineage grouped closest with *Pgt* samples from Pakistan, Czech Republic[47,48] and Australia due to the proposed movement of urediniospores on high-altitude westerly winds[46].

The Ug99 lineage on the other hand, first detected in South Africa in 2000 with the description of TTKSF[12], has expanded into five variants[13,14,21,35]. In contrast to the non-Ug99 lineage, all five South African variants and the original TTKSK[11] shared more than 85% genetic similarity and fall within the bigger Ug99 race group from east Africa[15]. In a recent study, Li et al.[48] provided genomic evidence of somatic hybridisation in *Pgt*, shedding light on the origin of Ug99 through the exchange of nuclei between standard race 21 and an unknown race. This is an important discovery to understand the formation of new diversity in the absence of sexual recombination.

The current South African *Pt* population consists of two primary genetic lineages[20], but at least five were evident according to *Pt* isolates collected during the previous century[49]. Three of these appear to be extinct while only one lineage is expanding.[27,28,30] Similar to *Pgt*, these new races probably represent exotic introductions as races with similar phenotypes and genotypes were found in countries to the north of South Africa.[23] Globally, the South African *Pt* races grouped significantly with isolates from the Middle East, Pakistan and New Zealand.[50]

Based on microsatellite analysis, the four *Pst* races described in South Africa represent a single, clonal lineage.[19] As opposed to these races, the recently identified *Pst* race in Zimbabwe was genetically very similar to two Kenyan isolates[45], indicating a southerly expansion of stripe rust diversity in Africa.

Due to the unique ability of markers to distinguish genotypes independently of their associated phenotypes, genetic screening of field isolates can detect variants before a new phenotype becomes evident. While *Pt* races 3SA38, 3SA10 and 3SA248 were first detected as phenotypic variants in 2016[27], their unique genotypes were already abundant in field isolates collected in 2015[49]. These markers also indicated that within each phenotype, significant genetic variation was present, making genetic markers an effective supplementary tool to race phenotyping.

Host resistance

Resistance phenotypes in wheat are typically growth stage mediated. All-stage resistance (ASR), conferred by major genes, is clearly expressed throughout the lifespan of the plant whereas adult plant resistance (APR), often polygenic and partial in manifestation, becomes effective at more mature growth stages.[51] Phenotypes commonly encountered on adult plants are shown in Figure 1. As some APR genes are considered durable, this resistance type is frequently preferred in breeding and selection. Wheat cultivars carrying the pleiotropic race non-specific APR genes *Sr2*/*Yr30*, *Lr34*/*Yr18*/*Sr57*, *Lr46*/*Yr29*/*Sr58* and *Lr67*/*Yr46*/*Sr55* have maintained moderate levels of rust resistance under epidemic field trial conditions in South Africa and might not provide adequate protection when deployed singly under high disease pressure. Soko et al.[52] recorded grain yield losses due to stem rust of between 10.1% and 19.5% for APR cultivars as opposed to a 6.4% loss in an ASR line. Previously, Pretorius et al.[8] mentioned losses as high as 65% for susceptible wheat cultivars infected with stripe rust and a 56% yield gain was obtained when leaf rust was controlled by fungicide application on a susceptible cultivar. Breeders are therefore encouraged to either combine APR sources or stack them with ASR genes, the latter especially in areas prone to early-season infection.

The damage potential of wheat rusts is a reality, and it remains important to verify the resistance status of local germplasm and embark on appropriate breeding and selection programmes. As part of risk assessment and compilation of production guidelines, all commercially recommended wheat cultivars in South Africa, as well as leading breeding lines, are tested annually against a panel of rust races. These tests comprise seedling assays for ASR and field tests under high inoculum pressure in carefully managed rust nurseries. The University of the Free State has implemented rust nurseries with great success at the research facilities of Corteva Agriscience™ at Greytown in KwaZulu-Natal since the early 1990s. The Greytown environment is highly conducive to the vigorous development of both spring and winter wheat types as well as rust development. In a typical year, stripe rust would be first to establish during the cooler months of August and September, followed by leaf rust in October and finally stem rust, which peaks at the end of the season.

Stem rust assessments for local germplasm are summarised in Figure 2. Only cultivars with seedling infection types <2 (0 to 4 scale)[53], and a coefficient of infection[54] <20, were considered to carry true ASR. Some cultivars regarded as resistant as seedlings showed an intermediate stem rust reaction in the field and were thus not classified as displaying true ASR. Inoculum loads in the Greytown field nursery are extremely high and not all ASR genes provide complete rust protection under such conditions. In most cases it is assumed that these cultivars will be acceptable in commercial fields where inoculum pressure is lower. The opposite was also observed where some cultivars were classified as intermediate in the seedling stage but stem rust resistant in the field. The effect of using race PTKST in the field from 2011 onwards is clear from the initial decrease in resistance before a gradual improvement in resistant entries as breeders adapted their selection and breeding strategies. Collectively such information, also for leaf and stripe rust, adds to an understanding of disease risk and management at production level. To support field data, protocols for accelerated and reliable greenhouse assays have been developed for stripe rust[16,55,56], leaf rust[57] and stem rust[58].

Genetic studies of host resistance provide information on the monogenic or polygenic nature of genes involved, their identity and chromosome location, association with known genes or quantitative trait loci (QTL), and molecular markers for tracking the resistance. Together this knowledge contributes to assumptions of durability and targeted attempts to achieve long-lasting resistance. Ramburan et al.[16] were the first to map rust resistance in a South African wheat cultivar. They identified three major stripe rust resistance loci in the spring wheat cultivar Kariega and paved the way for fine mapping and marker development for *QYr.sgi-2B.1* and *QYr.sgi-4A.1,* and confirmation of the pleiotropic resistance gene *Lr34*/*Yr18*/*Sr57*.[57,59] In a similar approach, the durable stripe rust resistance of the European wheat cultivar Cappelle Desprez was mapped[17] with subsequent identification of the major effect QTL *QYr.ufs-2A* along with three QTL of smaller effect, *QYr.ufs-2D*, *QYr.ufs-5B* and *QYr.ufs-6D*. Using histological techniques, Maree et al.[60,61] investigated fungal behaviour in lines containing different combinations of the stripe rust resistance

QTL characterised in Kariega and Cappelle Desprez, respectively. These studies confirmed the value of gene stacking and careful selection of lines with the best ability to mitigate fungal invasion.

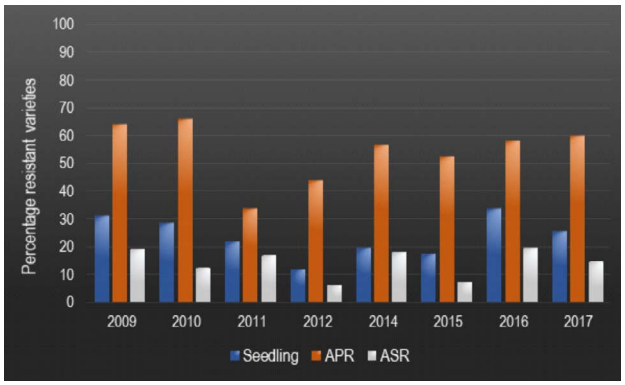


Figure 2: The frequency of South African wheat varieties expressing a low seedling response, adult plant resistance (APR) and true all-stage resistance (ASR) to stem rust over 8 years. In 2009 and 2010, entries were tested with *Puccinia graminis* f. sp. *tritici* pathotype UVPgt59 (TTKSP), and since 2011 with the more virulent UVPgt60 (PTKST) pathotype.

Prins et al.[62] assessed stem rust response in an African wheat collection and identified several marker-trait associations in a genome-wide study. Two lines with exceptional APR were identified and biparental mapping populations developed. Marker-trait associations on chromosomes 6AS and 3BS and the *Lr34/Yr18/Sr57* resistance locus were confirmed, along with stem rust resistance QTL not detected in the association study, one of which was the significant QTL *QSr.ufs-4D*. This emphasises the value of applying multiple approaches to unravel host resistance, particularly in cases where marker coverage in certain chromosomal areas is too low to detect QTL.

The availability of *Pgt* races with virulence attributes appropriate for targeting certain sources of resistance has contributed to several studies. These projects addressed phenotyping and genetics of resistance to Ug99 races[63-66], resistance characterisation of triticale[67] and lines with genes transferred from *Aegilops sharonensis*[68] and *Thinopyrum ponticum*[69,70]. Furthermore, Pretorius et al.[71] demonstrated the application of remote sensing and the normalised difference vegetation index in reliably phenotyping wheat stripe rust response in the field.

Breeding and selection

Marker-assisted selection (MAS) is widely accepted as a key strategy to pyramid resistance genes into wheat genotypes, in particular, genes that do not exhibit easily distinguishable phenotypes.[72] In South Africa, large-scale MAS was not implemented by breeding companies in the early 2000s[8], although it was routinely used to select for several traits in countries such as Australia, Mexico, USA, the UK and India[72]. In 2011, a proposal by CenGen (Pty) Ltd. titled 'Establishment of a molecular marker service laboratory for routine application of marker-assisted selection in South African wheat breeding programs' (WCT/W/2009/02), was approved for funding by the Winter Cereal Trust. The capital expense of establishing a MAS laboratory and routine maintenance justified a central facility at CenGen for all wheat breeding programmes. The project is based on (1) purity testing of donor lines and confirmation of the target trait, (2) planning of breeding schemes and crosses to transfer the new trait, and (3) tracking the trait in subsequent filial generations.

South African seed companies use different strategies to breed for rust resistance, dependent on their approach, resources and location. Yet there is a collective focus on pyramiding rust resistance genes, in particular those that confer durable APR, to uphold the international drive of gene stewardship. Sensako (Pty) Ltd., a private breeding company with headquarters in Bethlehem (Free State, South Africa), follows a strategy in which they combine target genes/QTL in doubled haploid donor lines. This is followed by a top cross with their elite lines or commercial cultivars and from the F_1-generation doubled haploid lines are developed to integrate the genes/QTL into better adapted backgrounds. This approach has proven to be successful in pyramiding rust resistance genes/QTL (Figure 3). They have managed to develop a line containing multiple genes for resistance to all three rust pathogens, which is now used as a key donor line to incorporate complex resistance into existing cultivars.

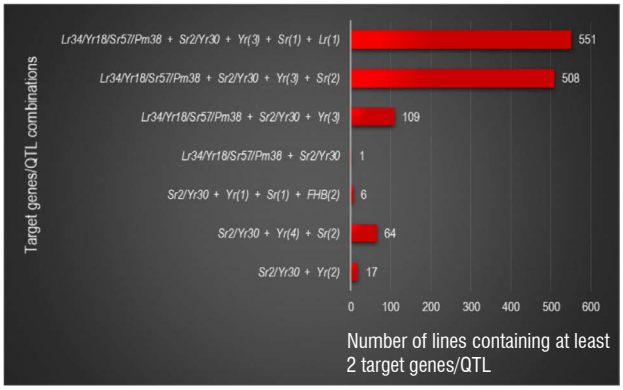


Figure 3: Complement of rust resistances incorporated in the Sensako doubled haploid marker-assisted selection programme. Numbers in brackets indicate the maximum number of genes/quantitative trait loci (QTL) screened for in the specific combination. For each combination, lines containing at least two target genes/QTL were selected for field evaluation in 2019. For all combinations, at least one line was recovered containing all targets.

The South African wheat breeding programme of Corteva Agriscience™ follows a more traditional approach of gradually incorporating multiple genes/QTL into their breeding lines. Gene enrichment is done at the F_2-generation, and the presence of the target genes is confirmed in the F_5-generation after three cycles of selection for agronomic traits. Pure lines containing the target genes are then either used to generate new resistance gene combinations or, if within the tolerance levels set for the different milling and baking quality criteria, are considered for commercial release. This programme has been successful in combining APR genes for stem, stripe and leaf rust resistance into elite breeding material that performs similarly to current commercial cultivars in yield trials (Table 1).

Table 1: Yield performance of selected marker-assisted selection (MAS) lines of Corteva Agriscience™ compared to commercial cultivars

Entry	Relative yield (%)[a]	Genes incorporated through MAS
Cultivar 01	102	Confidential[b]
Cultivar 02	97	Confidential
Cultivar 03	94	Confidential
Cultivar 04	100	Confidential
Cultivar 05	106	Confidential
Cultivar 06	106	Confidential
MAS Line 01	99	*Fhb1 Qfhs.ndsu-3BS; FHB Qfs.ifa-5A*
MAS Line 02	96	*Fhb1 Qfhs.ndsu-3BS; FHB Qfs.ifa-5A*
MAS Line 03	98	*Fhb1 Qfhs.ndsu-3BS; FHB Qfs.ifa-5A*
MAS Line 04	99	*Fhb1 Qfhs.ndsu-3BS; FHB Qfs.ifa-5A*
MAS Line 05	96	*Fhb1 Qfhs.ndsu-3BS; FHB Qfs.ifa-5A*
MAS Line 06	102	*Fhb1 Qfhs.ndsu-3BS; FHB Qfs.ifa-5A*
MAS Line 07	95	*Sr2/Yr30; Lr34/Yr18/Sr57/Pm38*

[a]*Yields measured in tons/ha are expressed relative to Cultivar 04 which was taken as the benchmark (100%).*

[b]*Developed through traditional breeding without MAS.*

The MAS programme commenced in 2011 with the capacity to screen for 19 genes/QTL, of which 13 were related to rust resistance. These targets included the popular APR genes *Lr34/Yr18/Sr57/Pm38* (*Pm* is the notation for powdery mildew resistance genes) and *Sr2/Yr30*, the leaf rust resistance gene *Lr19*[73] as well as QTL previously identified for stripe rust resistance in the cultivars Kariega[16] and Cappelle-Desprez[17]. Since its inception, the programme has grown to include 63 genes/QTL of which 29 are associated with rust resistance (Figure 4). These are obtained by breeders through international collaboration with organisations such as CIMMYT, or are newly identified sources from ongoing local research projects.[62]

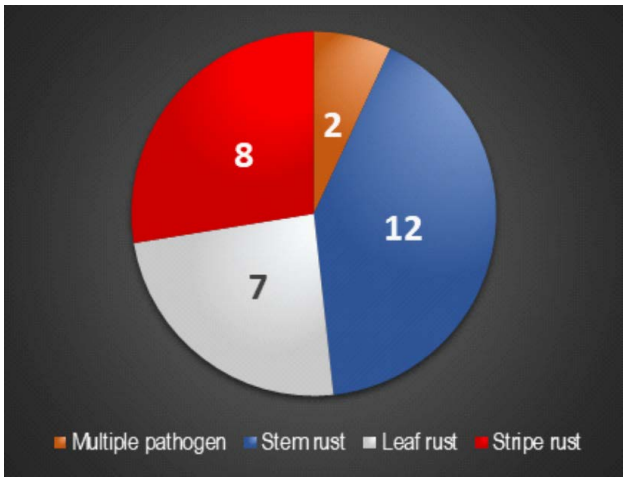


Figure 4: Targets of rust resistance genes screened for in the marker-assisted selection programme at CenGen.

Molecular markers for target genes/QTL are obtained from the public domain and research articles, or from in-house mapping projects by CenGen and collaborators. These include simple sequence repeat (SSR), sequence-tagged site (STS), cleaved amplified polymorphic site (CAPS) and single nucleotide polymorphism (SNP) markers. Since 2013, the implementation and upgrade of KASP™ SNPLine™ instruments (LGC, UK) at CenGen greatly enhanced high-throughput capacity. The number of data points (calculated as the number of samples x number of markers tested per sample) that are generated annually continues to increase (Figure 5) despite a decrease in industry funding.

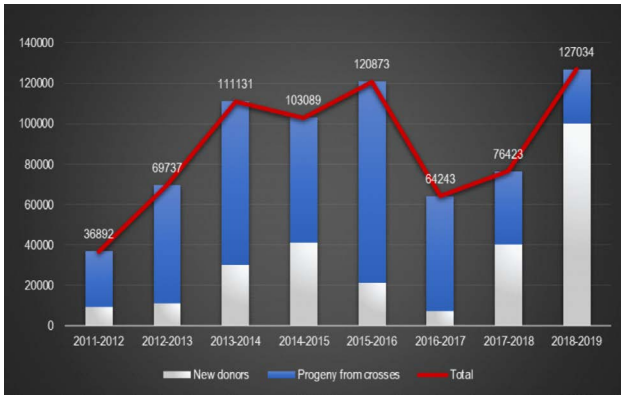
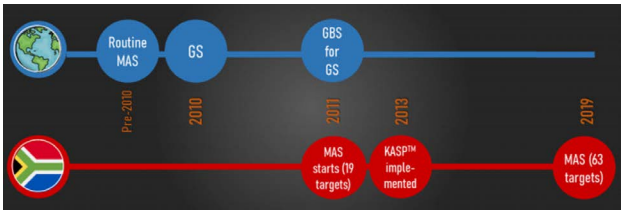


Figure 5: Number of data points generated since inception of the marker-assisted selection programme.

Notwithstanding the success of the implementation and application of the MAS programme for single locus traits such as rust resistance, the status of MAS in South Africa trails behind that of international programmes, which are exploring an integrated genomics-assisted breeding approach.[74] In 2010, crop geneticists started to investigate genomic selection in wheat to select for complex, multi-locus traits.[75] By 2012, reports of the value of genomic selection using genotyping-by-sequencing in wheat were published, creating yet another avenue for genomics-assisted breeding (Figure 6).[76] The challenge remains for South African breeders and geneticists to follow international trends in genomics-assisted breeding and sensibly implement selection strategies for multi-locus traits.



MAS, marker-assisted selection; GS, genomic selection; GBS, genotyping by sequencing

Figure 6: Timeline of MAS in South Africa (bottom) compared to international programmes (top).

Conclusions

The relatively frequent introduction of new rust races into South Africa strongly suggests the possibility of further incursions. Stem rust and stripe rust, in particular, are extremely damaging diseases and the description of highly virulent and aggressive *Pst* and *Pgt* races in other wheat regions[77,78] emphasises continued vigilance. The introduction of such races could impact severely on cultivar response with a consequent increase in production risk and cost. The survival of rust on off-season wheat crops and ancillary hosts such as wild rye (*Secale strictum* subsp. *africanum*) in the Roggeveld Mountains of the southwestern Karoo[79], requires further attention. Although samples collected from wild rye revealed *Pst*, the stem and leaf rust forms were those of cultivated rye and not bread wheat.[79] Wild rye is, however, moderately susceptible to *Pgt* and could serve as an inoculum source. The occurrence of both *Pt* and *Pgt* on a summer wheat crop in the eastern Free State in January 2020 (WHP Boshoff, unpublished) is of concern and supports the expansion of surveys to this period. Scientists should thus continue with surveillance, studies of pathogen variability, characterisation of cultivars, genetic analyses, resistance discovery, focused breeding and selection, and communication of research outcomes to producers. Overarching activities include international, regional and national collaboration; capacity building and training; embracing of new technologies; resistance gene stewardship; and sourcing sustained funding.

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Competing interests

We declare that there are no competing interests.

Authors' contributions

Z.A.P. developed the outline, wrote the Abstract, Background, 'Host resistance' section and Conclusions; R.P. and E.W. wrote the 'Breeding and selection' section including table and figures; C.M.B. provided long-term cultivar data; B.V. wrote 'Genetic analysis of *Puccinia* isolates'; W.H.P.B. wrote 'Rust surveillance and phenotypic analysis'. All authors contributed to editing of the final manuscript. Z.A.P. and W.H.P.B. provided photographs of rust phenotypes.

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INTERNATIONAL YEAR OF PLANT HEALTH 2020

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Progress in the management of Fusarium head blight of wheat: An overview

Fusarium head blight (FHB), also known as head scab, is a devastating fungal disease that affects small grain cereal crops such as wheat (*Triticum aestivum* L.). The predominant causal agent, *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schwein.) Petch), is ranked the fourth most important fungal plant pathogen worldwide. Apart from yield and quality losses, mycotoxin production can occur from FHB infection, resulting in harmful effects on human and animal health. Some level of disease control may be achieved by using certain fungicides and agronomic practices plus host resistance. In South Africa, there are currently no registered fungicides or bio-fungicides, no resistant wheat cultivars and only limited control is achieved by cultural practices. Because effective disease reduction cannot be achieved by using a single strategy, the integration of multiple management strategies can enhance disease control. We review possible strategies for reducing the risk for FHB infections that are relevant to the context of South Africa and other wheat growing areas in Africa.

Significance:

- The importance of the effect of FHB on wheat cannot be overemphasised. This review highlights and describes the various control options and their efficacies. It also describes the current state of research in an effort to control FHB and its associated mycotoxins.
- Wheat is one of the most produced crops worldwide and in South Africa, hence this review could promote and intensify research towards the development of more effective management strategies for FHB of wheat.

Introduction

Fusarium head blight (FHB), also known as head scab, is a devastating fungal disease that affects small grain cereal crops such as wheat (*Triticum aestivum* L.).[1-3] It is regarded as a major limiting factor in wheat and barley (*Hordeum vulgare* L.) production across the world.[4,5] The disease is caused by the FHB species complex which consists of more than 17 *Fusarium* species.[6-8] However, in South Africa, FHB is predominantly caused by *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schwein.) Petch).[7] The FHB pathogen is capable of causing head blight or scab on wheat, barley, rice (*Oryza sativa* L.) and oats (*Avena sativa* L.), and Gibberella stalk and ear rot disease on maize (*Zea mays* L.). The pathogen may infect other host genera without causing disease symptoms. These genera include *Agrostis, Bromus, Calamagrostis, Cortaderia, Cucumis, Echinochloa, Glycine, Lolium, Lycopersicon, Medicago, Phleum, Poa, Secale, Setaria, Sorghum, Spartina* and *Trifolium*. Apart from *F. graminearum*, the *Fusarium* species that occur in South Africa are: *F. acacia-mearnsii* O'Donnell, T. Aoki, Kistler & Geiser, *F. boothii* O'Donnell, T. Aoki, Kistler & Geiser, *F. brasilicum* T. Aoki, Kistler, Geiser & O'Donnell, *F. cortaderiae* O'Donnell, T. Aoki, Kistler & Geiser and *F. meridionale* T. Aoki, Kistler, Geiser & O'Donnell.[9]

F. graminearum is distributed worldwide, and is especially prominent in temperate regions where its hosts are mostly cultivated.[8] The pathogen infects spikelets at anthesis and thereafter colonises the entire head systemically, thus producing extensive blight symptoms.[10,11] This happens when the presence of favourable environmental conditions coincide with high disease pressure and susceptible host tissue.[8,11] Disease progress is accompanied by the production of trichothecene mycotoxins [primarily deoxynivalenol (DON), nivalenol (NIV)] and zearalenone (ZEA), which not only pose a threat to the health of humans and other animals, but also reduce grain quality.[12,13]

Challenges involved in the management of FHB are because the favourable conditions for disease development often coincide with the conditions that trigger anthesis. Moreover, the fast progress and epidemic development of FHB limits the effectiveness of certain control methods.[14] Nevertheless, some management strategies have been reported to provide certain levels of FHB and DON reduction on infected hosts.[15-21] There are no registered fungicides[1] and no completely resistant wheat cultivars[1,22] in South Africa or elsewhere, whilst only limited control is achieved by cultural control methods.[1,9] Therefore, the development of more effective FHB management strategies is essential.

Wheat production

Wheat is a cereal grain that is native to the Levant region of the Near East and Ethiopian highlands.[23] It is cultivated worldwide and is one of the three most produced cereal crops in the world.[23,24] Wheat is a good source of carbohydrates (78.10%), proteins (14.70%), minerals (2.10%), fat (2.10%), B-group vitamins and dietary fibre.[25] It can be consumed as an ingredient in foods such as bread, pasta, crackers, cakes, noodles and couscous.[23,25]

Epidemiology of *F. graminearum*

Wheat plants are mostly susceptible during anthesis, because during this stage the wheat anthers split to discharge pollen (a process known as anther extrusion), which serves as an opening and provides entry for the pathogen.[26,27] The favourable conditions for infection are prolonged periods (48–72 hours) of high moisture or relative humidity (<90%), moderately warm temperature (15–30 °C), frequent rainfall and the occurrence of air currents.[2,28,29] These conditions usually occur in spring. Trail et al.[30] reported that an increase in relative humidity results in a build-up of turgor pressure within the ascus and consequently the forcible discharge of ascospores. Rainfall has been reported to cause the rupturing of the ascus wall which consequently encourages the dispersal of ascospores.[14,30]

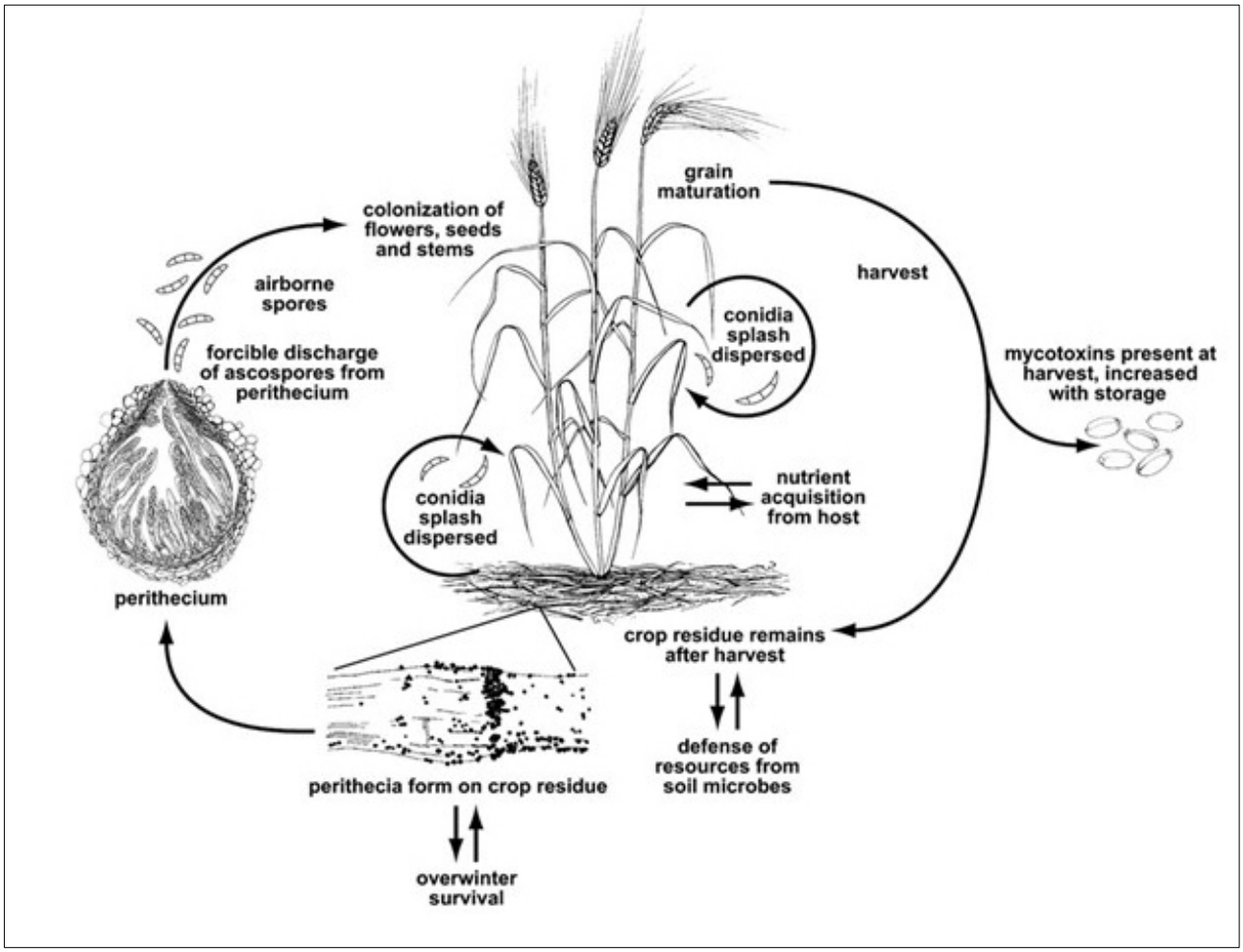
The occurrence of these conditions and the abundance of inoculum before, during and after anthesis of susceptible cultivars, therefore results in yield and quality losses as well as the development of severe epidemics.[2,26,31] The host remains susceptible throughout the flowering stage; however, late infections have been associated with reduced disease severity and high DON accumulation.[32] Due to differences in climatic requirements, and genetic and environmental adaptations within the FHB species complex, these species are capable of causing disease in a variety of conditions, resulting in the worldwide distribution of FHB (Figure 1).[2,8] For example, *F. culmorum* (Fc) (W.G. Smith) and *F. avenaceum* (Fr.) Sacc are more predominant in cooler regions (such as Western Europe) whereas *F. graminearum* predominates in warmer and more humid regions of the world (such as North America and Australia).[33]

Source: Reproduced from CABI[80] with permission.

Figure 1: The global distribution of *Fusarium graminearum* (orange dots) as per documented outbreaks.

Disease cycle and symptom development

During overwintering or over-summering, the pathogen survives as a precursor to perithecia from which ascospores (primary inoculum) are forcibly discharged under favourable environmental conditions (warm, wet and moist) (Figure 2).[11,30] The ascospores are dispersed by wind or rain splashes, land on susceptible plant tissue and colonise the plant surfaces (Figure 2).[8,11,33] After entry, *Fusarium* runner hyphae grow intercellularly and asymptomatically in the inner tissue of the spikelets (palea, lemma and glumes).[11,33]



Source: Reproduced from Trail[11] with permission.

Figure 2: The life cycle of *Fusarium graminearum*, the causal pathogen of Fusarium head blight disease of wheat.

Thereafter, the hyphae grow intracellularly, which leads to plant cell death.[11] This is accompanied by the production of mycotoxins such as DON which has virulent properties that lead to tissue necrosis.[34] Mycotoxins are capable of disabling the plant defence mechanisms and defending the fungus from other microorganisms, thus promoting infection.[35] A study by Boenisch and Schäfer[36] revealed that *F. graminearum* forms lobate appressoria and infection cushions during FHB pathogen infection in wheat tissue and trichothecene biosynthesis occurs in these structures. The authors further reported that trichothecene biosynthesis is not necessary for the formation of these structures nor the initial infection of wheat tissue.[36]

Initial disease symptoms include water-soaked lesions on spikelets which later appear whitened or bleached.[11,37] Thereafter, white or pinkish mycelia (Figure 3a) and pink or orange spore masses (Figure 3b) appear on the margin of the glumes of infected spikelets.[37,38] Small purple-like or black spherical structures (perithecia) are produced (Figure 3c)[37,38] which then sporulate and further infect healthy host tissue[2]. Infected kernels may appear shrivelled, shrunken and discoloured with a light-brown or pinkish-white appearance.[2] These Fusarium-damaged kernels (FDKs) are often associated with high mycotoxin concentrations, reduced seedling emergence and reduced seedling vigour, making them unusable as food, feed or seed.[38,39]

Economic and social importance of FHB

According to Dean et al.[40], FHB is currently ranked the fourth most scientifically and economically important plant fungal disease globally. Economic impacts (direct or indirect) caused by FHB are due to yield loss (production of FDKs), mycotoxin contamination, reduced animal productivity and human health costs.[41,42] In the USA, yield and quality losses due to FHB disease on wheat and barley in the 1990s amounted to more than USD3 billion (which equates to ZAR10.5 billion based on the average annual exchange rate in the aforementioned years).[43] Losses in Canada have ranged from USD50 million (ZAR183.5 million) to USD300 million (ZAR1.1 billion) annually since the early 1990s.[44] According to Scott et al.[45], a disease incidence of more than 70% was reported near Winterton in KwaZulu-Natal, South Africa.

FHB-infected grain can result in allergic reactions as well as breathing problems for handlers.[46] In non-ruminants (e.g. pigs), feed refusal and reduced feed consumption have been reported as side effects of DON-contaminated feed ingestion.[38,44,46] Ruminants (e.g. cattle) are reported to have a higher tolerance to DON concentrations than non-ruminants.[35,38] Moreover, adult beef cattle have a higher tolerance to DON concentrations than calves and pregnant cows.[44] DON has been reported to result in abortions, stillbirths and weak piglets, thus affecting pig markets.[41]

In addition to crop losses and mycotoxin production, the disease also leads to the selective loss of albumin and gluten proteins on contaminated grains.[47] This results in yield and quality (economic) losses due to a reduction in the market grade of grains intended for feed, malting, baking, milling, trade (exports), biofuel and brewing industries.[8,41,44,48]

Chemical and physical methods for the detoxification of mycotoxin-contaminated grains have been previously studied.[44] In a review by Jard et al.[49], various techniques of mycotoxin decontamination are discussed which can be achieved by either adsorption or transformation. Current regulations, however, prohibit both the decontamination of grains with mycotoxin levels above the acceptable limits and the chemical treatment of products intended for human consumption.[49] Moreover, obtaining an economical or commercially feasible method for the detoxification of contaminated grains has been unsuccessful thus far.[44]

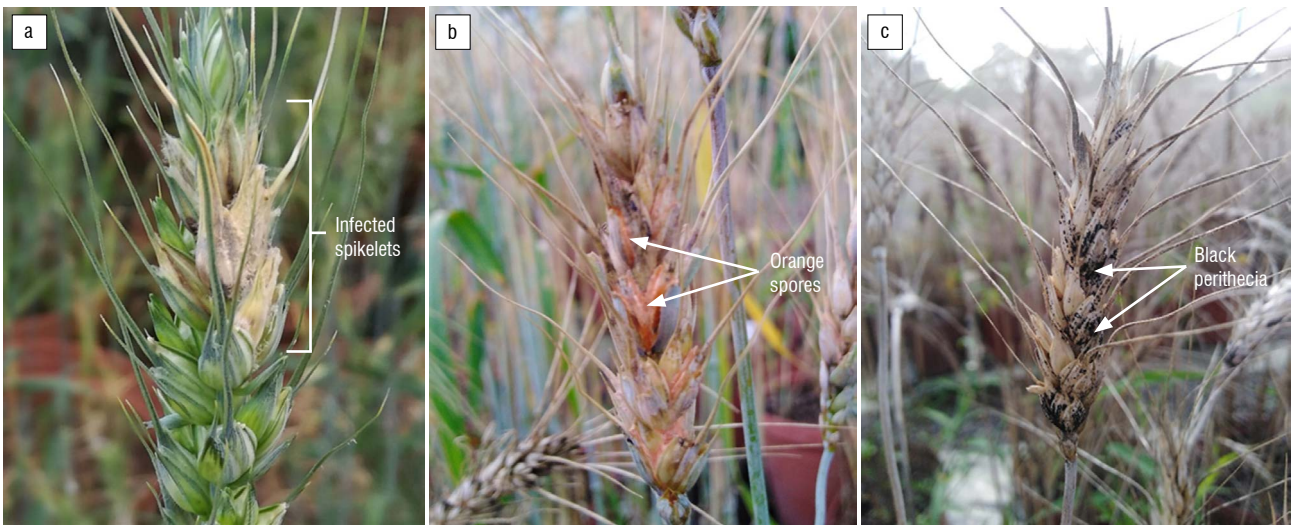
Management strategies

Agronomic practices

Certain agronomic practices have been reported to contribute to the reduction of FHB incidence and severity.[2,50,51] The most effective cultural control strategies that result in reduced pathogen inoculum and thus reduced FHB incidence and severity in succeeding seasons include: crop rotation with non-host crops; residue management; and tillage practices.[9,51,52]

Other practices that are of moderate or low efficacy in the control of FHB include: disease forecasting; early planting; the use of early maturing cultivars; the use of cultivars with agronomic traits that are unfavourable for FHB infection; weed control; irrigation management; and optimising crop nutrition.[9,51,53] Post-harvest storage practices such as increasing the combine's fan speed, reducing moisture and temperature in the silos, and sorting and discarding broken and damaged kernels have also been reported to be effective in reducing FHB and DON contamination in grain batches.[50,51] Because damaged kernels are lightweight and thus easily blown away, they can be separated by increasing the combine's fan speed.[50,51]

Environmental conditions can affect the efficacy of agronomic practices in the control of FHB. For example, the occurrence of rainy weather can encourage the dispersal of ascospores, thus resulting in FHB infections.[8,30] Nevertheless, agronomic practices can lower the amount of inoculum present in the field and thus lower FHB infections on host plants.



Photos: S.P.N. Shude

Figure 3: FHB-infected wheat heads showing bleached spikelets with white, pinkish fungal mycelia (a), orange spores (b) and black perithecia (c).

Chemical control

In most parts of the world, several fungicides have been tested for their efficacy in reducing FHB on wheat.[1,53] According to Haidukowski et al.[54], the use of certain fungicides resulted in reductions of 77% and 89% in disease severity and mycotoxin contamination of infected grains, respectively. Fungicides in the demethylation inhibitor (DMI) class are widely used to control FHB and DON contamination on grain crops.[53,55] In a study by Paul et al.[56], the application of DMI fungicides on wheat anthers at Feekes 10.5.1 growth stage was the most effective treatment in reducing FHB index and DON.

According to Salgado et al.[50] and Palazzini et al.[16], past research has reported on the successful reduction of FHB severity and DON concentrations and consequently reduced yield and quality losses from the timely application of triazole-based fungicides. Cromey et al.[17] observed a reduction up to 90% in FHB incidence and a 14% yield increase through the application of tebuconazole on FHB-infected wheat plants. Moreover, meta-analyses of fungicide trials conducted in the USA showed that metconazole, prothioconazole + tebuconazole, and prothioconazole were the best three fungicide treatments resulting in the highest increase in yield and test weight.[57,58]

Some fungicides used to control FHB have been reported to indirectly increase DON concentrations in grains.[56,59] These include fungicides of the quinone inhibitor (QoI) class.[56] In a study by Paul et al.[56], the application of QoI fungicides on wheat anthers at either Feekes 9 or Feekes 10.5 growth stages increased mean DON concentrations compared to the non-treated checks. Previous research reports that the use of azoxystrobin resulted in the reduction of FHB caused by *Microdochium nivale* var. *nivale* (Fries) Samuels and Hallet, and *M. majus* (Wollenw.) Glynn & S.G. Edwards, no reduction of *F. graminearum* and *F. culmorum* and high DON concentrations on harvested grains.[17,60] This could be attributable to the non-toxicity of *M. nivale* as mycotoxin production has been associated with increased virulence in *Fusarium* species.[51,61] Moreover, azoxystrobin could have slowed down and not prevented the disease[17] and also attacked other competitive microorganisms on wheat ears, thus encouraging the development of FHB[51,35].

The timing of fungicide application is crucial as fungicides are most effective when applied within a week of early anthesis.[1,62] Achieving this timing can be difficult due to the uneven flowering of tillers across cultivation fields as well as rainy weather.[62] Moreover, the erratic nature of FHB epidemics can reduce fungicide efficacy.[35] Regardless of the successful reduction of FHB and DON provided by certain fungicides, no fungicide has been reported to completely eradicate the disease on infected crops and even the best fungicides are not fully effective.[1] Therefore, fungicides are best used in combination with other control strategies (such as cultural methods).[1]

Biological control

Several bacterial, fungal and yeast strains have been reported to provide effective reduction of FHB severity and/or DON concentrations in infected grains.[15,63,64] These were reviewed by Legrand et al.[65] and presented in appropriate tables. Biological control agents (BCAs) can be applied as residue, seed, spikelets and/or post-harvest treatments.[65] According to Schmale and Bergstrom[35], BCAs have been reported to be potentiated with the ability to provide extended protection of spikes even after flowering, when most control strategies (e.g. fungicides) cannot be applied.

Mycotoxin-binding and bio-transforming microorganisms can also be used to reduce mycotoxin contamination in grains by binding the mycotoxins or by converting them to less toxic metabolites, respectively.[65] Unfortunately, the development of effective and safe detoxifying agents for use in grains intended for human consumption has been unsuccessful thus far.[49,65]

Bacteria as antagonists

Strains of *Bacillus* spp.[18,42], *Pseudomonas* spp.[64], *Streptomyces* spp.[15] and *Lactobacillus plantarum*[66] have been tested against *F. graminearum*. These bacteria were isolated from various environments, applied on anthers and/or residues of host plants, and employed various mechanisms of biological control (such as antibiosis, competition and mycoparasitism) against *F, graminearum*.[65]

In a study by Pan et al.[67], *Bacillus megaterium* reduced FHB incidence and severity, and DON production under field conditions by 93%, 54% and 89.3%, respectively. Furthermore, a study by Palazzini et al.[15] reported that *Streptomyces* sp. RC 87B reduced *F. graminearum* inoculum by 85% and 100% after 45 days and 90 days, respectively, when applied on wheat stubble. In a later study, Palazzini et al.[68] reported that *B. velezensis* RC 218 and *Streptomyces albidoflavus* RC 87B effectively reduced FHB incidence (up to 30%), severity (up to 25%) and deoxynivalenol accumulation (up to 51%) on durum wheat under field conditions.

Fungi and yeasts as antagonists

In a study by Xue et al.[63], significant reduction in mycelial growth (52.6%), spore germination (~100%), perithecial production (>99%), FHB index (58%), DON concentration (21%) and number of FDKs (65%) was obtained by using *Clonostachys rosea* strain ACM941 as a BCA against FHB under laboratory, greenhouse and field conditions. Resultantly, *C. rosea* strain ACM941 is believed to be a promising BCA of FHB. Other fungal species that have been tested against FHB include *Trichoderma* spp.[69] and *Microsphaeropsis* spp.[70] According to Gilbert and Haber[14], there are only a few yeast strains that have been reported to be effective against FHB compared to bacteria and fungi. Field trials of three strains of *Cryptococcus* spp. showed a reduction in FHB severity by as much as 50–60%.[71] Reduction in FHB severity by *Cryptococcus* spp. was also observed in other similar studies.[18,19,21,72,73]

Breeding for resistance

Many researchers believe that genetic resistance is the best, most cost-effective strategy that could provide meaningful, consistent and durable FHB control.[51,74] According to Mesterházy et al.[75], wheat resistance to FHB is not *Fusarium* species-specific, making it achievable by breeding for resistance to *Fusarium* species in general. Although there are variations in the susceptibility of different host plant species to FHB, there are no wheat or barley varieties that possess immunity against FHB.[1,75]

Recent wheat breeding programmes for FHB resistance focus on mapping quantitative trait loci (QTL) that confer a response on two or more types of FHB resistance[1,76], such as the *Fhb*1 derived from the Chinese wheat cultivar Sumai 3[1,76]. A list of wheat cultivars that have been evaluated for FHB resistance in China, the USA, Japan and Brazil are presented by Shah et al.[77] These landraces provide moderate to high resistance to FHB and some of them have been used as parents in breeding programmes.[77]

Nonetheless, resistance breeding programmes have been slow, resulting in only a few partially resistant cultivars being produced thus far.[1,53] This could be attributable to FHB resistance in small grains being complex and inherited quantitatively.[1,53] Consequently, there are no resistant cultivars commercially available in most parts of the world, including South Africa.[1,22] However, partially resistant cultivars can be used to reduce disease incidence and severity.[75]

Priming using resistance inducing chemicals

The use of resistance inducing chemicals such as jasmonic acid, ethylene and salicylic acid to enhance induced systemic resistance and systemic acquired resistance in wheat as means to control FHB has been previously studied.[19,20] According to past research, salicylic acid signalling is believed to be responsible for basal resistance to FHB whereas jasmonic acid signalling reduces further infection by the pathogen.[20,68] In a study by Palazzini et al.[68], salicylic acid signalling was induced early (12 hours) after the inoculation of wheat spikes with *F. graminearum* whereas jasmonic acid signalling was induced later (after 48 hours). Nevertheless, further research (such as formulation development, optimum concentration and application timing) is required for resistance-inducing chemicals to be employed in FHB management programmes.[19]

Integrated control strategies

As much as some control strategies provide certain levels of reduction in FHB severity and mycotoxin concentration, no single control strategy will provide significant control of FHB, especially under environmental conditions favourable for disease development.[1,19,21,53,73,78,79] Therefore, the use of integrated disease management strategies is considered the best way to control FHB on cereal crops due to the increased reduction of FHB severity and DON concentrations that could be achieved.[9,53,78]

In a study to test the efficacy of an integrated approach to FHB control, McMullen et al.[79] observed that the use of crop rotation, crop rotation + tolerant cultivar, crop rotation + tolerant cultivar + fungicide application resulted in 50%, 80% and 92% reductions in FHB, respectively. In a similar study, the combination of ploughing, a moderately resistant variety and triazole fungicide application at heading resulted in a 97% DON reduction on FHB-contaminated wheat grains.[78] BCAs can be combined with other control strategies (such as fungicides) or co-cultured with other BCAs in the integrated management of FHB.[19,21,73] In wheat field trials conducted by Schisler et al.[21], the co-culture of *C. flavescens* OH182.9 and *C. aureus* OH71.4 significantly reduced FHB severity compared to when each of the agents was applied alone. This shows that the integration of effective management strategies has the potential to enhance FHB reduction and should thus be further researched.

Way forward

Regardless of some reported efficacies, the inconsistency and lack of durability of BCAs[65], and the residue and resistance development concerns associated with fungicides[14,16] are major limitations in the development of FHB management strategies. Moreover, the use of agronomic practices in FHB management is not always feasible and/or economical in commercial farming systems. Some researchers believe that improving host genetic resistance could provide more meaningful, durable and consistent protection against FHB and its mainly produced mycotoxin, DON.[51,74] Therefore, future research can be aimed at improving host resistance to FHB either by resistance breeding or by the use of resistance inducers. The isolation and testing of more effective natural antagonists of *F. graminearum* that can be integrated with other management strategies could help improve FHB control and reduce the risks associated with fungicide use.

Conclusion

FHB remains a major threat to wheat production worldwide. Although some strategies have provided some level of disease reduction, the current dependency on fungicides in FHB management practices poses concerns regarding fungicide resistance as well as environmental, human and animal health. Therefore, further research in the development of more effective and more reliable FHB management strategies is necessary.

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Competing interests

We declare that there are no competing interests.

Authors' contributions

S.P.N.S.: Wrote the initial draft of the manuscript, implemented the comments after editing and revised the manuscript. K.S.Y.: Student supervision, project leadership and management, funding acquisition and editing of manuscript. N.C.M.: Student co-supervision, funding acquisition and proofreading of the final draft.

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INTERNATIONAL YEAR OF PLANT HEALTH 2020

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Effect of northern corn leaf blight severity on Fusarium ear rot incidence of maize

Northern corn leaf blight (NCLB) caused by *Exserohilum turcicum* and Fusarium ear rot caused by *Fusarium verticillioides*, are economically important maize diseases in South Africa. The effect of induced plant stress by NCLB on *F. verticillioides* ear rot and fumonisin production is unknown. Four field trials were conducted during 2016/2017 and 2017/2018 (November and December planting dates) at the Agricultural Research Council – Grain Crops in Potchefstroom (South Africa). Three maize cultivars with varying resistance levels to NCLB were selected (IMP50-10B – susceptible, BG3292 – moderately susceptible, DKC 61-94BR – resistant). NCLB severities were created through eight treatments: TMT1 – maximum control (three fungicide applications); TMT2 – standard control (two fungicide applications) and TMT3 – natural control (not inoculated or sprayed). The remaining treatments were inoculated with a cocktail of five NCLB races (Race 3, 3N, 23, 23N and 13N): TMT4 (five weeks after planting / WAP); TMT5 (five and six WAP); TMT6 (five, six and seven WAP); TMT7 (six and seven WAP); and TMT8 (seven WAP). Maize ears were naturally infected with *F. verticillioides*. Fifteen random plants were labelled at dent stage and NCLB severity (%), area under the disease progress curve, ear rot diseased area, ear rot severity (%), ear rot incidence (%) and total fumonisins ($FB^1+FB^2+FB^3$; ug/kg) were established. Low levels of cob rot severity and fumonisins were obtained in all four trials. NCLB severity did not affect ear rot related parameters measured. Mean fumonisin levels were below the South African tolerance levels. Fumonisin concentrations differed significantly between cultivars but was not affected by NCLB severity or the cultivar x treatment interaction.

Significance:

- This is the first study to investigate the effect of NCLB severity as a predisposing factor of ear rot incidence and severity of maize.
- The study confirmed that ear rot incidence and severity are not impacted by secondary stressors induced by NCLB, and that the cultivation of NCLB-resistant varieties would not bring about lower ear rot incidences.

Introduction

Northern corn leaf blight (NCLB), caused by *Exserohilum turcicum* (Pass.) K.J. Leonard and E.G. Suggs, is one of the most prominent leaf diseases of maize (*Zea mayze*) in South Africa. This disease occurs predominantly in the KwaZulu-Natal production areas and is particularly severe under irrigation systems.[1] Typical yield losses attributed to the disease generally range between 15% and 30%, but yield losses of up to 50% have been documented.[2,3] A potential yield reduction of 2–8% exists for every 10% increase in disease severity.[4,5]

Internationally, reference has been made to the development of secondary complications in maize due to severe leaf desiccation owing to infection by foliar pathogens. Latterell and Rossi[6] reported severe lodging and up to 100% yield loss due to stalk deterioration of maize brought about by grey leaf spot (*Cercospora zeae-maydis* Tehon & E.Y. Daniels). Stalk deterioration was attributed to the covering of the photosynthetic surfaces of the plant by lesions, which led to extreme water loss, but no report was given on whether stalk rot pathogens were conversely responsible for the stalk deterioration. NCLB has similarly been shown to potentially predispose maize plants to attack by both stalk[7,8] and root rot pathogens[9] when severe enough, by inducing sufficient stress in plants to weaken their natural defence mechanisms.

Despite the presence of Fusarium ear rot over the whole maize production area, the disease only gained importance when the mycotoxin-producing capabilities of its causal organism became evident.[10] Fusarium ear rot caused by *Fusarium verticillioides* (Sacc.) Nirenberg (syn. *Fusarium moniliforme* J. Sheldon, *Fusarium* section Liseola)[11], negatively affects crop yield and quality. The species can produce secondary metabolites (fumonisins) associated with a wide range of noxious effects on humans and livestock upon ingestion.[12] Locally, high natural infection rates of *F. verticillioides* and resulting fumonisin concentrations were reported in warmer production areas including the Northern Cape, North-West and Free State Provinces of South Africa.[13] South African regulations stipulate a tolerance of 4000 μg/kg for fumonisins in maize grain intended for further processing, while processed products that are ready for human consumption may not contain more than 2000 μg/kg of fumonisins.[14]

High temperatures, drought, poor fertilisation and stiff competition for nutrients are some of the conditions known to weaken the plant's natural defence, which predisposes the plant to increased ear rot infections.[15,16] These conditions can promote colonisation by mycotoxigenic *Fusarium* spp. in maize grain during the growing season. Although it is commonly accepted that severe leaf diseases can potentially result in an increase in stalk rot incidence, it is not yet established whether a similar association could be drawn for ear rot infections (such as *F. verticillioides*) and subsequent fumonisin production in maize grain.

In the course of 2016, the Agricultural Research Council – Grain Crops, initiated a project in which field trials were conducted over a 2-year period to ascertain to what extent NCLB severity would impact on the manifestation of secondary diseases in maize cultivars with differing NCLB resistance statuses. Key to these trials was that NCLB would be the only disease introduced artificially, whilst the response of the cultivars pertaining to the development of secondary diseases through natural infection would be monitored. Of interest in the current study was whether NCLB-resistant varieties would assist in minimising the risk associated with ear rot infections and

subsequent severity, and whether such a cultivar trait could be utilised in an integrated pest management strategy to not only reduce inoculum pressure, but also to minimise input costs. Weighing the cost associated with fungicide applications against the benefit of both natural resistance of NCLB-resistant varieties and the additional benefits of reduced ear rot infections potentially provided by NCLB-resistant varieties, will be useful to producers, allowing for informed decisions to be made regarding which cultivars to plant.

The current study reports on the observed influence of northern corn leaf blight severity on *F. verticillioides* ear rot infection and fumonisin production in the grain of three South African maize hybrids with varying NCLB disease resistance in the field.

Materials and methods

Inoculum preparation, field trials and treatment application

The five *E. turcicum* races (Race 3, 3N, 23, 23N and 13N) used in this study were ascertained through replicated growth chamber studies by means of differential sets of varying backgrounds.[17] NCLB races were inoculated into maize seedlings and re-isolated from lesions. Mycelial plugs of each race were grown on potato dextrose agar for 2 weeks before mycelial plugs were transferred to autoclaved maize kernels in fruit flasks prepared according to Flett and McLaren[18]. Flasks were incubated at room temperature and shaken daily. After 2 weeks, the contents of the flasks were dried for 3 days after which the maize kernels were ground in a standard maize mill.[19] The races were kept separate at all times and the mill was thoroughly cleaned after each isolate batch. After milling, equal amounts of each of the 10 isolates were added and thoroughly mixed to obtain an inoculation mixture.

Four field trials were conducted during 2016/2017 and 2017/2018. Two trials were planted during November and December, during each growing season, on the grounds of the Agricultural Research Council – Grain Crops (ARC-GC), Potchefstroom (North West Province; 26.743594.27 S, 27.069491 E). Three maize cultivars with varying resistance levels to NCLB were selected based on their performance in the national cultivar evaluation trials of ARC-GC under natural NCLB infection and included IMP50-10B (susceptible), BG3292 (moderately susceptible) and DKC 61-94BR (resistant). Various levels of NCLB were created through the application of eight treatments, including three control treatments: TMT1 – maximum control (three fungicide applications); TMT2 – standard control (two fungicide applications); TMT3 – natural control (not inoculated or sprayed). The remaining treatments were inoculated at various dates with the cocktail consisting of five NCLB races that included Race 3, 3N, 23, 23N and 13N: TMT4 – inoculated five weeks after planting (WAP); TMT5 – inoculated five and six WAP; TMT6 – inoculated five, six and seven WAP; TMT7 – inoculated six and seven WAP and TMT8 – inoculated 7 WAP. Each plant was inoculated with approximately 6 g inoculum placed in the whorl. TMT1 and TMT2 received two foliar fungicide formulations used in rotation every season i.e. Abacus® (pyraclostrobin/epoxiconazole – 1L/ha, BASF SA, Johannesburg, South Africa) and Sparta SC (flusilazole/carbendazim – 500 mL/ha, Villa Crop Protection, Johannesburg, South Africa) together with an adjuvant Picanta (150 mL/ha, Villa Crop Protection). Fungicides were applied at 3-week intervals, with TMT1 receiving its first fungicide application at V8 leaf stage and TMT2 at flowering. Fungicides were applied using a CO_2 gas operated knapsack sprayer and a four-nozzle (flat fan; 0.9 m spaced) boom. The knapsack sprayer was calibrated to a spray volume of 78 L/ha.

Each trial was planted in a split-plot design with treatment as the main plot and cultivar as the sub-plot, replicated three times. Each sub-plot consisted of two border rows flanking four rows per cultivar with 0.9-m inter-row spacing, 15 m in length. Intra-row spacing was 30 cm, with two kernels planted per hill. Four weeks after planting the plants were thinned out to one plant per hill. Fertiliser was applied according to soil analysis (150 kg/ha 3:2:1, 200 kg/ha LAN top dressing – 6 weeks after planting). Callisto (mesotrione – 480 g/L Syngenta SA, Centurion, South Africa) and Dual (s-metolachlor – 915 g/L, Syngenta SA) were applied pre-emergence and Basagran® (bendioxide – 480 g/L, BASF SA) was applied post-emergence to prevent weed encroachment. Directly after inoculation, approximately 15 mm water was applied through overhead irrigation over a 4-h period. Thereafter irrigation was supplied supplementary to rainfall as needed throughout the season to ensure that the trials received water weekly. Maize ear rot was initiated from natural infection by *F. verticillioides*. Weather data were captured by the ARC weather station situated on the Potchefstroom research farm.

Screening and sampling

Fifteen randomly selected plants were labelled in the first of the four middle rows of each plot and screened for NCLB development at V12, flower, milk, soft dough and dent stage.[20] Disease was quantified as the percentage infected leaf material per plant per plot using a modified scale of 0.0, 0.5, 1.0, 5.0 10.0, 25.0, 50, 70 and ≥85%.[19,21] Area under the disease progress curve (AUDPC) was determined for each plot.

At physiological maturity, ears from the 15 marked plants were harvested separately from the remaining plants in the allocated row and screened for ear rot severity. Ear rot incidence and area affected (cm^2) were established. Area affected (cm^2) was established by using a 1 cm x 1 cm transparent plastic grid placed over the ear and the number of squares in which diseased areas could be observed, were counted. The ears were threshed and the kernel weight determined. A representative milled sample from each plot was stored at -20 °C until determination of total fumonisin concentrations. Fumonisins were analysed using the HPLC-VICAM method.[22] Fumonisin standards were obtained from the Cape Peninsula University of Technology. A standard curve was generated by evaporating standards and reconstitution with a calibration standard solution ranging from 0.31 to 5 μg/kg. Fluorescence was performed at excitation and emission wavelengths of 335 nm and 440 nm, respectively, using a Waters 2475 multi λ fluorescence detector equipped with a Symmetry C18 (5 μm 3.9 x 150 mm) analytical column (Waters, Milford, USA). The LOD of the method used was 16 μg/kg and R^2 values were ≥99%. Total fumonisins were determined as the sum of FB1+FB2+FB3.

The remainder of the plants in the allocated row were harvested and yield established per plot by combining the kernel weight of the 15 marked plants and the remainder of the plants in the designated row. Yield was calculated at 12.5% moisture (t/ha).

Statistical analysis

Each trial was designed as a randomised block design with three replicates. The treatment design was a split-plot with the eight treatments and four cultivars randomised within each whole plot. Data of the various parameters measured from each trial were subjected to a split-plot analysis of variance to test for significant differences between treatments, cultivars and the interaction. Means of significant source effects were separated using Fisher's protected t-least significant difference (LSD) at a 5% significance level. In cases in which the interaction effect was non-significant, but either of the main effects indicated significant differences, treatment x cultivar interaction means were separated using Fisher's unprotected t-LSD.[23] All the analyses were conducted using GenStat for Windows 18th edition. Regression analyses were performed to ascertain whether a relationship (linear or non-linear) existed between NCLB disease and ear rot parameters measured. Regressions were performed per cultivar, per trial.

Results

As environmental conditions during the flowering period determine the potential for ear rot development, reigning conditions during this period were of interest in the current study. Temperature and rainfall data during January (2017 and 2018) coincided with the general flowering period of the November planting dates (2016 and 2017), whilst February (2017 and 2018) coincided with that of the December planting dates (2017 and 2018) (Table 1). The 2016/2017 season experienced higher rainfall (658 mm) than that of 2017/2018 (414.27 mm), with the majority recorded during the month of February (2017). Average maximum temperatures

were slightly higher during both January and February of 2018 than the same period during 2017. Temperatures for the remainder of the months for both seasons were very similar with the exception of December 2016, which was in general warmer than December 2017.

Ears of the 15 marked plants were inspected for all types of ear rot. *Fusarium verticillioides* ear rot was, however, the only type of ear rot present in all four trials. No Gibberella ear rot (*Gibberella zeae*) or Diplodia ear rot (*Stenocarpella maydis*) was observed.

Table 1: Weather data for the period October to July of the 2016/2017 and 2017/2018 seasons

		Temperature (°C)				Rainfall (mm)		
		Maximum		Minimum				
		2016/2017	2017/2018	2016/2017	2017/2018		2016/2017	2017/2018
October	Average	30.17	26.4	11.86	11.4	Total	55.12	56.13
	Highest	36.86	32.4	17.8	17.1	Highest	26.92	23.88
	Lowest	21.7	18	4.95	5.1			
November	Average	29.67	29.1	15.48	12.7	Total	94.74	69.34
	Highest	36.01	34.5	20.87	17.3	Highest	28.19	18.54
	Lowest	19.79	17.1	10.92	4.5			
December	Average	32.62	29.3	16.97	15.7	Total	73.3	62.48
	Highest	36.48	33.4	18.98	19.1	Highest	6.84	13.72
	Lowest	29.69	15.8	13.5	10.3			
January	Average	28.42	31	16.46	16.1	Total	53.33	47.24
	Highest	32.46	36.6	19.57	20.3	Highest	5.73	12.45
	Lowest	21.05	24.4	11.12	9.3			
February	Average	26.51	27.7	16.82	15.6	Total	225.55	68.33
	Highest	30.42	31.5	19.81	17.7	Highest	80.26	14.99
	Lowest	19.6	20.5	14.53	11.8			
March	Average	27.93	27.5	14.69	14.6	Total	33.78	58.93
	Highest	31.12	31.1	18.81	19.2	Highest	27.69	21.84
	Lowest	18.9	17.6	9.13	10.2			
April	Average	25.42	25.3	10.37	11.1	Total	46.23	35.56
	Highest	32.44	29	16.27	16.1	Highest	14.73	10.67
	Lowest	17.32	19.7	3.35	5.6			
May	Average	22.51	22.8	4.85	4.9	Total	10.67	11.18
	Highest	25.67	26.4	9.89	12.1	Highest	8.64	9.91
	Lowest	12.18	16.4	-1.14	1.3			
June	Average	21.91	21.6	3.15	1.5	Total	65.71	0
	Highest	25.47	25.6	8.43	4.6	Highest	2.55	0
	Lowest	14.19	17.5	-4.06	-2			
July	Average	22.19	19.3	3.47	1.1	Total	0.25	5.08
	Highest	26.1	26.3	9.73	7.8	Highest	0.25	2.03
	Lowest	16.88	14.2	-3.35	-6			
						Total seasonal rainfall (mm)	**658.68**	**414.27**

Northern corn leaf blight severity and AUDPC

Aside from the November planting of 2017/2018, the various treatments allowed for a range of NCLB severity levels to be produced within each trial (Tables 2–5) that allowed a comprehensive view of the possible impacts that different severity levels have on ear rot development. During 2017/2018, untimely or continuous rainfall was experienced, which resulted either in fungicide applications not being applied at the optimum time or fungicide that was applied being washed off after application. This resulted in little to no control in TMT1 and TMT2, especially in the November 2017/2018 planting (Table 4). Although DKC61-94BR was included as the resistant cultivar, the use of a mixture of NCLB races lead to similar NCLB severities in this hybrid compared to that of the more susceptible hybrids (BG3292 and IMP50-10B). Average NCLB disease severities realised within the eight treatments accordingly were in the ranges of 0.7–70.7% (Table 2), 6.7–60.1% (Table 3), 38.5–61.3% (Table 4) and 16.7–55.5 % (Table 5) in the various trials. Both cultivar and treatment differed significantly in all four trials, with the cultivar x treatment interaction differing significantly in the December (2016/2017) and November (2017/2018) trials. Of the three cultivars included, DKC61-94BR consistently gave the lowest NCLB severity, whist TMT5 yielded the greatest NCLB severities in three of the trials. The general trend for AUDPC data generated mirrored that of NCLB severities achieved at dent stage. With the exception of the November 2016/2017 trial (Table 2), cultivar differences were observed in the AUDPC data. In all three trials, DKC61-94BR produced significantly lower AUDPC values (Tables 3–5). Similarly to the NCLB severity, TMT5 yielded the highest AUDPC in three of the trials (Tables 2, 4 and 5). Average AUDPCs achieved within the eight treatments in the various trials were in the ranges 24–1465 (Table 2), 227–1005 (Table 3), 703–1198 (Table 4) and 103–771 (Table 5). Sufficient ranges of AUDPCs were generated to effectively evaluate the potential impact of NCLB on ear rot severity.

Ear rot affected area

In general, low levels of area affected were observed in all four trials. Cultivar differences were observed in three of the four trials (Tables 2–4). BG3292 attained significantly greater ear rot affected areas in all three trials, which varied between 3.7 cm^2 (November, 2016/2017 planting; Table 2) and 10.7 cm^2 (November, 2017/2018 planting; Table 4). The remaining two cultivars had similar ear rot affected areas in all three trials. Only in one trial (November, 2016/2017 planting; Table 2) did the treatments result in significant differences, with TMT2 yielding a significantly greater average ear rot affected area (2.9 cm^2) over the three cultivars included. A significant cultivar x treatment interaction was observed in the December 2017/2018 season, with TMT8, TMT1, TMT5, TMT2 and TMT6 of BG3292 achieving the highest area affected (Table 4).

Ear rot severity

Ear rot severity, similar to ear rot affected area, was very low in all four trials with trial means of 1.1%, 0.6%, 3.6% and 2.62%, respectively (Tables 2–5). Cultivar differences were observed in both the 2016/2017 trials as well as the November 2017/2018 planting trial, with BG3292 yielding significantly greater ear rot severity in all three trials (2.4%, 1.4% and 6.3% respectively; Tables 2–4). Neither the treatment effect nor the cultivar x treatment interaction was significant.

Ear rot incidence

Cultivar differences were observed in both the 2016/2017 trials as well as the November 2017/2018 trial. In all cases, BG3292 gave significantly greater ear rot incidence, which varied from 31.7% of the ears having some degree of ear rot (November 2017/2018 planting; Table 4) to 51% of the ears in the November 2016/2017 planting (Table 1).

Fumonisin

The average fumonisin concentration detected per trial in the sampled material ranged between 2 μg/kg (December 2016/2017 planting; Table 3) and 235 μg/kg (November 2017/2018 planting; Table 4). Cultivar differences occurred in the two 2016/2017 trials (Tables 2 and 3) as well as the November 2017/2018 planting (Table 4). BG3292 achieved the highest average fumonisin concentration in the grain in all three trials (3.8, 2.9 and 381 μg/kg, respectively). Significant differences between treatments in terms of fumonisin concentrations in the grain were only observed for the 2016/2017 November planting (Table 2), with TMT1 (5.3 μg/kg) followed by TMT8 (4 μg/kg). No significant cultivar x treatment interaction was observed. Fumonisin concentrations measured did not exceed 1407 μg/kg (Table 5) in any of the trials.

Regression analyses

Regression analyses were initially conducted against NCBL severity (at dent stage) and AUDPC for each of the ear rot related parameters. This was done per cultivar per season. As none of the regression analyses (either linear or non-linear) was significant (data not shown), the possibility was considered that external factors (other than NCLB severity) had contributed to the random effects observed over seasons. Data were accordingly pooled across the trials for each treatment, as pooling of data aids in minimising any effect that external factors, not linked to NCLB severity, might have had on the ear rot parameters measured. Linear, exponential and polynomial regression analyses were again conducted. Ear rot incidence was the only parameter that demonstrated a potential relationship with NCLB severity ($R^2 = 0.67$; Figure 1a) and AUDPC ($R^2 = 0.65$; Figure 2a) for IMP50-10B; however, the relationship was not significant in either circumstance.

Discussion

The objective of this study was to establish whether the ear rot severity observed in three maize cultivars with varying degrees of NCLB resistance, would be impacted by NCLB severity suffered during the growing season. Multiple season trials were conducted together with an intensive *E. turcicum* inoculation approach to ensure that different degrees of NCLB were created to assess whether NCLB would predispose the maize plant to greater ear rot infections and subsequent fumonisin production in maize grain. Despite the fact that high levels of NCLB were achieved in all four trials, very low levels of ear rot (less than 11% obtained in the November 2017/2018 planting) were nonetheless observed. Fumonisin levels detected in the grain were also well below the accepted 2000 μg/kg concentration for grain. The averages in the trials varied between 2 μg/kg and 235 μg/kg.

Internationally, it is accepted that *F. verticillioides* gains access to the ear by one or more of three main access pathways: (1) fungal spores germinating on the silks and then fungal mycelia growing down the silks to infect the kernels and the ear (rachis); (2) systemic infection of the ear through infected stalks that generate infected seeds and (3) through wounds on the ear generated by insects, birds or hail damage.[11,24] It is also common knowledge that ear rot incidence and severity as well as associations with mycotoxins vary with environmental conditions, genotype, and location.[11,25] In general, higher temperatures and drier weather during flowering (26 °C and higher), higher temperatures during kernel maturation, more rainfall before harvest, drought stress as well as insect damage stress are factors known to increase ear rot severity and fumonisin content at harvest.[11,26,27] Weather conditions during flowering are, however, considered critical for primary infection as well as for toxin synthesis in grain.[28-30] For the current study, it was imperative that moist conditions were maintained throughout the duration of trials to ensure effective NCLB infection and subsequent high NCLB disease severity. Although leaf blight data indicate high and variable levels of disease, the extremely low ear rot levels raised the question of whether these low levels were due to the absence of epidemiologically competent inoculum, the absence of predisposition or possibly the end result of inherent cultivar resistance.

Table 2: Northern corn leaf blight (NCLB) and ear rot related data generated for the first planting trial during 2016/2017

	TMT	Cultivar						*TMT mean*	
		BG3292		DKC61-94BR		IMP50-10B			
NCLB severity (%)*	1	2.0	j	2.3	j	1.5	j	*1.9*	*e*
F prob Treatment < 0.001	2	0.9	j	1.2	j	0.1	j	*0.7*	*e*
LSD Treatment $_{(P=0.05)}$ = 7.49	3	18.0	i	19.7	hi	40.9	ef	*26.2*	*d*
F prob Cultivar < 0.001	4	56.9	cd	58.0	cd	73.3	ab	*62.8*	*b*
LSD Cultivar $_{(P=0.05)}$ = 5.03	5	68.1	bc	64.4	bcd	76.9	ab	*69.8*	*ab*
F prob Cultivar x Treatment = 0.189	6	65.0	bcd	65.3	bcd	81.9	a	*70.7*	*a*
LSD Cultivar x Treatment $_{(P=0.05)}$ =13.47	7	52.8	de	34.0	fg	58.3	cd	*48.4*	*c*
	8	24.9	ghi	26.4	ghi	32.9	fgh	*28.1*	*d*
Cultivar mean		*36.1*	*b*	*33.9*	*b*	*45.7*	*a*	*38.6*	
AUDPC	1	8.6	g	149.1	fg	19.5	g	*59.1*	*d*
F prob Treatment <0.001	2	13.5	g	32.0	g	25.7	g	*23.7*	*d*
LSD Treatment$_{(P=0.05)}$ = 3.28	3	395.6	efg	311.9	fg	588.6	def	*432.0*	*c*
F prob Cultivar =0.48	4	1504.0	a	1287.3	abc	1544.0	a	*1445.1*	*a*
LSD Cultivar $_{(P=0.05)}$ =159.7	5	1568.3	a	1420.7	ab	1405.3	ab	*1464.8*	*a*
F prob Cultivar x Treatment = 0.983	6	1424.3	ab	1294.7	abc	1293.3	abc	*1337.4*	*a*
LSD Cultivar x Treatment $_{(P=0.05)}$ 447.8	7	964.2	bcd	848.9	cde	925.8	cd	*913.0*	*b*
	8	430.1	efg	208.9	fg	252.3	fg	*297.1*	*cd*
Cultivar mean		*789.0*		*694.0*		*757.0*		*746.5*	
Ear rot diseased area (cm²)	1	4.5	ab	0.3	d	0.5	cd	*1.7*	*b*
F prob Treatment = 0.047	2	5.1	a	0.1	d	3.4	ab	*2.9*	*a*
LSD Treatment $_{(P=0.05)}$ = 1.033	3	3.7	ab	0.6	cd	0.2	d	*1.5*	*b*
F prob Cultivar < 0.001	4	3.5	ab	0.3	d	0.3	d	*1.4*	*b*
LSD Cultivar $_{(P=0.05)}$ = 0.86	5	2.6	bc	0.5	cd	0.3	cd	*1.1*	*b*
F prob Cultivar x Treatment =0.786	6	3.1	ab	0.0	d	0.0	d	*1.1*	*b*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 2.193	7	3.5	ab	0.8	cd	0.3	d	*1.5*	*b*
	8	3.5	ab	0.1	d	0.5	cd	*1.4*	*b*
Cultivar mean		*3.7*	*a*	*0.7*	*b*	*0.3*	*b*	*1.6*	
Ear rot severity (%)	1	2.7	ab	0.2	ef	0.3	def	*1.1*	*b*
F prob Treatment = 0.013	2	2.8	ab	0.1	ef	4.1	a	*2.3*	*a*
LSD Treatment $_{(P=0.05)}$ =0.802	3	2.6	ab	0.5	def	0.1	ef	*1.1*	*b*
F prob Cultivar < 0.001	4	1.7	bcdef	0.2	ef	0.2	ef	*0.7*	*b*
LSD Cultivar $_{(P=0.05)}$ = 0.702	5	2.3	bc	0.6	cdef	0.3	ef	*1.1*	*b*
F prob Cultivar x Treatment = 0.19	6	1.8	bcde	0.0	ef	0.0	ef	*0.6*	*b*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 1.773	7	2.0	bcd	0.6	cdef	0.3	def	*1.0*	*b*
	8	3.1	ab	0.0	f	0.6	cdef	*1.2*	*b*
Cultivar mean		*2.4*	*a*	*0.7*	*b*	*0.3*	*b*	*1.1*	
Ear rot incidence (%)	1	50.9	a	21.5	bc	8.1	cd	*26.9*	
F prob Treatment = 0.577	2	50.0	a	5.1	cd	8.1	cd	*21.1*	
LSD Treatment $_{(P=0.05)}$ = 11.85	3	63.1	a	15.5	bcd	3.7	cd	*27.5*	
F prob Cultivar < 0.001	4	49.2	a	12.3	bcd	4.2	cd	*21.9*	
LSD Cultivar $_{(P=0.05)}$ = 6.91	5	27.7	b	16.9	bcd	7.2	cd	*17.3*	
F prob Cultivar x Treatment = 0.212	6	54.6	a	2.8	cd	0.0	d	*19.1*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 19.29	7	51.3	a	15.5	bcd	4.3	cd	*23.7*	
	8	60.9	a	6.0	cd	5.7	cd	*24.2*	
Cultivar mean		*51.0*	*a*	*12.0*	*b*	*5.2*	*b*	*22.7*	
Fumonisin (μg/kg)	1	9.6	a	0.8	e	5.5	abc	*5.3*	*a*
F prob Treatment = 0.007	2	1.0	de	0.8	e	2.0	cde	*1.3*	*c*
LSD Treatment $_{(P=0.05)}$ = 2.193	3	3.2	bcde	0.8	e	1.4	de	*1.8*	*c*
F prob Cultivar =0.002	4	5.0	bcd	0.3	e	0.3	e	*1.9*	*bc*
LSD Cultivar $_{(P=0.05)}$ =1.51	5	1.6	cde	0.3	e	4.1	bcde	*2.0*	*bc*
F prob Cultivar x Treatment = 0.124	6	0.9	e	0.3	e	0.6	e	*0.6*	*c*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 4.014	7	2.3	cde	2.1	cde	0.3	e	*1.6*	*c*
	8	7.1	ab	2.9	bcde	2.1	cde	*4.0*	*ab*
Cultivar mean		*3.8*	*a*	*2.1*	*b*	*1.0*	*b*	*2.3*	

**at dent stage*
AUDPC, area under disease progress curve; LSD, least significant difference

Table 3: Northern corn leaf blight (NCLB) and ear rot related data generated for the second planting trial during 2016/2017

	TMT	Cultivar						*TMT mean*	
		BG3292		DKC61-94BR		IMP50-10B			
NCLB severity (%)*	1	9.1	g	1.9	g	9.0	g	*6.7*	*e*
F prob Treatment < 0.001	2	29.7	de	6.2	g	15.3	fg	*17.1*	*d*
LSD Treatment $_{(P=0.05)}$ = 9.15	3	58.6	c	26.1	def	60.0	bc	*48.2*	*c*
F prob Cultivar < 0.001	4	66.9	abc	38.3	de	75.6	a	*60.3*	*a*
LSD Cultivar $_{(P=0.05)}$ = 5.1	5	73.3	ab	40.0	d	66.9	abc	*60.1*	*a*
F prob Cultivar x Treatment = 0.033	6	64.7	abc	35.6	de	75.6	a	*58.6*	*ab*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 14.46	7	61.1	abc	25.0	ef	65.6	abc	*50.6*	*bc*
	8	64.7	abc	32.5	de	67.2	abc	*54.8*	*abc*
Cultivar mean		*53.5*	*a*	*25.7*	*b*	*54.4*	*a*	*44.5*	
AUDPC	1	293.2	ef	41.4	g	345.7	e	*226.8*	*c*
F prob Treatment <0.001	2	604.1	d	101.4	fg	747.5	d	*484.3*	*b*
LSD Treatment $_{(P=0.05)}$ = 197.4	3	1115.0	bc	190.8	efg	1300.6	ab	*868.8*	*a*
F prob Cultivar < 0.001	4	1265.7	bc	247.1	efg	1501.3	a	*1004.7*	*a*
LSD Cultivar $_{(P=0.05)}$ = 67.5	5	1222.8	bc	317.7	ef	1279.3	abc	*939.9*	*a*
F prob Cultivar x Treatment < 0.001	6	1203.3	bc	205.6	efg	1259.0	abc	*889.3*	*a*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 243.6	7	1216.3	bc	209.0	efg	1210.3	bc	*878.6*	*a*
	8	1223.3	bc	346.1	e	1047.7	c	*872.4*	*a*
Cultivar mean		*1018.0*	*b*	*207.4*	*c*	*1086.4*	*a*	*771.0*	
Ear rot diseased area (cm^2)	1	5.0	abcde	0.0	e	0.0	e	*1.7*	
F prob Treatment = 0.253	2	3.8	bcde	1.7	de	8.0	abc	*4.5*	
LSD Treatment $_{(P=0.05)}$ = 3.936	3	3.6	bcde	0.0	e	0.0	e	*1.2*	
F prob Cultivar < 0.001	4	7.2	abcd	2.0	cde	0.0	e	*3.1*	
LSD Cultivar $_{(P=0.05)}$ = 2.119	5	10.4	a	1.7	de	3.7	bcde	*5.3*	
F prob Cultivar x Treatment = 0.405	6	8.4	ab	2.3	cde	4.7	abcde	*5.1*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 6.089	7	3.9	bcde	0.0	e	2.0	cde	*2.0*	
	8	9.0	ab	0.8	e	0.0	e	*3.3*	
Cultivar mean		*6.4*	*a*	*1.1*	*b*	*2.3*	*b*	*3.3*	
Ear rot severity (%)	1	0.7	bcd	0.0	d	0.0	d	*0.2*	
F prob Treatment =0.43	2	0.7	bcd	0.0	d	1.1	bcd	*0.6*	
LSD Treatment $_{(P=0.05)}$ = 0.8203	3	0.9	bcd	0.0	d	0.0	d	*0.3*	
F prob Cultivar < 0.001	4	2.7	a	0.2	d	0.0	d	*0.9*	
LSD Cultivar $_{(P=0.05)}$ = 0.4138	5	1.6	abc	0.1	d	0.3	d	*0.7*	
F prob Cultivar x Treatment = 0.094	6	1.6	ab	0.1	d	0.4	cd	*0.7*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 1.2198	7	0.6	bcd	0.0	d	0.1	d	*0.2*	
	8	2.5	a	0.1	d	0.0	d	*0.9*	
Cultivar mean		*1.4*	*a*	*0.1*	*b*	*0.2*	*b*	*0.6*	
Ear rot incidence (%)	1	17.8	de	0.0	f	0.0	f	*5.9*	
F prob Treatment = 0.121	2	33.2	bcd	3.3	ef	10.0	ef	*15.5*	
LSD Treatment $_{(P=0.05)}$ = 9.12	3	46.3	ab	0.0	f	0.0	f	*15.4*	
F prob Cultivar < 0.001	4	40.0	abc	5.6	ef	0.0	f	*15.2*	
LSD Cultivar $_{(P=0.05)}$ = 5.78	5	26.0	cd	3.3	ef	6.7	ef	*12.0*	
F prob Cultivar x Treatment = 0.234	6	40.6	abc	5.8	ef	7.5	ef	*18.0*	
LSD Cultivar x Treatment $_{(P=0.05)}$ 15.75	7	31.0	bcd	0.0	f	3.3	ef	*11.4*	
	8	49.9	a	9.1	ef	0.0	f	*19.7*	
Cultivar mean		*35.6*	*a*	*3.4*	*b*	*3.5*	*b*	*14.1*	
Fumonisin (μg/kg)	1	1.1	abc	0.4	abc	0.8	abc	*0.8*	
F prob Treatment = 0.683	2	4.2	abc	0.5	ac	6.0	ab	*3.6*	
LSD Treatment $_{(P=0.05)}$ = 4.229	3	0.3	abc	0.1	c	0.1	c	*0.2*	
F prob Cultivar = 0.014	4	4.9	abc	0.3	abc	0.2	abc	*1.8*	
LSD Cultivar $_{(P=0.05)}$ = 1.854	5	5.2	abc	0.2	c	3.5	abc	*3.0*	
F prob Cultivar x Treatment = 0.613	6	2.2	abc	0.2	abc	1.5	abc	*1.3*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 5.821	7	1.1	abc	0.9	abc	6.0	a	*2.7*	
	8	4.3	abc	0.1	c	2.8	abc	*2.4*	
Cultivar mean		*2.9*	*a*	*0.3*	*b*	*2.6*	*a*	*2.0*	

**at dent stage*

AUDPC, area under disease progress curve; LSD, least significant difference

Table 4: Northern corn leaf blight (NCLB) and ear rot related data generated for the first planting trial during 2017/2018

	TMT	Cultivar						*TMT mean*	
		BG3292		DKC61-94BR		IMP50-10B			
NCLB severity (%)*	1	47.2	cdef	38.3	ghijkl	42.8	defghij	*42.8*	*c*
F prob Treatment = 0.003	2	46.7	cdefgh	39.7	fgijkl	46.4	cdefgh	*44.3*	*c*
LSD Treatment $_{(P=0.05)}$ = 9.216	3	50.5	bcde	34.7	jkl	51.3	bcd	*45.5*	*bc*
F prob Cultivar < 0.001	4	43.1	defghij	41.0	efghijkl	44.4	cdefghij	*42.8*	*c*
LSD Cultivar $_{(P=0.05)}$ = 2.036	5	64.7	a	59.3	ab	59.8	ab	*61.3*	*a*
F prob Cultivar x Treatment = 0.046	6	54.5	bc	48.5	def	57.6	ab	*53.6*	*ab*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 10.105	7	41.5	defghijk	34.8	jl	39.3	fghijkl	*38.5*	*c*
	8	45.0	cdefghi	41.4	defghijkl	46.8	cdefg	*44.4*	*bc*
Cultivar mean		*49.1*	*a*	*42.2*	*b*	*48.6*	*a*	*46.6*	
AUDPC	1	1114.0	abcde	1064.0	acdef	1288.0	ab	*1155.0*	
F prob Treatment = 0.183	2	721.0	ef	680.0	f	707.0	ef	*703.0*	
LSD Treatment $_{(P=0.05)}$ = 410.1	3	1011.0	abcdef	916.0	abcdef	989.0	abcdef	*972.0*	
F prob Cultivar = 0.002	4	1168.0	abcd	1071.0	abcdef	1179.0	abcd	*1139.0*	
LSD Cultivar $_{(P=0.05)}$ = 63.5	5	1168.0	abcd	1111.0	bcde	1314.0	a	*1198.0*	
F prob Cultivar x Treatment = 0.775	6	1160.0	abcd	1063.0	abcdef	1212.0	abc	*1145.0*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 429.1	7	928.0	abcdef	756.0	def	805.0	cdef	*829.0*	
	8	1003.0	abcdef	932.0	abcdef	1018.0	abcdef	*985.0*	
Cultivar mean		*1034.0*	*a*	*949.0*	*b*	*1064.0*	*a*	*1016.0*	
Ear rot diseased area (cm²)	1	16.1	a	4.5	de	2.6	e	*7.7*	
F prob Treatment = 0.057	2	12.8	abc	5.9	cde	1.6	e	*6.8*	
LSD Treatment $_{(P=0.05)}$ = 4.232	3	4.2	e	3.0	e	3.2	e	*3.5*	
F prob Cultivar < 0.001	4	0.2	e	7.7	bcde	1.9	e	*3.3*	
LSD Cultivar $_{(P=0.05)}$ = 3.161	5	15.8	ab	1.5	e	1.6	e	*6.3*	
F prob Cultivar x Treatment = 0.036	6	12.6	abcd	7.1	cde	7.0	cde	*8.9*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 8.24	7	4.3	e	1.9	e	4.3	e	*3.5*	
	8	19.4	a	2.1	e	2.3	e	*7.9*	
Cultivar mean		*10.7*	*a*	*4.2*	*b*	*3.1*	*b*	*6.0*	
Ear rot severity (%)	1	8.9	abc	3.4	cde	0.9	e	*4.4*	
F prob Treatment = 0.127	2	4.2	bcde	6.9	abcd	1.4	de	*4.2*	
LSD Treatment $_{(P=0.05)}$,= 3.422	3	1.9	de	1.0	de	2.4	de	*1.8*	
F prob Cultivar < 0.001	4	3.2	cde	2.0	de	1.1	de	*2.1*	
LSD Cultivar $_{(P=0.05)}$ = 2.208	5	10.6	a	0.6	e	1.4	de	*4.2*	
F prob Cultivar x Treatment = 0.259	6	9.7	ab	4.6	bcde	4.9	abcde	*6.4*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 5.979	7	2.7	de	1.0	de	2.1	de	*1.9*	
	8	9.4	ab	2.0	de	0.6	e	*4.0*	
Cultivar mean		*6.3*	*a*	*2.7*	*b*	*1.9*	*b*	*3.6*	
Ear rot incidence (%)	1	35.6	abc	8.9	cf	13.3	cdef	*19.3*	
F prob Treatment = 0.134	2	33.3	abcde	24.4	bcdef	13.3	cdef	*23.7*	
LSD Treatment $_{(P=0.05)}$ = 10.07	3	22.2	bcdef	15.6	cdef	4.4	f	*14.1*	
F prob Cultivar < 0.001	4	15.6	cdef	13.3	cdef	4.5	f	*11.1*	
LSD Cultivar $_{(P=0.05)}$ = 9.53	5	44.4	ab	6.7	f	6.7	f	*19.3*	
F prob Cultivar x Treatment = 0.431	6	35.6	abcd	22.2	bcdef	15.6	cdef	*24.4*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 23.79	7	15.6	cdef	17.8	cdef	11.1	ef	*14.8*	
	8	51.1	a	2.2	f	4.4	f	*19.3*	
Cultivar mean		*31.7*	*a*	*13.9*	*b*	*9.2*	*b*	*18.2*	
Fumonisin (μg/kg)	1	262.3	bcd	154.1	d	71.8	d	*163.0*	
F prob Treatment =0.071	2	152.4	d	200.8	cd	127.0	d	*160.0*	
LSD Treatment $_{(P=0.05)}$ = 232.9	3	64.5	d	67.0	d	143.1	d	*92.0*	
F prob Cultivar = 0.02	4	665.8	abc	728.4	ab	54.0	d	*483.0*	
LSD Cultivar $_{(P=0.05)}$ = 183.5	5	406.5	abcd	116.8	d	189.1	d	*237.0*	
F prob Cultivar x Treatment = 0.493	6	428.4	abcd	158.4	d	187.3	d	*258.0*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 472.8	7	300.9	abcd	110.9	d	117.9	d	*177.0*	
	8	767.6	a	101.6	d	56.2	d	*308.0*	
Cultivar mean		*381.0*	*a*	*204.7*	*ab*	*118.3*	*b*	*235.0*	

**at dent stage*
AUDPC, area under disease progress curve; LSD, least significant difference

Table 5: Northern corn leaf blight (NCLB) and ear rot related data generated for the second planting trial during 2017/2018

	TMT	Cultivar						*TMT mean*	
		BG3292		DKC61-94BR		IMP50-10B			
NCLB severity (%)*	1.0	21.9	jklmno	11.3	lmnp	16.8	klmnop	*16.7*	*c*
F prob Treatment = 0.006	2.0	25.3	ghijklm	15.5	lnop	25.6	ghijklm	*22.1*	*c*
LSD Treatment $_{(P=0.05)}$ = 19.323	3.0	44.9	abcdefg	38.8	abcdefghij	42.3	abcdefghi	*42.0*	*ab*
F prob Cultivar < 0.001	4.0	47.8	abcde	40.4	abcdfghij	46.6	abcdef	*44.9*	*ab*
LSD Cultivar $_{(P=0.05)}$ = 2.203	5.0	58.1	a	49.9	bc	58.5	a	*55.5*	*a*
F prob Cultivar x Treatment = 0.595	6.0	52.9	ab	42.1	acefghi	48.4	abcd	*47.8*	*ab*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 19.8	7.0	42.5	abcdefgh	28.7	defgijl	36.4	bcdefghijk	*35.8*	*bc*
	8.0	24.7	hijklmn	20.9	jklmnop	21.7	jklmnop	*22.4*	*c*
Cultivar mean		*39.7*	*a*	*31.0*	*c*	*37.0*	*b*	*35.9*	
AUDPC	1.0	128.0	hi	67.0	i	114.2	i	*103.0*	*d*
F prob Treatment = 0.002	2.0	166.3	ghi	99.7	i	163.0	ghi	*143.0*	*d*
LSD Treatment $_{(P=0.05)}$ = 265.46	3.0	342.4	efghi	281.5	efghi	320.0	efghi	*314.6*	*bcd*
F prob Cultivar < 0.001	4.0	543.7	bcde	341.0	fghi	534.2	bcde	*473.0*	*bc*
LSD Cultivar $_{(P=0.05)}$ = 41.15	5.0	803.2	ab	685.6	c	823.3	a	*770.7*	*a*
F prob Cultivar x Treatment = 0.21	6.0	640.9	abcd	413.6	cefg	472.5	cef	*509.0*	*ab*
LSD Cultivar x Treatment $_{(P=0.05)}$ = 277.77	7.0	399.4	defgh	222.7	fgi	285.0	efghi	*302.4*	*bcd*
	8.0	248.4	fghi	209.9	fghi	192.8	ghi	*217.0*	*cd*
Cultivar mean		*409.0*	*a*	*290.1*	*c*	*363.1*	*b*	*354.1*	
Ear rot diseased area (cm²)	1.0	7.8		7.7		4.2		*6.6*	
F prob Treatment = 0.571	2.0	0.9		10.3		5.7		*5.6*	
LSD Treatment $_{(P=0.05)}$ = 5.611	3.0	2.7		5.6		1.5		*3.3*	
F prob Cultivar =0.463	4.0	2.4		2.8		3.8		*3.0*	
LSD Cultivar $_{(P=0.05)}$ = 2.767	5.0	1.6		0.4		2.0		*1.3*	
F prob Cultivar x Treatment = 0.526	6.0	8.0		1.2		1.8		*3.7*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 8.233	7.0	1.1		5.6		0.6		*2.4*	
	8.0	5.5		3.4		3.7		*4.2*	
Cultivar mean		*3.7*		*4.6*		*2.9*		*3.8*	
Ear rot severity (%)	1.0	6.3		7.5		2.6		*5.5*	
F prob Treatment = 0.475	2.0	0.4		5.8		2.8		*3.0*	
LSD Treatment $_{(P=0.05)}$ = 4.695	3.0	2.5		5.1		0.6		*2.7*	
F prob Cultivar = 0.273	4.0	0.8		2.0		1.2		*1.4*	
LSD Cultivar $_{(P=0.05)}$ = 2.268	5.0	0.7		0.2		0.7		*0.5*	
F prob Cultivar x Treatment = 0.491	6.0	7.4		0.4		0.6		*2.8*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 6.808	7.0	0.4		3.9		0.3		*1.5*	
	8.0	4.1		2.7		4.4		*3.7*	
Cultivar mean		*2.8*		*3.5*		*1.7*		*2.7*	
Ear rot incidence (%)	1.0	20.0		11.1		15.6		*15.6*	
F prob Treatment = 0.44	2.0	4.4		22.2		20.0		*15.6*	
LSD Treatment $_{(P=0.05)}$ = 10.05	3.0	8.9		8.9		4.4		*7.4*	
F prob Cultivar = 0.961	4.0	15.6		11.1		13.3		*13.3*	
LSD Cultivar $_{(P=0.05)}$ = 6.94	5.0	6.7		2.2		13.3		*7.4*	
F prob Cultivar x Treatment = 0.551	6.0	17.8		4.4		8.9		*10.4*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 18.43	7.0	11.1		24.4		6.7		*14.1*	
	8.0	13.3		6.7		8.9		*9.6*	
Cultivar mean		*12.2*		*11.4*		*11.4*		*11.7*	
Fumonisin (μg/kg)	1.0	55.0		69.0		15.0		*46.0*	
F prob Treatment = 0.437	2.0	58.0		42.0		35.0		*45.0*	
LSD Treatment $_{(P=0.05)}$ = 5.37	3.0	37.0		27.0		45.0		*36.0*	
F prob Cultivar = 0.466	4.0	817.0		35.0		37.0		*296.0*	
LSD Cultivar $_{(P=0.05)}$ = 337.5	5.0	84.0		32.0		27.0		*48.0*	
F prob Cultivar x Treatment = 0.522	6.0	153.0		20.0		11.0		*62.0*	
LSD Cultivar x Treatment $_{(P=0.05)}$ = 920.5	7.0	98.0		415.0		28.0		*180.0*	
	8.0	37.0		1407.0		195.0		*546.0*	
Cultivar mean		*167.0*		*256.0*		*49.0*		*158.0*	

**at dent stage*
AUDPC, area under disease progress curve; LSD, least significant difference

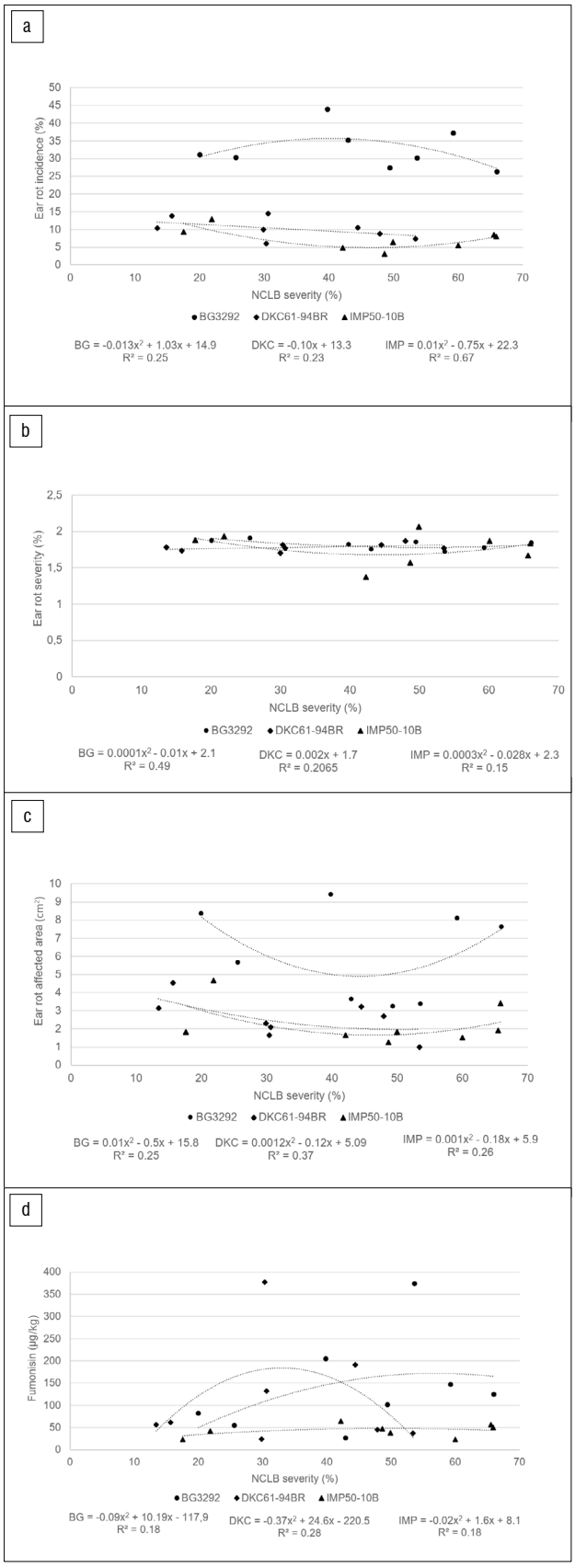

Figure 1: Average obtained over four trials for (a) ear rot incidence (%), (b) ear rot severity (%), (c) ear rot affected area (cm²) and (d) fumonisin concentration (µg/kg) in the grain, regressed against northern corn leaf blight (NCLB) severity achieved at dent stage of eight applied treatments.

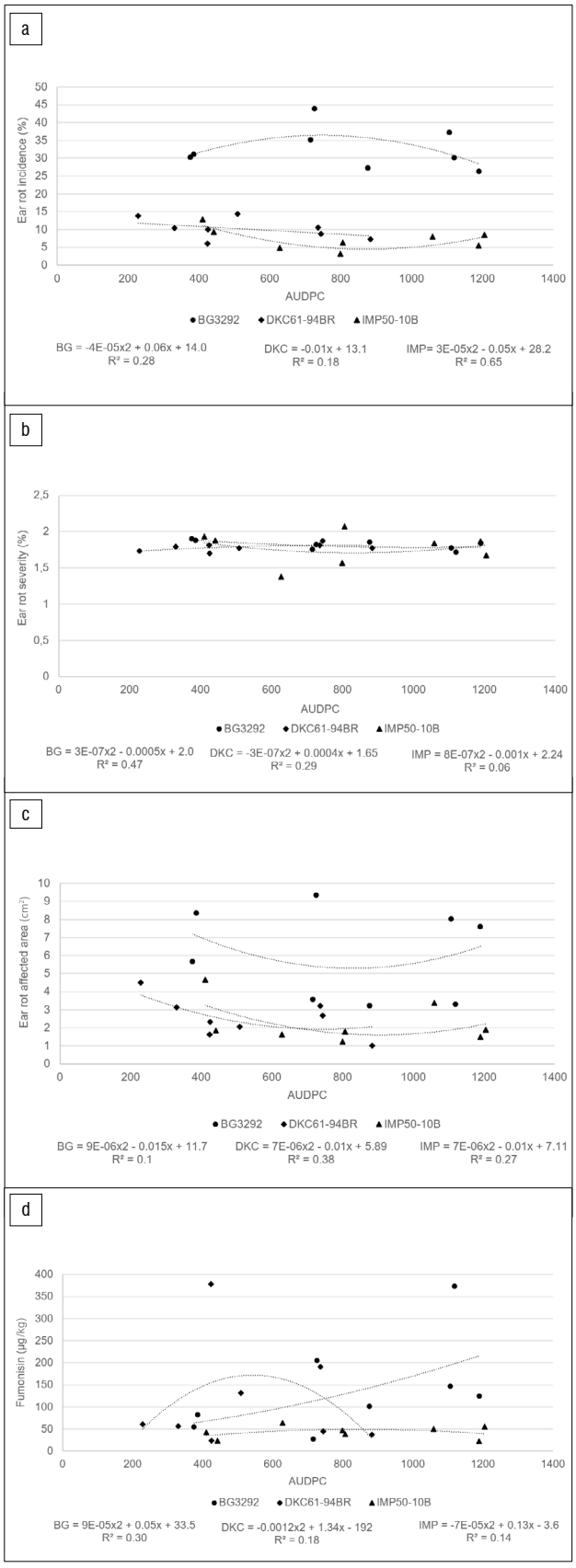

Figure 2: Average obtained over four trials for (a) ear rot incidence (%), (b) ear rot severity (%), (c) ear rot affected area (cm²) and (d) fumonisin concentration (µg/kg) in the grain, regressed against area under the disease progress curve (AUDPC) achieved at dent stage of eight applied treatments.

As all four trial sites of the current study were situated in the same area where ear rot related field experiments have been regularly conducted over numerous seasons, and entailed artificial inoculation with multiple *F. verticillioides* isolates[22,31], it was assumed that present-day isolates at the trial site area would be more than capable of infecting maize ears, provided environmental conditions were conducive for ear rot infection and development. Although maximum temperatures during all four trials were in the required range for Fusarium ear rot development, drier conditions (which would have enhanced ear rot development)[26,27] did not occur during flowering due to the irrigation applied to ensure NCLB development. The question to be addressed was whether NCLB severity would place the plant under sufficient stress to induce a water stress associated situation[6] in the plant, which would unlock a similar response in the plant as would drought stress. One way in which this could happen is if NCLB infection results in stalk rot develoment[7], which would hamper the plant's ability to access water and nutrients. NCLB severity at flowering stage was low with average NCLB severities of between 3% and 14% over the four trials (data not shown). Desiccation due to NCLB was accordingly most likely not severe enough at this critical stage to induce a form of water loss[6] that would aid colonisation by the *F. verticillioides* pathogen and result in fumonisin production[26].

Even though heritable resistance has been identified in maize[32,33], Small et al.[34] were the first to report potentially resistant maize inbred lines locally adapted to southern African production conditions. Very little is, however, known regarding the adoption rate of such lines by local breeding companies, especially as Fusarium ear rot resistance has been established to be a quantitative trait determined by polygenes.[35,36] The respective seed companies could not confirm the Fusarium ear rot resistance of the three cultivars included. Based on what is known internationally, it would nevertheless be highly unlikely that these cultivars would pose such high levels of resistance that could be linked to limited ear rot infection observed over multiple seasons for all three cultivars, as no highly resistant genotypes suited to the production regions in southern Africa exist.[37] A form of indirect resistance through the presence of the Bt gene, which would reduce damage by insects and subsequent infection by the pathogen, might have contributed to lower ear rots being observed. Of the three cultivars included, only DKC 61-94BR contains MON89034. BG3292, which accordingly does not contain Bt genes, consistently had the highest degree of ear rot, but never exceeded levels greater than 10.6% severity in any of the trials (Table 4). Irrespective of how the fungus infected, one would expect that – should stress induced by NCLB create favourable conditions for ear rot infection and growth – greater ear rot infections should have been observed in a cultivar such as BG3292, which consistently had high average NCLB severity over four trials.

Regression analyses conducted over multiple seasons and cultivars point to no significant association between NCLB and natural *F. verticillioides* infection. The possible exception is the fact that BG3292, which consistently had high NCLB severity over four trials, was identified as the cultivar with the highest degree of ear rot and fumonisin concentration observed in the ears (albeit at very low levels). The latter observation nevertheless speaks more to the hybrid's ability to cope with both the diseases individually, than to the link between the two diseases. In essence, the higher levels of NCLB in BG3292 did not result in an increase in ear rot or related parameters in any of the trials conducted.

It has lastly already been established that *F. verticillioides* can also infect through wounds on the ear[11,31]; hence artificial inoculations which make use of techniques which inject the pathogen into the ear are commonly used[22,31]. Although it has been established with the current study that NCLB severity was not able to induce greater ear rot incidence or severity under natural infection of *F. verticillioides*, follow-up research which includes artificial inoculation of *F. verticillioides* would shed additional light on the ability of NCLB to predispose the plant to greater ear rot infection in situations in which ears are damaged by insects, hail or birds.

Conclusion

In the current study, natural ear rot development was monitored in an area in which numerous field studies have been conducted in the past with epidemiological competent *F. verticillioides* ear rot isolates. Very low levels of ear rot severity were nonetheless obtained in all four trials. Without artificial interference, the local *F. verticillioides* isolates were not able to naturally infect the ears, most likely because conditions were too wet during flowering, which was a necessity to ensure sufficient NCLB development. Environmental conditions during flowering are determinant for ear rot development. Although high and variable degrees of NCLB severity were achieved in the current study, blight severity at flowering was not severe or sufficient enough to induce a stress response in the plants, which would simulate water stress conditions that would allow for greater ear rot development. Additional studies which include artificial inoculation of the ears, would aid in clarifying the potential effect of NCLB severity in scenarios in which ear rot development is brought about by insect, bird or hail damage. Based on fitted regression models, NCLB severity did not, however, affect natural ear rot development in three maize cultivars with varying NCLB resistance levels.

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Competing interests

We declare that there are no competing interests.

Authors' contributions

M.C.: Conceptualisation, methodology, data collection, writing – initial draft, funding acquisition. L.M.: Data analyses, validation, data curation. A.A.: Data collection, sample analyses, writing – revision, project leadership. H.N.: Data collection, sample analyses, writing – revision, project leadership. B.J.v.R.: Data collection, sample analyses, writing – revision.

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INTERNATIONAL YEAR OF PLANT HEALTH 2020

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Models for predicting pseudothecium maturity and ascospore release of *Phyllosticta* spp. in South African citrus orchards

Ascosporic infection plays a major role in the epidemiology of citrus black spot (CBS) in South Africa, a disease caused by *Phyllosticta citricarpa*. *Phyllosticta* pseudothecium maturation and ascospore release models have been integrated in infection models to predict the availability of the primary inoculum source. However, these models have not been validated on a broader data set and this study aimed to validate and improve these epidemiological models. New pseudothecium maturation and ascospore release models for *P. citricarpa* were developed, based on weather and ascospore trap data from 13 locations and up to five seasons. From the 29 data sets analysed, 3775 3-hourly periods with ascospore events were recorded on 1798 days; 90% of these events occurred between 16.0 °C and 32.1 °C (daily T_{min} and T_{max} of 15.4 °C and 33.5 °C, respectively) and 75% occurred above a relative humidity (RH) of 55.9% (daily RH > 47.9%). Rain was recorded during 13.8% of these ascospore events and 20.0% of ascospore days. Using logistic regression, a Gompertz model that best predicted pseudothecium maturation, or the probability of onset of ascospore release, was developed and was markedly more accurate than the previously described models. The model consisted of DDtemp [cumulative degree-days from mid-winter (1 July) calculated as (minimum + maximum daily temperature) / 2 – 10 °C] and DDwet (DDtemp accumulated only on days with >0.1 mm rain or vapour pressure deficit <5 hPa) as variables in the formula: probability of first ascospore event = exp(-exp(-(-3.131 + 0.007 × DDtemp - 0.007 × DDwet))). A Gompertz model [PAT = exp(-2.452 × exp(-0.004 × DDwet2))] was also developed for ascospore release; DDwet2 = DDtemp accumulated, from first seasonal ascospore trap day, only on days with >0.1 mm rain or vapour pressure deficit <5 hPa. Similar to the DDwet2 model described in a previous study, this model adequately predicted the general trend in ascospore release but poorly predicted periods of daily, 3-day and 7-day ascospore peaks.

Significance:

- We developed a new pseudothecium maturation model from 29 data sets, comprising different climatic regions in South Africa, and validated previously published models. The new model was markedly more accurate in predicting the onset of ascospore release and can be used to improve existing CBS epidemiological models and improve risk assessment and management of CBS in South African citrus orchards.

Introduction

Citrus black spot (CBS), caused by *Phyllosticta citricarpa* (McAlpine) van der Aa, is the most important fungal disease of citrus in South Africa, specifically due to the quarantine status of this pathogen in certain fruit export markets. The disease does not affect the internal fruit quality, but rather causes cosmetic lesions that reduce the fruit quality standard.[1,2] Fruit lesions form largely on maturing fruit from latent infections that occurred when fruit was immature.[1-4] The critical period for fruit infection in South Africa and Australia is the first 4–5 months after fruit set, whereafter fruit becomes more tolerant to infection.[1,5,6] In South Africa, Australia and Argentina, protective fungicide sprays are only required during this critical fruit infection period for effective control[3-8], but longer periods of protection are required under the highly CBS conducive conditions in São Paulo, Brazil[9]. Leaves are susceptible to latent infection during the 10 months after unfolding, but rarely show symptoms.[10]

Infection is caused by asexual pycnidiospores and sexual ascospores.[4] Pycnidiospores are produced in pycnidia formed in leaf litter and certain fruit, leaf or twig lesions. Pycnidiospores ooze from pycnidia in a gelatinous mass and are typically washed down, leading to infections occurring relatively short distances (<80 cm) from the source.[1,11-13] However, in regions with frequent storms such as Florida (USA), pycnidiospores have been reported to contribute to the dispersal of CBS across tree rows.[14,15] Ascospores, on the other hand, are formed in pseudothecia from which they are forcibly ejected and are wind-dispersed.[16] Whilst conditions required for germination are similar for both spore types (>12 h wetness at optimal temperature of 25–27 °C), ascospores play a more prominent role in CBS epidemiology in South Africa and Australia.[1,4,17]

Most citrus leaves drop naturally after 2 years on the tree, predominantly at the end of winter and in early spring.[18] *Phyllosticta citricarpa* is heterothallic[19,20] and mating occurs on decomposing leaf litter on the orchard floor to form pseudothecia[21,22]. Alternating wet and dry conditions at mild temperatures (21–28 °C) are required for pseudothecium maturation, whereas long wet periods are detrimental.[1,10,23] Ascospore discharge occurs after the onset of pseudothecium maturity, with ascospore peaks typically occurring during summer months, declining into early autumn.[3,24-26] Rainfall as little as 3 mm triggers ascospore release[3,4], but dew is also considered to trigger ascospore maturity and discharge[27]. Fourie et al.[25] reported ascospore release events of *Phyllosticta* spp. in the absence of a rainfall trigger and noted that other wetness factors, such as relative humidity, dew or irrigation, should be investigated.

Quantification of pseudothecium maturation and availability of *P. citricarpa* ascospores in orchards can be achieved by use of volumetric spore traps. This method can provide accurate measurement of cumulative

ascospore release, but it is labour intensive and time consuming. An important consideration when using ascospore trap data is the fact that *P. citricarpa* ascospores cannot morphologically be distinguished from those of the common endophyte *Phyllosticta capitalensis*.[28-30] *P. citricarpa* appears to prevail over *P. capitalensis* in South African citrus orchards in CBS prevalent areas[30], but further research is required to elucidate the relative prevalence of these species in citrus orchards in different climatic regions. Recently described species of *Phyllosticta*[29] are currently unknown in South African citrus orchards, but their relative proportion will also need to be investigated if they are found to exist.

Effects of environmental factors on pseudothecium maturation have been studied in different pathosystems, including apple scab (*Venturia inequalis*) and pear scab (*Venturia pirina*), as a basis for development of systems to forecast release of ascospores.[31-33] Models that relate pseudothecium maturation and cumulative ascospore release to cumulative degree-days have effectively been in use in many countries for *V. inequalis*.[34] In South Africa, results from *Phyllosticta* ascospore trapping by means of volumetric spore traps are routinely used by certain growers for decision support, to assess risk and improve CBS management. Ascospore trap data and weather data obtained for three areas over three seasons in the Limpopo Province of South Africa were previously used to model the effect of temperature and wetness on pseudothecium maturation and ascospore release.[25] These degree-day models were integrated into infection models used in pest risk assessment for *P. citricarpa*[17,35], as well as a web-based decision-support platform (www.cri-phytrisk.co.za) used by citrus growers in South Africa. The pseudothecium maturation and ascospore release models reported by Fourie et al.[25] were, however, built on a limited data set and needed to be validated using data from different geographical areas. In the present study, therefore, we aimed to validate and/or improve the models described by Fourie et al.[25] by using an extensive data set obtained from a diverse range of climatic regions in South Africa.

Materials and methods

Monitoring of ascospore release and weather parameters

The natural release of ascospores was recorded in 15 localities belonging to three provinces in South Africa: eight localities in Limpopo Province, six localities in the Eastern Cape Province and one locality in Mpumalanga Province. Ascospore release was monitored at 3-hourly intervals by use of volumetric spore traps (Interlock Systems, Pretoria, South Africa) as described by Fourie et al.[25] Monitoring of ascospore release was conducted over five seasons (2012–2016) in five localities in Limpopo (Letsitele A, Letsitele B, Letsitele C, Hoedspruit A and Hoedspruit B), three seasons (2014–2016) for the rest of the localities in Limpopo (Burgersfort, Ohrigstad, Musina A and Musina B), and over two seasons (2015–2016) in the Eastern Cape (Addo A, Addo B Sunland, Kirkwood A, Kirkwood B and Kirkwood C) and Nelspruit (Mpumalanga). Information on citrus type, GPS coordinates and prevalence of CBS at each location is presented in Table 1. In each location, hourly recordings of rainfall (mm), temperature (°C) and relative humidity (%) were provided by weather stations located in close proximity (<1 km) to the spore traps.

To investigate the relationships between the weather variables and the presence of ascospores (i.e. during the 3-hourly periods in which *Phyllosticta* ascospores were trapped), the hourly weather data were transformed into 3-hourly data as total rainfall, average temperature and relative humidity (RH). Thereafter, quantiles were estimated using the empirical distribution function in XLSTAT (version 2019.1.2; www.xlstat.com). Likewise, the data were summarised as daily data [minima, averages and maxima for temperature (T_{min}, T_{avg}, T_{max}) and RH (RH_{min}, RH_{avg}, RH_{max}), total rainfall and total number of ascospores trapped] and quantiles estimated.

Table 1: Information on the study sites including location, cultivar planted and prevalence of citrus black spot (CBS)

Location	Prevalence of CBS[a]	Cultivar planted	GPS coordinates
Limpopo Province			
Letsitele A	Bsh: arid, steppe, hot arid; high CBS prevalence	Midknight oranges	23°39'17.4"S, 30°38'22.0"E
Letsitele B	Bsh: arid, steppe, hot arid; high CBS prevalence	Delta Valencia oranges	23°52'07.9"S, 30°22'50.4"E
Letsitele C	Bsh: arid, steppe, hot arid; high CBS prevalence	Delta Valencia oranges	23°48'39.8"S, 30°26'38.5"E
Hoedspruit A	Bsh: arid, steppe, hot arid; high CBS prevalence		24°22'00.7"S, 30°44'02.8"E
Hoedspruit B	Bsh: arid, steppe, hot arid; high CBS prevalence	Valencia oranges	24°26'25.9"S, 30°49'10.4"E
Burgersfort	Bsh; high CBS prevalence	Nadorcott mandarins	24°50'33.6"S, 30°44'02.8"E
Ohrigstad	Bsh; high CBS prevalence	Unknown	24°39'08.0"S, 30°37'54.4"E
Musina A	Bwh: arid, desert, hot arid; low CBS prevalence	Delta Valencia oranges	22°38'12.1"S, 30°08'07.3"E
Musina B	Bwh: arid, desert, hot arid; low CBS prevalence	Unknown	22°09'42.6"S, 29°35'28.0"E
Mpumalanga Province			
Nelspruit	Cwa: warm, temperate, winter dry, hot summer; high CBS prevalence		25°25'32.1"S, 31°06'30.7"E
Eastern Cape Province			
Addo A	Bsh; moderate CBS prevalence	Eureka lemons	33°37'14.5"S, 25°41'38.7"E
Addo B	Bsh; moderate CBS prevalence	Eureka lemons	33°26'21.0"S, 25°42'29.4"E
Sunland	Bsh; moderate CBS prevalence	Eureka lemons	33°30'40.7"S, 25°39'20.8"E
Kirkwood A	Bsh; moderate CBS prevalence	Limoneira lemons	33°25'46.8"S, 25°26'56.9"E
Kirkwood B	Bsh; moderate CBS prevalence	Eureka lemons	33°25'14.5"S, 25°22'39.0"E
Kirkwood C	Bsh; moderate CBS prevalence	Eureka lemons	33°27'50.3"S, 25°34'01.9"E

[a]*Köppen-Geiger climate classification (http://stepsa.org/climate_koppen_geiger.html)*

Prediction of pseudothecium maturity and onset of ascospore release

Degree-day accumulation was used to determine the influence of weather variables (temperature, rainfall and relative humidity) on pseudothecium maturity and the onset of seasonal ascospore discharge. Onset of seasonal ascospore discharge was regarded as the date of the first meaningful discharge of *Phyllosticta* ascospores (>5 ascospores trapped per day). Cumulative degree-days were computed from daily weather data beginning on 1 July (biofix) as DDtemp = (T_{min} + T_{max}) / 2 - base temp, with a base temperature of 10 °C.[25] Degree-day accumulation was also calculated for rainy [DDrain = DDtemp accumulation only on days with measurable rainfall (>0.1 mm)], humid [DDvpd = DDtemp accumulation only on days with vapour pressure deficit (VPD) <5 hPa], as well as for rainy or humid days [DDwet = DDtemp accumulation only on days with measurable rainfall (>0.1 mm) or VPD <5 hPa].[25] Daily VPD was calculated as $(1 - RH_{avg}/100) \times 6.11 \times \exp[(17.47 \times T_{avg})/(239 + T_{avg})]$.[25,33]

Similar to Rossi et al.[33] and Fourie et al.[25], logistic regression analysis was performed on a subset of data for rainy or humid days (rainfall >3 mm or VPD <5 hPa) from 1 July to first meaningful ascospore discharge to model degree-day variables most predictive of onset of ascospore dispersal. The values 0 and 1 were used as dependent variables for when no ascospores were trapped, and when ascospores were trapped on that day, respectively. Independent variables were DDtemp, DDrain, DDvpd and DDwet. Best model was selected based on the coefficient of determination, adjusted following Nagelkerke, and root-mean-square error (RMSE). Model building was performed using data (594 cases in total) from the following locations and seasons: Letsitele C (2014 and 2015); Letsitele A, Letsitele B and Hoedspruit A (2012, 2014, 2015, 2016); Hoedspruit B (2012–2016); Ohrigstad and Musina B (2015 and 2016); Nelspruit (2015); Musina A, Addo A, Kirkwood C, Kirkwood A, Kirkwood B (2016). Data in Fourie et al.[25] were used for model evaluation (117 cases in total). Due to missing weather data and/or ascospore trapping data, data sets from the following areas and seasons were not considered in this analysis: Letsitele C (2012, 2013 and 2016); Letsitele A, Letsitele B and Hoedspruit A (2013); Musina A (2014 and 2015); Musina B and Ohrigstad (2014); Nelspruit (2016); Addo B and Sunland (2015 and 2016); Addo A, Kirkwood B, Kirkwood A and Kirkwood C (2015). The accuracy of the predictive model in distinguishing between true and false first ascospore events was determined by a receiver operating characteristic curve, which plots model sensitivity against specificity.

Modelling of ascospore release

Modelling of ascospore release was performed as described by Rossi et al.[33] and Fourie et al.[25] The relative ascospore dose was expressed as the daily proportion of ascospores trapped (PAT) and cumulated on a 0–1 scale.[33,36] The non-linear regression procedure in XLSTAT using a Gompertz function was then used to model PAT against DDtemp2, DDrain2, DDvpd2, or DDwet2 data, which were calculated as described for DDtemp, DDrain, DDvpd, and DDwet but using the first seasonal ascospore trap day as biofix.[25] Non-linear regression was conducted for the complete data set (data of all locations combined) with the various parameters. The best model (generic model) was selected using the coefficient of determination and RMSE. The generic model was compared with the respective data set specific models (site-specific models), as well as the ascospore release model proposed by Fourie et al.[25] The site-specific models were built by modelling PAT of each site against DDtemp2, DDrain2, DDvpd2, or DDwet2 data using non-linear regression. Following Fourie et al.[25], Pearson's correlation analyses of predicted and measured PAT were conducted to compare model performance. Additionally, daily, 3-day and 7-day ascospore peaks (accumulation in PAT) were correlated with predicted ascospore peaks for all data sets using Pearson's correlation analyses.

Results

Monitoring of ascospore release and weather parameters

Onset of ascospore release was generally earlier in the Northern parts of the country (Limpopo and Mpumalanga) in comparison to the Eastern Cape Province. The earliest ascospore release was recorded 62 and 83 days after 1 July in Limpopo and Mpumalanga, respectively, in comparison to 115 days in the Eastern Cape. The onset of release of *Phyllosticta* ascospores occurred as early as 1 September at Letsitele B during the 2016/2017 season and as late as 10 November at Kirkwood C during the 2016/2017 season (Table 2). DDtemp accumulated from 1 July until the first day of ascospore release ranged between 362.30 (Ohrigstad in 2015/2016 season) and 895.60 (Kirkwood C in 2016/2017) (Table 2), with a mean of 638.96. There were many days with measurable rain before first ascospore release in the Eastern Cape (ranged from 31 to 54) in comparison to 0 to 19 for the Northern areas (Table 2).

Ascospores were trapped throughout the day and night in this study. Greater numbers were captured between 9:00 and 15:00, but not at significantly higher levels (results not shown). Ascospore release was observed from September through to March, but large differences were observed in the number of ascospores trapped between localities and seasons (Table 3). Markedly higher numbers of ascospores were recorded in Hoedspruit A, particularly during the 2014/2015 season. Hoedspruit B had the second highest number of ascospores trapped, while the lowest number of ascospores was recorded in Ohrigstad, followed by Musina A during the 2016/2017 season. More ascospore events were recorded in Hoedspruit B than in Hoedspruit A.

From the 29 data sets analysed, a total of 3775 3-hourly periods with ascospore events were recorded; these were analysed separately for the 13 different locations before averages of the weather variables were calculated. The average median number of ascospores trapped per 3-h event was 510.0 spores/m^3, up to a 95th percentile of 3769.6 spores/m^3 and an average maximum of 36 997.2 spores/m^3 (Table 4). The average first and fifth percentiles for temperature at which ascospores were trapped were 14.0 °C and 16.0 °C, respectively. The average first and fifth percentiles for RH at which ascospores were trapped were 20.7% and 34.0%, and 25th percentile 55.9% (Table 4). Rainfall was sporadically (13.8%) measured during the 3-hourly ascospore release events.

Ascospore events were recorded on 1798 days. The average median for number of ascospores trapped per day was 875.9 spores/m^3, and the average maximum was 57 352.8 spores/m^3 (Table 5). Daily minimum temperature and relative humidity values recorded during ascospore days were lower than those observed for 3-hourly intervals (Table 4). The average first and fifth percentiles for T_{min} on days when ascospores were trapped were 13.7 °C and 15.4 °C, respectively. The 25th percentile values recorded on ascospore days for RH_{min}, RH_{avg} and RH_{max} were 47.9%, 58.5% and 64.1%, respectively (Table 5). Median values for daily T_{min}, T_{avg} and T_{max} were 20.6, 22.1 and 23.3 °C, respectively. Rainfall was measured on 359 days (20% of cases), and in most cases was <5 mm/day (the 95th percentile was 4.8 mm) (Table 5).

Prediction of pseudothecium maturation and onset of ascospore release

The logistic regression model that best predicted the probability of onset of ascospore release had an R^2 (Nagelkerke) value of 0.699 and consisted of DDwet and DDtemp as variables in the formula: probability of first ascospore event = exp(-exp(-(-3.131 + 0.007 × DDtemp - 0.007 × DDwet))). Using a probability of 0.5 to predict onset of ascospore release, this model (herein referred to as the DDwet pseudothecium maturation model) gave a true positive proportion of predicted first ascospore events (sensitivity) of 0.55, i.e. the model accurately predicted 21 of 38 actual first ascospore release events (Table 6). The model displayed a very high true negative proportion (specificity) of 0.98 as it predicted 544 of the 556 events without ascospore release. A sensitivity value of 0.95 (36 of the 38 actual ascospore discharges were accurately predicted) and specificity value of 0.81 (correctly predicting 64 of 79 events without ascospore release) were achieved by the model in the validation data set (Table 6). The area under the receiver operating characteristic curve was 0.975 (results not shown).

Table 2: Dates of first trapping of *Phyllosticta* ascospores at 13 locations in South Africa between 2012 and 2016, DDtemp accumulated until first ascospore trapping as well as amount of rain on first day of ascospore trapping and period from 1 July to first trapping

Location	Date of first trapping of ascospores	Rain (mm) on first ascospore trapping day	1 July to first ascospore trapping			
			DDtemp accumulated	Total rain (mm)	Number of days with measurable rain (≥0.1 mm)	Number of days with ≥3 mm
Limpopo Province						
Letsitele A	2012-09-05	4.40	514.20	4.40	1	1
Letsitele A	2014-09-16	0.00	635.50	1.00	3	0
Letsitele A	2015-09-24	0.10	650.70	14.20	10	2
Letsitele A	2016-09-24	0.00	655.75	1.60	2	0
Letsitele B	2012-09-05	9.00	521.75	9.00	1	1
Letsitele B	2014-09-05	0.00	458.05	1.40	2	0
Letsitele B	2015-09-07	0.00	529.70	34.60	4	2
Letsitele B	2016-09-01	0.00	466.05	13.80	4	2
Letsitele C	2014-09-15	0.00	562.75	3.20	3	0
Letsitele C	2015-09-19	1.00	612.35	32.40	4	2
Hoedspruit A	2012-09-03	0.00	503.15	0.00	0	0
Hoedspruit A	2014-09-02	0.00	484.95	0.00	0	0
Hoedspruit A	2015-09-03	0.00	569.45	0.20	1	0
Hoedspruit A	2016-09-21	0.00	738.75	17.80	5	2
Hoedspruit B	2012-09-15	0.00	659.30	40.20	7	2
Hoedspruit B	2013-09-24	0.00	773.95	24.80	5	2
Hoedspruit B	2014-10-01	0.00	853.40	6.60	3	1
Hoedspruit B	2015-09-06	0.00	629.20	21.40	12	1
Hoedspruit B	2016-09-21	0.00	800.50	20.80	5	2
Musina A	2016-09-24	0.00	775.80	1.10	3	0
Musina B	2015-09-04	9.20	540.20	9.20	1	1
Musina B	2016-09-30	0.00	839.95	1.40	2	0
Ohrigstad	2015-09-12	0.00	362.30	22.80	4	1
Ohrigstad	2016-10-11	0.00	590.70	20.00	10	1
Mpumalanga Province						
Nelspruit	2015-09-22	0.00	622.20	16.00	19	1
Eastern Cape Province						
Addo A	2016-10-24	0.40	713.55	85.00	39	11
Kirkwood A	2016-11-04	0.00	804.80	72.00	31	9
Kirkwood B	2016-11-02	0.20	765.40	130.40	54	7
Kirkwood C	2016-11-10	4.20	895.60	128.60	49	9

Table 3: Maximum cumulative DDwet2 values, cumulative ascospore trap numbers (spores/m^3) and final proportion of ascospores trapped (PAT) values predicted by the site-specific and generic DDwet ascospore release models, as well as a published DDwet model[23], for different locations and seasons. Correlation coefficients obtained between 1-day, sum of rolling 3-day (each particular day plus previous 2 days accumulation in PAT) and 7-day (each particular day plus previous 6 days accumulation in PAT) actual PAT and that predicted by site-specific and generic DDwet ascospore release models, as well as a published DDwet model[23] are also shown.

Location (season)			PAT[a]										Final predicted PAT[a] values reached		
	Max. DDwet2[b]	Cumulative ascospores trapped[c]	DDwet site-specific models[d]						DDwet ascospore release model[e]		Published DDwet model[f]		Site-specific model[d]	DDwet spore release model[e]	Published model[f]
			R^2[g]	a	b	Peak prediction[h]			Peak prediction[h]		Peak prediction[h]				
						1 d	3 d	7 d	3 d	7 d	3 d	7 d			
Limpopo Province															
Letsitele A (2012/2013)	799.6	41 525	0.96	3.568	0.005	0.29	0.43	0.45	0.34	0.34	0.38	0.38	0.955	0.901	0.928
Letsitele A (2014/2015)	371.8	53 184	0.85	7.258	0.010	0.11	0.12	0.10	0.07	-0.03	0.10	0.05	0.811	0.569	0.528
Letsitele A (2015/2016)	454.2	32 285	0.97	77.913	0.027	0.27	0.48	0.62	0.15	0.18	0.14	0.19	1.000	0.666	0.655
Letsitele A (2016/2017)	1273.7	22 340	0.93	3.174	0.002	0.05	-0.01	0.04	0.01	0.05	0.01	0.08	0.826	0.984	0.993
Letsitele B (2012/2013)	1179.2	83 740	0.99	2.909	0.004	0.32	0.38	0.50	0.34	0.47	0.40	0.53	0.964	0.977	0.989
Letsitele B (2014/2015)	811.8	57 940	0.96	1.875	0.006	0.26	0.33	0.37	0.21	0.19	0.14	0.10	0.990	0.906	0.932
Letsitele B (2015/2016)	833.8	116 746	0.99	5.288	0.007	0.12	0.43	0.67	0.28	0.47	0.39	0.61	0.987	0.913	0.939
Letsitele B (2016/2017)	1384.9	30 123	0.98	3.186	0.003	0.26	0.31	0.39	0.28	0.34	0.32	0.40	0.934	0.990	0.996
Letsitele C (2014/2015)	849.3	149 463	0.91	2.225	0.004	0.18	0.22	0.26	0.26	0.30	0.22	0.23	0.895	0.918	0.943
Letsitele C (2015/2016)	752.9	57 076	0.99	2.959	0.006	0.30	0.32	0.57	0.20	0.34	0.26	0.38	0.975	0.883	0.909
Hoedspruit A (2012/2013)	1174.5	548 128	0.98	2.267	0.007	0.31	0.41	0.50	0.33	0.39	0.25	0.34	0.999	0.977	0.989
Hoedspruit A (2014/2015)	1186.7	5 386 875	0.95	4.451	0.003	0.10	0.15	0.22	0.02	0.04	0.10	0.15	0.928	0.978	0.989
Hoedspruit A (2015/2016)	1089.2	510 078	0.97	5.066	0.004	0.19	0.34	0.55	0.10	0.22	0.15	0.27	0.935	0.968	0.982
Hoedspruit A (2016/2017)	1644.6	297 053	0.99	4.369	0.003	0.23	0.41	0.65	0.24	0.39	0.32	0.51	0.973	0.996	1.000
Hoedspruit B (2012/2013)	825.4	649 740	0.98	4.272	0.005	0.30	0.37	0.46	0.27	0.36	0.38	0.48	0.910	0.911	0.936
Hoedspruit B (2013/2014)	1065.6	285 955	0.99	4.074	0.005	0.40	0.51	0.64	0.37	0.51	0.52	0.65	0.974	0.964	0.980
Hoedspruit B (2014/2015)	652.3	605 348	0.98	4.280	0.006	0.21	0.30	0.33	0.17	0.15	0.27	0.31	0.929	0.830	0.855
Hoedspruit B (2015/2016)	863.6	235 653	0.95	3.620	0.004	0.18	0.21	0.30	0.18	0.21	0.20	0.26	0.865	0.923	0.941
Hoedspruit B (2016/2017)	844.5	134 906	0.98	5.619	0.005	0.25	0.28	0.35	0.10	0.03	0.19	0.20	0.921	0.917	0.942
Ohrigstad (2015/2016)	893.0	22 196	0.97	11.393	0.006	0.26	0.44	0.50	0.23	0.22	0.32	0.33	0.960	0.931	0.954
Ohrigstad (2016/2017)	981.5	6053	0.97	3.409	0.004	0.18	0.26	0.33	0.26	0.31	0.26	0.33	0.959	0.951	0.970
Musina A (2016/2017)	966.7	6630	0.94	3.152	0.004	0.03	0.01	0.19	0.07	0.23	0.00	0.21	0.928	0.948	0.968
Musina B (2015/2016)	627.4	53 184	0.95	1.944	0.016	0.06	0.06	0.28	-0.03	-0.06	-0.07	-0.13	1.000	0.815	0.837
Musina B (2016/2017)	922.8	10 377	0.90	2.756	0.003	0.12	0.20	0.24	0.23	0.26	0.22	0.28	0.853	0.938	0.960
Mpumalanga Province															
Nelspruit (2015/2016)	1166.5	116 602	0.97	3.759	0.003	0.38	0.47	0.56	0.35	0.42	0.44	0.51	0.929	0.976	0.988
Eastern Cape Province															
Addo A (2016/2017)	761.7	38 627	0.96	3.615	0.005	0.21	0.14	0.16	0.16	0.12	0.15	0.18	0.939	0.886	0.913
Kirkwood A (2016/2017)	742.4	46 698	0.82	1.505	0.005	0.33	0.25	0.14	0.15	-0.01	-0.03	-0.15	0.962	0.878	0.905
Kirkwood B (2016/2017)	768.5	14 990	0.98	4.458	0.006	0.37	0.59	0.78	0.51	0.70	0.61	0.81	0.966	0.889	0.916
Kirkwood C (2016/2017)	859.4	36 033	0.95	2.189	0.004	0.45	0.48	0.52	0.42	0.46	0.10	0.18	0.935	0.921	0.946

[a]PAT (proportion of seasonal ascospores trapped, on a 0 to 1 scale) was calculated from DDwet2 values, which were calculated as degree-days (using 10 °C as base temperature) from first seasonal ascospore release only on days with vapour pressure deficit <5 hPa or measurable rainfall (>0.1 mm) using DDwet ascospore release models [PAT= exp[-a × exp(-b × DDwet2).

[b]Maximum DDwet2 values reached.

[c]Cumulative ascospores trapped per cubic metre of air (spores/m^3).

[d]End values of PAT predicted by the site-specific DDwet ascospore release models [PAT= exp[-a × exp(-b × DDwet2)]

[e]End values of PAT predicted by the generic DDwet ascospore release model [PAT = exp(-2.452 × exp(-0.004 × DDwet2))].

[f]End values of PAT predicted by the published model [PAT = exp(-4.096 × exp(-0.005 × DDwet2))].[23]

[g]R^2 is the coefficient of determination adjusted following Nagelkerke.

[h]Peak prediction = Pearson's correlation between actual and predicted daily ascospore (PAT) peaks or 3- and 7-day peaks.

Table 4: Means and coefficients of variation (%) of quantiles estimated for temperature, relative humidity, rainfall and ascospore numbers measured during the 3775 3-hourly *Phyllosticta* ascospore release events recorded at 13 localities over one to five seasons

Variable	Minimum	1%	5%	10%	25%	50%	75%	95%	Maximum
Temperature (°C)	13.2 (2.5)	14.0 (2.0)	16.0 (1.4)	17.2 (1.4)	19.3 (1.4)	21.9 (1.4)	25.4 (1.6)	32.1 (2.0)	37.6 (1.8)
Relative humidity (%)	17.1 (5.9)	20.7 (5.3)	34.0 (7.1)	41.8 (6.7)	55.9 (7.7)	73.3 (7.0)	86.5 (7.8)	95.1 (5.7)	98.2 (2.8)
Rain (mm)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.02 (0.1)	2.5 (2.0)	21.3 (20.3)
Spores /m^3	144.1 (0.0)	155.2 (40.0)	177.4 (63.2)	243.9 (108.3)	354.8 (162.4)	510.0 (260.6)	964.6 (879.4)	3769.6 (6410.7)	36 997.2 (110 015.5)

Table 5: Means and coefficients of variation (%) of quantiles estimated for daily temperature (T), relative humidity (RH), rainfall and ascospore numbers measured on the 1798 days during which *Phyllosticta* ascospore release events were recorded at 13 localities over one to five seasons

Variable	Minimum	1%	5%	10%	25%	50%	75%	95%	Maximum
T_{min} (°C)	13.2 (2.5)	13.7 (2.1)	15.4 (1.7)	16.4 (1.7)	18.2 (1.5)	20.6 (1.8)	23.3 (20.)	29.3 (4.2)	35.0 (4.4)
T_{avg} (°C)	13.8 (2.5)	14.2 (2.1)	16.1 (1.7)	17.1 (1.7)	19.6 (1.4)	22.1 (1.6)	25.0 (1.9)	30.3 (2.9)	35.5 (3.2)
T_{max} (°C)	14.0 (2.3)	14.5 (2.1)	16.2 (1.8)	17.4 (1.7)	20.2 (1.6)	23.3 (1.7)	27.2 (1.7)	33.5 (2.1)	37.6 (1.8)
RH_{min} (%)	17.1 (5.9)	19.5 (4.8)	27.6 (6.2)	36.2 (6.0)	47.9 (5.7)	66.2 (6.2)	80.5 (7.7)	93.8 (5.1)	98.0 (2.7)
RH_{avg} (%)	22.5 (10.9)	24.2 (10.4)	36.4 (9.0)	45.5 (9.9)	58.5 (8.4)	71.9 (6.5)	83.9 (7.1)	94.1 (5.1)	98.0 (2.7)
RH_{max} (%)	23.7 (12.7)	25.5 (12.5)	39.3 (10.9)	48.6 (12.6)	64.1 (11.3)	80.6 (9.1)	90.3 (7.6)	96.0 (4.9)	98.2 (2.8)
Rain (mm)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.2)	4.8 (3.2)	29.4 (35.2)
Spores/m^3	166.3 (79.9)	177.4 (86.4)	232.8 (110.7)	310.4 (142.3)	421.3 (245.9)	875.9 (686.7)	2428.0 (3237.0)	9085.8 (17 424.7)	57 352.8 (169 540.9)

Table 6: Prediction of first seasonal release of *Phyllosticta* ascospores by the DDwet pseudothecium maturation model [probability of first ascospore event = exp(-exp(-(-3.131 + 0.007 × DDtemp - 0.007 × DDwet)))] in different citrus growing locations

Ascospore release observed	Ascospore release predicted at $p=0.5$		Total observations	Youden's index[a]	R^2 (Nagelkerke)
	No	Yes			
Model building data set					
No	544 (0.98)[b]	12 (0.02)[c]	556	0.53	0.669
Yes	17 (0.45)[d]	21 (0.55)[e]	38		
Total observations	561	33	594		
Model validation data set					
No	64 (0.81)[b]	15 (0.19)[c]	79	0.76	
Yes	2 (0.05)[d]	36 (0.95)[e]	38		
Total observations	66	51	117		

[a]*True positive proportion of predicted first ascospore event + true positive proportion of predicted first ascospore event - 1*

[b]*True negative proportion of predicted first ascospore event (model specificity)*

[c]*False positive proportion of predicted first ascospore event*

[d]*False negative proportion of predicted first ascospore event*

[e]*True positive proportion of predicted first ascospore event (model sensitivity)*

When compared with the temperature and temperature/moisture pseudothecium maturation models, described by Fourie et al.[25], in predicting the actual pseudothecium maturation date (i.e. first meaningful ascospore release date per season) using a probability of 0.5, the DDwet pseudothecium maturation model was generally more accurate. It accurately (within 14 days) predicted 19 of 29 actual ascospore release events, across all locations and years tested; on average across data sets, the DDwet pseudothecium maturation model predicted onset of ascospore release 1 day later than the actual. In cases in which the model was not very accurate, differences of up to 27 days occurred between the predicted and observed times of onset of pseudothecium maturity (Table 7). On the other hand, the temperature and temperature/moisture models[25] predicted 18 and 16 of the 29 actual ascospore release events, respectively; however, these models' predictions were on average, respectively, 10 and 16 days later than the actual (Table 7).

Modelling of ascospore release

The use of Gompertz equations in the non-linear regression analysis of PAT against DDrain2, DDwet2, DDvpd2 or DDtemp2 in the complete data set, revealed DDwet2 as the most suitable predictor of seasonal *Phyllosticta* ascospore release trends. Although the highest R^2 value of 0.820 (RSME = 0.148) was achieved in the non-linear regression analysis of PAT against DDtemp2, the model poorly predicted periods of ascospore release or their absence, due to the consistent increase in DDtemp2 (results not shown). PAT was poorly predicted from DDvpd2 (R^2 = 0.420; RMSE = 0.271). The DDrain2 (R^2 = 0.716; RMSE = 0.186) and DDwet2 (R^2 = 0.746; RMSE = 0.176) models, on the other hand, adequately predicted the general trend in ascospore release, with events predicted when DDrain2 or DDwet2 increased. The DDwet ascospore release model using DDwet2 as an explanatory variable was chosen as the best model based on its higher R^2 value and lower RMSE and also because it supports observations made during ascospore trapping, i.e. rain was not always a prerequisite for ascospore release: PAT = exp(-2.452 (standard error 0.0372) × exp(-0.004 (standard error 0.0005) × DDwet2)).

Table 7: Comparison of actual and predicted dates of first release of *Phyllosticta* ascospores as predicted by the DDwet pseudothecium maturation model, as well as the temperature and temperature/moisture pseudothecium models proposed by Fourie et al.[25], in different South African citrus growing regions between 2012 and 2016 seasons

Location	Date of actual ascospore release	Predicted first seasonal ascospore release at probability 0.5								
		DDwet pseudothecium maturation model[a]			Temperature model[b]			Temperature/moisture model[c]		
		Date[d]	Days[e]	PAT[f]	Date[d]	Days[e]	PAT[f]	Date[d]	Days[e]	PAT[f]
Limpopo Province										
Letsitele A	2012-09-05	2012-09-17	12	0.035	2012-09-28	23	0.042	2012-09-24	19	0.042
Letsitele A	2014-09-16	2014-09-14	-2	0.000	2014-09-27	11	0.022	2014-09-20	4	0.022
Letsitele A	2015-09-24	2015-09-28	4	0.004	2015-10-02	8	0.004	2015-09-23	-1	0.000
Letsitele A	2016-09-24	2016-09-24	0	0.032	2016-10-04	10	0.032	2016-09-24	0	0.032
Letsitele B	2012-09-05	2012-08-26	-10	0.000	2012-09-28	23	0.117	2012-09-13	8	0.022
Letsitele B	2014-09-05	2014-09-12	7	0.027	2014-10-04	29	0.119	2014-10-06	31	0.119
Letsitele B	2015-09-07	2015-09-14	7	0.022	2015-09-28	21	0.053	2015-09-29	22	0.053
Letsitele B	2016-09-01	2016-09-12	11	0.019	2016-09-27	26	0.029	2016-10-06	35	0.053
Letsitele C	2014-09-15	2014-09-14	-1	0.000	2014-10-04	19	0.015	2014-10-07	22	0.021
Letsitele C	2015-09-19	2015-09-21	2	0.003	2015-10-01	12	0.030	2015-09-24	5	0.013
Hoedspruit A	2012-09-03	2012-09-27	24	0.322	2012-09-29	26	0.334	2012-09-11	8	0.059
Hoedspruit A	2014-09-02	2014-09-17	15	0.003	2014-09-26	24	0.005	2014-09-10	8	0.002
Hoedspruit A	2015-09-03	2015-09-27	24	0.079	2015-09-22	19	0.068	2015-08-26	-8	0.000
Hoedspruit A	2016-09-21	2016-09-15	-6	0.000	2016-09-23	2	0.011	2016-09-10	-11	0.000
Hoedspruit B	2012-09-15	2012-09-02	-13	0.000	2012-09-24	9	0.013	2012-10-02	17	0.013
Hoedspruit B	2013-09-24	2013-09-08	-16	0.000	2013-09-23	-1	0.000	2013-09-27	3	0.001
Hoedspruit B	2014-10-01	2014-09-04	-27	0.000	2014-09-24	-7	0.000	2014-10-01	0	0.002
Hoedspruit B	2015-09-06	2015-09-07	1	0.018	2015-09-19	13	0.029	2015-10-04	28	0.057
Hoedspruit B	2016-09-21	2016-09-01	-20	0.000	2016-09-18	-3	0.000	2016-09-24	3	0.004
Musina A	2016-09-24	2016-09-16	-8	0.000	2016-09-23	-1	0.000	2016-10-07	13	0.109
Musina B	2015-09-04	2015-08-31	-4	0.000	2015-09-24	20	0.187	2015-09-28	24	0.249
Musina B	2016-09-30	2016-09-08	-22	0.000	2016-09-25	-5	0.000	2016-09-30	0	0.056
Ohrigstad	2015-09-12	2015-10-08	26	0.006	2015-09-18	6	0.006	2015-11-04	53	0.117
Ohrigstad	2016-10-11	2016-10-11	0	0.048	2016-10-26	15	0.048	2017-01-15	96	0.619
Mpumalanga Province										
Nelspruit	2015-09-22	2015-10-03	11	0.007	2015-10-03	11	0.007	2015-10-12	20	0.007
Eastern Cape Province										
Addo A	2016-10-24	2016-11-14	21	0.134	2016-10-29	5	0.034	2016-11-03	10	0.034
Kirkwood A	2016-11-04	2016-11-01	-3	0.000	2016-11-01	-3	0.000	2016-11-04	0	0.083
Kirkwood B	2016-11-02	2016-11-19	17	0.106	2016-11-02	0	0.038	2016-12-14	42	0.144
Kirkwood C	2016-11-10	2016-11-01	-9	0.000	2016-10-28	-13	0.000	2016-12-02	22	0.232

[a]Probability of ascospore event = exp(-exp(-(-3.131 + 0.007 × DDtemp - 0.007 × DDwet))), where DDtemp = accumulated degree-days (°C) using 1 July as biofix and 10 °C as base temperature, and DDwet = DDtemp accumulation only on days with measurable rainfall [> 0.1 mm] or vapour pressure deficit (VPD) < 5 hPa.

[b]Probability of ascospore event = exp(-exp(-(-2.725 + 0.004 × DDtemp))), where DDtemp = accumulated degree-days (°C) using 1 July as biofix and 10 °C as base temperature.

[c]Probability of ascospore event = exp(-exp(-(-3.238 + 0.008 × DDvpd + 0.004 × DDtemp - 0.009 × DDrain))), where DDvpd = DDtemp accumulation only on days with VPD < 5 hPa and DDrain = DDtemp accumulation only on days with measurable rainfall (> 0.1 mm).

[d]Predicted date of first release of ascospores at probability of 0.5.

[e]Difference in days between actual and predicted date of first ascospore release at probability of 0.5.

[f]Proportion of ascospores trapped (PAT) measured at the predicted dates of first ascospore release.

Non-linear regression of PAT against DDwet2 for each site and year resulted in good fits with coefficients of determination ranging from 0.821 to 0.993. The end values of PAT predicted by the site-specific models ranged from 0.811 to 1.000, and generally were >0.815 for the generic and published models; however, in two cases, the predicted final PAT values were as low as 0.569 and 0.528 (Letsitele A in 2014/2015 season) and 0.666 and 0.655 (Table 3). In both these cases, the PAT was predicted from markedly lower DDwet2 values (final DDwet2 values of 371.8 and 454.2), compared with the other data sets (627.4–1644.6). Final DDwet2 values did not correlate with cumulative ascospore counts, even when comparing per location across seasons.

The newly described generic DDwet ascospore release model behaved similarly in predicting PAT to the DDwet model described by Fourie et al.[25], as can be observed in Figure 1 (a–c), which displays the onset of ascospore release as predicted by the DDwet pseudothecium maturation model, observed seasonal ascospore data, daily rainfall and PAT predicted by both the generic and site-specific DDwet ascospore release models, as well as the published DDwet model[25]. Lag phases following onset of ascospore release until PAT began to increase to more than 0.1 ranged from 0 to 6 weeks. Onset of ascospore release was generally predicted during these lag phases by the DDwet pseudothecium maturation model (e.g. Figure 1a, b), and in some cases not (Figure 1c). At a probability of 0.5, the DDwet pseudothecium maturation model predicted onset of ascospore release when actual PAT was less than 0.1 in all cases, except for Addo A, Kirkwood B and Hoedspruit A (2012/2013 season) (Table 7, Figure 1). The trends of the lag phases and subsequent exponential increase in ascospore release were in most cases accurately predicted by the site-specific and generic DDwet ascospore release models, as well as the published model (Figure 1). The three DDwet ascospore release models followed the trend of measured ascospore release fairly accurately, but generally predicted ascospore peaks poorly. In all cases, the models correctly predicted ascospore peaks during certain days, missed ascospore peaks on others and also predicted false peaks (Figure 1). Graphs of the results from the remaining locations and/or seasons are not shown.

The models predicted trends in seasonal ascospore dispersal accurately: Pearson correlations between actual and predicted daily PAT ranged from 0.906 to 0.996 for site-specific models, whereas those for the generic DDwet ascospore release model and the model described by Fourie et al.[25] ranged from 0.829 to 0.995 and 0.789 to 0.995, respectively. Prediction of the actual daily ascospore peaks by the site-specific models was poor (0.018–0.448) (Table 3), and daily peak predictions were even poorer for the DDwet ascospore release model and the model described by Fourie et al.[25] (results not shown). The sum of rolling 3-day (each particular day plus previous 2 days accumulation in PAT) and 7-day ascospore peaks were also correlated with these ascospore peaks predicted by the models. This slightly improved the outcome of the correlations for some locations but correlation coefficients were poor in most cases, ranging from -0.007 to 0.594 and 0.039 to 0.784 for 3- and 7-day peaks for the site-specific models, respectively, and even poorer for the other models (Table 3).

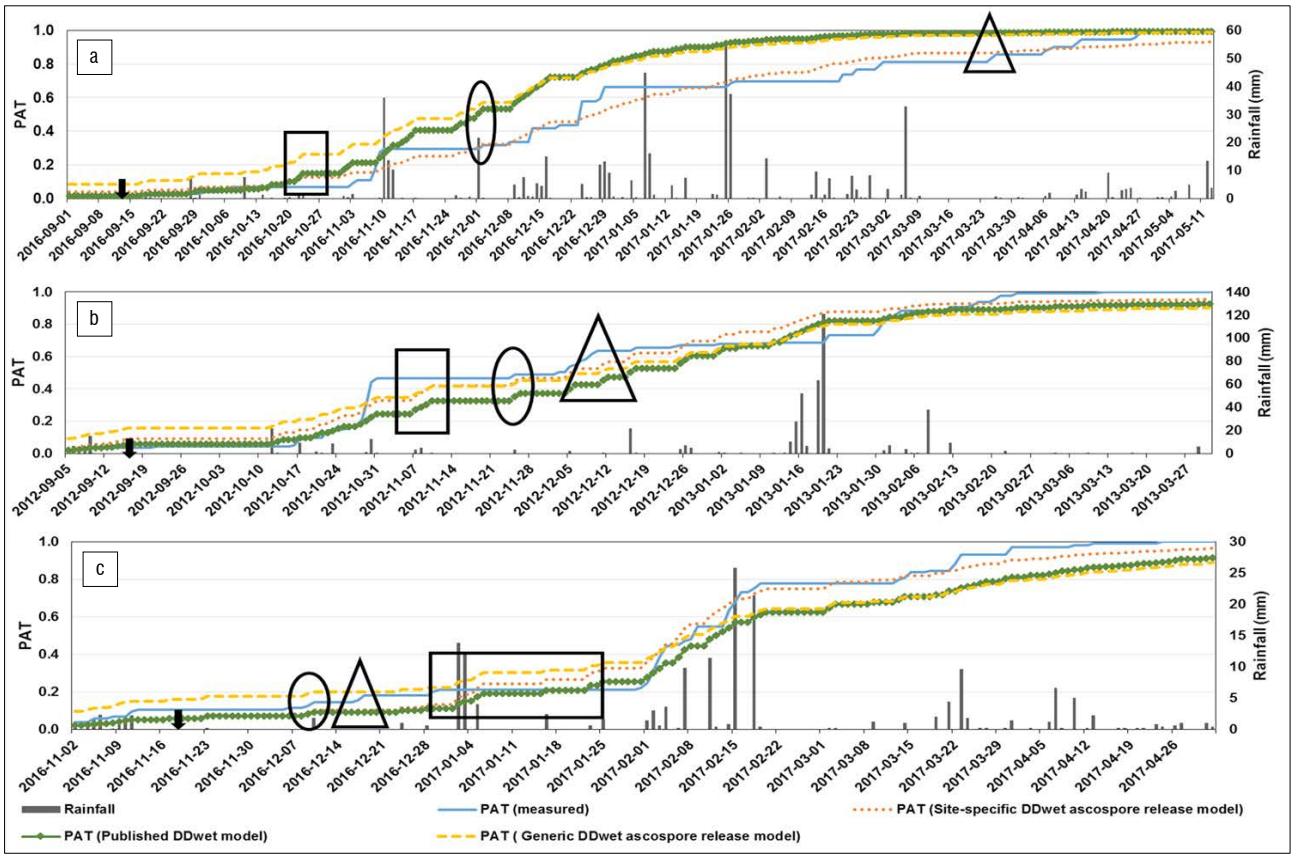

Figure 1: Observed cumulative proportion of airborne *Phyllosticta* ascospores trapped (measured PAT), the onset of ascospore release as predicted by the DDwet pseudothecium maturation model (black arrow) [P = exp(-exp(-(-3.131 + 0.007 × DDtemp - 0.007 × DDwet))), at p = 0.5], PAT predicted using the generic DDwet ascospore release model [PAT (generic DDwet model) = exp(-2.452 × exp(-0.004 × DDwet2))], a published model [PAT (published model) = exp(-4.096 × exp(-0.005 × DDwet2))][25], as well as the DDwet ascospore release model specific to: (a) Letsitele B during 2016/2017 [PAT (site-specific DDwet model) = exp(-3.186 × exp(-0.003 × DDwet2))]; (b) Letsitele A during 2012/2013 [PAT = exp(-3.568 × exp(-0.005 × DDwet2))] and (c) Kirkwood B during 2016/2017 seasons [PAT = exp(-4.458 × exp(-0.006 × DDwet2))]. DDtemp = accumulated degree-days (°C) using 1 July as biofix and 10 °C as base temperature, and DDwet = DDtemp accumulation only on days with measurable rainfall (>0.1 mm) or vapour pressure deficit (VPD) <5 hPa, and DDwet2 calculated from the first seasonal ascospore release date as biofix. Cases in which models missed measured ascospore peaks (triangle), predicted false peaks (rectangle) or accurately predicted ascospore peaks (circle) are indicated.

Further ascospore peak prediction comparisons involved classifying each day as '1' if one or more ascospore events occurred or as '0' if no ascospore event occurred. These binary data were then used to calculate 3-day and 7-day ascospore peaks. Pearson's correlation coefficients between actual PAT data and predicted PAT data were calculated, and similar to the previous peak prediction analysis, the correlation coefficients were generally poor (results not shown).

Discussion and conclusions

In South Africa, CBS is generally controlled by the repeated application of fungicides, targeted at the primary inoculum (ascospores). The use of mathematical models to estimate the maturity of pseudothecia of *P. citricarpa* is therefore important in the management of CBS because they predict the start of ascospore release, which is key in determining when fungicide applications need to begin in the field. Information on ascospore availability combined with infection model output better informs the decision on whether a protective or curative fungicide should be applied, and the number of infection periods and inoculum pressure informs the general CBS infection risk, as is contemplated in the CRI-PhytRisk application (www.cri-phytrisk.co.za). To date, the *Phyllosticta* ascospore availability models were published by Dummel et al.[26] and Fourie et al.[25], of which the models described by Fourie et al.[25] were subsequently used in CBS risk assessment studies[17,35] and in CRI-PhytRisk.

The present study evaluated the performance of models described by Fourie et al.[25] against new data obtained from several geographical locations with differing climatic conditions, and also described a more accurate pseudothecium maturation model. This newly described model considers both wetness and temperature as the two main weather factors that influence the maturation of pseudothecia of *Phyllosticta* spp., which is consistent with published literature.[1,3,4,10,23,25,26] The temperature model described by Fourie et al.[25] uses DDtemp as the sole variable and predicts pseudothecium maturation in the absence of wetness. This model was favoured for use in pest risk assessment studies[17,35], largely due to some aberrant predictions from the related temperature/moisture model (PH Fourie, personal observation). The model developed in the present study considers that the pseudothecium maturation process progresses when wet conditions occur in combination with moderate spring temperatures above a baseline of 10 °C. Alternate wetting and drying at temperatures between 21 °C and 28 °C is required for maturation of the pseudothecium of *P. citricarpa*.[1,3,4,10,23,25,26] The DDwet pseudothecium maturation model described here is a significant improvement on the temperature and temperature/moisture models described by Fourie et al.[25] and more accurately predicted onset of ascospore release.

Ascospore release occurred at lower temperatures in this study, compared to the values reported by Fourie et al.[25] Fourie et al.[25] reported that 90% of ascospore events occurred at temperatures between 17.8 °C and 33.0 °C (daily T_{min} and T_{max} of 15.1 °C and 35.5 °C), while 16.0 °C to 32.1 °C (daily T_{min} and T_{max} of 15.4 °C and 33.5 °C) is the range of temperatures at which 90% of ascospores were trapped in the present study. Reports on the relationship between ascospore trapping and rainfall have also been inconsistent. Previous studies found that rainfall was a requirement for ascospore release.[3,24] In this study, ascospore release did not always coincide with rainfall periods, which is in agreement with observations made by Fourie et al.[25] This indicates that other sources of moisture such as irrigation, dew and relative humidity may be playing a role in ascospore discharge.[1,26,27,37] Reis et al.[38] reported that ascospore release was more related to the duration of leaf wetness than the amount of rainfall. Similar to the 59.3% RH_{avg} reported by Fourie et al.[25], more than 75% of ascospores were released during 3-hourly periods with an RH_{avg} above 55.9% (and days with RH_{min} >47.9%), which supports the possible role of high RH in triggering ascospore release.[25] High humidity can prolong wetness of leaf surfaces which accelerates the maturation and opening of pseudothecia.[26] Contrary to our findings, Dummel et al.[26] reported that ascospore release started after a drop in RH after midday and postulated that leaf litter surfaces need to dry for a period of time to allow ascospores to be successfully ejected into the air.

Higher numbers of ascospores were captured during the day, reaching a peak at 12:00 to 15:00. Fourie et al.[25] and Dummel et al.[26] found greater ascospore numbers from 12:00 to 21:00 and 16:00 to 20:00, respectively, while no differences were found in the pattern of ascospore release during the day and night in Brazil[38]. No correlations were found between more humid seasons and the number of ascospores trapped, when comparing cumulative DDwet2 and ascospore trap numbers. Pseudothecium maturation is hindered in areas where the leaf litter is constantly dry or wet.[1,23] CBS is a polyetic epidemic, i.e. inoculum builds up over time, and the inoculum pressure and disease incidence is expected to differ among orchards and years. This could further explain the differences observed in the number of ascospores trapped and ascospore release events between seasons and localities in this study.

As expected, higher numbers of ascospores and ascospore events were observed in areas of high CBS prevalence, i.e. Hoedspruit A, Hoedspruit B, Letsitele B and Letsitele C compared to areas with moderate CBS prevalence (locations in the Eastern Cape) as well as areas of low CBS prevalence (Ohrigstad and Musina A). Ascospore release was observed from September through to March, but peaks were observed at different times among the years and locations, but generally followed trends reported previously.[3,25,26,38] There was no direct relationship between rainfall and number of ascospores captured, as was also found in previous studies.[25,26,38] Ascospore release is triggered by small amounts of rainfall and as long as leaf litter surfaces remain moist, a few ascospores will continue to be released.[25,37] This may explain the release of ascospores in small numbers, but with occasional considerable increases in numbers (peaks), often observed in this study.

The ascospore release model developed in this study, as well as that of Fourie et al.[25], used mild to warm temperatures on humid or rainy days (DDwet2) as the climatic driver of ascospore release and were accurate in predicting the general trends in ascospore release, and are useful to predict the lag phases at the start and end of the ascospore release cycle, as well as the period of exponential increase. However, the models poorly predicted daily, 3- and 7-day ascospore peaks, which limits their potential use, for example, in integration in infection models or forecasting platforms. It is possible that ascospore release patterns are influenced by microclimatic weather variables (including leaf wetness[26,27,38]), which are not necessarily correlated with mesoclimatic data, and this possibility should be investigated in future studies.

The DDwet pseudothecium maturation model, developed in this study, was markedly more accurate in predicting the onset of ascospore release and will undoubtedly benefit existing CBS epidemiological models and improve risk assessment and management of CBS in South Africa.

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Competing interests

We declare that there are no competing interests.

Authors' contributions

P.M. was responsible for data analysis and the first draft of the paper. S.d.R. was responsible for ascospore trapping, and the compilation and preparation of data sets. P.H.F. conceptualised the study, and participated in data analyses and finalisation of the paper.

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INTERNATIONAL YEAR OF PLANT HEALTH 2020

Occurrence and spread of the banana fungus *Fusarium oxysporum* f. sp. *cubense* TR4 in Mozambique

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Fusarium wilt, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc), poses a major threat to banana production globally. A variant of Foc that originated in Southeast Asia, called tropical race 4 (TR4), was detected on a Cavendish banana export plantation (Metocheria) in northern Mozambique in 2013. Foc TR4 was rapidly disseminated on the farm, and affected approximately half a million plants within 3 years. The fungus was also detected on a second commercial property approximately 200 km away (Lurio farm) a year later, and on a small-grower's property near Metocheria farm in 2015. Surveys in Mozambique showed that non-Cavendish banana varieties were only affected by Foc race 1 and race 2 strains. The testing of Cavendish banana somaclones in northern Mozambique revealed that GCTCV-119 was most resistant to Foc TR4, but that GCTCV-218 produced better bunches. The occurrence of Foc TR4 in northern Mozambique poses a potential threat to food security on the African continent, where banana is considered a staple food and source of income to millions of people. Cavendish somaclones can be used, in combination with integrated disease management practices, to replace susceptible Cavendish cultivars in southern Africa. The comprehensive testing of African cooking bananas for resistance to Foc TR4 is required, along with the improvement of biosecurity and preparedness of growers on the African continent.

Significance:

- This paper presents the first official report of the invasive pest Foc TR4 in Africa.
- The spread of Foc TR4 on Cavendish banana farms in Mozambique was documented.
- Banana varieties that could replace susceptible Cavendish bananas were identified.

Introduction

Fusarium wilt of banana was first observed in 1874[1], but gained prominence when it severely affected the Gros Michel based international banana export industry in Latin America in the 1900s[2]. Despite a plethora of control measures tried and tested, the disease could never be brought under control. In the end, the export banana industry was forced to replace Gros Michel (AAA) bananas with a resistant variety that satisfied the international consumer market, the Cavendish (AAA) banana. Cavendish bananas soon became popular, and today constitute almost 45% of bananas grown worldwide.[3]

Cavendish bananas did not entirely escape Fusarium wilt, which is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc). Reports of Fusarium wilt of Cavendish bananas were first received from the Canary Islands in the 1920s, followed by losses of Cavendish banana in South Africa, Australia and Taiwan.[4,5] Yet, Cavendish bananas did not succumb to Fusarium wilt in severely infested fields in Latin America where Gros Michel was previously planted. This resulted in the designation of races in Foc, of which Foc race 1 affects Gros Michel and dessert banana varieties such as Pisang Awak (ABB) and Silk (AAB), race 2 affects Bluggoe (ABB), and race 4 affects Cavendish bananas, initially in the sub-tropics only.[6]

In the 1990s, reports were received of an Asian Foc strain that severely affected newly planted commercial Cavendish plantations in Indonesia and Malaysia.[6] This strain, commonly referred to as Foc TR4 (abbreviation for 'tropical race 4'), soon became the most devastating of all Foc strains, as it not only affected Cavendish bananas in the tropics and sub-tropics, but also many banana varieties susceptible to Foc races 1 and 2. For years, Foc TR4 was restricted to five Asian countries (Malaysia, Indonesia, Philippines, mainland China and Taiwan) and the Northern Territory state of Australia, but in 2011 it was detected outside Asia for the first time when it was identified in the Sultanate of Oman (Al-Kaabi S 2019, written communication, September 18).

Banana Fusarium wilt is difficult to control. Prevention of introduction is thus important to sustain the production of susceptible varieties.[7] Once Foc is introduced into a plantation the fungus can survive in soil for decades by producing survival structures called chlamydospores.[1] Chlamydospores are difficult to target with fungicides, while soil disinfestation techniques such as fumigation and flood fallowing have only been marginally successful.[2,8] Replacing susceptible with resistant varieties thus remains the only option for growers to continue growing banana in infested fields. However, the replacement of Cavendish bananas as a popular fresh fruit has significant challenges. Cavendish bananas are difficult to breed, and most export markets do not accept genetically modified food.[9] Mutation breeding by the prolonged multiplication of plants in tissue culture has successfully produced Cavendish clones with improved Foc TR4 resistance.[5] These somaclones may not be well adapted to new environments, and thus require further selection to improve their production traits.[7]

Banana Fusarium wilt was first detected in Africa when it was reported in West Africa in 1924.[2] This introduction most likely resulted from contaminated Gros Michel plants brought to the continent from Latin America.[10] A second introduction occurred when Indian workers brought sweet dessert bananas to East Africa.[10] While the Foc strains

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introduced into West and East Africa had not been identified at the time, it can be accepted that they belonged to Foc races 1 and 2. Surveys in East and Central Africa have since confirmed the dominance of vegetative compatibility groups (VCGs) in Foc races 1 and 2.[11] The most popular bananas on the continent, the East African Highland bananas (EAHBs) and African plantains, are not affected by Foc races 1 and 2. Cavendish bananas are also not affected by Foc races 1 and 2, but are seriously affected by a 'sub-tropical' strain of Foc race 4 (VCG 0120), referred to as Foc sub-tropical race 4 (STR4), in South Africa.[12]

In Mozambique, bananas are grown by commercial and small-scale farmers on approximately 68 000 ha.[13] In 2017, the country produced almost 580 000 tons of bananas, of which 75% were produced by smallholder farming households.[14] Banana varieties produced in Mozambique mostly include Cavendish cultivars such as Williams, Grand Naine and Chinese Cavendish, although cooking and dessert bananas can be found.[15] Most (85%) of the bananas grown are intended for trade in local markets, and the remainder is exported to neighbouring African countries like South Africa, as well as Eastern Europe and the Middle East.[16,17] Commercial plantations in the Boane District in southern Mozambique, western Manica Province and northern Mozambique almost exclusively produce Cavendish bananas. The international banana export industry, therefore, has increased earnings from USD4.5 million in 2008 to USD37.7 million in 2013.[16]

In February 2013, conspicuous yellowing and wilting of Cavendish banana plants were observed at a commercial export plantation in northern Mozambique. The plantation was established merely 4 years earlier and had experienced a number of production challenges. These challenges included excessive climatic fluctuations under the semi-tropical conditions, heavy clay soils that made fertilisation difficult, and the absence of proper draining systems that led to compaction problems and the salinisation of soils. The summer of 2013 was also preceded by an extreme drought, followed by flooding during the rainy months of January and February. The appearance of yellow and wilted plants, initially, did not appear out of the ordinary. When the symptoms continued after the heavy rains, however, production managers suspected that the cause of the yellowing was banana Fusarium wilt.

In recent years, papers have reported on the presence of Foc TR4 in Mozambique, but without scientific evidence.[18] The current paper, therefore, for the first time provides scientific proof that Foc TR4 was detected in northern Mozambique. It further reports on the spread of the fungus at the affected farms and into Mozambique between 2013 and 2018, and investigates Cavendish somaclones and mutants as potential replacement varieties for susceptible Cavendish cultivars.

Materials and methods

Identification of the Fusarium wilt fungus

Collection of isolates and primary isolation

In March 2013, pseudostem samples collected from symptomatic Cavendish cv Williams banana plants at Metocheria farm near Namialo in Northern Mozambique (14°41′45.29″S; 40°04′05.46″E) were sent to Eduardo Mondlane University in Maputo. The affected plants all showed progressive yellowing of the older leaves and, when the pseudostems were split open, a reddish-brown discolouration of the vascular tissue (Figure 1). A second sample was sent to Eduardo Mondlane University in April 2013, and three samples to Stellenbosch University in June 2013. In November 2013, samples were collected from eight more banana plants at Metocheria farm and sent to Stellenbosch University, and samples from a commercial Cavendish banana farm called Lurio farm, approximately 200 km from Metocheria farm, were sent in May and July 2014. Lurio farm is situated next to the Lurio River that separates the Nampula and Cabo Delgado Provinces (13º42'43.56"; 39º41'44.23"). The pseudostem samples were all placed in sterile paper towels and sent to Maputo and Stellenbosch in parcels with information about the collection sites and dates.

Figure 1: Symptoms of banana Fusarium wilt at Metocheria farm, northern Mozambique: (a) external symptoms of leaf yellowing, progressing from the older to younger leaves, and (b) internal symptoms of reddish-brown discoloration of vascular tissue in the pseudostem.

For primary isolation of the causal agent, pseudostem samples from each plant were cut into 5-mm pieces, surface disinfected and plated onto potato dextrose agar (PDA) amended or not with 0.4% streptomycin. The plates were then incubated for 4–5 days at room temperature, and the developing fungal colonies purified and single-spored.[19] Bacteria that were isolated on nutrient agar were common contaminants (data not presented), and were excluded from further analysis. At Stellenbosch University, the isolates of *Fusarium* were stored at -80 °C in 30% glycerol at the facilities of the Department of Plant Pathology.

Morphological and VCG identification

For morphological identifications, single-spore isolates were plated out on PDA and carnation leaf agar (CLA), and incubated at 25 °C for 14 days. Isolates of *F. oxysporum* were then identified based on their colony colour and morphological characteristics.[20] From each of the *F. oxysporum* isolates, *nit*-mutants were generated, and the mutants typed according to the methodology described by Leslie and Summerell[19]. *Nit*-1 mutants of isolates collected in Mozambique were thereafter paired on minimal media with *nit*-M testers of Foc available in the *Fusarium* culture collection at Stellenbosch University. The VCG group of the unknown isolates was identified if a heterokaryon was formed with a known tester strain.

Molecular identification

DNA was extracted from the mycelia of Foc strains collected in Mozambique using the protocol provided by Wizard SV Genomic DNA Purification System Kit (Promega, Madison, USA). The DNA was then amplified by PCR using Foc TR4-specific primers developed by Dita et al.[21] and Li et al.[22] An isolate of VCG 01213/16 obtained from Queensland Department of Agriculture and Forestry in Brisbane, Australia, was used as a positive control, and nuclease-free water served as the negative control.

Pathogenicity testing

Two-month-old Cavendish bananas were inoculated with two representative Foc isolates collected in Mozambique, using the method described by Viljoen et al.[23] The positive control included in the experiment was the Foc TR4 isolate from Australia, whereas the negative control was inoculated with sterile millet seeds. After inoculation, the plants were kept in a quarantine greenhouse in Stellenbosch at a 25 °C daytime and 20 °C night-time temperature, until symptoms developed. The plants were inspected for external and internal symptoms after 6 weeks, and the inoculated organism re-isolated to complete Koch's postulates.

Spread of Foc TR4 in Mozambique

On-farm disease development

Cavendish banana plants with Fusarium wilt symptoms were identified at Metocheria farm between April 2013 and October 2015. The number of plants affected were recorded weekly for each plantation block. The total number of cases per plantation were then accumulated every 3 months. The counting of plants was terminated in October 2015 when the number of dead plants became too many to accurately ascertain infections of new plants. At Lurio farm, diseased plants were also counted on a weekly basis from May 2014 to December 2016.

National surveillance

Surveys were conducted in Mozambique in April and October 2015. In April, banana plantings were visited in the vicinity of Metocheria and Lurio farms in the Nampula Province, whereas samples were collected from plantings in northern (Nampula Province), central (Manica Province) and southern (Maputo Province) Mozambique in October. Manica, Nampula and Maputo are the highest banana-producing provinces in Mozambique. During each visit, pseudostem strands were collected from plants showing Fusarium wilt-like symptoms, and information on the location, variety and planting history was obtained.

A total of seven districts in Nampula (Murrupula, Ribaue, Mecuburi, Rapale, Muecate, Erati and Monapo) was visited in April, and 24 samples were collected. In October, 56 samples were collected, of which 23 samples came from Nampula Province, 21 from Manica Province, and 12 from Maputo Province. Three districts were selected per province, with two administrative posts per district and five plantations per administrative posts. In Nampula Province, the districts included Monapo, Muecate and Erati, in Manica Province it included Sussudenga, Manica and Macate Districts, and in Maputo Province it included Boane, Manhica and Moamba Districts. In Nampula Province, some samples were also collected from the Rapale and Mecuburi Districts.

Both commercial and small-scale farms were included in the survey, following a zig-zag transect strategy. GPS coordinates for each plant sample were collected. Sample collections and identifications were undertaken as described earlier. In addition to Foc TR4-specific primer sets, Foc Lineage VI-specific primers[24] were used for the molecular identification of collected strains. Foc Lineage VI[25] includes all the Foc races 1 and 2 isolates found in East and Central Africa[11].

Evaluation of Cavendish somaclones in northern Mozambique

Five Cavendish banana clones were evaluated for resistance to Foc TR4 and production traits at Metocheria and Lurio farm in northern Mozambique, respectively. These clones included four Giant Cavendish somaclones developed by the Taiwan Banana Research Institute (TBRI) (GCTCV-106, GCTCV-119, GCTCV-218 and GCTCV-247) and DPM-25, a Dwarf Parfit (Cavendish) variety mutated by gamma-ray irradiation in Australia. The GCTCV somaclones were sourced from Bioversity International's International Transit Centre in Leuven, Belgium. The Cavendish banana cultivar Nandi, an elite selection made from Grande Nain by DuRoi Laboratory in South Africa, was used as the susceptible control. All clones were multiplied by DuRoi Laboratory, and were planted in a randomised complete block design with 40 plants per block and five replications of each clone. Fertilisers and irrigation were applied according to standard operational practices. Trials were conducted over two seasons, and experimental data were collected monthly for Fusarium wilt resistance and when required for production traits.

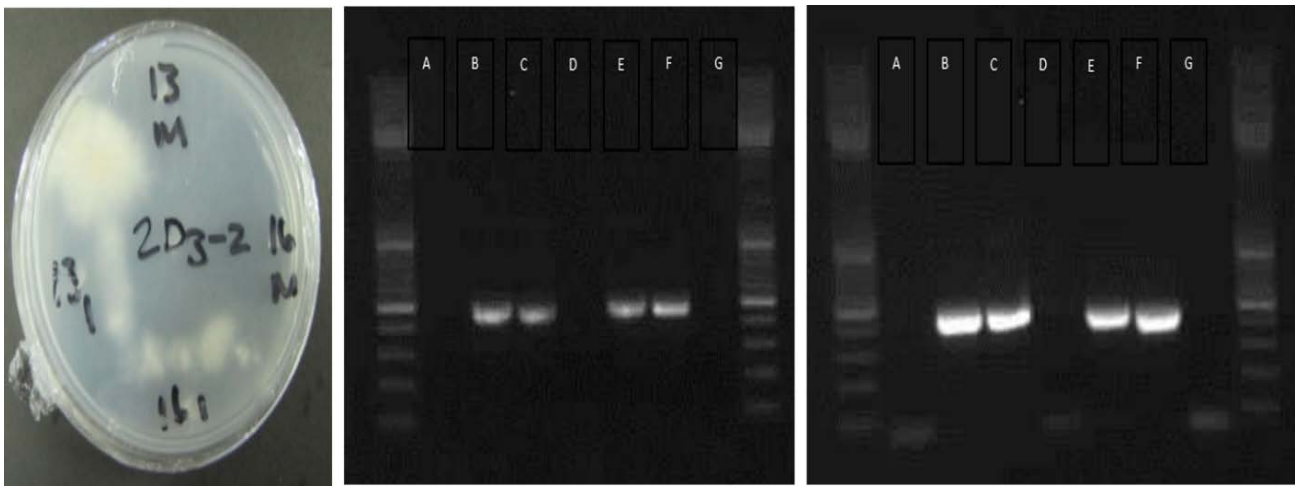
Field evaluation of Fusarium wilt severity was based on external leaf symptom development, measured on a scale of 1–5, with 1 = no symptoms and 5 = plant dead.[23] Susceptibility of the clones to Foc TR4 was determined by comparing the area under the disease progress curve.[26] For production traits, data were collected for the number of weeks from planting to flowering and harvest: plant height and pseudostem diameter at flowering, the number of leaves at flowering, gross bunch weight and number of hands at harvest, and finger length of the lower and upper hand at harvest. After data collection, the plot means were calculated and analysed at a 95% confidence level using analyses of variance (ANOVA). Pairwise comparisons were made using Fisher's protected 95% least significant difference. All analyses were conducted in XLSTAT-Premium (2018) for Windows.

Results

Identification of the Fusarium wilt fungus

The fungal isolates obtained from samples collected at Metocheria and Lurio farms in northern Mozambique were morphologically identified as *F. oxysporum*. They produced white to creamy aerial mycelia on PDA, with a tinge of purple in the colony centres. On CLA, microconidia were produced abundantly in false-heads on short monophialides, and macroconidia were produced sparsely in sporodochia. Single or pairs of chlamydospores were formed inside hyphae or macroconidia after 10–14 days.

VCG tests showed that the isolates from the two commercial Cavendish farms paired with tester strains belonging to Foc VCGs 01213/16 (Figure 2). This VCG is commonly known to be a member of Foc TR4. The primer set of Dita et al.[21] produced a 463-bp amplicon, and the one of Li et al.[22] a 455-bp fragment, thereby confirming that the Mozambican isolates indeed belonged to Foc TR4 (Figure 2). All subsequent collections from Metocheria and Lurio farms also showed that the Foc strains causing disease to Cavendish bananas on commercial farms in northern Mozambique were Foc TR4 (VCG 01213/16).



Lane A: Fusarium inquisetti-incarnatum; Lane B: Unknown plant sample 1; Lane C: Unknown plant sample 2; Lane D: F. inquisetti-incarnatum; Lane E: Unknown plant sample 3; Lane F: Foc TR4 control; Lane G: water control

Figure 2: Identification of isolates collected from a commercial banana plantation in northern Mozambique as *Fusarium oxysporum* f. sp. *cubense* (Foc) TR4. (a) Heterokaryon (barrier) formation between *nit*-1 mutants of a *Foc* isolate collected at Metocheria farms and *nit*-M mutants of Foc TR4 (VCG 01213/16). (b) PCR amplification using Foc TR4-specific primer set developed by Dita et al.[21] (c) PCR amplification using a Foc TR4-specific primer set developed by Li et al.[22]

The Cavendish banana plantlets inoculated with *F. oxysporum* isolates from both Metocheria and Lurio farms developed yellow leaves within 4 weeks, which became more pronounced in the following 2 weeks until the plants wilted and died. When the pseudostems were cut open, the inner rhizomes were dark brown, and the inoculated fungus could be re-isolated from the edges of the necrotic tissue to confirm Koch's postulates. The symptoms caused by the Mozambican isolates were similar to those developing on plants inoculated with an isolate of Foc TR4 from Australia. Plants inoculated with pathogen-free millet seed did not develop any internal or external symptoms.

Spread of Foc TR4 in Mozambique

On-farm disease development

After the first symptoms of Fusarium wilt were observed in February 2013, and before the identity of the fungus was confirmed in July 2013, the management at Metocheria farm started to notice more symptomatic plants. Initially, diseased plants were observed in Plantation 2 on an area of approximately 265 ha (Table 1). The worst affected blocks of 50 ha were removed by August that same year, and the area was fenced in an effort to contain the disease (Figure 3). None of the plants in the other plantations developed any symptoms until August 2013, when a few plants next to farm roads turned yellow and died.

By July 2014, the disease incidence increased almost three-fold in all plantations. By then the disease was present in all Plantation 1 and 2 blocks (data not presented), and containment became difficult, forcing a strategy to fence the entire Plantation 2. Fusarium wilt cases continued to increase, and by April 2015 the number of diseased plants in the six plantations increased between 7 and 20 times (Table 1). By the end of October 2015, more than 500 000 plants were killed by Fusarium wilt TR4, which meant that almost 20% of all plants at Metocheria farm were affected. Containment, at this stage, was no longer possible.

Fusarium wilt TR4 was first observed at Jacaranda Company's Lurio farm in April 2014. The first symptoms developed at the edge of the plantation, followed by new outbreaks next to the road 2 months later (Figure 4). Due to an early and active scouting and containment strategy, the disease was slowed down compared to that observed at Metocheria. Counting was discontinued in December 2016 when 42 of the 50 blocks were affected by Fusarium wilt TR4.

National surveillance

Samples collected in the Nampula and Capo Delgado Provinces in April 2015 resulted in the identification of five isolates belonging to Foc Lineage VI (VCG complex 0124/5/8/22, and VCGs 0124, 0128, 01220), and three belonging to Foc TR4 (VCG 01213/16). Of the Foc TR4 isolates, two were collected at Lurio farm and one isolate was obtained from a Cavendish banana planted on a small-grower's homestead near Metocheria farm. The plant was destroyed and the other banana plants at the property removed. All Foc Lineage VI VCGs were obtained from 'Macua' (Bluggoe) bananas.

Of the 56 samples collected in October 2015, 23 were morphologically identified as *F. oxysporum*. Of these, 13 were either Foc Lineage VI or Foc TR4. Four of the Foc Lineage VI isolates were collected from 'Apple' (Silk) and 'Macua' bananas in Nampula Province, and three from 'Apple' banana in Maputo Province (Figure 5). Foc TR4 was associated with Cavendish banana cultivars at Metocheria and Lurio farms only. VCG testing revealed that the Foc TR4 isolates belonged to VCG 01213/16, and the Foc races 1 and 2 isolates collected in the Nampula and Maputo Provinces belonged to VCGs 0124, 0125 and 01220.

Evaluation of Cavendish somaclones in northern Mozambique

Field evaluation of Cavendish somaclones and DPM-25 showed that the Taiwanese somaclones were significantly more resistant to Foc TR4 than the susceptible Cavendish control and DPM-25 (Figure 6). There was no significant difference in the area under the disease progress curve among the four GCTCV somaclones tested. The most resistant of the TBRI somaclones was GCTCV-119, which developed symptoms with an average disease rating of less than 2 over two disease cycles. GCTCV-218 was second most resistant with an average disease rating of less than 2 in the plant crop, and less than 2.5 in the ratoon crop (Figure 6). Disease development was also faster in Nandi and DPM-25 than in the somaclones. For instance, disease severity often progressed from Level 2 to Level 5 in less than a month, whereas the disease developed much slower in the somaclones. Nandi and DPM-25 showed symptoms 2–4 months after planting, whereas the disease in the somaclones developed from 6 months after planting until flowering (Figure 6).

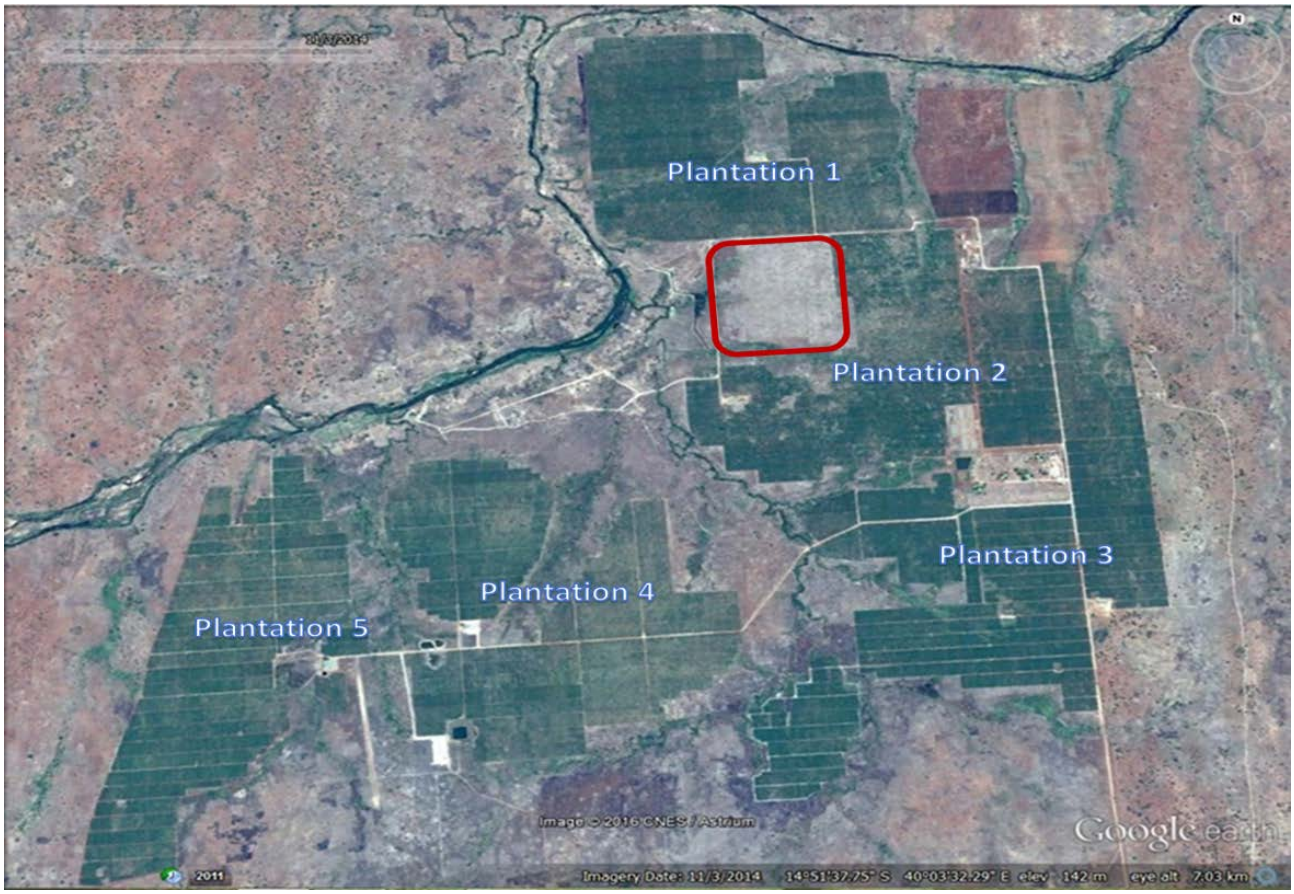


Figure 3: Metocheria farm consisted of five plantations. More than 50 hectares of banana was killed after the discovery of Fusarium wilt in 2013 (inside red square), and the area has been replanted with Formosana somaclones from Taiwan since 2016.

Table 1: The number of plants affected by banana Fusarium wilt TR4 at Metocheria farm between August 2013 and July 2016

Date	Plantation					Total
	1	2	3	4	5	
April 2013	0	780	0	0	0	780
July 2013	0	4756	0	0	0	4756
October 2013	55	5650	25	10	30	5770
January 2014	143	6187	129	46	91	6596
April 2014	1164	7888	445	215	239	9951
July 2014	4476	19 858	2736	1150	503	28 723
October 2014	7121	45 680	6315	2538	852	62 506
January 2015	11 286	87 096	18 852	7645	4539	129 418
April 2015	22 082	142 480	43 919	24 954	13 743	247 178
July 2015	56 816	198 843	88 235	49 611	25 722	419 227
October 2015	76 280	237 556	111 426	67 004	31 918	524 184

In the plant crop, more than 75% of Nandi and DPM-25 plants were infected at the end of the crop cycle (data not presented), with a disease severity of more than 4 on a rating scale of 1–5. Less than 25% of the somaclones were infected, with an average disease severity of less than 2.5 (Figure 6). In the ratoon crop, about 50% of the surviving Nandi and DPM-25 plants were infected, with a disease severity of more than 4.5 out of 5. Less than 25% of GCTCV-119 and GCTCV-218 were infected, with a disease severity of less than 2.6. About 60–70% of somaclones that showed symptoms at an early stage, recovered to produce bunches. Internal discolouration of the susceptible and the partially resistant somaclones also differed. When Nandi and DPM-25 were split longitudinally, vascular discolouration was visible to the centre of the pseudostem, whereas pseudostem discolouration in the somaclones was limited to the outer leaf blades. More disease developed in the second than in the first crop cycle.

Nandi and DPM-25 grew significantly faster than the somaclones, with a period from planting to harvest of less than 51 days compared to most somaclones that took more than 60 days (Table 2). In the plant crop, Nandi was the fastest grower, but had a great variation in bunch weights between the 2 years. In the plant crop, the biggest bunches were produced by DPM-25 and GCTCV-218, while the largest bunces in the ratoon drop were produced by Nandi and DPM-25. In the ratoon crop, the somaclones produced smaller bunches and grew into taller plants, making harvesting more difficult to manage compared to Nandi and DPM-25 (Table 2).

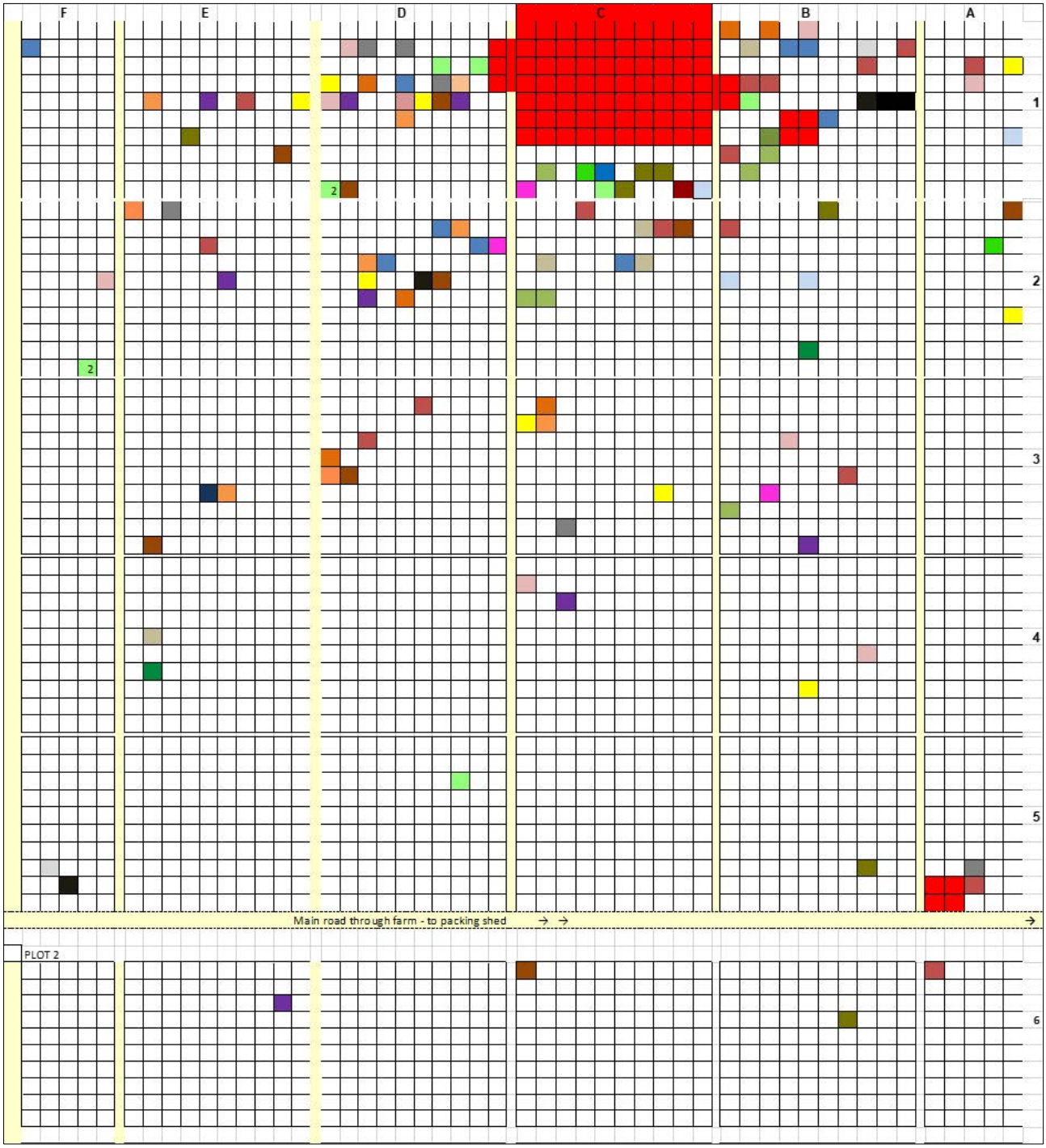


Figure 4: An illustration of the spread of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 at Lurio farm in northern Mozambique by July 2015. Each block represents 1 ha, and each colour indicates a different week. The yellow lines are farm roads. The red blocks indicate the areas where banana Fusarium wilt TR4 was first detected, whereafter production in block C1 was terminated.

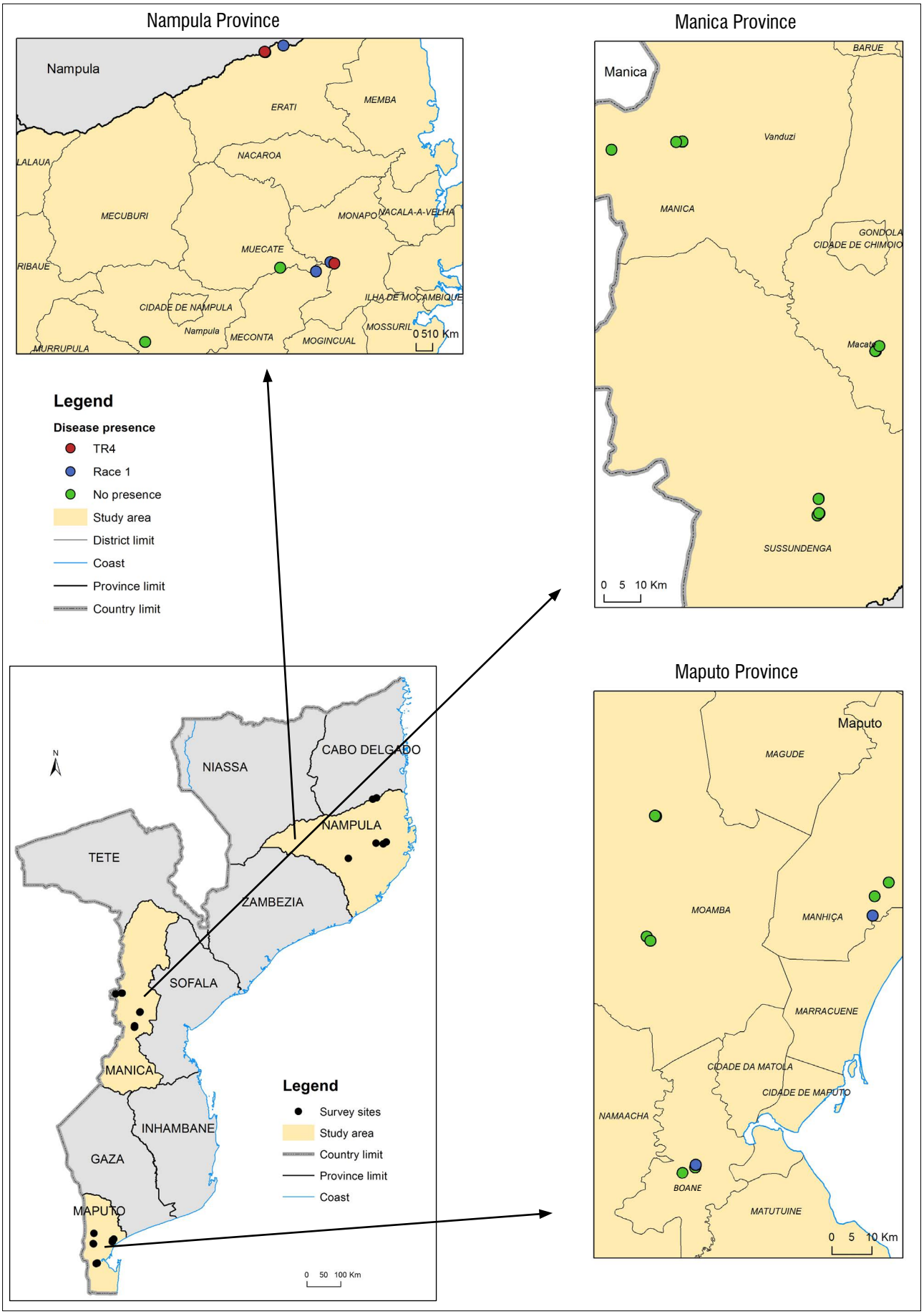


Figure 5: The distribution of *Fusarium oxysporum* f. sp. *cubense* in Mozambique.

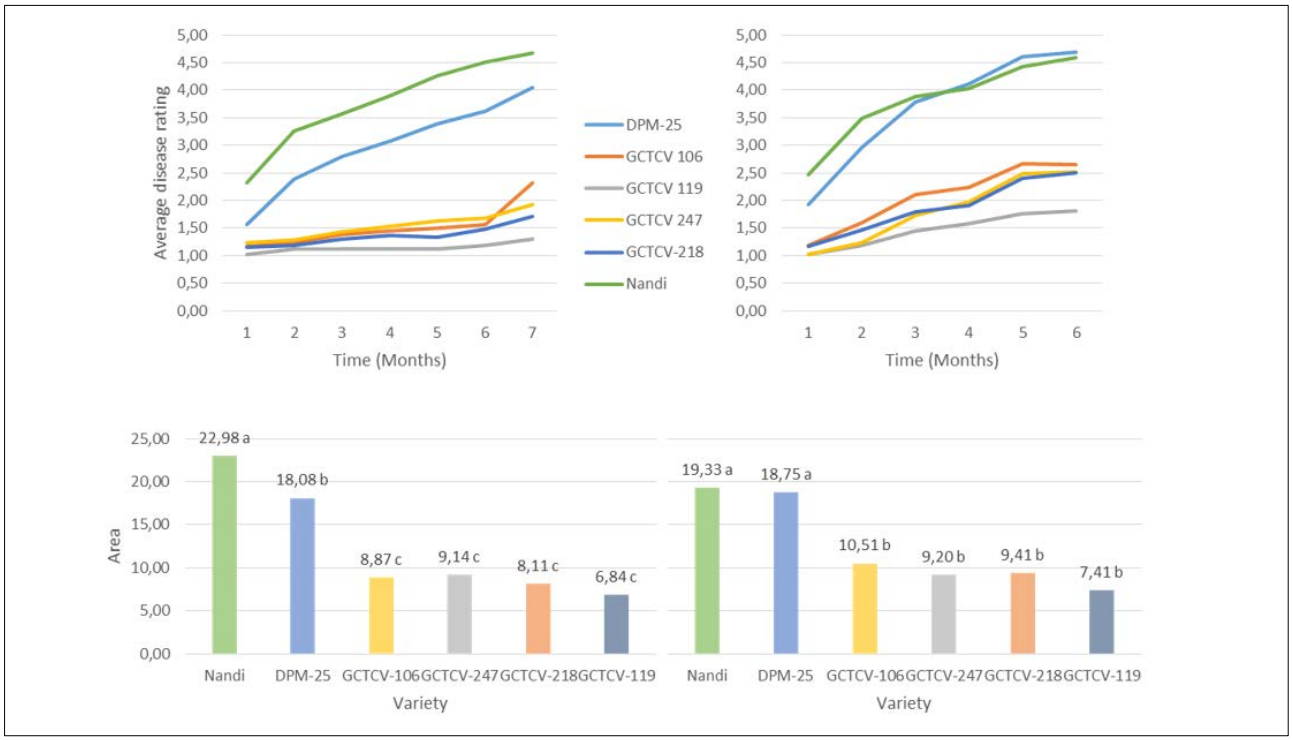


Figure 6: Field evaluation of Cavendish banana somaclones for resistance to Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* TR4, over two seasons. The figures at the top represent the average disease rating over two seasons, and the figures at the bottom represent the area under the disease progress curve. The figures on the left represent the disease development in the plant crop, and those on the right show disease development in the ratoon crop.

Table 2: Agronomic properties recorded for the plant crop and first ratoon of Cavendish clones at Jacaranda's Lurio farm, northern Mozambique

Plant crop

Variety	Weeks from planting to flowering	Weeks from planting to harvest	Height at flowering (cm)	Pseudostem diameter at flowering (1 m)	Number of leaves at flowering	Gross bunch weight	Number of hands	Finger length: Lower hand	Finger length: Upper hand
'Nandi'	38.078 f	47.853 f	193.742 e	49.102 d	11.845 b	16.149 d	6.020 c	19.849 b	22.723 b
DPM-25	39.998 e	50.905 e	223.940 d	50.829 c	12.879 a	19.546 a	6.888 a	20.494 a	23.374 a
GCTCV106	49.283 c	60.236 c	240.120 c	49.720 d	11.778 b	17.472 c	5.904 c	18.733 d	20.965 d
GCTCV247	44.558 d	57.679 d	253.255 b	47.324 e	11.370 c	17.546 bc	5.954 c	19.821 b	22.517 b
GCTCV218	54.027 b	62.806 b	244.169 c	53.093 b	12.096 b	19.710 a	6.524 b	19.898 b	22.483 b
GCTCV119	64.899 a	72.578 a	281.198 a	54.852 a	10.837 d	18.242 b	5.151 d	19.364 c	21.379 c

First ratoon

Variety	Weeks from planting to flowering	Weeks from planting to harvest	Height at flowering (cm)	Pseudostem diameter at flowering (1 m)	Number of leaves at flowering	Gross bunch weight	Number of hands	Finger length: Lower hand	Finger length: Upper hand
'Nandi'	73.762 e	82.867 d	220.965 d	57.475 de	12.724 a	20.220 ab	6.263 a	20.394 a	22.595 a
DPM-25	76.122 e	86.612 c	279.453 c	59.889 b	12.703 a	19.644 bc	6.096 a	19.753 ab	22.157 ab
GCTCV106	84.968 c	93.658 b	292.056 b	56.875 e	11.848 b	19.217 c	5.769 b	18.908 c	20.913 c
GCTCV247	80.673 d	91.492 b	313.267 a	58.263 cd	11.944 b	18.229 d	5.754 b	19.383 bc	21.779 b
GCTCV218	88.322 b	98.962 a	281.965 c	61.319 a	11.993 b	20.535 a	6.241 a	19.176 bc	21.694 bc
GCTCV119	91.127 a	97.940 a	308.091 a	59.350 bc	10.989 c	19.602 bc	5.170 c	19.494 bc	21.798 b

Values followed by the same number in each column do not differ significantly from each other ($p<0.05$).

Discussion

The discovery of banana Fusarium wilt TR4 on a commercial Cavendish banana farm in northern Mozambique signifies the first occurrence of Foc TR4 on the African continent. It was initially believed that the disease could have been caused by Foc STR4, a strain present in South Africa.[12] International staff and workers employed at Metocheria farm included South Africans, and the farm was frequently visited by South African service providers. Planting material used at the farm was not believed to be the source of introduction, as these plants were obtained from reputable banana tissue culture companies in South Africa and Israel. Banana plantlets produced in vitro are also known to be Foc-free. The eventual identification of the causal agent as Foc TR4 came as a massive surprise. The only areas where Foc TR4 was known to occur by February 2013 were Southeast Asia, the Northern Territory of Australia, and Oman.[9] It is thus believed that Foc TR4 was most likely introduced into Metocheria from Asia, but it is unclear when and how this happened. Circumstantial evidence has subsequently implied that Foc TR4 might have been present at Metocheria farm as early as 2010 when several plants with leaf yellowing were noticed and destroyed. The fungus was introduced from Metocheria farm onto Lurio farm most likely by visitors or trucks visiting both farms before TR4 was known to occur at Metocheria farm.

The early outbreaks of Fusarium wilt TR4 at Metocheria farm's Plantation 2, and the progression of the epidemic, suggest that this field was the source from where Foc TR4 spread to the rest of the farm. Google Earth images also clearly show the spots where plants were first affected by the disease. The spread of Foc TR4 in this plantation, if present in 2010 already, might have been caused by the movement of plantation workers. The heavy clay soil can easily attach to shoes, plantation tools and machinery and, if infested with Foc TR4, could have moved the fungus into new areas.[2,9] The first cases of the disease in other plantations were initially found on road sides, and could have been spread by vehicles or the rotation of farm workers between plantations. Another possible source of dissemination could have been irrigation water, as water from infested plantations was drained into the river from where it was pumped back onto the plantations. The rapid increase of the disease by mid-2015 is most likely due to severe flooding in northern Mozambique in February 2015, which disseminated the soil-borne fungus everywhere. After this, all efforts to contain Foc TR4 on the farm became obsolete.

Fusarium wilt turned into a full-blown epidemic at Metocheria within 3 years, which prompted discussions of alternative options to deal with the losses. One option was to replace dead plants with new ones after 3–6 months, or the replanting of previously diseased areas at higher densities. The use of Cavendish somaclones and DPM-25 as potential replacement of susceptible Cavendish cultivars, and the selection of survivor plants in severely infested fields, were discussed, and trials initiated. The conversion of banana to other crops was considered, but the financial investment, logistics and market opportunities obstructed this option. Metocheria farm also invested in measures to prevent the spread of Foc TR4 off the farm. Sanitation processes and farm security were improved, standard operating procedures altered, and the movement of people and vehicles on the farm restricted.

Foc TR4 at Lurio farm was most likely introduced from Metocheria farm. In 2014, Lurio farm employed only one non-African worker who came from Latin America where Foc TR4 was not known to occur. The farm, however, was often visited by trucks transporting fruit, or by service providers and donor organisations, after they had visited Metocheria farm. Not many of the visitors drove into the plantation with their own vehicles. They did, however, visit banana fields with the owners without cleaning their shoes, as Foc TR4 had not yet been detected at Metocheria farm at the time. After the detection of Foc TR4 at Lurio farm, a scouting and containment strategy implemented at the farm significantly slowed down the spread of the disease compared to that observed at Metocheria. The property of 50 ha, also, was smaller and easier to manage. Still, the disease could not be contained, and by 2016 led to the systematic killing of many susceptible Cavendish banana plants.

The presence of Foc TR4 in northern Mozambique prompted the Department of Agriculture and Food Security to introduce quarantine measures that restricted the movement of plants and planting material off the affected farms, and the transport of fruit outside the Nampula and Cabo Delgado Provinces. Strict containment measures were introduced at both Metocheria and Lurio farms, which included gate control, the use of disinfectants and the issuing of footwear to all workers and visitors. Only farm vehicles were allowed to enter plantations. Farm workers, communities and plant protection officials were trained in symptom identification, and awareness programmes were launched. The single case of Foc TR4 affecting a Cavendish banana plant on a small-grower's homestead near Metocheria farm could be tracked back to planting material taken off the farm before Foc TR4 was discovered in northern Mozambique. Foc TR4 was not detected in local cooking bananas found in small patches everywhere in northern Mozambique, even though the variety was shown to be susceptible when tested at Metocheria farm in 2015 (Viljoen, unpublished data).

The national survey conducted in 2015 in Mozambique confirmed that Foc races 1 and 2 strains are spread across Mozambique, as previously documented.[10] Their presence, however, is limited to areas where farmers grow non-Cavendish local cultivars such as 'Apple' and 'Macua' bananas. The absence of Fusarium wilt in Manica Province can be attributed mainly to the widespread planting of Cavendish cultivars in the province. These Cavendish bananas are also not affected by Foc TR4 that is present in Nampula Province, or Foc STR4 that is present in South Africa.[12] The occurrence of Foc TR4 in Mozambique has become of great concern to growers in Mozambique, its neighbours and other African countries as the pathogen does not only affect Cavendish cultivars, but also all other cultivars susceptible to Foc races 1 and 2.[6,9]

The Cavendish somaclones evaluated in this study were significantly more resistant to Foc TR4 than Nandi and DPM-25 over two planting seasons, even though they were slower growing, generally taller, and produced smaller bunches in the ratoon cycle. While GCTCV-119 was most resistant to Foc TR4, it performed worst in production properties. GCTCV-218, however, was moderately resistant but produced excellent bunches. GCTCV-218 is registered as Formosana, a banana cultivar developed by TBRI in Taiwan. This cultivar is now also replacing susceptible Cavendish varieties in the Philippines. Mozambique, and southern Africa in general, have a climate different from that in Taiwan, and it is suggested that further selection be made of GCTCV-218 for higher-yielding clones adapted to local climatic conditions. The other GCTCV somaclones could also be considered for field selection of clones with superior production properties. This has been the case with the selection of GCTCV-219 from GCTCV-119 in the Philippines.[27]

The detection of Foc TR4 in northern Mozambique resulted in significant concerns about the threat of the fungus to bananas on the African continent, as the crop is considered a staple food in many countries. Most bananas grown in Mozambique, like the rest of southern Africa, are Cavendish bananas known to be highly susceptible to Foc TR4.[9] In East and Central Africa, banana provides an income to over 20 million resource-poor farmers, and is more profitable than any other crop grown in the region.[28] These bananas include a group of unique cooking and beer bananas, called East African Highland bananas (EAHBs-AAA). West Africa produces primarily African plantains (AAB), another type of cooking banana that evolved on the continent after being introduced almost 3000 years earlier.[29] West Africa also produces Cavendish bananas for export, while Cavendish plantations are being expanded in many countries on the east coast of Africa. The testing of Cavendish somaclones in this study was therefore of great significance to the replacement of susceptible Cavendish plantings if necessary. A small group of EAHBs and plantains had been evaluated in Asia for resistance to Foc TR4[30,31], but the testing of a much larger set of EAHBs and plantains is required to fully understand their response to the fungus.

In May 2019, the owners of Metocheria farm filed for insolvency. The primary reason was a limitation in cash flow to deal with Foc TR4 and not because Formosana, which was widely replanted at the time, had failed as a replacement variety. Jacaranda Agricultura has since purchased Metocheria farm, and has renamed it 'Monapo River farm'.

Containment measures have been strengthened, and fields are being replanted with Formosana. The availability of this plant has changed the lives and livelihoods of thousands of Mozambicans employed at Monapo River farm and living in the surrounding communities. Surveillance efforts to establish Foc TR4 distribution in northern Mozambique and in southern Africa are continuing. Urgent priorities to deal with Foc TR4 in southern and eastern Africa were also identified at a Foc TR4 strategy meeting in Maputo in 2019. These include the testing of African banana varieties for resistance to Foc TR4 at Monapo River farm, the enhancement of Formosana's resilience to Foc TR4 with integrated disease management practices, an assessment of the impact of Foc TR4 in Mozambique, and the improvement of biosecurity and preparedness on the African continent.

The possibility of Fusarium wilt spreading from the infested properties in northern Mozambique into other parts of the country and to neighbouring countries remains. To deal with this possibility, three strategies to manage the pathogen should be employed. These include the prevention of introduction into new areas by proper awareness campaigns, farm border control, and the use of clean planting and field materials; the early detection and containment of new outbreaks, which involves routine scouting and the appropriate isolation of newly infested field sites; and the management of the disease by planting disease-resistant varieties and reducing inoculum levels through good agricultural practices, and soil and water management. Given the difficulties in managing the disease, these measures should be a collective effort involving policymakers, growers and the scientific community, and should be implemented on local and regional levels. A proper pest risk analysis for Foc TR4 in Mozambique and Africa is required in the event that the fungus spreads beyond the borders of the two affected farms. Banana cultivars and production areas that can be affected need to be identified, potential pathways of spread determined, and the risk of the fungus spreading to these areas managed. Resistant banana varieties in Mozambique and Africa still need to be identified, and the possible spread of Foc TR4 in Mozambique needs to be continuously investigated. The strict implementation and effectiveness of quarantine measures announced by the Mozambican government should also be continuously monitored.

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Competing interests

We declare that there are no competing interests.

Authors' contributions

A.V.: Conceptualisation, methodology, data collection, validation, writing the initial draft, student supervision, project leadership, project management and funding acquisition. D.M.: Conceptualisation, methodology, sample analysis, validation, data curation, writing revisions, student supervision and project management. T.C.: Sample analysis, data analysis, writing revisions, student supervision, and funding acquisition. I.B.: Sample analysis and data analysis. C.F.: Methodology, data collection and writing revisions. J.D.; H.M.: Methodology, data collection, validation, writing revisions, project management and funding acquisition. J.A.: Methodology, data collection, validation and writing revisions. E.L.M.: Data collection and writing revisions. G.T.: Data collection, data analysis, validation and project management. O.M.A.: Methodology, data collection, data analysis, validation, data curation and writing revisions. A.M.: Methodology, data analysis, validation, student supervision and funding acquisition. A.V.: Writing revisions, project management and funding acquisition. A.P.; S.B.: Data collection and data curation. L.J.R.: Student supervision and writing revisions. F.B.; A.B.M.; C.-P.C.: Conceptualisation and writing revisions. F.D.: Writing revisions, project management and funding acquisition.

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INTERNATIONAL YEAR OF PLANT HEALTH 2020

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Establishment of *Beauveria bassiana* isolates as endophytes in rice cultivars and their biocontrol efficacy against rice stem borer, *Sesamia calamistis*

Possible endophytic colonisation of rice cultivar parts (leaves, stem and roots) by *Beauveria bassiana* isolates and their potential as biocontrol agents against *Sesamia calamistis* Hampson (African pink stem borer) were investigated. Five promising *B. bassiana* isolates were evaluated for their endophytic colonisation, the effectiveness of the inoculation methods and the efficacy of the isolates as biocontrol agents against *S. calamistis*. The plant part colonised is often dependent on the inoculation method. Colonisation of plant parts was assessed at 30 and 60 days after seed inoculation and foliar spray. For the pathogenicity activity, third instar larvae of *S. calamistis* were fed with rice stems that were previously inoculated with endophytic isolates of *B. bassiana*. Both inoculation methods led to the colonisation of the rice cultivar tissues, but were affected by the interactions of cultivars x isolates x inoculation methods. The colonisation of the cultivar plant parts varied over time (30- and 60-day intervals), and was affected by the inoculation method used. For both inoculation methods, highly significant differences were observed in the roots and the leaves over time ($p=0.0001$). However, with seed treatment, there was no significant difference in levels of colonisation in stems by the isolates x time ($p=0.32$). The *B. bassiana* isolates were pathogenic on the third instar larvae of *S. calamistis*, causing mortalities of more than 50% at 28 days after treatment. However, the virulence of the isolates varied. According to the isolates and the inoculation methods, *B. bassiana* formed an endophytic relationship with rice plants, and produced various mortality rates.

Significance:

- *Beauveria bassiana* could be a potential biocontrol agent of rice stem borer, *S. calamistis* as there is no report of endophytic isolates of *B. bassiana* for the control of rice borers.
- Currently there is no commercially registered biocontrol agent against rice borers; hence further studies into *B. bassiana* could lead to the registration and commercialisation of *B. bassiana* as a bio-pesticide for rice stem borers.

Introduction

Rice (*Oryza* spp L.) is one of the world's most important crops, providing food for more than half of the world's population.[1-3] Rice and wheat (*Triticum* spp L.) together contribute about 21% of the total energy consumed by humans.[4] In West Africa it has become the main source of calories for low-income households.[5] Two *Oryza* spp. are cultivated globally: Asian rice (*Oryza sativa* L.) and African rice (*Oryza glaberrima* S.), for which the cultivation is limited to tropical West Africa.[6] Rice is now grown and consumed in more than 40 countries on the African continent.[7] Its consumption has increased rapidly in Africa, making it the second largest source of carbohydrates in sub-Saharan Africa.[4] Imports of rice account for nearly 40% of the total rice consumption of the region.[8,9]

Losses caused by biotic factors such as pests, diseases and weeds reduce yields of rice worldwide. According to estimates of the Food and Agriculture Organization of the United Nations (FAO), diseases, insects and weeds cause as much as 25% yield losses annually in cereal crops.[2] The most serious pests of rice plants worldwide are rice stem borers, which belong to three families (Noctuidae, Pyralidae and Diopsidae). *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) is one of the major pests that attacks grain crops including rice, maize (*Zea mays* L.), pearl millet (*Pennisetum* sp L.), wheat, sorghum (*Sorghum bicolor* L.) and sugarcane (*Saccharum officinarum* L.). The control of this borer by commercial farmers has mainly relied on the application of synthetic insecticides.[10-12] However, control of *S. calamistis* using chemicals is difficult because of a prolonged emergence pattern, multiple generations and a cryptic feeding behaviour.[13] A further issue is that, as with many other stem borers, *S. calamistis* has developed resistance to chemicals. In addition to the insecticides' high costs and their inefficacy against the borers, they may also cause ecological problems, and are usually unaffordable for small-scale farmers.[14-17]

The need for alternative methods for the control of major pests has driven research to develop biological control products. Indigenous predators, parasites and entomopathogens are the most commonly used biological control agents in tropical Asia and Africa to control stem borers.[18] Entomopathogenic fungi are important among biological control agents due to their broad host range, their diverse mechanisms of pathogenicity, and their environmental safeness.[19-21] Some strains of the entomopathogen *B. bassiana* have been introduced into several plant species [maize, banana (*Musa* spp L.), tomatoes (*Solanum lycopersicum* L.), sorghum, coffee (*Coffea arabica* L.), wheat and pumpkins (*Cucurbita* spp D.)] to control various insects.[22-25] Various inoculation methods (seed treatments, soil drenches, foliar and flower sprays, and stem injections) have been used for their establishment as endophytes in those crops. The main reason for conducting this study was to determine if endophytic strains of *B. bassiana* in rice cultivars might provide protection against *S. calamistis*, the major rice stem borer prevalent in West Africa.

Materials and methods

Five isolates of *B. bassiana* previously identified as endophytes in sorghum were evaluated for their potential establishment as endophytes in rice cultivars plant tissues. Third larval instars of *S. calamistis* were used as the test insects.

Production of conidial suspensions of B. bassiana *isolates*

Five *B. bassiana* isolates (Bb3, Bb4, Bb10, Bb21 and Bb35) were used for the study. These had been isolated from various soils, including soil samples collected from the rhizosphere of mangoes (*Mangifera indica* L.), rooibos tea (*Aspalathus linearis* Burm. f.) and wheat. They were characterised by the Plant Protection Research Institute (Pretoria, South Africa). In prior research, they were selected for their endophytic abilities in sorghum plant tissues (leave, stem and root).[18] Conidial suspensions used for the study were prepared following the method of Parsa et al.[18] The strains were cultured in 90-mm diameter plastic Petri dishes containing potato dextrose agar (PDA) supplemented with antibiotics (100 mg/L of ampicillin and streptomycin), and incubated at 28 °C. The cultures were allowed to grow for 14–18 days, after which the conidia were harvested. The conidia were harvested under sterile conditions by gently scraping the fungal growth from the surface of the agar with a sterile spatula, and rinsing with sterile distilled water. The resulting suspensions were filtered using sterile cheese cloth to remove mycelia and agar debris. Conidial density was determined using an improved Neubauer haemocytometer, and adjusted to $2x10^6$ conidia/mL with sterile distilled water containing Tween-80 (1 mL/L). The viability of the conidia for all the experiments was evaluated by taking a 100-μL sample of each strain, spreading it on PDA and incubating at 25 °C. Conidia germination was assessed after 24 h of incubation. The percentage germination of conidia was determined from 100 randomly selected conidia under a light microscope. The germination of conidia was assumed when the hyphae were visible or the germ tube was about twice the length of the conidium. For each strain, the mean of three replicates was used to assess the viability of the conidia. The final inoculum was used for seed treatment and foliar spray experiments.

Production of rice plants for greenhouse studies

Three African rice cultivars (NERICA1, NERICA8 and NERICA-L19)[26] were used as the host plants as there may be differential interactions between host plants and endophytic strains. Seeds of each cultivar were separately surface sterilised in 3% sodium hypochlorite for 3 min followed by 70% ethanol for 2 min. They were rinsed three times with sterile distilled water, air dried on a laminar flow bench and then divided into two sets. The first set was used for seed inoculation and the second for foliar spray experiments. The second set of seeds used for foliar spray experiment were sown in Speedling® 24 trays filled with Composted Pine Bark (CPB) seedling mix growing medium. The seeds were watered with tap water and placed under greenhouse conditions at 20–28 °C day and night. Two weeks after germination, the seedlings were transplanted into 30-cm diameter pots filled with CPB seedling mix growing medium and placed under greenhouse conditions at 20–28 °C day and night. The plants were allowed to grow for 7 days before being used in the foliar spray experiment. Plants were irrigated three times a day with irrigation water containing NPK fertiliser [3: 1: 3 (38)] (50%) together with calcium nitrate (50%) and trace elements.

Inoculation of B. bassiana *isolates for endophytic colonisation in rice cultivars*

Seed treatment

The seeds of the three rice cultivars were surface sterilised as previously described. After surface sterilisation, the seeds for each cultivar were separately soaked in the conidial suspension of each *B. bassiana* isolate [5 mL of the prepared inoculum (2×10^6 conidia/mL)], allowed to stand overnight, then removed and air dried on a laminar flow bench. The seeds were then planted in Speedling® 24 trays filled with CPB seedling mix growing medium. The control plants consisted of non-inoculated seeds treated in a similar manner using sterile distilled water. After 2 weeks, the emerging seedlings were transplanted into 30-cm diameter pots filled with CPB seedling mix growing medium and placed under greenhouse conditions at 20–28 °C day and night. Three plants per pot were arranged in the greenhouse in a randomised complete block (RCB) design with three replicates. Plants were irrigated three times a day with irrigation water containing NPK fertiliser [3: 1: 3 (38)] (50%) together with calcium nitrate (50%) and trace elements. The plants were grown for 30 or 60 days before they were harvested, and the roots, stems and leaves were evaluated for evidence of endophytic colonisation.

Foliar spray

The seedlings of the three rice cultivars were sprayed 15 days after transplanting into pots. A hand spray was used to inoculate the rice plant leaves with the inocula of the *B. bassiana* isolates. A volume of 50 mL inoculum of each *B. bassiana* isolate was used per plant. Before the leaves were sprayed, the base of each pot was covered with aluminum foil, with a hole to allow the plant to emerge. This was to stop inoculum running off the leaves onto the roots and creating a root drenching situation. Plastic bags were used to cover the entire plant for 24 h to increase humidity. For the control plants, sterile distilled water was applied in a similar manner as described for the *B. bassiana* treatments. The treated plants (three plants per pot) were then placed in a greenhouse (20–28 °C day and night) using a RCB design with three replicates. Plants were irrigated three times a day with irrigation water containing NPK fertiliser [3: 1: 3 (38)] (50%) together with calcium nitrate (50%) and trace elements. The roots, stems and leaves of each treated plant were harvested after 30 and 60 days, for evaluation for endophytic colonisation.

Evaluation of endophytic colonisation of the B. bassiana *isolates*

The colonisation of rice plant tissues by *B. bassiana* was determined 30 and 60 days after inoculation with each *B. bassiana* isolate. From each rice cultivar x *B. bassiana* treatment combination, plants were carefully removed from their pots and sampled into leaves, stems and roots. The roots were gently washed with tap water to remove residues of CPB. The plant tissues were surface sterilised by immersing them in 3% sodium hypochlorite for 3 min, followed by 70% ethanol for 2 min. They were rinsed three times with sterile distilled water. The surface sterilised samples were placed on sterile tissue paper under a laminar flow cabinet for air drying. After drying, six pieces of each of the samples (leaves, stem and roots) from each treated plant were randomly taken and plated separately onto a *B. bassiana* selective medium (39 g/L PDA +2 g yeast extract + 1.1 g Dodine + 100 mg/L of streptomycin and ampicillin)[27] and incubated for 15 days at 25 °C. To confirm that the surface sterilisation was effective, 10 mL of the sterile distilled water used to rinse the samples during the surface-sterilisation procedure was spread onto Petri dishes containing the *B. bassiana* selective media. The plates were incubated for 10–15 days at 25 °C to count the colony forming units. However, the sterilisation resulted in clean plates. Therefore, any *B. bassiana* mycelium emerging from surface-sterilised plant tissues was assumed to have originated from within the plant tissues as an endophyte. The plates that contained the plant samples were monitored every 2–3 days for the emergence of fungal mycelia. After 10–15 days, the presence or absence of *B. bassiana* colonies were recorded. The fungal colonies grown from the samples were confirmed to be *B. bassiana* based on morphological characteristics.

Mass rearing of S. calamistis *larvae*

A suitable number of *S. calamistis* pupae collected from a maize field were placed into cages that contained cotton soaked in sugar/honey water that served as food for the moths once they emerged from the pupae. Sheets of transparent paper were wound around wooden rods (40 cm) to create a slot for the female moths to lay their eggs. These eggs were harvested from the slots by scraping the wooden rods with a sterile spatula. The eggs were placed into plastic containers on a sterile paper towel. The containers were incubated at 26 °C with a relative humidity of 60% (±10%), and a photoperiod of 12-h light and 12-h dark. The containers were monitored daily until the larvae

hatched, creating the stage called 'black heads' (first instars). These young larvae were transferred into other transparent plastic containers whose lids were perforated but covered with mosquito netting to ensure permanent ventilation, and were incubated at 26 °C. Fresh maize stalks were harvested and placed into the plastic containers to serve as food for the black head larvae. After 3 days, the maize stalks were dissected and the larvae were extracted and transferred into new containers that contained fresh maize stalks (Figure 1), and kept in an incubator at 26 °C. This procedure was repeated until larvae of the desired third instar larval stage had developed. The number of days for each stage varied from one larval stage to the next. Development from the second to the third instar took 5 days, while development from the third to the fourth instar took 8–10 days.

Production of endophytic stems of rice plants

Seeds of a rice cultivar (NERICA 1) were surface sterilised in 3% sodium hypochlorite for 3 min, followed by 70% ethanol for 2 min. They were then rinsed three times with sterile distilled water and air dried. The surface-sterilised seeds were then dipped separately in a conidial suspension of each of the five *B. bassiana* isolates [5 mL of the prepared inocula (2×10^6 conidia/mL)] and left overnight before air drying under a laminar flow cabinet. The seeds were then planted in Speedling® 24 trays filled with CPB seedling mix growing medium. After 2 weeks, the seedlings were transplanted into 30-cm diameter pots filled with CPB seedling mix growing medium and placed under controlled greenhouse conditions set at 20–28 °C day and night. Three plants per pot were arranged in the greenhouse in three replicates per treatment, using a RCB design. Plants were irrigated three times a day with irrigation water containing NPK fertiliser [3: 1: 3 (38)] (50%) together with calcium nitrate (50%) and trace elements. The plants were allowed to grow for 30 days before one plant per treatment from each crop cultivar was harvested and sampled (stems) to confirm their endophytic colonisation by *B. bassiana* isolates. The stems were separately surface sterilised by immersing them in 3% sodium hypochlorite for 3 min, followed by 70% ethanol for 2 min. They were separately rinsed three times with sterile distilled water and placed on a sterile paper towel in a laminar flow cabinet to air dry. After drying, six pieces of each treated stem were randomly selected and plated separately onto *B. bassiana* selective medium.[27] The inoculated plates were incubated for 15 days at 25 °C. The plates were monitored every 2–3 days for the emergence of fungal mycelia. After colonisation of the stems by *B. bassiana* isolates was confirmed, the remaining inoculated plants were harvested, washed with tap water, and the stems were then used for pathogenicity testing on the stem borer, *S. calamistis*.

Efficacy of B. bassiana *isolates against* S. calamistis

The endophyte positive stems produced as previously described were harvested and washed with distilled water, before being fed to the third instar larvae of *S. calamistis*. A total of 10 third instar larvae of *S. calamistis* were placed into plastic containers of 10 g of *B. bassiana* infected rice stems. The containers were placed in an incubator at 28 °C. Mortality of the larvae was recorded after 7, 14, 21 and 28 days. For the control, larvae were fed with non-inoculated rice stems. Dead larvae were collected at 7, 14, 21 and 28 days and were maintained in plastic containers on Whatman filter paper previously wetted with sterile distilled water. Two to three days after collection, the dead larvae were surface sterilised in 3% sodium hypochlorite for 1 min followed by 70% ethanol for 1 min. They were then rinsed three times with sterile distilled water for 15 s. The surface-sterilised dead larvae were placed on sterile paper towels under a laminar flow cabinet for air drying. The dried dead larvae were plated onto Petri dishes that contained a *B. bassiana* selective media[27] (Figure 2). The plates were kept in an incubator at 26–28 °C and monitored every 2–3 days. Fungi that appeared on the surface-sterilised larvae of *S. calamistis* were harvested and sub-cultured onto fresh PDA plates for pure culture and identification. After 15 days, the colonies were compared to the endophytic *B. bassiana* isolates that were initially inoculated onto the rice seed. The experiment was performed three times to confirm the pathogenicity of the *B. bassiana* isolates.

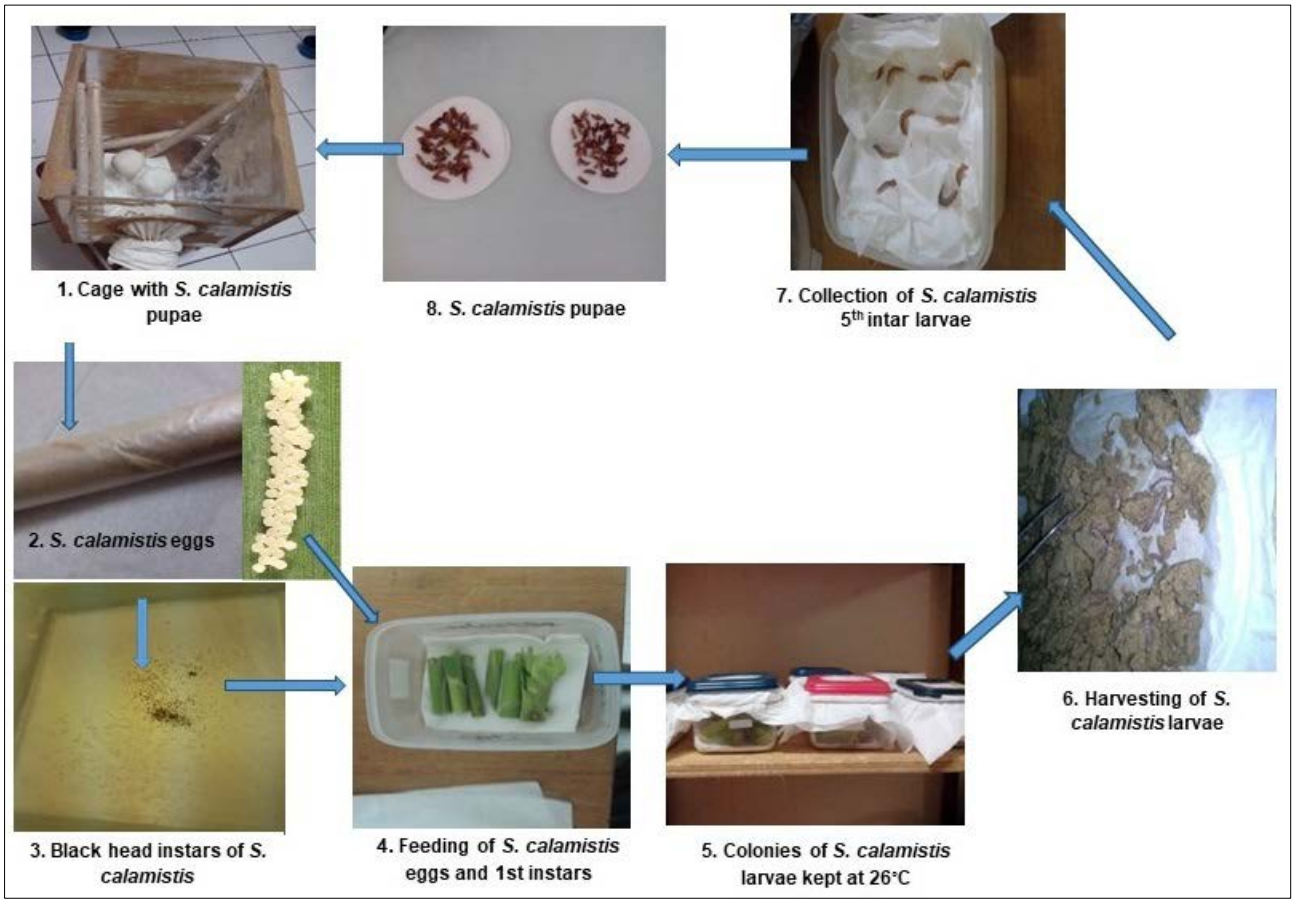

Figure 1: Steps in the artificial rearing of *Sesamia calamistis.*

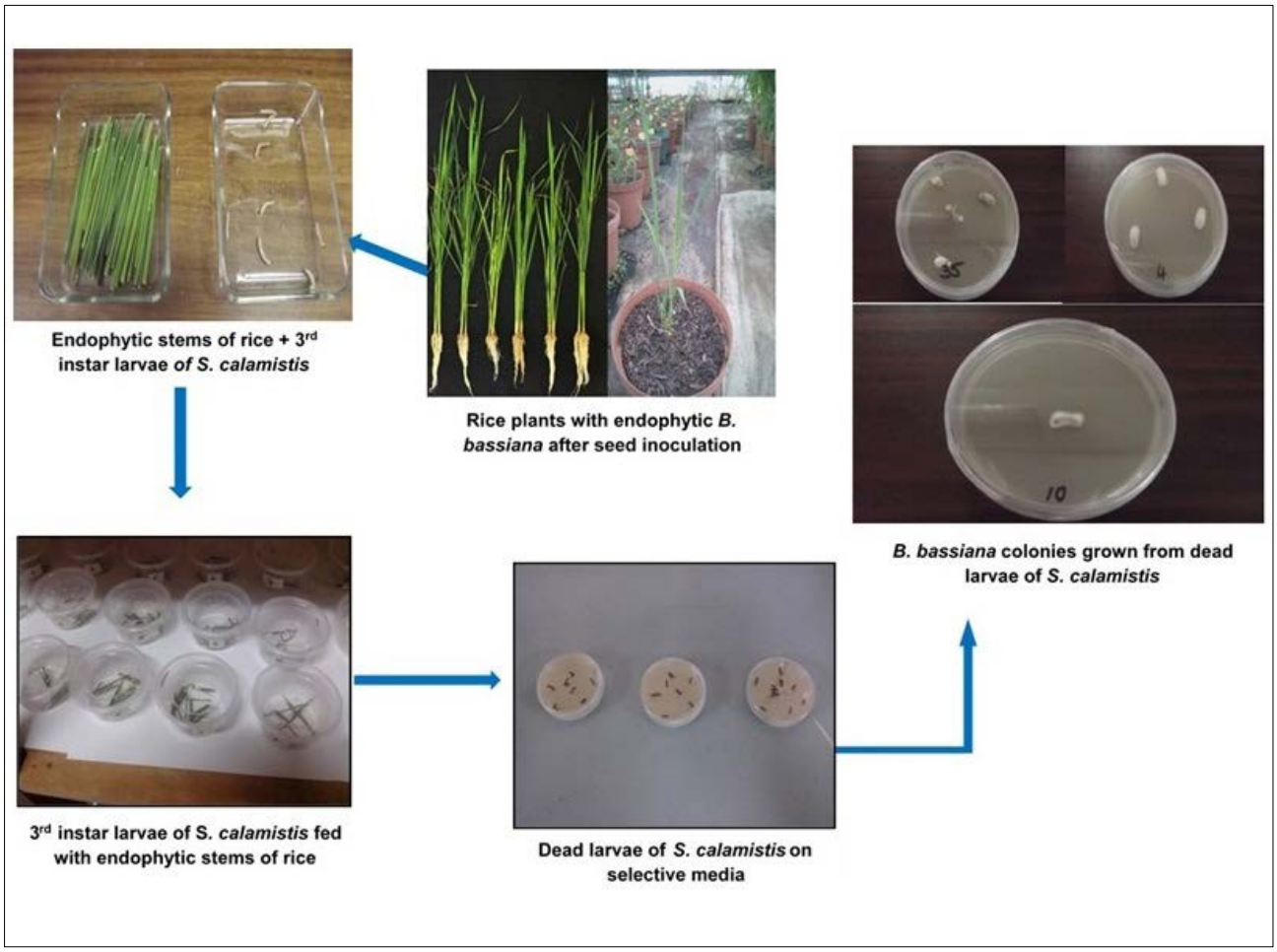

Figure 2: In-vivo screening of endophytic isolates of *Beauveria bassiana* for pathogenicity activity against third instar larvae of *Sesamia calamistis.*

Data analysis

Colonisation of the rice plant tissues resulting from the various treatment combinations was analysed using SAS (version 9.4). A general linear model was used for the analysis of variance (ANOVA). If the ANOVA F-test was significant ($p<0.05$), then treatment means were separated using the Duncan Multiple Range Test.

The cumulative percentage of mortality of *S. calamistis* was recorded, and the area under the mortality progress curve (AUMPC) was calculated. The data collected were analysed using GenStat (18th edition). A two-way ANOVA was run with *B. bassiana* isolates and time (days) as the main factors.

Results

The rice plants were colonised by *B. bassiana* isolates using both inoculation methods. The colonisation of each rice plant tissue (root, stem and leaf) was both isolate and inoculation method dependent and varied also with time (30–60 days) (Tables 1 and 2).

Following seed treatment, highly significant differences were observed in the colonisation of the tissues (roots, stem and leaves) x *B. bassiana* isolates ($p=0.0001$) and x rice cultivar ($p=0.0001$). At both 30 and 60 days for all interactions [(isolates x cultivars, isolates x time, cultivars x time and isolates x cultivars x time] there were highly significant differences in the colonisation of the roots and the leaves ($p=0.0001$). There was no interaction between strain x time ($p=0.32$) (Table 1).

After foliar sprays of inoculum, highly significant differences were observed in the levels of colonisation of the roots between *B. bassiana* isolates, rice cultivars, *B. bassiana* isolates x cultivars, *B. bassiana* isolates x time, cultivars x time, and *B. bassiana* isolates x cultivars x time ($p=0.0001$; Table 2). Highly significant differences were observed in colonisation of the leaves between *B. bassiana* isolates, cultivars, time (30–60 days), *B. bassiana* isolates x cultivars, *B. bassiana* isolates x time, cultivars x time, and *B. bassiana* isolates x cultivars x time ($p=0.0001$). In the stem, significant differences were observed between strains ($p=0.005$), time ($p=0.02$) and cultivars x time ($p=0.0018$). The interactions of *B. bassiana* isolates x cultivars, and *B. bassiana* isolates x cultivars x time were highly significant ($p=0.0001$ and $p=0.0002$, respectively). No colonisation by *B. bassiana* isolates was observed in the tissues of the control plants, with either inoculation method (Tables 1 and 2).

There were highly significant differences between the pathogenicity of the five *B. bassiana* isolates that were used against the third instar larvae of *S. calamistis* ($p=0.001$; Table 3). *B. bassiana* isolates Bb4 and Bb35 were the most effective strains, killing 93.3% and 76.6% of *S. calamistis* larvae at 28 days, respectively (Figure 3). The AUMPC data revealed that the *B. bassiana* isolates Bb4 vs Bb35, Bb35 vs Bb10 and Bb10 vs Bb3 showed similar levels of pathogenicity (Table 3).

Table 1: Evaluation of endophytic behaviour of *Beauveria bassiana* strains in rice plants following seed inoculation

B. bassiana isolate	Rice cultivar	Time (days)	No. of root sections colonised	No. of stem sections colonised	No. of leaf sections colonised
Bb3	NERICA1	30	6.0 a	6.0 a	4 b
Bb3	NERICA8	30	6.0 a	6.0 a	2 c
Bb3	NERICA.L.19	30	3.33 c	2 c	0 e
Bb4	NERICA1	30	4 b	4 b	2 c
Bb4	NERICA8	30	6.0 a	4 b	4 b
Bb4	NERICA.L.19	30	4 b	4 b	2 c
Bb10	NERICA1	30	6.0 a	6.0 a	2 c
Bb10	NERICA8	30	6.0 a	6.0 a	6.0 a
Bb10	NERICA.L.19	30	3 d	2 c	0.66 d
Bb21	NERICA1	30	6.0 a	6.0 a	0 e
Bb21	NERICA8	30	4 b	4 b	4 b
Bb21	NERICA.L.19	30	0 g	0 d	0 e
Bb35	NERICA1	30	6.0 a	6.0 a	6.0 a
Bb35	NERICA8	30	6.0 a	6.0 a	2 c
Bb35	NERICA.L.19	30	0 g	0 d	0 e
Bb3	NERICA1	60	6.0 a	6.0 a	2 c
Bb3	NERICA8	60	6.0 a	6.0 a	2 c
Bb3	NERICA.L.19	60	2 e	0 d	0 e
Bb4	NERICA1	60	6.0 a	6.0 a	2 c
Bb4	NERICA8	60	6.0 a	6.0 a	6.0 a
Bb4	NERICA.L.19	60	4 b	2 c	0 e
Bb10	NERICA1	60	2 e	2 c	0 e
Bb10	NERICA8	60	6.0 a	6.0 a	0 e
Bb10	NERICA.L.19	60	6.0 a	6.0 a	2 c
Bb21	NERICA1	60	6.0 a	6.0 a	2 c
Bb21	NERICA8	60	2 e	0 d	0 e
Bb21	NERICA.L.19	60	6.0 a	2 c	4 b
Bb35	NERICA1	60	6.0 a	6.0 a	2 c
Bb35	NERICA8	60	1 f	0 d	0 e
Bb35	NERICA.L.19	60	2 e	2 c	0 e
Control 1	NERICA1	60	0 g	0 d	0 e
Control 2	NERICA8	60	0 g	0 d	0 e
Control 3	NERICA.L.19	60	0 g	0 d	0 e

Effect	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value
Isolates	176.91	0.0001***	3.87	0.0054**	310.23	0.0001***
Cultivars	1050.54	0.0001***	9.95	0.0001**	2225.67	0.0001***
Time	1	0.3213 NS	5.26	0.0235*	1444.96	0.0001***
Isolate x cultivar	245.02	0.0001***	9.55	0.0001***	1129.84	0.0001***
Isolate x time	89.16	0.0001***	1.18	0.3213 NS	634.5	0.0001***
Cultivar x time	493.85	0.0001***	6.68	0.0018**	1264.83	0.0001***
Isolate x cultivar x time	252.87	0.0001***	4.18	0.0002**	1039.79	0.0001***
% CV	**4.74**		**46.41**			

*Means with the same letter are not significantly different (*p*=0.05) according to the Duncan Multiple Range Test.*

****Highly significant; **and *significant; NS, not significant; Bb,* B. bassiana *isolate*

Table 2: Evaluation of endophytic behaviour of *Beauveria bassiana* strains in rice plants using foliar sprays inoculation

B. bassiana isolate	Rice cultivar	Time (days)	No. of root sections colonised	No. of stem sections colonised	No. of leaf sections colonised
Bb3	NERICA1	30	0 g	6.0 a	6.0 a
Bb3	NERICA8	30	6.0 a	6.0 a	6.0 a
Bb3	NERICA.L.19	30	2 f	2 c	6.0 a
Bb4	NERICA1	30	0 g	0 d	2 c
Bb4	NERICA8	30	0 g	4 b	4 b
Bb4	NERICA.L.19	30	4 dc	4 b	6.0 a
Bb10	NERICA1	30	4 dc	4 b	4 b
Bb10	NERICA8	30	4 dc	6.0 a	6.0 a
Bb10	NERICA.L.19	30	0 g	4 b	6.0 a
Bb21	NERICA1	30	4 dc	4 b	4 b
Bb21	NERICA8	30	0 g	0 d	0 e
Bb21	NERICA.L.19	30	0 g	4 b	4 b
Bb35	NERICA1	30	2 f	6.0 a	6.0 a
Bb35	NERICA8	30	0 g	0 d	0 e
Bb35	NERICA.L.19	30	4 dc	4 b	4 b
Bb3	NERICA1	60	0 g	4 b	4 b
Bb3	NERICA8	60	2 f	6.0 a	6.0 a
Bb3	NERICA.L.19	60	2 f	6.0 a	6.0 a
Bb4	NERICA1	60	6.0 a	6.0 a	6.0 a
Bb4	NERICA8	60	5.33 b	6.0 a	6.0 a
Bb4	NERICA.L.19	60	4 dc	4 b	6.0 a
Bb10	NERICA1	60	4 dc	6.0 a	4 b
Bb10	NERICA8	60	3.33 e	6.0 a	6.0 a
Bb10	NERICA.L.19	60	4.33 c	6.0 a	6.0 a
Bb21	NERICA1	60	6.0 a	6.0 a	6.0 a
Bb21	NERICA8	60	0 g	0 d	0 e
Bb21	NERICA.L.19	60	0 g	6.0 a	1.33 d
Bb35	NERICA1	60	6.0 a	6.0 a	6.0 a
Bb35	NERICA8	60	0 g	0 d	0 e
Bb35	NERICA.L.19	60	3.66 d	4 b	2 c
Control 1	NERICA1	60	0 g	0 d	0 e
Control 2	NERICA8	60	0 g	0 d	0 e
Control 3	NERICA.L.19	60	0 g	0 d	0 e

Effect	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value
Isolates	209	0.0001***	3.87	0.0054**	3302.81	0.0001***
Cultivars	232.79	0.0001***	9.95	0.0001**	1683.04	0.0001***
Time	600.4	0.3213 NS	5.26	0.0235*	16.01	0.0002**
Isolate x cultivar	572.81	0.0001***	9.55	0.0001***	1998.31	0.0001***
Isolate x time	336.57	0.0001***	1.18	0.3213 NS	495.88	0.0001***
Cultivar x time	232.83	0.0001***	6.68	0.0018**	555.78	0.0001***
Isolate x cultivar x time	205.28	0.0001***	4.18	0.0002**	270.94	0.0001***
% CV	**8.28**		**46.41**		**2.44**	

Means with the same letter are not significantly different at p=*0.05 according to the Duncan Multiple Range Test.*

****Highly significant; **and *significant; NS, not significant; Bb,* B. bassiana *isolate*

Table 3: Evaluation of the pathogenicity of five *Beauveria bassiana* strains against *Sesamia calamistis*

B. bassiana isolate	AUMPC mean after 28 days	
Bb3	1540 c	
Bb4	793 a	
Bb10	1225 bc	
Bb21	2007 d	
Bb35	1108 ab	
Effect	***p*-value**	**Significance**
Isolates	0.001	**
Bb4 vs Bb35	0.06	NS
Bb4 vs Bb10	0.02	**
Bb4 vs Bb3	0.0005	***
Bb4 vs Bb21	0.0001	***
Bb35 vs Bb10	0.45	NS
Bb35 vs Bb3	0.02	**
Bb35 vs Bb21	0.0001	***
Bb10 vs Bb3	0.06	NS
Bb10 vs Bb21	0.0004	***
Bb3 vs Bb21	0.01	**
% CV	**13.6**	

Means with the same letter are not significantly different (p=0.05).
AUMPC, area under the mortality progress curve
****Highly significant; **significant; NS, not significant*

Discussion

Beauveria bassiana has been reported to colonise many plants as an endophyte[24-30], which supports the results of our study. Colonisation of plants by *B. bassiana* depends on the inoculation method, fungal isolate and plant species. Some isolates of *B. bassiana* were able to colonise maize plants via the epidermis, thereafter persisting in the plant throughout the entire growing season, and reducing tunnelling by *Ostrinia nubilalis* Hubner (Lepidoptera: Pyralidae).[31,32] As demonstrated in this study, *B. bassiana* can become established as an endophyte in rice when seeds or seedlings are inoculated with conidia of *B. bassiana* strains. Similar results have been demonstrated in other studies.[25,33,34] Successful *B. bassiana* colonisation of coffee leaves[21], banana roots[35] and maize plants[29] has been reported. These studies used inoculation techniques such as leaf injection, seed treatment, root drench and foliar sprays. All these techniques led to successful *B. bassiana* inoculation and colonisation.

The level of colonisation of the various plant tissues (leaf, root and stem) differed according to the *B. bassiana* isolates and the rice cultivars used in this study. The inoculation methods used conferred good colonisation of the rice stem by some of the *B. bassiana* isolates. Our study confirmed that there are several possible pathways to inoculation and recovery of *B. bassiana* from plant tissues.[36] Both inoculation methods (seed treatment and foliar spray) resulted in high levels of leaf and root colonisation. The inoculation method did not appear to favour a specific pattern of local colonisation of the rice cultivars. This is contrary to the results of Posada et al.[21] who reported that foliar sprays favoured leaf colonisation, whereas soil drenching favoured root colonisation in coffee. Similar findings were demonstrated for the common bean.[25]

The systemic spread of each *B. bassiana* isolate differed over the two sampling time periods (30 and 60 days) used in this study. A reduction in level of colonisation over time may have been caused by a host resistance response to the heterotrophic fungi or because of competition from other endophytes in the rice tissues.[23] The colonisation of the rice cultivars by the *B. bassiana* isolates did not cause any apparent negative effects on the growth of the rice plants, as was reported by Van Bael et al.[37]

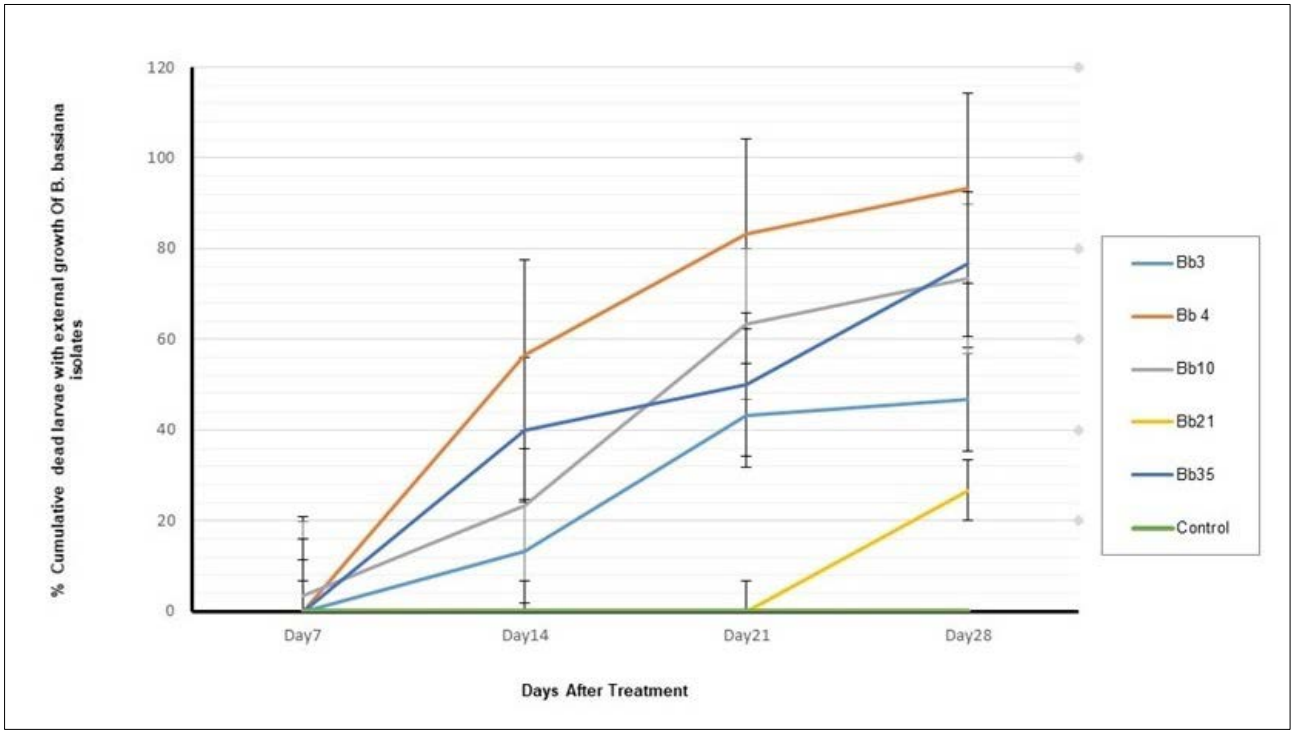

Figure 3: Cumulative mortality (%) of third instar larvae of *Sesamia calamistis* caused by five endophytic *Beauveria bassiana* isolates.

Gurulingappa et al.[38] reported that fungal isolates from different insect hosts possess varying degrees of virulence to the different insects. In this study, the five *B. bassiana* isolates showed differential pathogenicity against the third instar larvae of *S. calamistis*. Mortalities of 93.3% and 76.6% were achieved on third instar larvae of *S. calamistis* with two of the five selected *B*. *bassiana* strains used in this study. Similarly, a lower frequency of *S. calamistis* was recorded in *B. bassiana* treated maize plants compared to non-inoculated maize plants.[39]

Research from Valda et al.[40] and Godonou et al.[41] proved the effectiveness of *B. bassiana* strains on a diamondback moth population, *Plutella xylostella* L. (Lepidoptera: Plutellidae). Similarly, the survivorship and development of banana weevil larvae, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) were significantly affected by endophytic *B. bassiana* strains[35] as were the adult banana weevils as reported in Ghana.[41] Tesfaye et al.[42] also isolated different strains of *B. bassiana* and found that they caused mortalities greater than 75% of adults of *Myzus persicae* Sulzer (Homoptera: Aphididae). Bing and Lewis[31,32] found that 60% of *O. nubilalis* larvae collected from maize plants inoculated with *B. bassiana* were controlled by the fungus. A reduction in feeding is one of the reported altered behaviours by insects when infected by *B. bassiana*. For example, Tefera and Pringle[26] showed that there was a significant reduction in feeding by *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) as a result of 1–4 days inoculation with *B. bassiana*. The results reported in this study differ from that previously reported by Cherry et al.[39] The difference in the results may be due to the different strains of *B. bassiana* and inoculation methods used in the two studies. The results from this study therefore indicate that two of the five *B*. *bassiana* isolates used in this study have potential as biological control agents against *S*. *calamistis* in rice.

Conclusion

This study revealed that rice cultivars could be colonised by strains of *B. bassiana*. The five *B. bassiana* isolates tested in this study were endophytic with various degrees of colonisation and pathogenicity against the rice stem borer, *S. calamistis*. The results of this study indicate that two of the five tested *B. bassiana* isolates hold promise as biological control agents of rice stem borers. Further studies under field conditions at different sites and seasons are needed to ascertain the potential of these isolates. From this study, seed treatment seems to be the most appropriate and practical way to introduce the best *B*. *bassiana* strains during field studies. The field experiments will be implemented where rice is grown on a large scale using an experimental formulation of the best two *B*. *bassiana* strains.

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Competing interests

We declare that there are no competing interests.

Authors' contributions

W.B.A.B. wrote the initial manuscript, collected the samples, isolated the endophytic fungus, and performed all the morphological, in vitro and in vivo bioassays. A.T. provided guidance and the protocol for the rearing of the borer, *Sesamia calamistis*. M.D.L. and K.S.Y. provided student supervision, project leadership and management, acquired the funding, and edited the manuscript.

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INTERNATIONAL YEAR OF PLANT HEALTH 2020

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Application of plant extracts and *Trichoderma harzianum* for the management of tomato seedling damping-off caused by *Rhizoctonia solani*

Seedling production under smallholder farming systems can be negatively affected by both abiotic and biotic factors. Seedling damping-off caused by *Rhizoctonia solani* is one of the major biotic factors which causes significant yield reduction. Management is mainly based on the application of synthetic fungicides and cultural practices. However, both methods have limitations which result in their inefficiency. Several studies have reported on the use of plant extracts and biological control to manage plant diseases. The aim of this study was to formulate an effective and practical approach to manage tomato seedling damping-off using extracts of *Monsonia burkeana* and *Moringa oleifera* and a biological control agent *Trichoderma harzianum*. The efficacy of both extracts was investigated under laboratory conditions to determine the most suppressive concentration to *R. solani* growth. Methanolic extracts from both plants significantly suppressed pathogen growth at different concentrations. *M. burkeana* significantly reduced *R. solani* growth at 8 g/mL (71%) relative to control whilst *Moringa oleifera* extract reduced pathogen growth by 60% at a concentration of 6 g/mL. The highest suppressive concentrations were further evaluated under greenhouse conditions to test their efficacy on seedling damping-off. In damping-off treatments, both plant extracts and *T. harzianum* also significantly reduced ($p=0.5$) pre- and post-emergence damping-off incidence. *M. burkeana* recorded the highest suppression at 78%, followed by *M. oleifera* at 64%. *Trichoderma harzianum* reduced incidence of damping-off by 60% and this was higher than both plant extract treatments.

Significance:

- The use of *M. burkeana* and *M. oleifera* extracts and *T. harzianum* effectively suppressed pathogen growth and disease incidence and can be used to reduce the use of synthetic pesticides that are harmful to the environment and human health.
- Application of plant extracts and biological control agents as possible alternatives to synthetic fungicides is considered a sustainable and affordable practice for smallholder farmers.

Introduction

Vegetable production is a major farming activity for smallholder farmers in the Limpopo Province of South Africa as it contributes to food security and improved livelihoods for rural communities. Production can however be negatively impacted by both biotic and abiotic factors, with diseases causing major yield losses. High incidences of soil-borne diseases such as damping-off and root rot that occur during seedling stage can cause crop losses of 60–90%.[1] *Rhizoctonia solani* Kuhn is an important soil-borne fungal pathogen that is capable of causing diseases on a wide range of plants under favourable environmental conditions.[1] It is a facultative parasite that is very competitive against other soil-borne organisms.[2] Its survival in infected soils is mainly due to the formation of sclerotia for long-term survival without a host.[3] Germination of sclerotia which takes place in the presence of a susceptible host, results in the infection and spread of disease.[1] The tomato plant is highly susceptible to *R. solani* during different growth stages, with seedlings being particularly susceptible to attack by this pathogen.[4,5] At seedling stage, plants are more susceptible to *R. solani* infection due to reduced resistance mechanisms which normally emerge at the adult stage of plant growth.[3] Seedling blight, root and hypocotyl rots are typical symptoms of *R. solani* infection in highly susceptible plants, especially when planted under suitable environmental conditions.[2]

Management of damping-off is mainly through growth media treatment with chemicals or heat[6], seed treatment, use of cultural practices and planting of resistant cultivars[7]. However, all control measures have limitations which result in their inability to provide significant disease control. For example, the detrimental effect of soil fumigants on the environment and human health has resulted in their ban in agricultural production systems.[8] Also, development of resistant cultivars against damping-off has proven difficult due to the diversity of soil pathogens involved in seedling infection.[9] Seed treatment, on the other hand, is more efficient during seed germination[7] and this is normally lost during seedling stage[10]. For these reasons, there is a growing need to identify and develop new approaches for the control of *R. solani* damping-off based on the sustainable management of crops and application of environmentally friendly compounds, especially for smallholder farmers.

In recent years, alternative control measures such as plant-based bioactive compounds in the form of extracts have been studied and have provided promising results, especially against soil- and seed-borne diseases.[11-13] Plants produce compounds which have been shown to inhibit the growth and development of diseases caused by bacteria, fungi and other disease-causing organisms.[14,15] Plant extracts have the ability to induce defences in plants, resulting in an effective tolerance against pathogen attack.[16] Therefore, the presence of these antimicrobial compounds in plants provides an opportunity for their use in the management of pests and diseases as environmentally safe alternatives to synthetic pesticides.[17]

In previous studies, *Monsonia burkeana*, also known as special tea, and *Moringa oleifera* extracts displayed strong antimicrobial activity against Fusarium wilt of tomato and its causal agent *Fusarium oxysporum* f. sp. *lycopersici.*[11,18] Reports further show that both plants have high contents of secondary metabolites such as alkaloids, flavonoids, glycosides and many more which are responsible for pathogen suppression.[19-21] Despite promising application in the management of other soil-borne plant diseases, reports are still lacking on the application of both plants in the management of tomato seedling damping-off caused by *R. solani*. Both plants are also easily available and accessible to smallholder farmers in most rural communities in Limpopo Province.

Application of biological control agents such as *Trichoderma* spp. and *Bacillus* spp. have also been used successfully in the management of various plant diseases, especially those caused by soil-borne pathogens.[22,23] For example, *Trichoderma* spp. has been used as a seed treatment and soil inoculant to prevent pathogen establishment and suppress disease development in various crops[24], whilst *Bacillus* spp. has been used to control both soil-borne and foliage diseases[25]. Biological control agents employ various modes of action in the suppression and control of plant pathogens and these can include competition, antibiosis, hyperparasitism and many more.[24]

The objective of this study was to evaluate the efficacy of *M. burkeana* and *M. oleifera* extracts and the biocontrol agent *T. harzianum* in their ability to suppress *R. solani* induced seedling damping-off in tomato plants. Both plant extracts were applied as seed treatment to control pre-emergence damping-off and soil drenching to suppress post-emergence damping-off.

Materials and methods

Study location

The in-vitro and greenhouse experiments were conducted in the Plant Pathology Laboratory and the Green Biotechnologies Research Centre greenhouse of the University of Limpopo, South Africa, respectively. The average maximum/minimum temperatures in the greenhouse were 28/21 °C in summer, whereas in winter the average temperatures were 24/16 °C.

Fungal isolates preparation

Isolates of *R. solani* (PPRI 13845) and *Trichoderma harzianum* (PPRI 8230) used in this study were provided by the Mycology Division of the Agricultural Research Council – Plant Protection Research Institute (Pretoria, South Africa). *R. solani* was isolated from a diseased maize seedling showing characteristic symptoms of damping-off and the biological control agent *T. harzianum* used in this study was isolated from cabbage roots. Fungal isolates were maintained on potato dextrose agar (PDA; Lab-M Neogen Company) and stored at 4 °C. Before use, both fungal isolates were grown on PDA and incubated at ±25 °C for 7–8 days.

Plant collection and extract preparation

Healthy whole plants of *M. burkeana* (including roots, leaves, flowers, stems) and *M. oleifera* leaves were collected from the University of Limpopo experimental farm in Mankweng, Limpopo Province, South Africa (23°53'10" S, 29°44'15" E). A representative specimen for each plant was taken to the University of Limpopo herbarium for confirmation of identification before use. Collected plant materials were dried under shade for 7 days and then milled into a fine powder with a laboratory mill (Model FZ-102, Zhongxingweiye Instrument Ltd, China). *M. burkeana* and *M. oleifera* powders (100 g) were added separately to 700 mL methanol and placed on a rotary shaker for 24 h. Methanol was then evaporated on a rotary evaporator under reduced pressure at 64 °C. The obtained extract was oven dried for 21 days at 35 °C to constant weight, yielding a green solid suspension. Prepared plant extracts were kept at 4 °C until further use.

Effect of M. burkeana *and* M. oleifera *extracts on mycelial growth of* R. solani

Amounts of 0, 2, 4, 6, 8 and 10 g of the resultant suspension of each plant extract were separately dissolved in 10 mL sterile distilled water and thoroughly mixed before being added to 200 mL bottles and content containing PDA. Bottles were then autoclaved at 121 °C for 15 min. In the previous studies[11], heat was found to have no effect on the ability of both extracts to suppress pathogen growth. The extract amended PDA was poured into 80-mm Petri plates and left to solidify overnight. Disks of 5 mm in diameter were cut from 7-day-old actively growing *R. solani* cultures and were placed at the centre of extract-amended PDA Petri plates. Inoculated PDA Petri plates were then incubated at 25 °C under aseptic conditions for 7 days. Non-amended PDA plates served as control treatments. Pathogen colony growth diameter was measured using a transparent ruler[17] after ±7 days. Mycelia growth inhibition was calculated using the formula:

$$\text{Relative treatment effect (RTE)} = [(T/C) - 1] \times 100, \qquad \text{Equation 1}$$

where C is the average diameter of the fungal colony in control plates and T is the average diameter of the fungal colony in extracts-amended plates.

Greenhouse experiment

Fungal inoculation and treatment preparation

Rhizoctonia solani inoculum was prepared by soaking 240 g clean quartz in 75 mL of sterile, distilled water for 24 h in 500-mL Erlenmeyer flasks. Thereafter, 6.0 g yellow maize meal and 75 mL tomato juice were added to the flasks and autoclaved twice for two consecutive days. The autoclaved mixture was then inoculated with 20 discs of 7-day-old pure *R. solani* culture and incubated for 14 days at 25 °C. After incubation, the inoculum was oven dried at 30 °C for 14 days.

Tomato (cv. Money maker) was used as a test plant against *R. solani* seedling damping-off. Plant extracts were first tested for their effect on seed germination by soaking surface-sterilised tomato seeds in different concentrations of *M. burkeana* and *M. olifera* extract solutions used in the laboratory and then determining the number of germinated seeds. The plant extracts were then confirmed to have no effect on seed germination and were further used for the greenhouse experiment.

Plastic pots (250 mm in diameter) were filled with pasteurised sand and Hygromix in a 3:1 (v/v) ratio. Four holes, 80 mm deep and 50 mm wide, were made and the media was artificially inoculated with 20 g *R. solani* inoculum in each hole. Inoculated growth media was moistened with 200 mL sterile distilled water and left to stand for 7 days before planting to allow the pathogen to establish in the soil. After 7 days, five surface sterilised seeds were planted in each pot, followed with soil drenching with plant extract concentrations. Concentrations of plant extracts that displayed pathogen growth suppression[6] under in-vitro evaluation were used. These were 0.4, 0.6 and 0.8 g/mL for *M. burkeana* and 0.2, 0.4 and 0.6 g/mL for *M. oleifera*. Soil treatment with each plant extract solution was done once after 7 days of inoculation. Irrigation with tap water was applied once a week for 4 weeks. Each treatment was replicated four times and damping-off was determined by counting the number of non-geminated seeds and dead seedlings for pre- and post-emergence damping-off, respectively. Pathogen re-isolation was done from dead seedlings to confirm the presence of *R. solani*.

Biocontrol inoculum and treatment preparation

Trichoderma harzianum inoculum was prepared following the same procedure used for *R. solani* and received the same amount of dried biocontrol treatment 7 days after *R. solani* was established in the soil. Pots treated with *T. harzianum* and control treatment were irrigated with 100 mL tap water once every 2 days. Pre- and post-emergence damping-off were assessed and recorded as described:

$$\text{Pre-emergence damping-off (\%)} = \text{Number of diseased seeds / total number of seeds planted x 100} \qquad \text{Equation 2}$$

Post-emergence damping-off (%) = Number of diseased seedlings / total number of seedlings x 100 Equation 3

Efficacy of plant extracts of each treatment was evaluated as:

Relative treatment effect (RTE) = [(treatment / untreated control) –1] × 100, Equation 4

where reduction was expressed with a negative sign and stimulation or increase was expressed by a positive sign.

Data analysis

The experiment was laid down in a completely randomised design with six treatments and four replicates. Data were subjected to a partial ANOVA using SAS statistical program. Mean separation was achieved by using Fisher's least significant difference at a probability level of 5%. Mean suppression level (*y*-axis) and *M. burkeana* or *M. oleifera* concentration levels (*x*-axes) were subjected to the lines of the best fit using MS Excel v. 2016. The responses of mean suppression to increasing *M. burkeana* or *M. oleifera* concentration level were modelled by the regression curve estimations resulting in a quadratic equation: $Y=b_2x^2+b_1x+a$, where Y is mean suppression level and x is *M. burkeana* or *M. oleifera* concentration level using $x=-b_1/2b_2$ relation for the saturation point for each extract.

Results

Effect of M. burkeana *and* M. oleifera *extracts on mycelial growth of* R. solani

All tested concentrations of both *M. burkeana* and *M. oleifera* reduced mycelial growth of *R. solani* when compared to control (Table 1). *M. burkeana* displayed the highest mycelia growth inhibition at a concentration of 8 g/mL (Table 2; Figure 1). With *M. oleifera* treatments, the highest pathogen growth suppression was obtained at 6 g/mL (Table 2; Figure 2). The maximum growth inhibition was measured at 71% and 60% for both *M. burkeana* and *M. oleifera,* respectively (Table 2).

Table 1: In-vitro effect of *Monsonia burkeana* and *Moringa oleifera* plant extract concentrations for optimal mycelia growth suppression of *Rhizoctonia solani*

Plant extracts	Formula	R^2	*x*	*Y*	*p*
M. burkeana	$y = 1.0134x^2 – 16.234x + 88.179$	0.97	8.01	23.16	0.05
M. oleifera	$y = 1.1384x^2 – 13.513x + 88.321$	0.69	5.94	48.22	0.05

Table 2: Effect of *Monsonia burkeana* and *Moringa oleifera* on mycelia growth suppression of *Rhizoctonia solani in vitro*

Treatment (g/mL)	*M. burkeana*		*M. oleifera*	
	Mean (mm)	RTE (%)	Mean (mm)	RTE (%)
0	85[a]	–	85[a]	–
2	68[b]	–20	74[ab]	–13
4	34[c]	–60	52[cd]	–54
6	26[d]	–69	34[d]	–60
8	25[d]	–71	67[abc]	–21
10	27[d]	–68	63[bc]	–26

Means in the same column followed by the same letter were not significantly different (p>0.05) according to Fisher's least significant difference test.

RTE, relative treatment effect = (treatment/untreated control) × 100

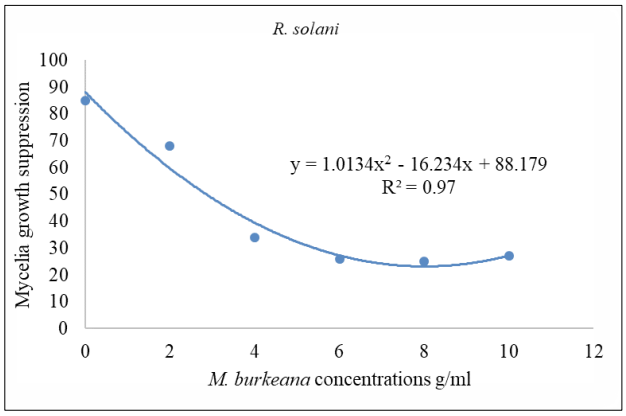

Figure 1: Quadratic relationship between mycelia growth of *Rhizoctonia solani* and *Monsonia burkeana* plant extract concentration.

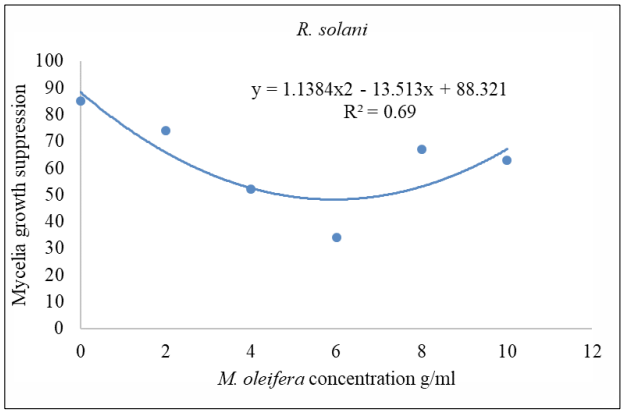

Figure 2: Quadratic relationship between percentage mycelia growth of *Rhizoctonia solani* and *Moringa oleifera* plant extract concentration.

Greenhouse experiment

Amendment of *R. solani* inoculated soil with different concentrations of *M. burkeana* and *M. oleifera* had a varying effect on damping-off incidences with treatments displaying low, moderate and high suppressive effect (Table 3 and Table 4). The same trend was also observed for *T. harzianum* treatments, in which the levels of suppression were different for both pre- and post-emergence damping-off. However, despite these variations, suppression was still higher than in the inoculated non-amended control. For example, at 0.6 g/mL, *M. burkeana* significantly reduced pre-emergence damping-off (78%), whilst the highest post-emergence damping-off reduction of 69% was recorded for 0.8 g/mL treatments. At a concentration of 0.4 g/mL, *M. burkeana* had no effect on damping-off incidence, resulting in high incidences of both pre- and post-emergence damping-off. Treating *R. solani* inoculated soil with *T. harzianum* also resulted in a relative reduction of pre-emergence (60%) and post-emergence damping-off (38%) (Table 3). However, reduction was lower when compared to *M. burkeana* treatment.

The three tested *M. oleifera* concentrations displayed a significant difference in their ability to reduce both pre- and post-emergence damping-off (Table 4). Treatment of infected soil with 0.2 g/mL of *M. oleifera* extract solution resulted in a significant reduction in both pre- and post-emergence damping-off incidence whilst 0.6 g/mL was only effective in reducing pre-emergence damping-off. Soil treatment with 0.4 g/mL had no effect on either pre- or post-emergence damping-off of tomato as there was no significant difference for this concentration compared with the non-amended control. A significant reduction in damping-off was also recorded where *R. solani* inoculated soil was amended with *T. harzianum,* resulting in 60% and 39% pre- and post-emergence damping-off reduction, respectively. To confirm differences between treatments, a relative treatment effect was also carried out

against the untreated control (Table 4). The results show that there was a significant difference amongst the concentrations of *M. oleifera* and biocontrol *T. harzianum* and their ability to reduce damping-off incidences. For example, although damping-off incidence in *T. harzianum* treated pots was slightly reduced with RTEs of 18% and 13% for pre- and post-emergence damping-off, respectively, these were still significantly higher than that for the control treatment. The RTE for *M. oleifera* treatments further shows that pre-emergence damping-off was reduced by 64% with a concentration of 0.2 g/mL, whilst post-emergence damping-off was reduced by 31% in 0.6 g/mL relative to untreated control (Table 4).

Table 3: Comparing the effect of *Monsonia burkeana* extract and *Trichoderma harzianum* on pre- and post-emergence damping-off caused by *Rhizoctonia solani* under greenhouse conditions

Treatment (g/mL)	Pre-emergence damping-off		Post-emergence damping-off	
	Mean	RTE (%)	Mean	RTE (%)
Untreated control	0.4^{ab}	–	0.7^{a}	–
0.4 g/mL	0.4^{ab}	-16	0.6^{a}	-7
0.6 g/mL	0.1^{c}	-78	0.5^{a}	-23
0.8 g/mL	0.4^{ab}	-14	0.2^{bc}	-69
T. harzianum	0.2^{bc}	-60	0.4^{b}	-38

Means in the same column followed by the same letter were not significantly different (p≥0.05) according to Fisher's least significant difference test.

RTE, relative treatment effect = (treatment/untreated control) × 100

Table 4: Comparing the effect of *Moringa oleifera* extract and *Trichoderma harzianum* on pre- and post-emergence damping-off caused by *Rhizoctonia solani* under greenhouse conditions

Treatment (g/mL)	Pre-emergence damping-off		Post-emergence damping-off	
	Mean	RTE (%)	Mean	RTE (%)
Untreated control	0.6^{a}	–	0.3^{b}	–
0.2 g/mL	0.2^{c}	-64	0.5^{a}	-21
0.4 g/mL	0.5^{a}	-12	0.4^{ab}	5
0.6 g/mL	0.3^{bc}	-51	0.3^{b}	-31
T. harzianum	0.5^{ab}	-18	0.4^{ab}	-13

Means in the same column followed by the same letter were not significantly different (p≥0.05) according to Fisher's least significant difference test.

RTE, relative treatment effect = (treatment/untreated control) × 100

Discussion

Monsonia burkeana and *M. oleifera* plant extracts separately reduced the incidence of damping-off under greenhouse conditions. *T. harzianum* as a biological control agent was also found to be effective in reducing damping-off in vivo. The efficacy of the treatments corroborates previous studies which demonstrated the ability of *M. burkeana*[21], *M. oleifera* and *T. harzianum*[26] to reduce the disease severity and disease incidence of fungal soil-borne diseases. Besides reducing disease severity, plant extracts have also been shown to increase shoot and root mass.[27]

Most medicinal plants – including *M. burkeana* and *M. oleifera* – contain a number of phytochemicals that exhibit antimicrobial activity.[12,17,23] Most of these phytochemicals include secondary metabolites and compounds such as flavonoids and tannins[19,28], which are the main antifungal components associated with disease suppression. Furthermore, these secondary metabolites form complexes with the polysaccharides and proteins associated with the external layer of fungal cells that might result in possible death of the pathogen.[10] However, additional work is necessary to determine the mode of action exhibited by the plant extracts on *R. solani*.

The effectiveness of *T. harzianum* might be due to a number of factors including competition, production of antifungal metabolites with fungicidal capabilities, toxic antibiotics and mycoparasitism.[26] The degree of reduction of damping-off by *T. harzianum* is possibly attributed to the secretion of antibiotics by the antagonist[10] or other inhibitory substances produced by the antagonistic chemical compounds such as geodin, terricin and terric acids.[29] For example, certain *Trichoderma* species colonise and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, which are considered to be part of the plant defence responses, which eventually lead to an induced systemic resistance in the entire plant.[26]

Although the main focus of the current study was on the effects of plant extracts and *T. harzianum* on disease incidence, it was also observed that the level of suppression differed between extracts. For example, *M. burkeana* extract was more effective in suppressing both pre- and post-emergence damping-off incidence than the extract obtained from *M. oleifera*. Despite their ability to suppress pathogen growth to reduce disease incidence, many reports have shown that this occurs to varying degrees and is mainly dependent on the plant species and its interaction with the pathogen at physiological and molecular levels. For example, a report by Hassanein et al.[30] indicated greater efficacy of neem (*Azadirachta indica*) extracts when compared to other extracts, probably due to different chemical compounds in neem that had greater antifungal activities. This phenomenon also applies to biocontrol agents, with reports showing that their degree of effectiveness varies according to the nature, quality and quantity of antibiotics or inhibitory substances secreted.[6]

Conclusion

The current findings demonstrate the effectiveness of both *M. burkeana* and *M. oleifera* extracts and *T. harzianum* in the management of soil-borne diseases in seedling production. The tested plant extracts are easily accessible to smallholder farmers, they are easy to process and are environmentally friendly; they can therefore be used as possible parts of an integrated control measure against seedling damping-off. In this study, both plant extracts and *T. harzianum* were applied separately; further studies on their combined application are recommended to determine their synergistic relationship. Further research is also recommended to determine the impact of plant extracts on the soil and rhizosphere microbiome, especially on beneficial microorganisms.

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Competing interests

We declare that there are no competing interests.

Authors' contributions

M.T.P.H.: Methodology; data collection and analysis; sample analysis; validation; writing. M.A.K.: Project leader; conceptualisation; student supervision; funding acquisition; assistance with data collection and analysis; writing and correction; final approval for submission. D.N.M.: Student co-supervision; data analysis; writing.

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INTERNATIONAL YEAR OF PLANT HEALTH 2020

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Benefits of maize resistance breeding and chemical control against northern leaf blight in smallholder farms in South Africa

Maize underpins food security in South Africa. An annual production of more than 10 million tons is a combination of the output of large-scale commercial farms plus an estimated 250 000 ha cultivated by smallholder farmers. Maize leaves are a rich source of nutrients for fungal pathogens. Farmers must limit leaf blighting by fungi to prevent sugars captured by photosynthesis being 'stolen' instead of filling the grain. This study aimed to fill the knowledge gap on the prevalence and impact of fungal foliar diseases in local smallholder maize fields. A survey with 1124 plant observations from diverse maize hybrids was conducted over three seasons from 2015 to 2017 in five farming communities in KwaZulu-Natal Province (Hlanganani, Ntabamhlophe, KwaNxamalala) and Eastern Cape Province (Bizana, Tabankulu). Northern leaf blight (NLB), common rust, Phaeosphaeria leaf spot, and grey leaf spot had overall disease incidences of 75%, 77%, 68% and 56%, respectively, indicating high disease pressure in smallholder farming environments. NLB had the highest disease severity (LSD test, $p<0.05$). A yield trial focused on NLB in KwaZulu-Natal showed that this disease reduced yields in the three most susceptible maize hybrids by 36%, 71% and 72%, respectively. Eighteen other hybrids in this trial did not show significant yield reductions due to NLB, which illustrates the progress made by local maize breeders in disease resistance breeding. This work highlights the risk to smallholder farmers of planting disease-susceptible varieties, and makes recommendations on how to exploit the advances of hybrid maize disease resistance breeding to develop farmer-preferred varieties for smallholder production.

Significance:

- Northern leaf blight, grey leaf spot, Phaeosphaeria leaf spot and common rust diseases were widespread in KwaZulu-Natal and Eastern Cape smallholder maize fields where fungicides were not applied.
- NLB was the most severe maize leaf disease overall.
- NLB caused maize leaf blighting, which reduced grain yields by 36–72% in susceptible maize hybrids.
- Maize resistance breeding has produced locally adapted hybrids that do not have significant yield losses under NLB disease pressure.

Introduction

Food security is at the forefront of global political and economic agendas with estimates that the food supply must double by 2050.[1] However, yield increases of the four major crops, including maize, are not on track to reach this target.[2] Pests and diseases are a significant threat to production. Global yield losses of maize due to these biotic factors was recently estimated to be 23%.[3]

In South Africa, maize is critical for food security as both a staple food (consumption of 86 kg/capita/year)[4] and a source of animal feed. The total annual maize production in the 2018/2019 season was 12 million tons.[4] Commercial farmers account for 96% of production with average yields of 4.2 ton/ha under mostly dryland conditions (2015 estimate).[5] In addition, rural communities throughout South Africa are dependent on maize for food security. Eastern Cape and KwaZulu-Natal Provinces have a high proportion of households (20%) that are involved in agricultural activities, underpinned by maize smallholder production.[6]

There are many factors contributing to the lower average yields of 1.5 ton/ha from maize smallholder farms.[5] Socio-economic factors are perhaps the most important, and these factors have an impact on the production constraints for smallholder farmers, which include access to fertilisers and pesticides.[7] Smallholder farmers growing maize for their own consumption often choose low input production practices (e.g. saved seed, minimal chemicals), as opposed to farmers growing maize on a larger scale for profit.[8]

Foliar diseases caused by fungi are a persistent challenge to maize production locally, especially in the wetter climes of KwaZulu-Natal and the Eastern Cape.[9] Grey leaf spot (GLS), northern leaf blight (NLB) and common rust (CR) are three of the main foliar diseases.[10,11] The trend towards minimum tillage to conserve soil quality has been touted as a reason for resurgence of GLS and NLB in recent years, because the fungi form structures that allow them to over-winter on maize stubble, creating fresh inoculum in spring.[12,13]

Grey leaf spot in South Africa is caused by the fungus *Cercospora zeina* Crous & U. Braun[9,13], which forms matchstick-like lesions parallel to maize leaf veins which are grey-brown in colour. There is some confusion in the literature because another fungal species, *Cercospora zeae-maydis* Tehon & E.Y. Daniels, also causes GLS with similar symptoms. *C. zeae-maydis* was first described in 1925 in the USA. It was only in the early 2000s that it was split into two sibling species, Type I and Type II, after which a morphological and molecular taxonomic study retained Type I as *C. zeae-maydis*, and named Type II as *C. zeina*.[14] Extensive survey work has shown no evidence

for *C. zeae-maydis* in South Africa[9,10], although some authors use this name, especially in publications prior to 2006.

Northern leaf blight, also known as northern corn leaf blight or Turcicum leaf blight, is a disease of both maize and sorghum caused by the fungus *Exserohilum turcicum* (Pass.) Leonard and Suggs.[11,15] This fungus also undergoes a sexual phase named *Setosphaeria turcica*, a rare form observed in the laboratory. Some publications refer to the fungus by this name. Foliar symptoms are characterised by cigar-shaped lesions with pointed ends which are not constrained by vein margins, and therefore are wider and larger than GLS lesions.[16] The fungi causing GLS and NLB appear to exploit different ecological niches in maize leaves with *C. zeina* entering through stomata and proliferating between cells[17], whereas *E. turcicum* colonises the xylem and uses this as a 'pathway' to move through the maize leaf[15].

Common rust of maize is caused by the basidiomycete fungus *Puccinia sorghi* Schwein. This disease was regarded as a minor problem in South Africa until 2004, when an increase in the incidence and severity of CR was observed.[18] *P. sorghi* is an obligate pathogen and therefore requires a living host plant for survival, requiring an alternate host to complete its life cycle, which is mostly fulfilled by weeds in the *Oxalis* genus in maize fields of South Africa.[18] GLS, NLB and CR are managed by farmers with fungicides when (1) conditions are highly conducive for disease and/or (2) host resistance in commercial hybrids is not sufficient.[19]

Phaeosphaeria leaf spot (PLS) is characterised by leaf symptoms that develop as white spots; however, its aetiology remains controversial as different authors have attributed the disease to either a fungus or a bacterium or both.[20] PLS disease development is similar to GLS and NLB in that lesions develop and reduce photosynthetic potential during grain filling, and it remains a resistance breeding target in South Africa.[21]

This study was initiated to fill the knowledge gap on the severity and impact of maize fungal foliar diseases in smallholder farms in higher rainfall regions of KwaZulu-Natal and Eastern Cape. The work was focused on demonstration plots of maize hybrids in rural communities in different agro-ecological areas of the two provinces to assess the level of current disease pressure over a 3-year period. NLB, CR, PLS and GLS were present at significant levels in all fields. NLB was found to have the highest disease severity. This led to an assessment of its impact on yield in a controlled field trial. NLB-susceptible hybrids had significant yield reductions, but other hybrids harboured sufficient genetic resistance to withstand NLB disease pressure.

Methods

Plant material

Field survey

Seed of maize hybrids from Pannar Seed (Pty) Ltd, a company in the Corteva Agriscience group of companies, was provided to selected farmers in community farming cooperatives in KwaZulu-Natal Province (Hlanganani, Ntabamhlophe, KwaNxamalala) and Eastern Cape Province (Bizana, Tabankulu) over three seasons (2014/2015; 2015/2016; 2016/2017). The GPS coordinates for the KwaZulu-Natal sites are recorded in Nsibo et al.[9] The Bizana and Tabankulu sites were at -30.892500; 29.843056 and -30.892750; 29.526972, respectively. Details of the agro-ecological zones of the sites are provided in Supplementary table 1. During the 2014/2015 and 2016/2017 growing seasons, maize was planted from the end of October until mid-December, but was planted later in the drier 2015/2016 growing season (from the end of November until the beginning of January). Comparisons of disease scores between different hybrids is not presented here and therefore hybrid codes are not provided for the field survey data. A total of 39 diverse hybrids were planted over the 3 years, but not all hybrids were planted at each site due to availability of seed. Support for land preparation, planting and fertiliser regimes was provided by Pannar Seed (Pty) Ltd. Each smallholder plot was planted to several maize hybrids with at least four replicate rows of each genotype and at least 20 plants per row with a plant spacing of 0.3 m and row spacing of 0.9 m. No fungicides were applied during the season. Standard dryland maize agronomic practices were followed.

Maize yield trial

The aim of this trial was to assess the impact of NLB disease on maize yields by comparing fungicide treated maize with untreated maize planted in a controlled field trial in a hotspot for NLB. A total of 21 maize hybrids (coded H1–H21) from different commercial sources were planted at Redgates Farm, Greytown, KwaZulu-Natal, South Africa on 12 January 2017 in a randomised block design. There were three replicates of each hybrid that were not sprayed with fungicide, and three replicates of each hybrid that were subject to a fungicide spray programme. Each hybrid within a treatment block was planted as two adjacent rows 4.4 m long with 0.76 m spacing between plants. Standard dryland maize agronomic practices were followed. The fungicide treatment was AMISTAR TOP® (Syngenta SA Pty (Ltd), Centurion, South Africa) at 500 mL/ha at 48 days after planting (dap) and ARTEA® (Syngenta SA Pty (Ltd), Centurion, South Africa) at 500 mL/ha at 68 dap. AMISTAR TOP® is a combination of azoxystrobin (strobilurin) and difenoconazole (triazole) active ingredients. ARTEA® contains two triazoles (propiconazole and cyproconazole). Grain yield (tons/ha) was evaluated at the end of the season by the method that adjusts for moisture content.[22]

Foliar disease assessments

Foliar diseases were quantified for both the field survey and the maize yield trial.

Field survey

Disease severity was scored on a per plant basis for the foliar diseases grey leaf spot (GLS), northern leaf blight (NLB), Phaeospaeria leaf spot (PLS) and common rust (CR). Disease data were obtained once per season for 12–16 plants per hybrid at each smallholder farm plot at the KwaZulu-Natal and Eastern Cape sites listed above. Plants separated by at least three plants in a row were selected for scoring. Disease severity scores for each of the four foliar diseases on the same plant were recorded at anthesis. The final disease severity data set was made up of 1124 plant observations per disease from the three seasons. Disease severity was scored using 1–9 scales adapted for each disease from the GLS scale described in Berger et al.[23] GLS, NLB, PLS severity scale: 1 = no disease lesions; 2 = a few lesions visible; 3 = lesions only below the earleaf; 4 = lesions visible on leaves just above earleaf; 5 = a few lesions visible on top leaves; 6 = many lesions visible on top leaves; 7 = half of maize leaf area diseased; 8 = three quarters of maize leaf area diseased; 9 = whole plant diseased. CR disease severity scale: 1 = no disease seen; 2 = a few rust pustules; 3 = several pustules visible; 4 = first rust band near base of leaf visible; 5 = first rust band with pustules on rest of leaf; 6 = second rust band visible closer to leaf tip; 7 = two rust bands clear with additional pustules; 8 = rust bands and pustules coalesce; 9 = leaves necrotic from rust. Disease incidence for each disease was quantified as the percentage of the 1124 plants (or a subset) that was positive for that disease.

Disease symptom identification was confirmed by isolation of the causal fungi as follows: (1) single spore isolations were made from GLS and NLB lesions; (2) conidial morphology was assessed by light microscopy (40X magnification), and (3) ITS (internal transcribed spacer) sequencing was conducted as described.[11,13] *Puccinia sorghi*, the causal agent of common rust is an obligate pathogen and cannot be cultured. Therefore, samples were collected directly from rust pustules for light microscopy (40X magnification), DNA extraction and ITS sequencing. The causal agent(s) of PLS have not been established unequivocally, therefore this disease was identified only by the distinct brown water soaked or white spot lesions on maize leaves.

Maize yield trial

Northern leaf blight disease severity of the 21 hybrids planted at Greytown was scored using the 1–9 scale described above on a per row basis for each of the treatment replicates separately at 2-weekly intervals

(82, 97 and 112 dap). These time points corresponded to late vegetative stage, anthesis and early reproductive stage of maize development. The NLB disease severity scores at the three time points for each treatment replicate were used to calculate the area under the disease progress curve (AUDPC) values.[24]

Statistical analysis

The field survey maize disease data for NLB, GLS, PLS and CR were subjected to an analysis of variance (ANOVA) using the general linear models procedure (PROC GLM) in SAS version 9.4 statistical software.[25] The ANOVAs were done on the original disease scores and on the ranks of the disease scores. Fisher's protected t-least significant differences (LSDs) were calculated ($\alpha=0.05$) to compare treatment means of significant effects on the original scores and Tukey's studentised range test on the ranks of the scores.[26]

The maize yield and NLB disease severity data from the field trial held at Greytown were analysed separately using a two-way ANOVA and a Tukey's honestly significant difference (HSD) multiple comparison test ($\alpha=0.05$), considering two factors (hybrid and fungicide treatment), their interaction and a (replicate) blocking factor. R version 3.5.1 was used for the ANOVA and post-hoc analysis, as well as for data visualisation using boxplots.

Results and discussion

Survey of maize foliar diseases

A maize foliar disease survey was carried out to determine the prevalence of four foliar diseases (GLS, NLB, PLS and CR) in smallholder farms over three seasons (2015–2017). Disease was scored from 1124 plants at on-farm demonstration plots at Hlanganani, Ntabamhlophe and KwaNxamalala (KwaZulu-Natal), and Bizana and Tabankulu (Eastern Cape). These sites are on average 100 km apart and represent different agro-ecological zones (described in Supplementary table 1).

All four diseases were present at all sites, and typical disease symptoms were obvious and readily scorable. Images of symptoms are shown within the bars in Figure 1. GLS was characterised by matchstick-like lesions parallel to leaf veins. NLB had larger cigar shaped lesions with pointed ends that were not confined to leaf veins. PLS had white spot lesions. CR had bands of pustules across the leaf blade that were reddish in colour. The fungus *C. zeina* was isolated from more than 100 GLS lesions tested.[9] The species identity was confirmed by the expected conidial morphology described previously[13], and ITS sequences matched the *C. zeina* type strain sequence (data not shown). The fungus *E. turcicum* was isolated from all 10 NLB lesions tested. Cultures had characteristic conidia with a hilum at one end[15], and ITS sequences matched the *E. turcicum* type strain (data not shown). *P. sorghi* teliospores were obtained from several rust pustules that were collected, and the ITS sequence confirmed the species identity (data not shown).

Overall disease incidence data from the 1124 plant observations for 2015–2017 indicated that NLB (75% incidence) and CR (77%) were the most prevalent, followed by PLS (68%) and GLS (56%)(Table 1). Multiple infections on the same plants were common (20% with all four diseases, up to 37% with three diseases, and up to 61% with two diseases; data not shown). As all four diseases were widespread, disease severity values were investigated in detail. The highest overall disease severity observed in the survey was caused by NLB (Figure 1). This was significantly greater than the overall disease severity values for PLS or CR (LSD, $p<0.05$; Figure 1). GLS showed the lowest disease severity in the field survey (Figure 1). Average disease severity values shown in Figure 1 (ranging from 2.0 to 2.7) were relatively low on the 1–9 scales. This is most likely due to the time of data collection prior to anthesis or during early anthesis when lesions were only present on lower leaves. Higher disease scores are given when lesions are present on upper leaves, which tends to occur as maize plants mature and allocate resources to reproduction (grain filling).[13] In addition, some of the hybrids may exhibit different levels of disease resistance.

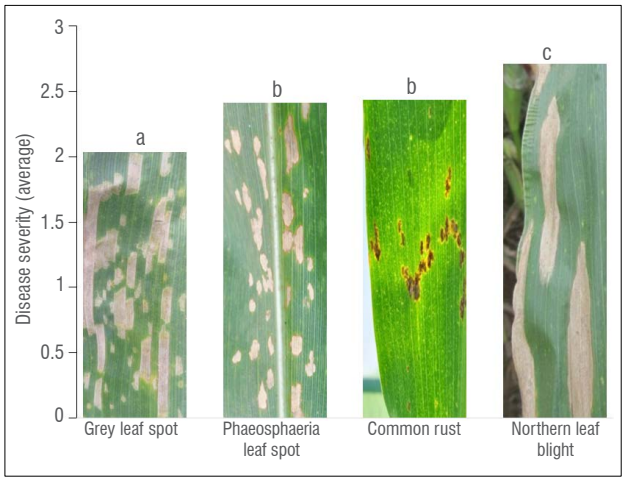


Figure 1: Severity of four maize foliar diseases in smallholder plots in KwaZulu-Natal and Eastern Cape Provinces. Disease severity data for maize grey leaf spot, Phaeosphaeria leaf spot, common rust, and northern leaf blight from maize field sites in KwaZulu-Natal (Hlanganani, Ntabamhlophe, KwaNxamalala) and Eastern Cape (Bizana, Tabankulu). Data presented are average disease severity (on a scale of 1–9) for each disease from 1124 plant observations made at anthesis in 2015, 2016 and 2017. Typical disease symptoms are shown within each bar. Disease severity values that are not significantly different from one another are denoted by the same letter (LSD=0.07; $p<0.05$).

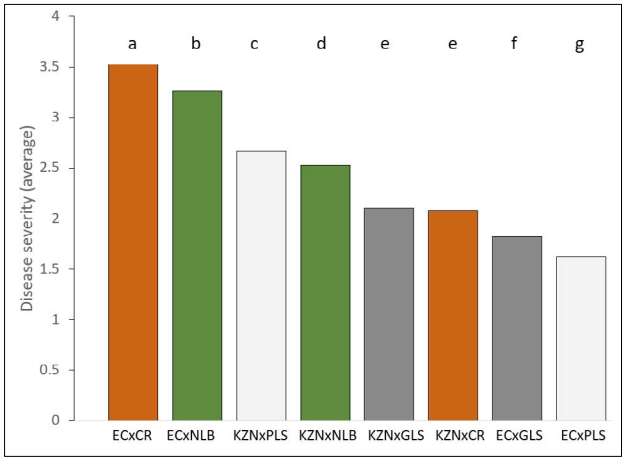
Table 1: Maize foliar disease incidence (%) at five smallholder sites† (2015–2017)

Year	Grey leaf spot	Phaeosphaeria leaf spot	Common rust	Northern leaf blight
2015	71	62	90	79
2016	2	91	87	78
2017	64	65	67	72
Total (2015–2017)	56	68	77	75

†Hlanganani, Ntabamhlophe, KwaNxamalala (KwaZulu-Natal); Bizana, Tabankulu (Eastern Cape)

Seasonal variation in overall disease severity was observed with significantly lower foliar disease in the 2015/2016 season which experienced a drought ($p<0.05$). The average overall disease scores were 2.4, 2.0 and 2.5 for scores taken in March of each year (2015, 2016 and 2017, respectively). One of three major El Niño events in the Pacific Ocean since 1982 occurred in the 2015/2016 season, resulting in lower rainfall across southern Africa, including KwaZulu-Natal and Eastern Cape.[27] Indeed, KwaZulu-Natal had the worst drought in this season since 1921.[28] High humidity is required for optimal development of these diseases[12,16], and therefore less disease is consistent with the drought season of 2015/2016. Furthermore, the 2014/2015 season was also subject to drought[28], and therefore the ranking and significant disease differences between the seasons is consistent with rainfall levels. Interestingly, disease incidence did not vary greatly with season (Table 1), except for GLS which only had a 2% incidence in the 2016 drought season. This is consistent with the requirement for prolonged humidity for development of this disease.[12]

Northern leaf blight was consistently one of the top two diseases in both the Eastern Cape and KwaZulu-Natal (Figure 2). CR had significantly higher disease severity at the Eastern Cape sites than at the KwaZulu-Natal sites (Figure 2). The causal fungus *Puccinia sorghi* undergoes its sexual phase on *Oxalis* spp.[29], which are a common weed in maize fields in South Africa. The greater severity in the Eastern Cape may reflect less weed control in this province. The orange urediniospores on the underside of *Oxalis* leaves were evident in the fields during the disease survey; however, quantitative data are required to confirm a difference between provinces. PLS and GLS had greater disease severity in KwaZulu-Natal than the Eastern Cape (Figure 2). In the Eastern Cape, the more humid coastal site of Bizana had a significantly higher average GLS disease severity (2.26) than Tabankulu, a drier inland site (1.01) (data not shown).



EC, Eastern Cape; KZN, KwaZulu-Natal; GLS, grey leaf spot; PLS, Phaeosphaeria leaf spot; CR, common rust; NLB; northern leaf blight

Figure 2: Disease severity of four foliar maize diseases at the KwaZulu-Natal sites compared to the Eastern Cape sites. Average severity of each disease from KwaZulu-Natal (Hlanganani, Ntabamhlophe, KwaNxamalala) and the Eastern Cape (Bizana, Tabankulu) are shown. Data are from 1124 plant observations made at anthesis in 2015, 2016 and 2017. Disease severity values that are not significantly different from one another are denoted by the same letter (LSD=0.11; $p<0.05$).

Field trial to assess impact of NLB on maize yield

Grain yield is the main priority for maize farmers; therefore, it is important to ascertain the impact of diseases on yield under South African growing conditions. NLB was chosen for a controlled field trial based on the importance of this disease in smallholder plots from the disease survey (Figure 1), as well as its increasing prevalence throughout sub-Saharan Africa.[30] A site in Greytown which is a hotspot for NLB was chosen for a field trial in the 2016/2017 season in which 21 maize hybrids were planted to compare yield between (1) unsprayed plots which would develop NLB, and (2) foliar fungal disease-free plots that were treated with fungicides.

Natural inoculum levels of the fungal pathogen *E. turcicum* at the Greytown site were high and thus NLB disease development proceeded without any need for artificial inoculation (Supplementary figure 1). No other foliar diseases were evident during the course of the trial. NLB disease severity of each hybrid treatment was scored at three time points during the reproductive phase of maize development and represented as AUDPC units. An ANOVA of disease severity showed that there were highly significant treatment effects ($p<0.001$) due to hybrid, fungicide and hybrid X fungicide, but no effect of block (Table 2).

There was a range of NLB disease scores amongst the 21 hybrids in the unsprayed treatment, with the most susceptible hybrids (H1, H5 and H7) showing a three-fold greater average AUDPC disease score than the hybrids with the least disease (H9 and H17)(Figure 3a). All hybrids showed higher NLB disease on average in the unsprayed treatment (turquoise boxes) compared to their corresponding fungicide spray treatment (pink boxes), as illustrated by the boxplots in Figure 3a. Of the 21 hybrids, 14 showed significantly higher NLB disease in the unsprayed treatments ($p<0.05$)(Figure 3a). One anomaly was H18, which had similar average disease severity in treated and untreated samples. Observations during the field trial were that H18 harboured genetic resistance to NLB because lesions did not fully develop and were a reddish colour indicative of a resistant hypersensitive response which limits further spread of the fungus in the lesion.[31,32]

Table 2: Analysis of variance of factors affecting northern leaf blight disease severity in a field trial at Greytown, KwaZulu-Natal

Factor	d.f.	Sum of squares	Mean square	F-value	Pr(>F)	Significance
Hybrid	20	159 553	7 978	52	<2e-16	***
Fungicide	1	74 898	74 898	485	<2e-16	***
Block	4	1 116	279	2	0.14	
Hybrid × fungicide	20	8 449	422	3	8E-04	***
Residuals	80	12 347	154			

Pr(>F) is the probability that a random F-value can exceed the observed F-value for the null hypothesis that there is no effect on disease severity due to the factor.

****Pr(>F) < 0.001*

Factors that significantly affected grain yield of the hybrids in the Greytown trial were hybrid ($p<0.001$), hybrid X fungicide ($p<0.001$) and block ($p<0.01$) (Table 3). The maximum average yield attained in this field trial was 3.28 tons/ha (for H20 – fungicide sprayed) and the lowest yield was 0.77 tons/ha (for H7 – unsprayed)(Figure 3b). As can be seen in Figure 3b, most of the hybrids do not show a significant yield difference between fungicide sprayed (pink boxes) and unsprayed treatments (turquoise boxes). This is consistent with the ANOVA result that fungicide treatment was not a significant factor (Table 3). However, the factor hybrid X fungicide was significant (Table 3), indicating that some hybrids responded to chemical treatment. There were three hybrids that showed a large improvement in yield due to fungicide treatment, namely H5, H1 and H7 that showed yield differences of 37%, 71% and 72%, respectively (Figure 3b). The higher grain yields of the maize hybrids H1 and H7 were significantly different ($p<0.001$) (Figure 3b).

Table 3: Analysis of variance of factors affecting maize yield in a field trial at Greytown, KwaZulu-Natal

Factor	d.f.	Sum of squares	Mean square	F-value	Pr(>F)	Significance
Hybrid	20	33	1.7	9.1	2E-13	***
Fungicide	1	0	0.3	1.5	2E-01	
Block	4	3	0.7	3.6	9E-03	**
Hybrid × fungicide	20	20	1.0	5.5	2E-08	***
Residuals	80	15	0.2			

***Pr(>F) < 0.01; ***Pr(>F) < 0.001*

Taking the results of NLB disease severity (Figure 3a) and maize yield (Figure 3b) together, it can be seen that the three most susceptible hybrids (H1, H5 and H7) were the ones that had the highest yield gain due to fungicide treatment. We therefore conclude that in susceptible maize hybrids, infection with *E. turcicum* causing NLB can reduce yields in the field by 37–72%. These figures are consistent with 31–70% yield losses measured for sweetcorn hybrids in Florida and Illinois in the USA[33], and 40% yield losses of maize varieties in Tanzania[34].

A second observation was that for the remaining 18 hybrids there was no significant difference in yields between fungicide-treated and untreated plots (Figure 3b). Seven of these hybrids showed no significant difference in NLB disease between the treatments (H2, H4, H9, H12, H14, H17 and H18)(Figure 3a). The genetic background of these hybrids is proprietary information; however, a plausible explanation is that these hybrids carry genes for quantitative or qualitative resistance to NLB. In six of these hybrids, the average disease severity was lower with chemical control (Figure 3a), indicating partial resistance, possibly due to different combinations of quantitative resistance alleles. The seventh hybrid (H18), as indicated above, may carry a qualitative disease resistance gene.

The remaining 11 hybrids showed no significant yield differences with and without chemical control (H3, H6, H8, H10, H11, H13, H15, H16, H19, H20, H21; Figure 3b), but showed significantly greater NLB disease without chemical control (Figure 3a). They appear to compensate for lower photosynthetic potential from foliar disease lesions, resulting in sufficient grain filling. Alternatively, some of these hybrids may not have developed sufficient NLB disease to have had an effect on yield. This could be the case for H11, H13, H19 and H20 (Figure 3a) and is consistent with previous work in which sweetcorn plants with NLB disease below a certain threshold (25% in their case) did not show a significant yield loss.[33]

Conclusion

Our data have shown that the four foliar diseases NLB, GLS, PLS and CR are widespread in smallholder maize farms in the higher rainfall regions of KwaZulu-Natal and the Eastern Cape. In the absence of chemical control, disease pressure remained high over the 3-year period of the survey. Favourable environmental conditions for disease development are a major factor, as shown by significantly reduced disease in the drought season 2015/2016. NLB was the most severe disease in both provinces, indicating that this should be a priority target for management practices. Representative yield losses caused by NLB were quantified, and this quantification showed that planting of susceptible varieties can result in 36–72% loss of the grain crop. The yield trial also illustrated that NLB resistance breeding efforts have been successful, as a range of hybrids did not show a significant yield deficit under NLB disease pressure.

Farmer participatory surveys have indicated that for their own consumption, farmers prefer low input varieties that taste good, have yield stability under a range of stresses (including foliar diseases) and produce seed that can be saved.[8] To take advantage of the yield benefits and resistance breeding success of hybrid maize[23], four factors have to be considered: (1) minimising or subsidising the cost of seed and input costs; (2) paying attention to the local maize milling and taste preferences of communities; (3) developing regional disease and pest monitoring systems so that agricultural extension officers and farmers can respond effectively to disease outbreaks[35]; and (4) maintaining genetic diversity within smallholder farming systems by ensuring mixtures of genotypes[36].

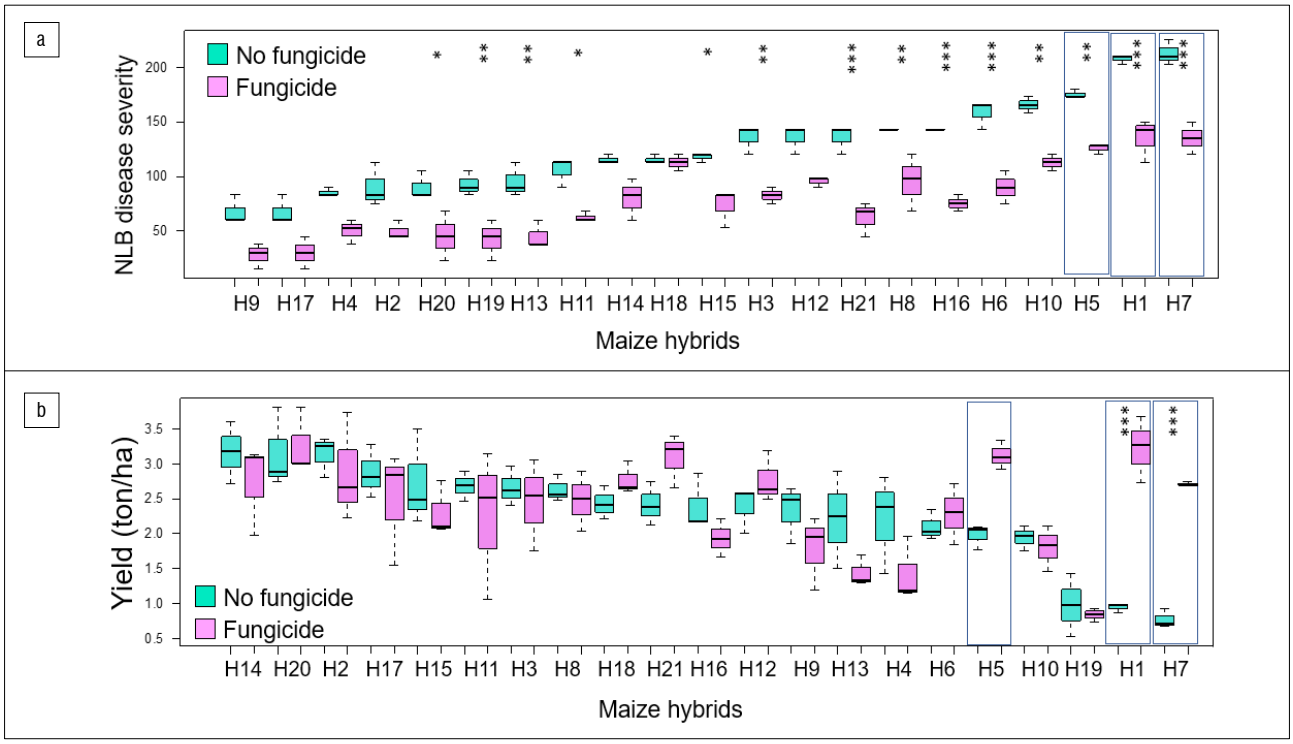


Figure 3: Northern leaf blight (NLB) disease severity and yield in the field trial at Greytown, KwaZulu-Natal. **(a)** Boxplots of NLB disease severity (area under the disease progress curve) for 21 maize hybrids that were either sprayed with fungicide (pink) or not sprayed (turquoise). Data of the three most susceptible hybrids (H1, H5, H7) are indicated with open boxes. Asterisks shown between pairs of boxes indicate significantly greater disease severity for each hybrid between unsprayed and sprayed plots (Tukey's HSD test following a two-way ANOVA; $p<0.05$ (*); $p<0.01$ (**); $p<0.001$ (***)). **(b)** Boxplots of maize yield (tons/ha) for 21 maize hybrids that were either sprayed with fungicide (pink) or not sprayed (turquoise). Open boxes indicate data of three hybrids (H5, H1, H7) that show yield reductions of 37%, 71% and 72%, respectively. Asterisks indicate significantly greater yields for each hybrid between sprayed and unsprayed plots (Tukey's HSD test following a two-way ANOVA; $p<0.001$ (***)). Maize hybrids are labelled as H1–H21 on the *x*-axis of each panel.

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Competing interests

We declare that there are no competing interests.

Authors' contributions

D.K.B.: Study conception, coordination and design; data analysis and interpretation; student supervision; funding acquisition; wrote the manuscript. T.A.S.A.: Study coordination and design; student supervision; edited the manuscript. K.d.R. and T.M.: Data collection and analysis. N.C.: Data analysis and interpretation.

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