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

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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/m³) 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)	Max. DDwet2[b]	Cumulative ascospores trapped[c]	PAT[a]										Final predicted PAT[a] values reached		
			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^{2g}	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/m³).*

[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).

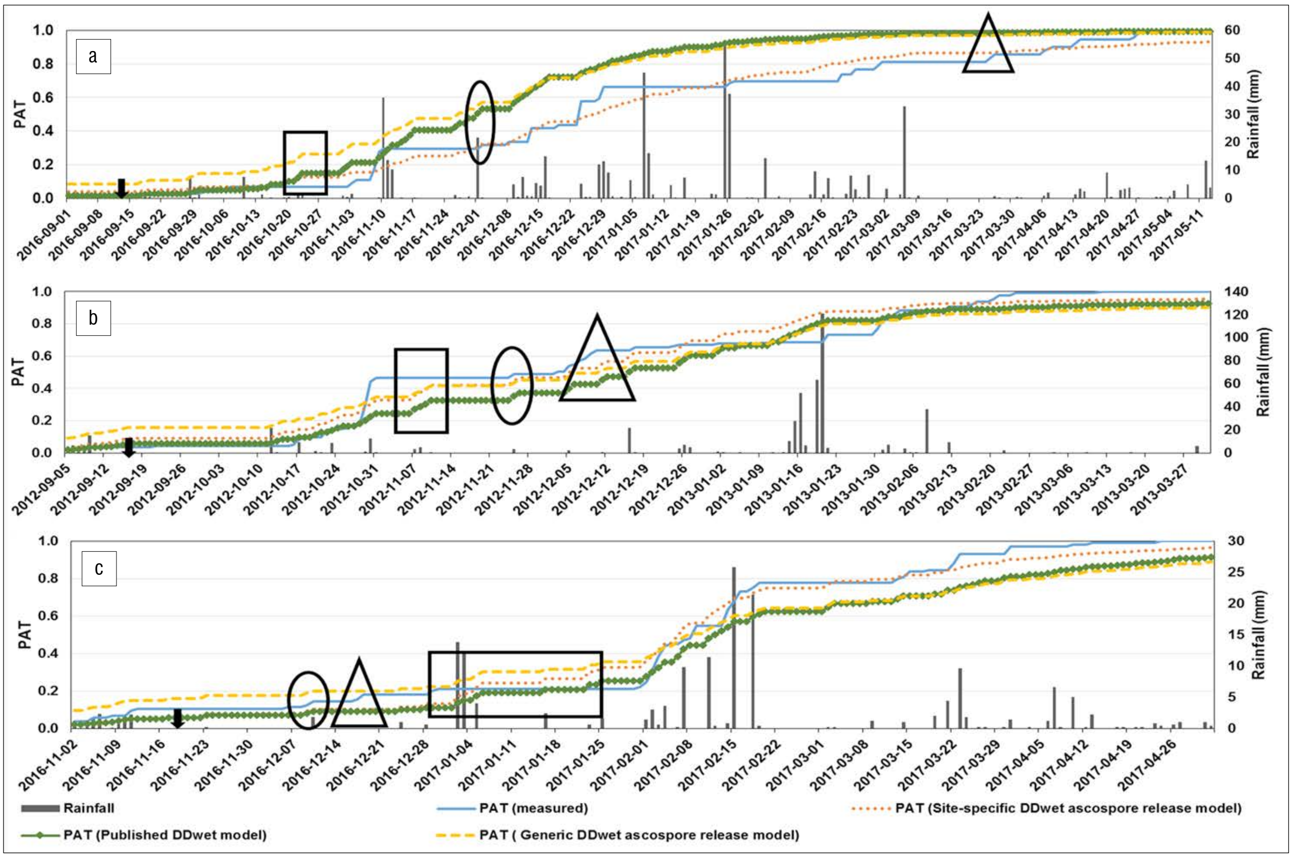

Figure 1: Observed cumulative proportion of airborne *Phyllosticta* ascospores trapped (measured PAT), the onset of ascospore release as predicted by the DDwet pseudothecium maturation model (black arrow) [P = exp(-exp(-(-3.131 + 0.007 × DDtemp - 0.007 × DDwet))), at $p = 0.5$], PAT predicted using the 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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