New Thesis Research Contributions to Plant Disease Epidemiology

Spatiotemporal Analysis of Epiphytotics of Downy Mildew of Oilseed Poppy in Tasmania, Australia

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ABSTRACT

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Downy mildew, caused by *Peronospora arborescens*, has become the major disease affecting oilseed poppy (*Papaver somniferum*) since its first record in Tasmania in 1996. Two field trials conducted in 2000 and 2001 studied the progression and spatial distribution of downy mildew epiphytotics. The logistic and exponential models best described the progression of disease incidence and severity, respectively. Incidence and severity increased rapidly following canopy closure. In 2001, incidence

increased from 0.16%, prior to canopy closure, to 100% at late flowering (40 days). Spatial analyses of epiphytotics were conducted by fitting the beta-binomial and binomial distributions, median runs analysis, and the spatial analysis by distance indices. All analyses demonstrated that the distribution of incidence and severity was strongly spatially aggregated from canopy closure until at least late flowering. These results suggest that secondary spread from a few primary infections is the major factor in epiphytotics.

Additional keyword: quantitative epidemiology.

Oilseed poppy (*Papaver somniferum* L.) is grown commercially in Tasmania, Australia, for the production of morphine, codeine, and thebaine, which are used in the production of a variety of medicines. The pharmaceutical poppy industry constitutes an important component of Tasmania's primary production, with approximately 21,000 ha grown per annum, and an export value of approximately US\$100 million.

Downy mildew of oilseed poppy is caused by the oomycete *Peronospora arborescens* (Berk.) Casp. (2). This disease has become a major concern to the Tasmanian poppy industry since its discovery in 1996 (1), and it is considered one of the most serious diseases of poppy worldwide (16). In wet and humid conditions, the fungus can spread rapidly throughout the crop, reducing the yield of opiates, and it has been shown by studies in Europe to have the potential to cause complete crop destruction (16). The disease is now prevalent in Tasmania, occurring in most crops each year, necessitating control strategies that are currently based on fungicides. Despite the importance of this pathogen, little is known about the epidemiology and how local conditions influence disease severity. *Peronospora arborescens* is known to reproduce both sexually in the form of oospores and asexually as conidia (16). At present, little is known about the relative importance of oospores as a primary inoculum source. Current understanding is primarily based on knowledge of downy mildew on other crop species.

Spatial and temporal analysis of epiphytotics can be used to develop hypotheses on the dynamics of epiphytotic development, including the importance of primary inoculum and the mecha-

nisms of pathogen dispersal. One approach to temporal analysis of epiphytotics is through fitting standard disease progress models to data (6). Spatial analysis techniques can be divided into two broad categories: point-pattern and correlation type groups of analyses (13). Distribution fitting, a point-pattern type analysis, is used to measure the heterogeneity of counts in sampling units, independent of the spatial structure of those sampling units. Median runs analysis (3), a correlation type analysis, assesses the relationship between the individual disease status of spatially referenced sampling units, although not directly utilizing the disease incidence or disease severity measurements. Spatial analysis by distance indices (SADIE) is a second correlation type analysis that differs from other correlation analyses in that it uses data in the form of point patterns and is therefore conditioned upon the inherent heterogeneity within a data set (10). Recently, an extension of SADIE that assesses spatiotemporal relationships was described (14). This methodology accounts for spatial association to assess the relationship between spatial patterns within a field at differing time periods.

The objectives of this work were to characterize the temporal dynamics of epiphytotics of poppy downy mildew, quantify the spatial pattern of disease at different time periods in the crop development, and assess the spatiotemporal association between time periods. This information will assist in selecting important time periods in crop development for the implementation of control strategies for the disease.

MATERIALS AND METHODS

Field trials. Field trials were established in the 2000/2001 (2000) and 2001/2002 (2001) growing seasons. The 2000 trial consisted of 50 plots (each 8 by 12 m) in a commercial field that received a single eradicated fungicide application 67 days after

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Fig. 1. Temporal change in **A**, incidence and **B**, proportion of total crop leaf area affected by downy mildew in 2000 and 2001. Arrows indicate the timing of fungicide application in 2000. Bars represent the standard error of the mean. Equations of curves for **A**, incidence are $y = -0.844 + 0.0179x$ in 2000 ($R^2 = 0.992$) and $y = 1/(1 + 3.58 \times 10^9 e^{-0.273x})$ in 2001 ($R^2 = 1.00$), and **B**, severity are $y = 4.45E - 4e^{0.0524x}$ in 2000 ($R^2 = 0.953$) and $y = 8.70 \times 10^{-7}e^{0.125x}$ in 2001 ($R^2 = 0.994$).

sowing (DAS). The 2001 trial consisted of 64 plots (each 5 by 5 m) that were not treated with fungicides. Field trials were sown on 26 and 14 September 2000 and 2001, respectively. A single leaf was sampled from the lowermost five nodes of 30 arbitrarily selected plants from each plot at six times during each season. Samples were collected at 53, 63, 73, 83, 93, and 103 DAS in 2000 and 45, 55, 65, 75, 85, and 95 DAS in 2001. Leaves were scored for severity of symptoms with percentage of leaf area diagrams. Leaves were incubated in plastic food containers over a wet tissue in the dark at 12°C and 100% relative humidity for at least 12 h. Downy mildew infection was confirmed by microscopic observation of sporulation (×50 magnification).

Disease progress. The disease progress within each trial was assessed by fitting the exponential, monomolecular, logistic Gompertz and linear epidemic models to the data and selecting the model of best fit (9). The linear form of each model was used to assess goodness-of-fit through regression to find the coefficient of determination (R^2), and visual comparison of the plots of the observed versus expected values. To determine the model of best fit, data were back-transformed, and the new R^2 values were compared (9).

Spatial analyses. Spatial analyses of epiphytotic data for each season were conducted using three separate analysis techniques: distribution fitting, median runs analysis, and SADIE. Spatial analyses were conducted only when the overall disease incidence, or severity, ranged between 5 and 95%. Spatial pattern of incidence was assessed at five time periods between 53 and 93 DAS in 2000 and between 75 and 85 DAS in 2001 (Fig. 1A). For disease severity, only 103 DAS in 2000 and 95 DAS in 2001 were analyzed.

The beta-binomial and binomial distributions were fitted to the available incidence data for each season to assess the heterogeneity using the beta-binomial distribution (BBD) software (7). A good fit of a data set to the binomial distribution was suggestive of random disease distribution. A good fit to the beta-binomial distribution was suggestive of overdispersion (spatial aggregation). The log-likelihood ratio statistic (LRS) was used to test for the distribution of best fit, under a null hypothesis of no significant difference between the two distributions (8). Rejection of the null hypothesis was evidence for the beta-binomial distribution (7). The index of dispersion, D , which is the ratio of the observed variance to the expected variance under the binomial distribution, was also calculated to provide a measure of the degree of aggregation (7).

Median runs analysis, an adaptation of ordinary runs analysis (3), was conducted on the available incidence and severity data for each season. A run was defined as a series of one or more plots with the same disease status bordered by plots with a different disease status (3). To adapt the data sets for median runs analysis,

TABLE 1. Spatial analyses of incidence and severity of downy mildew of oilseed poppy at intervals during the 2000 and 2001 seasons using spatial analysis by distance indices (SADIE), distribution fitting, and median runs analysis^a

Season	Data type	Days after sowing ^b	SADIE ^c		Distribution fitting ^d		Median runs ^e	
			I_a	J_a	LRS	D	Column runs	Row runs
2000	Incidence	53	0.71	0.999	-0.084	1.078	0.4408	0.4408
		63	2.24****	...	-22.04****	2.266	0.1429	-1.858**
		73	1.46*	...	-81.05****	3.930	-1.277*	-3.280****
		83	1.43*	1.05**	-159.3****	5.626	-2.135**	-4.425****
		93	1.55**	...	-59.92****	3.239	-2.071**	-3.280****
	Severity	103	1.74**	...	n.a.	n.a.	-3.001***	-3.572****
2001	Incidence	75	1.81**	1.08*	-110.5****	4.282	-2.533***	-1.182
		85	2.98****	...	-261.1****	6.444	-4.911****	-2.489***
	Severity	95	2.44****	1.32****	n.a.	n.a.	-5.198****	-2.458***

^a *, **, ***, and **** indicate the level of significance at $P < 0.1$, < 0.05 , < 0.01 , and < 0.001 , respectively.

^b Time periods in which the overall incidence (or severity) was < 5 or $> 95\%$ were not assessed.

^c I_a and J_a are the indices of aggregation under clustering and crowding, respectively, from SADIE; J_a , listed when one or less disease patches were present in a data set.

^d LRS is the log-likelihood ratio test statistic and D is the index of dispersion under distribution fitting; n.a. = not applicable as distribution fitting and indices of dispersion are only applicable to incidence (binary) data.

^e Column runs and row runs are the Z statistics of observed against expected in median runs analysis.

Fig. 2. Contour plots of spatial analysis by distance indices (SADIE) clustering indices for the 2000 season for **A**, incidence at 63 days after sowing (DAS), **B**, incidence at 73 DAS, **C**, incidence at 83 DAS, **D**, incidence at 93 DAS, and **E**, severity at 103 DAS. Gap clusters defined as areas with $v_i < 95\%$ of v_j values under randomization, where v_j is the clustering index of sampling units with values less than the field mean. Patch clusters defined as areas with clustering indices of $>95\%$ of v_i values under randomization, where v_i is the clustering index of sampling units with values greater than the field mean.

sampling units with values greater than the data set median were given the disease status 1 (3). All other sampling units were assigned a 0. The individual rows and columns of each data set were combined into a single row and column, respectively. Significance was tested using a standard Z test statistic to compare the observed number of runs with the expected number of runs under a null hypothesis of randomness. Observed runs significantly lower than expected runs suggested spatial aggregation (3).

The spatial distribution of incidence and severity data for each season were also analyzed using SADIE (10,11). SADIE uses a transportation algorithm to rearrange spatially referenced data and determine the shortest distances individual count units need to travel to achieve both “regular” and “crowded” spatial patterns, while maintaining the overall number of individuals (10). These distances are then summed for each pattern to give the overall “distance to regularity” and “distance to crowding.” Random reallocation of sampling unit values and recalculating distances to regularity and crowding are then used to find expected (mean) and percentile values based on frequency distribution of distances. Indices of aggregation are then calculated for both regularity (I_a) and crowding (J_a). I_a is equal to the ratio of the observed and expected distances to regularity. J_a is equal to the ratio of expected to observed distances to crowding. For both indices, values greater than one indicate spatial aggregation, less than one indicate regularity and equal to one indicate randomness (10). A one-sided test for aggregation was used. For all analyses, the maximum of 5,967 randomizations were used. To accommodate the requirement within SADIE for discrete integer values, disease severity (percent) data was transformed by multiplying by 10 and rounding to the nearest integer. The clustering indices were plotted in contour plots using SigmaPlot software (version 7.0, SPSS Inc., Chicago) to map patch and gap clusters within field. Clustering indices are a measure of the net distance needed to travel by individuals at each sampling unit to achieve regularity (11). Net movement of individuals out of a sampling unit, by definition those sampling units with counts greater than the field mean, are termed outflows (v_i), whereas net movement into a sampling unit, occurring when counts are less than the field mean, are termed inflows (v_j) and are negative in value. Patch clusters, areas of significantly high individual counts, were defined as areas with v_i greater than the 95th percentile of v_i from the randomizations (11). Gap clusters, areas of significantly low individual counts, were defined as areas with v_j less than the 95th percentile of v_j from randomizations (11). J_a was only used when one or less patch cluster was present in a given data set (10).

Spatiotemporal analysis. Spatiotemporal analysis in SADIE uses the assumption that once spatial pattern has been established for the individual time periods, the relationship between pairs of time periods can be analyzed by measuring their spatial association (14). Spatial association in SADIE first measures local association (χ_k) for a sampling unit by comparison of the clustering indices for each time. The overall spatial association (X) is the mean of the individual local associations. The significance of X is determined through the random reallocation of clustering indices among the sampling units, after correction for small-scale autocorrelation in either population (14).

In our work, spatial-temporal association was assessed between all time periods assessed for spatial pattern and between incidence and severity data sets for each season. In all cases, the maximum of 9,999 randomizations were conducted. A 2-tailed test was used with a null hypothesis of no association against the alternatives of spatiotemporal association (positive values of X) or spatiotemporal disassociation (negative values of X) (14).

RESULTS

Temporal analysis. In 2000, the proportion of plants infected by downy mildew (incidence) increased from 0.09 ± 0.003

($\pm$ standard error of mean) at 53 DAS to 0.99 ± 0.002 at 103 DAS (Fig. 1A). The proportion of leaf area affected (severity) increased from $0.005 \pm 8 \times 10^{-4}$ to 0.10 ± 0.002 (Fig. 1B). In 2001, no infection was observed at 45 DAS, whereas incidence increased from $0.002 \pm 9 \times 10^{-4}$ at 55 DAS to $0.998 \pm 8 \times 10^{-4}$ at 95 DAS (Fig. 1A), and severity increased from $4 \times 10^{-5} \pm 2 \times 10^{-5}$ to 0.12 ± 0.01 (Fig. 1B).

Temporal changes in incidence were best described by the linear disease model ($R^2 = 0.992$) in 2000 and the logistic model ($R^2 = 1.000$) in 2001 (Fig. 1A). For disease severity, in both

seasons the model of best fit was the exponential model with $R^2 = 0.953$ and 0.994 for 2000 and 2001, respectively (Fig. 1B).

Spatial analysis. Spatial aggregation was detected by all spatial analyses for all data sets tested, with the exception of disease incidence at 53 DAS in 2000 (Table 1).

Under distribution analysis for disease incidence in 2000, the beta-binomial distribution provided a significantly ($P < 0.001$) better fit than the binomial distribution for all assessments between 63 and 103 DAS (Table 1). At 53 DAS in 2000, there was no evidence to suggest that the beta-binomial distribution provided a better fit than the binomial distribution. Values of D increased from 1.1 at 53 DAS to a maximum of 5.6 at 83 DAS and then decreased to 3.2 at 93 DAS (Table 1). In 2001, disease incidence at 75 and 85 DAS was better described by the beta-binomial distribution than the binomial ($P < 0.001$). Values of D increased from 6.3 at 75 DAS to 6.4 at 85 DAS (Table 1).

Using median runs analysis, significant spatial aggregation was detected in incidence in 2000 along rows (running east to west) at 63 ($P < 0.05$), 73, 83, and 93 DAS ($P < 0.001$; Table 1). For incidence, significant aggregation along columns (running north to south) was detected at 73 ($P < 0.1$), 83, and 93 ($P < 0.05$) DAS. For severity, significant aggregation was detected both along rows ($P < 0.001$) and along columns ($P < 0.01$) at 103 days. In 2001, significant aggregation for incidence was detected along columns (north to south) at 75 ($P < 0.01$) and 85 DAS ($P < 0.001$), while significant ($P < 0.01$) aggregation along rows (east to west) was detected at 85 DAS (Table 1). For severity at 95 DAS, significant aggregation was detected along columns ($P < 0.001$) and along rows ($P < 0.01$). In 2000, the Z statistics for aggregation of incidence decreased from 0.44 (both along rows and along columns) at 53 DAS to minimums of -4.4 (along rows) and -2.14 (along columns) at 83 DAS, and then increased to -3.3 (along rows) and -2.07 (along columns) at 93 DAS (Table 1). In 2001, the Z statistics for aggregation of incidence decreased from -2.5 (along columns) and -1.2 (along rows) at 75 DAS to -4.9 (along columns) and -2.5 (along rows) at 85 DAS (Table 1).

Significant I_a values indicated that aggregation was obtained by SADIE analysis for incidence at 63, 73, 83 and 93 DAS and for severity at 103 DAS in 2000 (Table 1). In 2001, aggregation was indicated by I_a at 75 and 85 DAS for incidence and 95 DAS for severity. For incidence in 2000, I_a reached a maximum at 63 DAS (2.24; Table 1). For incidence in 2001, I_a increased from 1.81 at 75 DAS to 2.98 at 85 DAS (Table 1). The index of aggregation under crowding, J_a , was valid only at 53 and 83 DAS for incidence in 2000 and 75 DAS for incidence and 95 DAS for severity in 2001. In all these cases, J_a was in agreement with I_a (Table 1).

The spatial position of the disease foci was identified by plotting the clustering indices output from SADIE for each period in which spatial aggregation was detected (Figs. 2 and 3). For incidence at 63 DAS in 2000, patch clusters were situated at the eastern end of the trial site with a gap cluster at the western end (Fig.

Fig. 3. Contour plots of spatial analysis by distance indices (SADIE) clustering indices for the 2001 season for **A**, incidence at 75 days after sowing (DAS), **B**, incidence at 85 DAS, and **C**, severity at 95 DAS. Gap clusters defined as areas with $v_j < 95\%$ of v_j values under randomization, where v_j is the clustering index of sampling units with values less than the field mean. Patch clusters defined as areas with clustering indices of $>95\%$ of v_j values under randomization, where v_j is the clustering index of sampling units with values greater than the field mean.

TABLE 2. Spatiotemporal analysis of incidence and severity of downy mildew of oilseed poppy using spatial analysis by distance indices (SADIE) associations for 2000 and 2001 seasons^a

Season	Days after sowing	Incidence					Severity	
		63	73	83	85	93	95	103
2000								
Incidence	53	0.0592	-0.0159	-0.0579	...	0.1126	...	0.0108
Incidence	63	...	0.3842***	0.3428**	...	0.2392*	...	-0.1806
Incidence	73	...	...	0.4965****	...	0.5965****	...	0.2113
Incidence	83	...	...	...	...	0.6894****	...	0.2648
Incidence	93	...	...	...	...	...	...	0.5013***
2001								
Incidence	75	...	...	...	0.8462****	...	0.2773*	...
Incidence	85	...	...	...	...	...	0.4345***	...

^a Values are the overall level of association (X) for this test. *, **, ***, and **** indicate the level of significance at $P < 0.05$, <0.025 , <0.005 , and <0.0005 , respectively, according to the 2-tailed test.

2A). At 73, 83, and 93 DAS for incidence, patch clusters were present in the center of the trial site with a gap cluster at the western end (Fig. 2B, C, and D). For severity at 103 DAS in 2000, patch clusters were present in the center of the field with a gap cluster at the eastern end (Fig. 2E). For 2001, patch clusters were present in the northern and central areas of the trial site with gap clusters in the southeastern corner at all time periods for both incidence and severity (Fig. 3A, B, and C). At 95 DAS for severity, a second gap cluster was present at the western end of the trial site (Fig. 3C).

Spatiotemporal analysis. Strong significant spatial associations ($P < 0.005$) were detected between all spatially aggregated time periods and the successive time period in each season (Table 2). Significant spatial association was also detected between 63 and 83 DAS for incidence ($P < 0.025$), 63 and 93 DAS for incidence ($P < 0.05$), and 73 and 93 DAS for incidence ($P < 0.005$) time periods in 2000 (Table 2). Strongly significant spatial association was also detected between disease incidence at 93 DAS and disease severity at 103 DAS ($P < 0.005$). No significant spatial association was observed between the random spatial pattern of disease incidence at 53 DAS and any of the subsequent time periods in 2000. In 2001, significant spatial association was observed between 75 DAS for incidence and 95 DAS for severity ($P < 0.05$) and 85 DAS for incidence and 95 DAS for severity ($P < 0.005$; Table 2).

DISCUSSION

To our knowledge, this is the first record of the use of temporal and spatial analysis techniques to quantify the development of downy mildew epiphytotics in oilseed poppy.

Temporal analysis of disease development suggested that epiphytotics are best described by polycyclic disease models (6). Disease severity was best described by the exponential curve in both seasons. In 2001, temporal progression of disease incidence was defined by a typical logistic curve, whereas in 2000, a linear curve provided the best fit. This inconsistency between seasons may be attributed to the fungicide application at 67 DAS in 2000. Visual observation of the 2000 curve (Fig. 1A) suggests that the epiphytotic may have been in, or entering, the exponential phase of development when the fungicide was applied, with the resultant effect of suppressing disease spread. This change in disease dynamics delayed disease incidence reaching a maximum in the course of this study. Observation of the 2000 severity curve (Fig. 1B) also revealed a flattening of the curve after fungicide application.

With the exception of the linear increase in disease incidence in 2000, observation of the disease progress curves showed rapid development in downy mildew epiphytotics following canopy closure (63 and 65 DAS in 2000 and 2001, respectively). Incidence of downy mildew has also been closely correlated with the stage of crop growth in lettuce (15). This may be attributed to the microclimatic changes occurring in the crop at different growth stages. Canopy closure in poppies would result in increased humidity within the crop, allowing the development of greater levels of secondary inoculum.

The temporal results of this study also highlight the ability of the pathogen to propagate itself rapidly under favorable conditions. In uncontrolled conditions in 2001, disease incidence increased from 0.002 to 1.0 in a period of 40 days, a greater than 500-fold increase. This is consistent with the results obtained for onion downy mildew (*Peronospora destructor* [Berk.] Casp.), whereby incidence increased from 0.00 to 1.0 in approximately 43 days or four infection cycles (4).

In both seasons, all spatial analysis methodologies agreed when indicating either aggregated or random spatial patterns for all data sets assessed. Spatial aggregation was detected in assessments conducted after canopy closure. Prior to canopy closure in 2000, a

random spatial pattern was observed. This dynamic nature to spatial pattern has also been observed for other downy mildews, in which the early stages of epiphytotics exhibit random distribution or low levels of aggregation, increasing spatial aggregation until midepiphytotic, and then decreases in aggregation at high disease incidences (8,12). This same pattern was observed in *D* under distribution fitting and *Z* under median runs analysis in 2000 incidence data, but not in *I_a* with SADIE. The decrease in the level of aggregation detected by SADIE following the application of a fungicide control, in contrast to the results of median runs analysis and distribution fitting, suggested that SADIE may be more sensitive to aggregation than these other forms of analysis. In 2001, when no fungicide application occurred, all three analyses indicated increasing aggregation of disease incidence from 75 to 85 DAS.

Spatiotemporal analysis using SADIE demonstrated that following the development of spatial aggregation within a field, the spatial pattern of one time period was strongly associated with the spatial pattern of the previous time period. This strong association between spatial patterns suggested that new disease foci were not developing within the field over time or were of lesser importance to epiphytotic progress than those already established. This suggested that localized secondary spread, presumably in the form of conidia (16), is the predominant mechanism behind epiphytotic development.

The evidence collected in this study suggests that the predominant mechanism in epiphytotic development for downy mildew in oilseed poppy is localized secondary spread. However, microclimatic conditions, which are influenced by crop development, also play a role in regulating spread. Work is continuing in the development of a disease forecasting model based on the weather conditions favorable for the development of conidia, similar to the model "Downcast" developed for onion downy mildew (5). This may enable more strategic timing of fungicide applications in the field and improved disease control.

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