Spatiotemporal Spread of Cucurbit Downy Mildew in the Eastern United States

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ABSTRACT

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The dynamics of cucurbit downy mildew, caused by *Pseudoperonospora cubensis*, in the eastern United States in 2008 and 2009 were investigated based on disease records collected in 24 states as part of the Cucurbit downy mildew ipmPIPE monitoring program. The mean season-long rate of temporal disease progress across the 2 years was 1.4 new cases per day. Although cucurbit downy mildew was detected in mid-February and early March in southern Florida, the disease progressed slowly during the spring and early summer and did not enter its exponential phase until mid-June. The median nearest-neighbor distance of spread of new disease cases was ≈ 110 km in both years, with $\approx 15\%$ of the distances being >240 km. Considering disease epidemics on all cucurbits, the epidemic expanded at a rate of 9.2 and 10.5 km per day in 2008 and 2009, respectively. These rates of spatial spread are at the lower range of those reported for the annual spread of tobacco blue mold in the southeastern United States, a disease that is also aerially dispersed over long distances. These results suggest that regional spread of cucurbit downy

mildew may be limited by opportunities for establishment in the first half of the year, when fewer cucurbit hosts are available for infection. The *O*-ring statistic was used to determine the spatial pattern of cucurbit downy mildew outbreaks using complete spatial randomness as the null model for hypothesis testing. Disease outbreaks in both years were spatially aggregated and the extent of spatial dependence was up to 1,000 km. Results from the spatial analysis suggests that disease outbreaks in the Great Lakes and mid-Atlantic regions may be due to the spread of *P. cubensis* sporangia from outbreaks of the disease near the Georgia/South Carolina/North Carolina border rather than from overwintering sites in southern Florida. Space-time point pattern analysis indicated strong ($P < 0.001$) evidence for a space-time interaction and a space-time risk window of ≈ 3 to 5 months after first disease outbreak and 300 to 600 km was detected in both years. Results of this study support the hypothesis that infection of cucurbits by *P. cubensis* appears to be an outcome of a contagion process, and the relative large space-time window suggests that factors occurring on a large spatial scale ($\approx 1,000$ km) facilitate the spread of cucurbit downy mildew in the eastern United States.

Additional keywords: spatial point pattern analysis, space-time clustering.

Downy mildew of cucurbits, caused by *Pseudoperonospora cubensis*, is among the most damaging diseases of cucurbitaceous crops. Although up to 50 cucurbit species are known hosts of *P. cubensis* (25), *Cucumis sativus* (cucumber), *Cucumis melo* (cantaloupe and muskmelon), *Cucurbita pepo* (pumpkin and winter and summer squashes), and *Citrullus lanatus* (watermelon) are economically the most important host crops. The disease has a worldwide distribution but it is more severe in areas characterized by warm and humid weather that favors disease development (30), such as in the southeastern United States. In the absence of control measures, complete crop losses have been reported (20). Since the 1960s, resistance in *Cucumis sativus* had adequately controlled the disease in the United States, with minimal use of fungicides (20). However, in 2004, there was a resurgence of the disease on cucumber in the United States that devastated the cucumber crop in North Carolina, Virginia, Delaware, Maryland, and New Jersey, resulting in a 40% region-wide loss of the crop. For cucumber alone, the yield loss for the entire region in 2004 was estimated at \$20 million (20). In cucurbits, host plant resistance alone is no longer sufficient to control downy mildew (25). Thus, fungicides are now routinely applied in commercial cucurbit fields throughout the eastern United States to adequately control the disease.

Under favorable environmental conditions, sporangia of *P. cubensis* from diseased cucurbit crops in the southern United States can be dispersed aerially over long distances on a regional scale (27,28). The presumed lack of overwintering oospores for *P. cubensis* coupled with the sensitivity of cucurbitaceous plants to frost implies that the pathogen cannot survive in areas where temperatures during the winter are low enough to destroy cucurbit crops (21,43). As such, *P. cubensis* can only survive in frost-free southern areas of the continental United States ($<30^\circ$ latitude) in southern Florida and along the Gulf of Mexico, as active mycelium in cultivated or wild species of cucurbits (5,10). Thus, in areas $>30^\circ$ latitude, cucurbit downy mildew (CDM) epidemics will depend on the aerial dispersal of *P. cubensis* sporangia from subtropical overwintering sources (28) or protected greenhouse cultivation of cucurbit crops. However, quantitative information on the rates of temporal and spatial spread of the disease in the eastern United States is lacking. Although the disease occurs annually in the eastern United States, its spread from overwintering sites and, subsequently, when and where disease outbreaks will occur in areas $>30^\circ$ latitude is still the most uncertain within the framework of predicting an epidemic of the disease. This uncertainty is expected because regional disease spread tends to be curtailed more often by limited opportunities for pathogen establishment (i.e., absence of a susceptible host) than by atmospheric transport of inoculum (4). Further, although disease outbreaks in areas $>30^\circ$ latitude depend on seasonal dispersal of sporangia from overwintering sites, there is no evidence to support the hypothesis that disease outbreaks, for example, in the

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Great Lakes region are directly due to the spread of *P. cubensis* from overwintering sources in southern Florida.

Spatial pattern is a fundamental property of disease dynamics because it reflects the environmental forces acting on the spread and life cycle of a pathogen (35). In botanical epidemiology, spatial patterns have mainly been analyzed using quadrat counts and distance-based methods (8,26) on a limited spatial scale. Because plants do not move from one environment to another, they do not respond to some spatial average of the properties of the overall community but, rather, to their immediate neighborhood (44). Thus, although spatial pattern might be expected to contain information about the underlying processes, identical spatial patterns may be generated by several substantially different processes (46). One approach for inferring underlying processes from spatial patterns is to contrast measured patterns with null models derived from specific assumptions about the underlying process (39,47). A useful framework for doing this is provided by point pattern analysis, a set of tools for analyzing the spatial distribution of discrete points (e.g., infected plants or diseased fields mapped to Cartesian coordinates within a study area) (13,26).

Briefly, a point pattern is a realization of a process (17) and is simply a collection of points distributed in some region, R , which may be of any shape or dimension. Each point is defined by some set of coordinates and each such point can be referred to as an "event". In the simplest case, the event set will be made up solely of these locations. However, additional information is often attached to each event, and event sets labeled with such information are termed "marked" patterns (17). Thus, event sets typically take the form $\{x_i, y_i, m_i\}$, giving the locations (x_i, y_i) and the mark m_i (if included) in the region of observation (41). In a botanical epidemiology setting, (x_i, y_i) might represent the position of an infected plant or a field, and m_i might represent characteristics such as crop cultivar, species, or disease severity. Although often categorical, marks can be continuous, especially when representing temporal phenomena (e.g., time of disease onset or crop establishment). A fundamental property of every point process is its intensity $[\lambda(r)$ or $\lambda(x_i, y_i)]$ (i.e., the expected number of events per unit area at the point r or $[x_i, y_i]$). The simplest spatial process is that of complete spatial randomness (CSR). Spatial point patterns can be characterized in terms of their first-order and second-order properties, each being the focus of a different analysis. First-order properties are related to the mean number of events per unit area, while second-order properties are related to the (co)variance of the number of events per unit area. Thus, in the same way that the mean (μ) and variance (σ^2) are the first and second moments of a regular probability distribution, the intensity $[\lambda(r)]$ and covariance structure $[\kappa(r)]$ are the first and second moments, respectively, for a two-dimensional distribution (11). The first-order properties of a point pattern are characterized using kernel estimation, while the second-order properties are estimated using the K function (17,34).

Spatial point pattern methods based on kernel estimation and the K function have been used extensively in medical epidemiology to infer the underlying processes from patterns of a variety of human and animal diseases (14,23,37). In botanical epidemiology, however, these methods have only been applied more recently to plant pathosystems (15,18,24,26,33,40) and one contributing factor for this was the unavailability of a good software. The scale of interest in all these plant disease studies has been limited to either a field (15,18,33,40) or a state (24). One exception is the study of the invasive plant pathogen *Phytophthora ramorum* in the United Kingdom (49). In addition, except for the study by Xu et al. (49), the above plant disease studies examined a nonmarked approach in their spatial analysis. The marked approach gives better insight into the underlying biological processes than the nonmarked approach (32). For

example, the relative location of an infected plant (or field) with respect to its neighbors can always be associated with crop species, susceptibility to a disease, soil characteristics, or environmental conditions. Here, we apply a marked point pattern analysis to gain understanding of the processes that drive dynamics of CDM in the eastern half of the United States (henceforth referred to as the eastern United States) following the resurgence of the disease in 2004. Our working hypothesis was that disease outbreak within the eastern United States is a contagion process as a result of aerial transport of inoculum within the region. Thus, the objectives of this study were to (i) quantify the regional patterns of the temporal spread of CDM in the eastern United States, (ii) determine the distribution and rate of spread of downy mildew in the eastern United States, and (iii) determine the scale and extent to which outbreaks of CDM are spatially clustered in time and space.

MATERIALS AND METHODS

Data source. The CDM ipm-PIPE forecasting website was the source of the data used in this study (29). This database is part of the United States Department of Agriculture (USDA) Pest Information Platform for Extension and Education (PIPE) program. Records of the chronology of cases (i.e., confirmed outbreaks of CDM) were collected as part of the CDM sentinel and non-sentinel plot monitoring network, where registered users of the forecasting website reported to the website outbreaks of the disease during the cropping season. Records for 24 states (Table 1) in the eastern United States were obtained in 2008 and 2009. The 24 states were selected for detailed analysis because they appeared to form a contiguous epidemic unit based on the general pattern of disease progress in the 2 years (Fig. 1). The assumption of a contiguous epidemic unit is suggested by earlier reports on aerial transport of inoculum from the subtropical regions in the south to the northern Atlantic states (28).

When the database was accessed at the end of each growing season, 326 and 313 disease cases had been reported in 24 states in 2008 and 2009, respectively, and the following variables contained within the database were retained for analysis: (i) date of disease outbreak, (ii) state, (iii) county, (iv) host type (i.e., cucumber or other cucurbit species [watermelon, pumpkin, and squashes]), and (v) plot type that was classified as either a sentinel or nonsentinel plot. Sentinel plots were fixed plots, generally planted early and designated a priori for regular surveillance (at least once a week) within the disease monitoring network. Nonsentinel plots consisted of commercial fields, research plots, and home gardens. The customized "Reporting of Disease Outbreaks" section of the CDM ipmPIPE website was used to generate the latitude and longitude data associated with the location of each reported case. For reports where no geo-referenced data were available (<1% of the total), latitude and longitude data corresponding to the county centroid (extracted from U.S. Census Bureau 1990 Gazetteer Files) were used as the approximate geo-referenced data.

Temporal progress. The following four variables were related to the regional-scale temporal progress of CDM: (i) total number of cases, (ii) total number of affected counties, (iii) total duration of the epidemic, and (iv) season-long average rate of increase in disease prevalence. The total duration of the epidemic (days) was calculated as the difference in time between the first and the last observed case in each year, while the season-long average rate of increase in disease prevalence (disease cases per day) was calculated on a linear scale by dividing the total number of cases by the corresponding duration of the epidemic. These variables were considered separately for each state and for the 24 states combined. In addition, based on the corresponding epidemic durations, the rate of increase in disease prevalence was computed for all host plots and separately for (i) cucum-

ber, (ii) other cucurbit species, (iii) sentinel plots, and (iv) non-sentinel plots.

Spatial spread. The maximum spatial extent of the epidemic was calculated as the largest linear distance (in kilometers) between two cases that occurred in that year. These distance calculations were based on latitude and longitude information for each case. The rate of spatial spread (kilometers per day) was calculated by dividing the maximum spatial extent with the corresponding duration of the epidemic. In addition, we determined the nearest-neighbor distances (NND) of spread of new infections in each year (18). Computations of NND rested on the assumption that each new case originated from the closest previously recorded case, as long as the previously recorded case serving as inoculum source was at least 7 days old. The latter restriction was applied to account for an average latent period of 7 days (range 4 to 12 days depending on temperature and photoperiod) for CDM (43). Affected plots were assumed to remain infectious for 85 days because cucurbits can be infected at any growth stage and 85 days represent the average time from emergence to senescence (range is ≈ 60 to 120 days). Preliminary results showed that calculated distances of spread were relatively insensitive to the choice of the latent period and crop life span. All distance calculations were carried out using Excel 2003 software (Microsoft, Redmond, WA). The PROC UNIVARIATE procedure of SAS (version 9.2; SAS Institute, Cary, NC) was used to generate the frequency distributions of nearest-neighbor distances of spread for each year.

Spatial point pattern analysis. The marked point pattern analysis was used to establish the spatial pattern of CDM in the eastern United States. Here, the mark was the “cucurbit crop type” on which disease outbreak was reported, but time of disease outbreak, though not applied in this study, can also be used as the mark. Prior to the analysis, the latitude and longitude coordinates of locations of disease outbreaks were first transformed into plane coordinates with a universal transverse Mercator (UTM) projec-

tion in ArcInfo (version 9.3.1; Environmental Systems Research Institute, Redlands, CA). The UTM coordinates were then converted to kilometer coordinates for use in all subsequent statistical analyses.

The expected density of points at radius r around a randomly chosen point (in our case, around locations of CDM outbreaks) is a commonly used characterization of spatial point patterns. The pair-correlation function $g(r)$, viewed as a function of radius (spatial scale r), multiplied with the intensity λ of the pattern is known as the O -ring statistic, $O(r) = \lambda g(r)$ (47). The O -ring statistic is a neighborhood-density function and it is an appropriate method for detecting patterns across scales because it can isolate specific distance classes of interest, while cumulative statistical methods of point pattern analysis such as Ripley's K function (34) may confound effects at larger distances with ones at shorter distances (47). The mark-correlation coefficient function, $g_{12}(r)$, is the analogue of Ripley's $K_{12}(r)$ when replacing a circle of radius r with a ring (or annulus) of radius r . The O -ring statistic $O_{12}(r)$ gives the expected number of points of pattern 2 at distance r from an arbitrary point pattern 1 [i.e., $O_{12}(r) = \lambda_2 g_{12}(r)$]. In this context, pattern 1 refers to the pattern of the crop, plant, or tree, while pattern 2 refers to the pattern of the infected crop, plant, or tree (or simply the pattern of observed disease cases). Neither pattern needs to be random because randomness is only considered in hypothesis testing (null hypothesis). The correlation coefficient function, $g_{12}(r)$, is related to the Ripley's K function, $K_{12}(r)$, as follows: $g_{12}(r) = [(1/2\pi r) \times (dK_{12}[r]/dr)]$. For random patterns, $O_{12}(r) = \lambda_2$ whereas, for regular and aggregated patterns, $O_{12}(r) < \lambda_2$ and $O_{12}(r) > \lambda_2$, respectively.

The univariate O -ring statistic $O(r)$ is calculated by setting pattern 2 equal to pattern 1. By conditioning results for pattern 2 on pattern 1, one is adjusting for the heterogeneity of the spatial plane. For CSR, $O(r) = \lambda$, while $O(r) > \lambda$ and $O(r) < \lambda$ indicate an aggregated and regular pattern, respectively, at distance r . Based on point-to-point distances, the O -ring statistic describes clump-

TABLE 1. Summary statistics for the regional spread of cucurbit downy mildew (*Pseudoperonospora cubensis*) in the eastern United States in 2008 and 2009

State	2008					2009				
	No. of cases	No. of counties ^a	Duration (days) ^b	Cases per day ^c	Distance (km) ^d	No. of cases	No. of counties ^a	Duration (days) ^b	Cases per day ^c	Distance (km) ^d
Alabama	...	...	...	...	...	15	10	107	0.14	446
Connecticut	10	6	20	0.50	121	1	1	...	...	...
Delaware	8	3	61	0.13	117	10	3	56	0.18	114
Florida	38	13	207	0.18	680	20	8	200	0.10	697
Georgia	21	7	116	0.18	435	8	5	101	0.08	471
Illinois	...	...	...	...	...	9	6	78	0.12	306
Indiana	3	2	54	0.06	240	8	3	58	0.14	311
Kentucky	2	2	12	0.17	284	11	5	75	0.19	345
Louisiana	14	4	118	0.12	290	8	6	119	0.07	328
Massachusetts	5	2	29	0.17	10	5	2	71	0.07	15
Maryland	8	2	68	0.12	111	10	5	61	0.16	117
Michigan	26	15	64	0.41	235	35	21	57	0.61	354
Mississippi	3	2	91	0.03	159	7	6	106	0.07	398
North Carolina	48	11	78	0.62	441	45	20	76	0.59	555
New Jersey	9	2	79	0.11	138	7	4	49	0.14	130
New York	26	8	71	0.36	565	15	9	75	0.18	539
Ohio	22	8	58	0.38	301	16	11	62	0.26	320
Oklahoma	15	4	44	0.34	283	3	3	17	0.18	244
Pennsylvania	19	9	61	0.31	387	20	6	52	0.39	422
South Carolina	12	5	108	0.11	273	19	9	117	0.16	306
Tennessee	10	3	31	0.32	338	5	4	58	0.10	344
Texas	9	5	173	0.05	704	5	5	141	0.04	950
Virginia	17	5	70	0.24	171	16	10	83	0.19	324
Wisconsin	2	2	5	0.40	117	10	5	51	0.20	178
All 24 states	326	120	244	1.34	2,232	313	161	218	1.44	2,283

^a Number of counties affected.

^b Duration of epidemic: time between the first and last reported case.

^c Temporal progress, obtained by dividing the total number of cases by the corresponding duration of the epidemic.

^d Maximum distance between cases refers to the largest linear distance between two reported cases regardless of the time when the two cases occurred relative to each other.

ing [$O(r) > 1$] and regularity [$O(r) < 1$] at a given radius r using a simulated density, creating an upper and a lower confidence limit for the null model. Consequently, values of $O(r)$ above the upper confidence limit indicate significant aggregation, whereas $O(r)$ below the lower confidence limit indicates significant regularity. Values of $O(r)$ within the confidence interval cannot be distinguished from randomness and, thus, characterize spatially uncorrelated points at scale r . Significant departure from an underlying null model was tested by 999 Monte Carlo simulations of the null model. The highest and lowest values of these simulations represent $\approx 99.9\%$ upper and lower confidence limits, respectively, of the null model.

The calculation of the O -ring statistic involves a technical decision on the radius of the ring, r . Rings that are too narrow produce jagged plots because fewer points will fall into different distance classes. If the rings are too wide, then the O -ring statistic will not be able to isolate specific distance classes. In this study, we used a circular moving window with a fixed bandwidth $r = 6$ km to estimate $\lambda(x, y)$. The moving window with radius assumes a random distribution of disease outbreaks only within this window. The fixed bandwidth of $r = 6$ km was chosen based on the size of each cell of the grid. In this study, the cell size was 26 by 26 km and it is recommended that r be not more than half the width of the grid cell to avoid bias due to edge effects (47). Point pattern analysis of the spatial distribution of the disease outbreaks was performed using the software PROGRAMITA (47).

Additional information on the spatial pattern is also provided by the cumulative distribution function of the nearest neighbor distances y , $G(y)$, because point processes with identical $g(r)$ may have different $G(y)$ (13,45). The quantity $\lambda g(r)$ is the mean number of points located within a given distance r of each marked

point. However, the same mean [i.e., $\lambda g(r)$], may arise if many points have no neighbors, few points have many neighbors, or all points have more or less the same number of neighbors. Thus, the distribution of nearest-neighbor distances provides complementary information of how the number of points located within a given distance r of each point is distributed. The empirical $G(y)$ of the nearest-neighbor distances and the Monte Carlo simulations of the $G(y)$ with the 99% confidence limits under the null hypothesis of CSR and corresponding test statistics were generated using the SAS macro *ghatenv* (38).

Space-time clustering analysis. To determine whether there was a space-time interaction for the outbreaks of CDM, the approach of Diggle et al. (13) was followed, in which the hypothesis tested was that of complete spatiotemporal randomness. In the case of complete spatiotemporal randomness, the space-time K function, $K(r, t)$, should be the product of the separate K functions for space, $K(r)$, and time, $K(t)$. Thus, theoretically, under the null hypothesis of independence between time and space, we expect $K(r, t) = K(r) \times K(t)$. To examine the space-time interactions, one can use the function

$$\hat{D}_0(r, t) = \frac{\hat{K}(r, t) - \hat{K}(r) \cdot \hat{K}(t)}{\hat{K}(r) \cdot \hat{K}(t)}$$

Space-time interaction is indicated by observing peaks (positive or negative) on the surface of $\hat{D}_0(r, t)$, which are unitless when plotted over space and time (17). Space-time clustering implies more than just clustering in space and clustering in time but, specifically, that events that are relatively close in space are also relatively close in time.

Space-time clustering analysis was performed using the SPLANCS library (7) in R to test for the hypothesis of complete spatiotemporal randomness for CDM. Space-time interaction is determined to be present if the following are found to occur: (i) peaks on the surface of $\hat{D}_0(r, t)$ plotted over space and time, (ii) values of the standardized residuals > 2 units above or below the expected value of zero, and (iii) the test statistic from the Monte Carlo simulation is significant. To formally assess the significance of $\hat{D}_0(r, t)$, 999 Monte Carlo simulations created for the space and time K functions were used to calculate $\hat{D}_i(r, t)$ for $i = 1$ to 999. In this case, time (t) was considered the date when a new case of CDM was reported. The overall sum of $\hat{D}_0(r, t)$ over all r and t was compared with the frequency distribution of the sums of each $\hat{D}_i(r, t)$. If the overall sum of $\hat{D}_0(r, t)$ is $> 95\%$ of the simulated values, then there is evidence of overall space-time interaction (14). The same permuted $\hat{D}_0(r, t)$ were also used to create a three-dimensional prediction envelope for examining whether the observed peaks of $\hat{D}_0(r, t)$ were due to the underlying distribution of outbreaks of CDM or if the peaks might be indicative of further space-time interactions within the disease pattern. Values of $\hat{D}_0(r, t) > 0$ indicate the presence of a space-time interaction, and values of $\hat{D}_0(r, t) > 1$ indicate at least a doubling of the observed cases relative to the expected number of cases (37).

RESULTS

Extent of disease epidemic. The overall extent of the CDM epidemic in the eastern United States was greater in 2008 than in 2009. The total number of reported cases was 4% higher in 2008 than in 2009, while the elapsed time between the first and last reported case (i.e., duration of the epidemic) was 12% longer in 2008 than in 2009. The maximum linear distance between cases was slightly lower in 2008 than in 2009 (Table 1). For example, the maximum distance between affected counties was 2,232 km in 2008 (between Miami-Dade, FL and Columbia, WI) and 2,283 km in 2009 (between Miami-Dade, FL and Portage, WI). Despite the relatively larger number of cases in 2008 (326 versus

Fig. 1. Location of sites with confirmed outbreaks of cucurbit downy mildew, caused by *Pseudoperonospora cubensis*, in the eastern United States in 2008 and 2009.

313 in 2009), the longer duration of the epidemic (244 days versus 218 days in 2009) in that year resulted in a season-long average rate of temporal increase of 1.34 cases per day which was lower than the season-long rate of 1.44 cases per day observed in 2009 (Table 1). The season-long rate of temporal disease progress was higher in the northern-tier states (e.g., Connecticut, Michigan, North Carolina, Ohio, and Wisconsin) than in southern states (e.g., Alabama, Florida, Georgia, Louisiana, and South Carolina), and this was primarily due to the longer epidemic duration (>90 days) in the latter states (Table 1).

Based on reported cases of downy mildew in each state, the main differences between 2008 and 2009 were a lower number of cases in Florida, Georgia, New York, Ohio, Oklahoma, and Tennessee in 2009; the higher number of cases in Kentucky,

Michigan, South Carolina, and Wisconsin in 2009; and the reports of cases in Alabama and Illinois for the first time in 2009 (Table 1). In both 2008 and 2009, cases from North Carolina, Florida, and Michigan accounted for the majority (32 to 34%) of all the disease reports. Additional states that also dominated the disease reports were Georgia, New York, and Ohio (21% of total) in 2008 and South Carolina and Pennsylvania in 2009 (13% of total) (Table 1).

Temporal progress. In both years, epidemics of CDM started early in the year (in February or March) and progressed slowly (Fig. 2A) until June. Epidemics entered the “exponential” phase in late June in 2008 and early June in 2009, during which a rapid increase in the reports of number of disease cases was observed. After mid-July, the disease progress curves for both years were very similar. However, the epidemic leveled off much earlier in 2009 (early September) than in 2008, where the epidemic leveled off in early October. In 2008, the first case of the epidemic was on cucumber, whereas the first case in 2009 was reported on squash. Prior to the exponential phase of the epidemic in 2008, CDM was primarily reported on a cucurbit species other than cucumber, while the epidemic was mainly driven by infections on cucumber during the exponential phase until the end of the season (Fig. 2B and C). However, the entire epidemic of CDM in 2009 was primarily driven by disease outbreaks on cucurbit species other than cucumber (Fig. 2B and C).

In both years, disease outbreaks occurred sporadically until early June in both sentinel and nonsentinel plots, and then increased rapidly thereafter (Fig. 3). The first case of CDM was consistently reported from sentinel plots (Fig. 3A). The lag period

Fig. 2. Temporal progress of cucurbit downy mildew caused by *Pseudoperonospora cubensis* on **A**, all hosts, **B**, cucumber, and **C**, other cucurbits in 2008 and 2009 in the eastern United States. Other cucurbits are cantaloupe, watermelon, pumpkin, and squashes.

Fig. 3. Temporal progress of cucurbit downy mildew caused by *Pseudoperonospora cubensis* in **A**, cucurbit sentinel plots and **B**, nonsentinel cucurbit plots in 2008 and 2009 in the eastern United States.

between the first detection in sentinel plots and nonsentinel plots was 55 days in 2008 and 28 days in 2009. Disease increase in nonsentinel plots was very rapid as soon as the first case was reported from those plots (Fig. 3B). This rapid increase in disease prevalence in nonsentinel plots was also reflected in the average rates of temporal disease increase, particularly in 2009, which were higher for nonsentinel plots compared with sentinel plots (Table 2).

Spatial spread. The most common nearest-neighbor distance of spread between CDM cases was >0 to 30 km, followed by 30 to 60, 60 to 90, and 90 to 120 km (Fig. 4). The maximum nearest-neighbor distance was slightly larger in 2009 (879 km between Suwannee County, FL and Vermilion County, LA) than in 2008 (737 km between Charleston County, SC and Monroe County, MI). However, the calculated distances of spread within the 5th and 95th percentiles were reasonably comparable in both years (Table 3). For example, 10% of the distances were <10 and >200 km, while the median nearest-neighbor distance was ≈80 km (Table 3). In 2008, the largest linear distance (2,232 km) was between a case reported in Miami Dade County, FL and a case reported in Columbia County, WI whereas, in 2009, the largest linear distance (2,283 km) was between a case reported in Miami Dade County, FL and a case reported in Portage County, WI. The corresponding season-long average rate of CDM spread across the eastern United States was 9.2 and 10.5 km per day in 2008 and 2009, respectively (Table 2).

Spatial point pattern analysis. In both years, the spatial distribution of disease outbreaks showed significant ($P < 0.01$) aggregation relative to the distribution expected from the null hypothesis of complete spatial randomness (Fig. 5). The shapes of the O -ring statistics were very similar in both years and the observed univariate O -ring statistic was above the 95% upper limit for the null model distances of ≤1,000 km, indicating spatial aggregation up to this distance. Based on the univariate statistics, the intensity of the point process, λ , in 2008 was 0.065 (Fig. 5A), while a slightly lower intensity, $\lambda = 0.055$, was observed in 2009 (Fig. 5C). The higher λ in 2008 compared with 2009 was primarily due to the higher number of cases reported in 2008 compared with 2009. Bivariate O -ring statistics, $O_{12}(r)$, indicated that outbreaks of CDM on cucumber were also significantly ($P < 0.01$) spatially associated disease outbreaks reported on other cucurbit hosts in 2008 (Fig. 5B) and in 2009 (Fig. 5D). The shapes of the observed bivariate $O_{12}(r)$ and the extent to which spatial aggregation was evident were similar to those of the univariate process, except that the O -ring statistic and λ were lower for the bivariate process than for the univariate process. Thus, extent of spatial aggregation of disease outbreaks was insensitive to the cucurbit species on which the disease was reported.

In both years, the presence of a strong spatial aggregation was also apparent when plots of the cumulative distribution function of nearest-neighbor distance were examined (Fig. 6). Evidence for spatial aggregation up to a scale of 1,000 km is further supported by the significant ($P < 0.01$) departure from the null model of complete spatial randomness for nearest-neighbor

distances in 2008 (Fig. 6A and B) and 2009 (Fig. 6C and D). Further, the paired correlation function, $G(y)$, of the data was above the 95% upper confidence limit for the Monte Carlo simulation envelopes for the theoretical distribution function of nearest-neighbor distance under the null model of a complete spatial random process (Fig. 6B and D).

Space-time clustering. In both years, there was evidence for a space-time interaction for outbreaks of CDM (Fig. 7). In 2008, $\hat{D}_0(r, t)$ was -0.2 to 12.6 and the D_0 perspective plot showed a well-defined peak at distances ≈300 km and times of ≈3.5 months after the first disease outbreak in mid-February (Fig. 7A). In

Fig. 4. Frequency distribution of nearest-neighbor distances of spread of cucurbit downy mildew in the eastern United States in 2008 and 2009. Distance calculations are based on the assumption that each new case originated from the closest previously recorded case, with the restriction that the previously recorded case serving as an inoculum source was 7 to 85 days old. Not shown are two extreme values of 737 and 879 km recorded in 2008 and 2009, respectively.

TABLE 3. Quantiles for observed distribution of nearest-neighbor distances of spread of cucurbit downy mildew (*Pseudoperonospora cubensis*) in the eastern United States in 2008 and 2009

Percentile	Distance of spread (km) ^a	
	2008	2009
5	5.8	3.3
10	8.3	6.1
25	33.2	27.5
50	80.3	73.9
75	168.2	135.6
90	266.2	225.8
95	301.9	323.1

^a Nearest-neighbor distances are calculated based on the assumption that each new case originated from the closest previously reported case as long as the previously reported case serving as a source of inoculum was 7 to 85 days old.

TABLE 2. Average rate of temporal progress and spatial spread of cucurbit downy mildew (*Pseudoperonospora cubensis*) in the eastern United States in 2008 and 2009

Host/plot	No. of cases		Duration of epidemic (days)		Cases per day ^a		Spatial spread (km/day) ^b	
	2008	2009	2008	2009	2008	2009	2008	2009
Sentinel cucurbit plots	195	169	246	218	0.79	0.81	...	...
Nonsentinel cucurbit plots	131	144	211	179	0.62	0.85	...	...
Cucumber	167	133	244	181	0.68	0.78	...	...
Other cucurbit species	159	180	224	218	0.71	0.86	...	...
All hosts/plots	326	313	244	218	1.34	1.51	9.2	10.5

^a Rate of increase in disease prevalence, obtained by dividing the total number of cases by the corresponding duration of epidemic.

^b Rate calculated by dividing the maximum linear distance between two cases across the 24 states (Table 1) by the respective duration of the epidemic in each year.

2009, $\hat{D}_0(r, t)$ was -0.1 to 1.3 and the perspective plot indicated a defined peak at a distance of ≈ 600 km and 5 months after the initial disease outbreak in late March (Fig. 7B). Further, in both years, there were several instances where the standardized residual values were outside the range of ± 2.0 (data not shown), providing further evidence of space–time clustering. Results of the Monte Carlo simulation tests showed a significant ($P = 0.001$) test statistic for the observed space–time patterns in both years (Fig. 7C and D). In both years, the observed statistic, 14.35 and 9.01 for outbreaks in 2008 and 2009, respectively, was more extreme than the test statistic obtained from the simulations (Fig. 7C and D).

DISCUSSION

Dynamics of the early temporal and spatial spread of CDM in the eastern United States following the resurgence of the disease in 2004 were examined using disease records collected as part of the USDA's CDM ipmPIPE surveillance and monitoring program. The cucurbit ipmPIPE database was considered ideal for such epidemiological analyses because of its dense network of fixed-site sentinel plots and nonsentinel survey plots across the cucurbit-growing region in the eastern United States, the frequent and coordinated monitoring conducted in these plots, and the consistent methodology used to ascertain the presence of CDM (29). Understanding the early temporal and spatial dynamics of *Pseudoperonospora cubensis* is important for predicting spread and damage potential during the growing season (19). Results from this study indicate that early outbreaks of CDM are

aggregated in space and there is a specific space–time risk window associated with outbreaks of the disease. Further, the primary mechanism of disease spread appears to be a contagion process mostly likely due to aerial transport of sporangia.

The first case of CDM in both years was reported from early-planted sentinel plots. The lag period between the first detection in sentinel plots and that in nonsentinel plots was ≈ 2 months and 1 month in 2008 and 2009, respectively. Although this lag period was not consistent between years, it is still sufficiently long to serve as an early warning for commercial cucurbit fields within the region. Under favorable environmental conditions, CDM can spread very rapidly. Thus, the advance warning afforded by the cucurbit sentinel plots is especially important given that the increase in prevalence of the disease across nonsentinel plots (which includes commercial and research fields planted at regular times) was very rapid as soon as the first case was recorded in these plots. Disease onset in nonsentinel plots was similar in the 2 years (mid-April) but there was a slight delay of disease onset in sentinel plots in 2009 (late March) compared with 2008 (mid-February). This slight delay may have been related to the weather. For example, in the beginning of 2009, parts of southern Florida (where first reports occurred) were affected by a moderate to severe drought and lower temperatures that were less conducive for the cucurbit crop (1). Regardless of reasons for the delay in disease onset, the average rate of temporal progress of the prevalence of CDM in sentinel plots was similar in both years.

Even though CDM was detected in late winter in southern Florida, the spread of the disease northward across the eastern United State progressed slowly during the spring and early

Fig. 5. O-ring statistics and associated simulation envelopes for cucurbit downy mildew outbreaks in the eastern United States. Univariate statistics, $O(r)$, from spatial analysis of outbreaks of cucurbit downy mildew in **A**, 2008 and **C**, 2009 in the eastern United States. Bivariate statistics, $O_{12}(r)$, for disease outbreak in **B**, 2008 and **D**, 2009, where the pattern of outbreaks on cucumber is contrasted with that of other cucurbit species. Closed circles are the observed mean $O(r)$ or $O_{12}(r)$ for a ring of radius r with 6-km lags. The solid gray line is the intensity of the point pattern, while dashed lines represent the 99% confidence envelopes with 199 replicates about the null hypothesis of random spatial structure. Values of $O(r)$ or $O_{12}(r)$ above and below the simulation envelopes indicate significant clumping and regularity, respectively.

summer and did not enter its exponential phase until June. This long phase of relative inactivity despite the availability of inoculum may have been due to several factors and, in particular, to the limited host availability for infection by *P. cubensis*. Except in Florida, the spring crop of cucurbits is typically planted between April and June in the eastern United States if frost is not an issue (2). Thus, fewer cucurbits are available for infection prior to June. Indeed, the end of the planting phase in June coincided with the exponential increase of the disease in both years. It has been reported that regional spread of diseases caused by obligate pathogens tends to be curtailed more often by limited opportunities for pathogen establishment (i.e., absence of host tissue or unavailability of susceptible host) than by atmospheric transport of inoculum (4). Thus, the long phase of relative inactivity of CDM from the first detection in mid-February to the exponential phase in early June may be due to the limited opportunities for disease establishment because most of the cucurbits would not have been planted during this period. A similar observation has been reported for soybean rust in the southeastern United States, whereby the long lag phase from when the disease is first detected on kudzu in late winter until it enters its exponential phase in mid-June is due to the dieback of kudzu and reduced susceptibility of kudzu to *P. pachyrhizi* during this period (9).

The season-long average rate of spatial spread of CDM in the eastern United States was ≈ 10 km/day in both years. This rate of spread of the disease is at the lower end of the 9 to 18 km/day range reported for the annual spread of tobacco blue mold, caused by *Peronospora tabacina*, from the Caribbean Basin through the southeastern United States and along the eastern seaboard (4,12).

Similarly, the rate of spread of CDM reported in the present study is much lower than that reported either for wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici*, in the Mississippi Basin, which has a range of 16 to 76 km/day (4), or soybean rust, which has a range of 9 to 18 km/day (9). Different analytical techniques were used to calculate the rates of spread of these three diseases. For example, the rate of spread of tobacco blue mold is represented by disease-onset wave-front trajectories in distance-time space and the mean slope of the trajectory is the rate of spread of the disease front (4), whereas the rates of spread of soybean rust and CDM are based on the maximum linear distance between two cases and the duration of their respective epidemics. Despite the differences in the analytical procedures, direct comparison of these rates of spread is still useful. The higher rate of the spread for tobacco blue mold compared with CDM may be due to the seasonal northward advance in the planting (and, thus, availability) of a susceptible host (3,4). In the study by Aylor (4), planting date of tobacco in the eastern United States starts from February and continues until the end of June whereas, in the present study, the planting of the spring crop of cucurbits, excluding the planting in Florida, was from April until June (2). Thus, there is a much longer period available for effective blue mold establishment on tobacco than downy mildew on cucurbits. Because cucurbits and tobacco can be affected by CDM and blue mold, respectively, during most stages of plant development, the respective pathogens are expected to leap northward at approximately the same rate as new plantings of the hosts at successively higher latitudes as the season progresses (4). However, because cucurbits are planted much later than tobacco, the velocity of spread of CDM will be proportionately much

Fig. 6. Univariate analysis of pattern of outbreaks cucurbit downy mildew depicting the empirical cumulative density function [$G(y)$] of nearest-neighbor distances spread in **A**, 2008 and **C**, 2009. Dashed line is the simulated cumulative density function under the null hypothesis of complete spatial randomness. The Monte Carlo simulations under the null hypothesis of complete spatial randomness depicting the 99% upper and lower simulation envelopes of the nearest neighbor distances (dotted lines) against the observed $G(y)$ (solid line) in **B**, 2008 and **D**, 2009. Dashed line is the expected cumulative density function under the null hypothesis of complete spatial randomness.

slower than that of tobacco blue mold. The higher rate of spread of wheat stem rust compared with either CDM or tobacco blue mold may be explained by the ability of pigmented rust spores to better survive exposure to sunlight (4). For example, based on an exponential decay of spores with increasing cumulative solar radiation dose, the critical dose for less pigmented sporangia of *P. cubensis* is 9 MJ/m² (22), whereas that of more pigmented rust spores such as *U. appendiculatus* is 25 MJ/m² (3).

Although overwintering sources for *Pseudoperonospora cubensis* at <30° latitude in southern Florida provide inoculum for seasonal infection of cucurbits in areas at >30° latitude, there was no direct link of the inoculum sources in the south and infections occurring in the Great Lakes or mid-Atlantic states. Spatial point pattern analysis indicated that disease outbreaks in the eastern United States were aggregated regardless of the type of cucurbit crop, and the spatial extent of aggregation of disease outbreaks was ≈1,000 km in both years. This extent of spatial aggregation can be interpreted as the mean distance of dispersal of CDM (42). If inoculum originates from southern Florida, then a distance of 1,000 km is the linear distance between Miami Dade County and a location near the Georgia/South Carolina/North Carolina border. The linear distance between a county in central North Carolina and central Michigan is also ≈1,000 km. Thus, it is likely that overwintering sources in southern Florida contribute directly to CDM outbreaks from Florida up to North Carolina while disease outbreaks in the Great Lakes region and mid-Atlantic states are more likely due to infections around the Georgia/South Carolina/North Carolina border. It is also likely that infections

around the Great Lakes and mid-Atlantic states may be due to other sources of inoculum. For example, production of cucumber in year-round greenhouses in Michigan and Canada could be potential sources of inoculum during the season, particularly when disease control measures are either inadequate or not effective. In addition, a perennial host (*Bryonia dioica*) of *P. cubensis* was recently reported that could overwinter in temperate climates (36). Although *B. dioica*, which occurs naturally in Europe, has not been reported as a host of *P. cubensis*, laboratory studies show that it could also serve as an overwintering host for *P. cubensis*. It is not known how these alternative sources of inoculum could affect the epidemiology of the disease, and studies need to be conducted to determine the role of these other potential inoculum sources in the temporal and spatial spread of the disease.

Space-time analyses of CDM cases support the idea that disease outbreaks vary over different spatial and temporal scales. Although we cannot infer the nature of the process from the observed patterns, the analyses provide a new starting point to either for examining environmental heterogeneity that could explain the pattern or in allowing one to consider biological functions that could produce such a pattern. Preferably, the spatial aggregation pattern of the cucurbit crop, rather than complete spatial randomness, would have been used as the baseline for evaluating aggregation pattern of downy mildew outbreaks. However, the location of cucurbit fields is not uniform, and geo-referenced data for the locations of the cucurbit crop in the eastern United States are not available to allow us to establish the

Fig. 7. Summary plots for the space-time point pattern analysis for cucurbit downy mildew in the eastern United States. Time was defined as the date when a new outbreak of the disease was reported in a cucurbit field plot. The $\hat{D}_0(r, t)$ (D_{zero}) plot is a perspective plot that examines the difference between the space-time K function and the product of the space and time K functions in **A**, 2008 and **B**, 2009. Evidence of space-time interaction is indicated by positive peaks in the D_{zero} value that is also above the 95th quartile value for that time and distance for 999 simulations. Monte Carlo simulation results summarize all of the test statistics, where the time data for each location of cucurbit downy mildew outbreak was randomly assigned to fixed locations for the outbreaks of the disease in **C**, 2008 and **D**, 2009; the vertical thick line is the test statistic calculated from the observed data.

spatial pattern of the crop. It would have been interesting to see how the spatial pattern of the cucurbit crop influenced the spatial pattern of disease outbreaks reported in this study.

Clustering of outbreaks in space and time is considered to be an indicator of the infectious nature of a disease (6,31). For example, the pattern of spatiotemporal interaction has been used to describe the transmission characteristics of infection processes in humans and animals (14,37,48). The distance component of the $\hat{D}_0(r,t) = 1$ risk contour identifies the extent of spatiotemporal interaction of infection risk in space (colloquially termed “contagiousness”) and the temporal component of the $\hat{D}_0(r,t) = 1$ risk contour identifies the extent of contagiousness in time. In this study, these two components were not static throughout the disease epidemic in both years. Increases in the maximum distance component of the $\hat{D}_0(r,t)$ is indicative of increases in local-spread distances (48) and this implies a need to reassess the effectiveness of control measures (mainly fungicide sprays) aimed at reducing local spread of the disease. Increases in the maximum temporal position of the $\hat{D}_0(r,t) = 1$ risk contour suggest that affected fields remain infectious for a long period and, thus, there is need to enhance monitoring for new disease outbreaks (48). This is consistent with results of the temporal analysis where the length of epidemic duration was very long, particularly in southern-tier states. Based on the space–time analysis, we would argue that disease outbreaks that are relatively close in space are also close in time. Thus, disease outbreaks within 3 to 5 months after the first disease outbreak and 600 km of where a new outbreak occurred would likely have a greater chance of interacting with another disease outbreak (16). This relatively large space–time window suggests that regional factors, for example, aerial transport of sporangia of *P. cubensis* and host availability, most likely facilitate the spread of CDM (4). As indicated above, the relative impact of being close in space and time to an infected field differed considerably between and within years. This large heterogeneity suggests that the efforts to control CDM on a regional scale may benefit from indicators of local spread (31) which could be used to complement regional predictive efforts in guiding local decisions on the need to apply fungicides for disease control. It appears that a significant increase in the level of CDM control will be required to decrease the frequency of disease outbreaks and the rate of spread of the disease in the eastern United States.

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