J Gen Plant Pathol (2014) 80:430–434
DOI 10.1007/s10327-014-0539-x

FUNGAL DISEASES

Spatiotemporal distribution of tomato plants naturally infected with leaf mold in commercial greenhouses

A. Kawaguchi · H. Suenaga-Kanetani

Received: 26 November 2013 / Accepted: 24 March 2014 / Published online: 3 July 2014

Abstract The distribution of tomato plants infected by *Passalora fulva*, causal agent of leaf mold, was analyzed by using Taylor's model and Iwao's model to assess the patterns of spatial distribution within the greenhouses. In Taylor's model, the sample variance (s^2) of diseased plants newly recognized at each observation increased only slightly with mean density (m). In Iwao's model, the mean crowding (m^*) of newly recognized diseased plants at each observation increased with m of diseased plants. The statistical analysis in this study suggests that new infections in greenhouses observed during this investigation tended to cluster around a diseased plant and that secondary infections occurred as independent cluster points.

Keywords Spatiotemporal distribution · Tomato leaf mold · Taylor's power law · Iwao's m^*–m regression · Epidemiology

Tomato leaf mold caused by fungus *Passalora fulva* (synonym: *Fulvia fulva*) affects the foliage of tomatoes, particularly those grown in greenhouses. It is a serious disease of tomatoes and is most often a problem when greenhouses are crowded with foliage. In recent years, outbreaks of tomato leaf mold have emerged in commercial greenhouses in Okayama Prefecture, Japan.

Electronic supplementary material The online version of this article (doi:10.1007/s10327-014-0539-x) contains supplementary material, which is available to authorized users.

A. Kawaguchi (✉) · H. Suenaga-Kanetani
Research Institute for Agriculture, Okayama Prefectural Technology Center for Agriculture, Forestry and Fisheries, 1174-1 Koudaoki, Akaiwa, Okayama 709-0801, Japan
e-mail: akira_kawaguchi@pref.okayama.lg.jp

The general strategy to control the disease is by breeding resistant cultivars. Controlling the disease using resistant cultivars is not easy because it is a long-term disease management strategy, and the resistance of the cultivar can be overcome by the fungus. The successful use of resistance genes thus depends upon the dynamics of the different physiological races of the pathogen. Among tomato cultivars in Japan that carry genes conferring resistance to *P.Fulva* (*Cf-2*, *Cf-4*, *Cf-9*, and *Cf-11*), resistance has been overcome by nine races of *P. fulva* (Enya et al. 2009; Iida et al. 2010; Kishi 1962; Ozaki and Shirakawa 1996; Satou et al. 2005; Yamada and Abiko 2002).

According to reports on the epidemiology of tomato leaf mold, *P. fulva* is dependent on high relative humidity and temperature for disease development (Jones and Jones 1991). The fungus survives as a saprophyte on crop residue and as conidia or sclerotia in the soil. Tomato seeds can be contaminated and serve as a primary source of inoculum (Jones and Jones 1991). Conidia in the soil are readily disseminated by rain or wind and can survive at least 1 year. However, it is unclear what role primary and secondary inocula play in the disease cycle in commercial greenhouses. Clarifying the role of the primary and secondary inocula is very important and could allow farmers to control the disease more efficiently. To assess the role of the primary and secondary inocula, we investigated the spatial distribution of tomato plants naturally infected with leaf mold in commercial greenhouses after the first detection of the disease.

From 2009 to 2010, we investigated the spatial distribution and spread of tomato leaf mold within greenhouses in Okayama Prefecture, Japan. We investigated 11 commercial greenhouses in four locations in 2009 and 10 commercial greenhouses in four locations in 2010 (Online Resources Tables S1, S2; Fig. S1). The details of each

greenhouse are outlined in Online Resource Table S1. Each greenhouse contained three beds, with two rows of tomato plants per bed. The rows were spaced 60 cm apart. Self-rooted tomato seedlings (cv. Momotaro-8) were planted each year. The plants were spaced 45 cm apart in greenhouses H to Q, 50 cm apart in greenhouses A to C, and 60 cm apart in greenhouses D to G. All the greenhouses were unheated.

Disease incidence was mapped in contiguous quadrats, which consisted of two rows of 17–19 plants. Plants with leaf mold symptoms were counted in each commercial greenhouse on one to four different dates after the disease was initially detected. To confirm the identification of *P. fulva*, we examined the lesions of five leaves from each greenhouse under the microscope and checked that the conidia were those of *P. fulva*. To confirm the visual examination, we isolated the fungus from about three plants from each greenhouse on potato sucrose agar medium (1 L of boiled extract of 300 g potato tubers, 20 g sucrose, 15 g agar, pH 5.8) and confirmed the identification by microscopic observation of the conidia.

The sample variance (s^2) of the number of individuals per quadrat usually increases with increasing sample mean (m). Bliss (1941) used the following equation to describe the relation between s^2 and m:

$$s^2 = am^b, \tag{1}$$

where a and b are constants. Equation 1 expressed in linear form on a logarithmic scale is called Taylor's power law (Taylor 1961):

$$\log s^2 = \log a + b \log m. \tag{2}$$

Equation 2 represents the relationship between s^2, a, b, and m. Equations (3) and (4) express the definitions of m and s^2:

$$m = \sum_{i=1}^{n} x_i/n, \tag{3}$$

$$s^2 = \sum_{i=1}^{n} (m - x_i)^2/n, \tag{4}$$

where $\sum_{i=1}^{n} x_i$ is the total number of diseased tomato plants, n is the number of quadrats, and x_i is the number of diseased tomato plants in the ith quadrat ($i = 1,\ldots,n$). The index b is a measure of the dispersion of individuals in a population: $0 \leq b < 1$ indicates a uniform distribution; $b = 1$, a random distribution; and $b > 1$, an aggregated distribution (Taylor 1961). The value of b remains constant for the same organisms in the same environment and can be considered an "index of aggregation" that is characteristic of the species because samples collected from different localities and at different times can be fit to a single regression line by Taylor's power law (Taylor 1961; Taylor et al. 1978). Accordingly, sets of data obtained from different greenhouses, locations, and years were combined and analyzed.

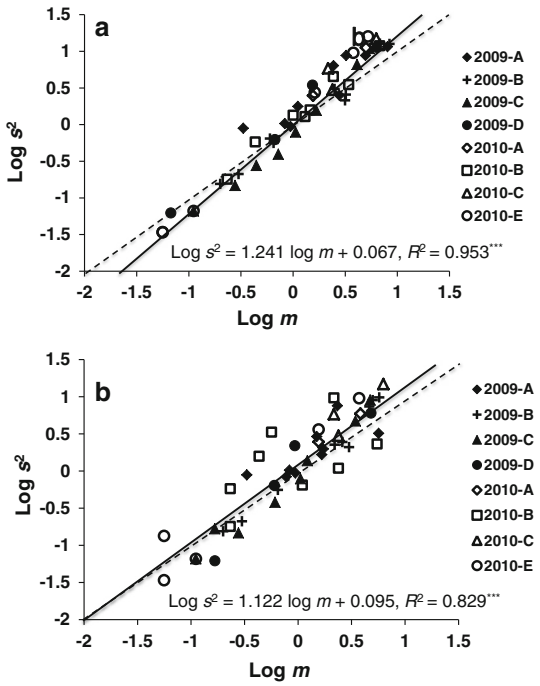

Fig. 1 Taylor's power law regression (Taylor 1961) for diseased tomato plants naturally infected with *Passalora fulva* in commercial greenhouses. **a** Data for all diseased plants recognized at each observation. **b** Data for diseased plants newly recognized at each observation. Coefficient of determination (R^2) of the two different regressions was significant (***$P < 0.001$). The *solid line* shows the Taylor's power law regression line. The *dotted line* shows log $s^2 = \log m$ ($a = 0$, $b = 1$), indicating a random distribution. *Symbols* indicate year and location as in Online Resource Table S1

Bliss (1941) used another equation to describe the relation between s^2 and m:

$$s^2 = cm + dm^2, \tag{5}$$

where c and d are constants. Equation 5 expressed in a linear form is called Iwao's m^*–m regression method (Iwao 1968). It is used to quantify the relationship between the mean crowding index (m^*) (Lloyd 1967) and mean density (m) using data from several sets of observations as follows:

$$m^* = \alpha + \beta m, \tag{6}$$

$$m^* = \sum_{i=1}^{n} x_i(x_i - 1)/\sum_{i=1}^{n} x_i, \tag{7}$$

Where α and β are constants: α is defined as an index of basic contagion and is related to the size of clusters of diseased plants; β is defined as a density–contagiousness coefficient, and indicates the patchiness of clusters (Iwao 1968). The indices α and β are measures of dispersion of individuals in a population: $-1 < \alpha < 0$ indicates a

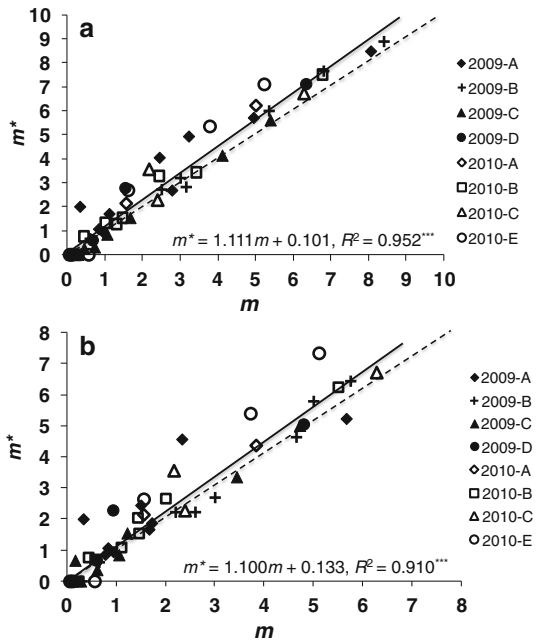

Fig. 2 Iwao's m^*–m regression (Iwao 1968) for diseased tomato plants naturally infected with *Passalora fulva* in commercial greenhouses. **a** Data for total diseased plants at each observation. **b** Data for diseased plants newly recognized at each observation. Coefficient of determination (R^2) of the two different regressions was significant (***$P < 0.001$). The *solid line* is the m^*–m regression line. The *dotted line* shows $m^* = m$ ($\alpha = 0$, $\beta = 1$), indicating a random distribution

uniform distribution; $\alpha = 0$, a random distribution; and $\alpha > 0$, an aggregated distribution of diseased plants within clusters, while $0 \leq \beta < 1$ indicates a uniform distribution; $\beta = 1$, a random distribution; and $\beta > 1$, an aggregated distribution. Samples collected from different localities and at different times can be fit to a single regression line by Iwao's m^*–m regression (Iwao 1968). Accordingly, sets of data obtained from different greenhouses, locations, and years were combined and analyzed.

The goodness of fit of Taylor's model was compared with that of Iwao's model by using coefficient of determination (R^2) based on methods reported previously (Ali et al. 1998; Mollet et al. 1984; Soemargono et al. 2008; Yamamura 2000).

The results of Taylor's model when applied to the combined data for the total number of recognized diseased plants observed in each greenhouse showed that the sample variance (s^2) increased with mean density (m) (Fig. 1a). Taylor's regression formula was $\log s^2 = 1.241 \log m + 0.067$, with coefficient of determination (R^2) = 0.953 ($P < 0.001$) and $b = 1.241$ (95 % confidence interval [CI]: 1.161–1.321). Thus, b was significantly greater than 1.0 ($P < 0.01$). On the other hand, the sample variance of newly recognized diseased plants at each observation increased only slightly with the mean density of diseased plants (Fig. 1b). Taylor's regression formula was $\log s^2 = 1.122 \log m + 0.095$, with $R^2 = 0.829$ ($P < 0.001$) and $b = 1.122$ (95 % CI: 0.980–1.264). Thus, b was not significantly different from 1 ($P > 0.05$).

The results of Iwao's m^*–m regression when applied to the combined data for total number of recognized diseased plants observed in each greenhouse showed that mean crowding (m^*) increased with mean density (m) (Fig. 2a). The m^*–m regression formula was $m^* = 1.111\ m + 0.101$ with $R^2 = 0.952$ ($P < 0.001$), $\alpha = 0.101$ (95 % CI: −0.144–0.346), and $\beta = 1.111$ (99 % CI: 1.014–1.208). Thus, α was not significantly different from 0 ($P < 0.05$), and β was significantly greater than 1.0 ($P < 0.01$). On the other hand, the mean crowding of newly recognized diseased plants at each observation increased with the mean density of diseased plants, too (Fig. 2b). The m^*–m regression formula was $m^* = 1.100\ m + 0.133$ with $R^2 = 0.910$ ($P < 0.001$), $\alpha = 0.133$ (95 % CI: −0.135–0.401), and $\beta = 1.100$ (95 % CI: 1.001–1.201). Thus, α was not significantly different from 0 ($P > 0.05$), and β was significantly greater than 1.0 ($P < 0.05$).

Taylor's power law and Iwao's m^*–m regression are two statistical methods commonly used in population research to analyze the distribution of organisms. Taylor's model is a colony expansion model, and Iwao's model is a colony increase model (Yamamura 2000; Fig. 3). When applying Taylor's model to the total diseased plants observed in the combined greenhouse data, the index b was significantly greater than 1.0, suggesting that there was an aggregated distribution of diseased plants when the mean density of diseased plants was much greater than 0. When applying Iwao's model to the total of all diseased plants observed in the combined greenhouse data, α was not significantly different from 0, and β was significantly greater than 1.0. These results suggest that there was a random distribution of all recognized diseased plants at each observation when the mean density of diseased plants was very low ($m \approx 0$) and that there was an aggregated distribution of diseased plants when the mean density of the diseased plants was much greater than 0. For the data of all diseased plants observed in the combined greenhouse data, Iwao's model and Taylor's model each fit the data well, because the R^2 values of Iwao's model and Taylor's model were almost the same at 0.952 and 0.953, respectively.

On the other hand, when applying Taylor's model to newly recognized diseased plants at each observation, the index b was not significantly different from 1.0, suggesting that the newly recognized diseased plants were randomly distributed when the mean density of diseased plants was much greater than 0. When applying Iwao's model to newly recognized diseased plants at each observation, α was not significantly different from 0, and β was significantly greater than 1.0. These results suggest that the newly

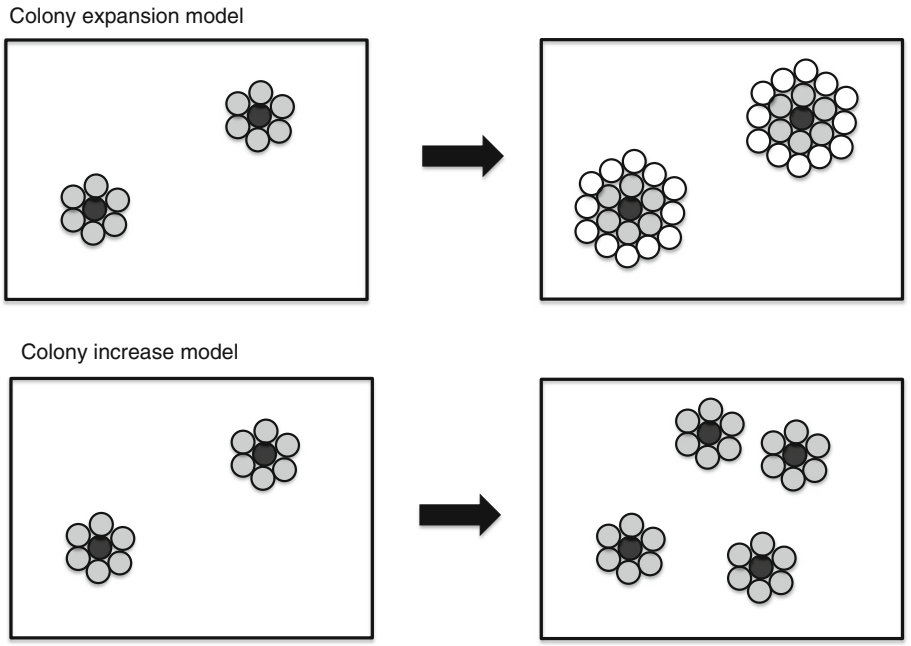

Fig. 3 Schematic illustrations of the assumptions of the models. *Circles* indicate individuals (diseased plants, in this study), and *aggregated circles* indicate colonies. In Taylor's model (Taylor 1961), population increase is described by the development of each colony while the number of major colonies is fixed (colony expansion model, *upper*). In Iwao's model (Iwao 1968), by contrast, population increase is described by an increase in the number of colonies while the colony size is fixed (colony increase model, *lower*)

recognized diseased plants at each observation are randomly distributed when the mean density of diseased plants was very low ($m \approx 0$) and that there was an aggregated distribution of diseased plants when the mean density of the diseased plants was much greater than 0. Iwao's model fit the data of newly recognized diseased plants at each observation better than did Taylor's model because R^2 of Iwao's model and Taylor model were 0.910 and 0.829, respectively. Therefore, the distribution of newly recognized diseased plants with leaf mold at each observation best fit the colony increase model (Fig. 3). The newly recognized diseased plants at each observation showed an aggregated distribution, suggesting that newly infected diseased plants formed new independent foci during outbreaks. This is the colony increase model, in which new independent foci often form during outbreaks.

Similar results have been reported for Fusarium head blight of wheat (Ohsaki et al. 2009). In that investigation, populations of the pathogen formed new independent clusters, suggesting that ascospores from the primary inoculum started new infections over long periods in fields and that conidia had a limited role in secondary infection in the disease cycle (Ohsaki et al. 2009). In the case of tomato leaf mold, our present statistical analysis showed that the data best fit Iwao's model (i.e., the colony increase model; Fig. 3), with populations of the pathogen forming new independent clusters during our investigation. This result indicates that conidia, which are formed in the lesions of diseased plants, have a major role in secondary infection in the disease cycle.

Our statistical analysis showing that populations of the pathogen formed new independent clusters during outbreaks suggests two hypotheses for the disease cycle: (A) conidia from the primary inoculum initiate new infections over long periods in greenhouses, and each new infection initiates other new infections, and (B) conidia from diseased plants spread randomly throughout the greenhouse and initiate new infections. The two hypotheses are not mutually exclusive, but hypothesis (B) may be the most appropriate because the greenhouses investigated in this study (Online Resource Table S1) were much smaller than a field, and conidia could be easily spread throughout a greenhouse of that size. The sizes of the greenhouses investigated in this study are typical of those in the mountainous areas of western Japan, so these results will be applicable to similarly sized commercial greenhouses for growing tomato plants. Examining the spatial distribution of *P. fulva* conidia in commercial greenhouses may support our results and hypothesis; however, in our preliminary examination during the early to middle periods of leaf mold outbreak, hardly any *P. fulva* conidia were trapped in glycerin jelly on microscope slides (data not shown).

Additionally, we observed that many leaf mold lesions formed on newly developing leaves, and we hypothesized that the scale of colony expansion of *P. fulva* is at the level of the same tomato individual. We are now pursuing this part of our investigation and will report our results in a forthcoming paper.

There have so far been no reports on the pattern of spread of tomato leaf mold infection in greenhouses. In general, the spread of diseased plants in fields tends to follow an aggregated distribution pattern (Madden and Hughes 1995), but the pattern of spread of diseased plants

in greenhouses is unknown. In a recent report on the pattern of spread of a plant disease in greenhouses, our laboratory group (Kawaguchi et al. 2013) investigated the spatiotemporal distribution pattern of tomato bacterial canker caused by *Clavibacter michiganensis* subsp. *Michiganensis* within a greenhouse. We found that the bacteria from the primary inoculum can cause new infections over long periods. Thus, our current study provides fundamental information on the pattern of spread of diseased plants infected with fungi in greenhouses.

In conclusion, *P. fulva* appears to initiate new infections from the primary inoculum at the onset of an outbreak, and the conidia of *P. fulva*, which form in lesions of diseased plants, have a major role in secondary infections in the disease cycle in commercial greenhouses. This study shows that statistical analysis of the spatiotemporal distribution provides clues to infer the source and the mode of spread of the fungus causing tomato leaf mold.

Acknowledgments The authors are grateful to Dr. K. Ishiguro (NARO Tohoku Agricultural Research Center, Iwate, Japan), Dr. H. Tanaka (Research Institute of Environment, Agriculture and Fisheries, Osaka Prefecture, Osaka, Japan) and Dr. K. Yamamura (National Institute of Agro-Environment Sciences, Ibaraki, Japan) who gave us much useful advice.

References

Ali A, Gu WD, Lobinske RJ (1998) Spatial distribution of chironomid larvae (Diptera: chironomidae) in two central florida lakes. Environ Entomol 27:941–948

Bliss CI (1941) Statistical problems in estimating populations of Japanese beetle larvae. J Econ Entomol 34:221–232

Enya J, Ikeda K, Takeuchi T, Horikoshi N, Higashi T, Sakai T, Iida Y, Nishi K, Kubota M (2009) The first occurrence of leaf mold of tomato caused by races 4.9 and 4.9.11 of *Passalora fulva* (syn. Fulvia fulva) in Japan. J Gen Plant Pathol 75:76–79

Iida Y, Iwadate Y, Kubota M, Terami F (2010) Occurrence of a new race 2.9 of leaf mold of tomato in Japan. J Gen Plant Pathol 76:84–86

Iwao S (1968) A new regression method for analyzing the aggregation pattern in animal populations. Res Popul Ecol 10:1–20

Jones JB, Jones JP (1991) Leaf mold. In: Jones JB, Jones JP, Stall RE, Zitter TA (eds) Compendium of tomato diseases. APS Press, St Paul, p 18

Kawaguchi A, Tanina K, Inoue K (2013) Spatiotemporal distribution of tomato plants naturally infected with bacterial canker in greenhouses. J Gen Plant Pathol 79:46–50

Kishi K (1962) Studies on the physiological specialization of *Cladosporium fulvum* cooke (in Japanese with English summary). Ann Phytopath Soc Japan 27:189–196

Lloyd M (1967) Mean crowding. J Anim Ecol 36:1–30

Madden LV, Hughes G (1995) Plant disease incidence: distributions, heterogeneity, and temporal analysis. Annu Rev Phytopathol 33:529–564

Mollet JA, Trumble JT, Sevacherian V (1984) Comparison of dispersion and regression indices for *Tetranychus cinnabarinus* (Boisduval) (Acari: tetranychidae) populations in cotton. Environ Entomol 13:1511–1514

Ohsaki M, Tomimura K, Urano S, Yoshida M, Nakajima T (2009) Spatiotemporal distribution of diseased heads with fusarium head blight in naturally infested wheat fields (in Japanese with English summary). Jpn J Phytopathol 75:307–313

Ozaki K, Shirakawa T (1996) Pathogenic races of Fulvia fulva in iwate prefecture (in Japanese with English summary). Ann Rept Plant Prot North Jpn 47:62–64

Satou M, Shinozaki T, Nishi K, Kubota M (2005) Leaf mold of tomato caused by races 4 and 4.11 of Passalora fulva in Japan. J Gen Plant Pathol 71:436–437

Soemargono A, Ibrahim Y, Ibrahim R, Osman MS (2008) Spatial distribution of the asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: psyllidae) on citrus and orange jasmine. J Biosci 19:9–19

Taylor LR (1961) Aggregation, variance and the mean. Nature 189:732–735

Taylor LR, Woiwod IP, Perry JN (1978) The density-dependence of spatial behavior and the parity of randomness. J Anim Ecol 47:383–406

Yamada K, Abiko K (2002) Race composition of Fulvia fulva in Japan during 1997–1998 (in Japanese with English summary). Jpn J Phytopathol 68:36–38

Yamamura K (2000) Colony expansion model for describing the spatial distribution of populations. Popul Ecol 42:161–169