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Agronomical management influence on the spatiotemporal progress of strawberry dry wilt in Michoacan, Mexico

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The spatiotemporal distribution of strawberry wilt, caused by *Fusarium oxysporum*, *Phytophthora* sp., *Pythium aphanidermatum* and *Rhizoctonia fragariae*, was studied with the aim to establish the effect of some technological components of the strawberry crop cv. Camarosa (*Fragaria* x *ananassa* Duch.) and sustain their use in an integral management of the disease. Epidemics were characterized in two cropping seasons at three localities in Valle de Zamora, Michoacan, Mexico, in commercial plantations with plastic mulch and drip irrigation (A+G), and non-mulch and gravity irrigation (T) on a 100 m^2 area per site. Temporal parameters were contrasting between both management techniques. A+G plantations had significantly lower final incidence (Y_f =12.8±5.6%) than T (22.5±5.9%) (p=0.05) and were consistent with estimators of area of curve (ABCPEa and ABCPEe). The range of epidemic intensity reduction induced by A+G was 22.21 to 76.7% day, which was reflected in lower apparent infection rates (b^{-1}=0.0015-0.0027, R^2=0.92-0.99). Lloyd's Index of Patchiness and Morisita Index (1.01 to 1.17) indicated a slightly aggregated dispersion pattern. Autocorrelation and geostatistical analysis confirmed lower aggregates in A+G (up to 5 plants) vs. T (8 plants), but an apparent higher mobility of inoculum in A+G up to 6.5 m. Plastic mulch and drip irrigation are proposed as technological components of an eventual integrated management program of dry wilt in Michoacan.

Key words: Epidemiology, plastic mulch, drip irrigation, strawberry dry wilt.

INTRODUCTION

Black root rot is a worldwide disease that limits the yield of strawberry and is a serious and common problem that has been reported and studied in mayor producing countries such as Japan, Israel, South Africa, Italy, Spain and the United States (Kohmoto et al., 1981; Yigal et al., 1981; Wing et al., 1994; Botha et al., 2003; Manici et al., 2005; Avilés et al., 2008; Ellis, 2008). Despite its significance, the etiology of black root rot has not yet been fully resolved, and appears to vary according to the site on which it occurs (Botha et al., 2003). In Mexico, strawberry black root rot is commonly known as strawberry dry wilt, caused by the complex *F. oxysporum*, *Phytophthora* sp., *Pythium aphanidermatum* and *Rhizoctonia fragariae* (Ceja-Torres et al., 2008), and reaches incidences of 40 to 80% of the major strawberry producing states in Mexico (Mendoza and Romero, 1989; Castro and Dávalos, 1990). The development of effective strategies for disease management is limited due to the

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success of methyl bromide as a soil sterilant, currently restricted. In addition, the management includes cultivar selection, use of certified planting stock, replacement of plants annually, biological control, rotation crops, soil fumigation prior to planting, soil solarization and use of systemic fungicides during the crop cycle (Yigal et al., 1981; Yuen et al., 1991; Elmer and LaMondia, 1999; Benlioğlu et al., 2005). Moreover, the use of new production technologies such as plastic mulch and drip irrigation influence on the population structure of microorganisms associated with the disease by modifying the soil microenvironment. Therefore, it is important to sustain recent etiological studies of the dry wilt and have a to better knowledge of the development of disease over time and space for identifying options for management of soil-borne diseases (Gilligan, 2002), with additional work to determine the influence of agricultural practices, with emphasis on land cover and irrigation system, and on the spatiotemporal behavior of the disease in order to design management strategies that enhance the possible suppressive effects of conventional crop technologies. This research has been established for this purpose and under the assumption that attributes of intensity of epidemics and pathogen dispersal patterns are strongly influenced by the mulch and the drip irrigation.

MATERIALS AND METHODS

Field experiments

This research was carried out during the 2003-04 and 2004-05 crop cycles in three localities of the Valley of Zamora, Michoacan, Mexico: Ario de Rayón, Tamándaro and Villafuerte. In each locality two commercial plots of 2 ha were chosen for the variety Camarosa; one with plastic mulch and drip irrigation (A+G) and the other with non-mulch soil with gravity irrigation (T). In all cases, the soil was clay. The density was 90 thousand plants per ha set out in zig-zag, double row manner, every 18 cm. The monthly average temperature of the study area was obtained from the Irrigation District 061, Zamora, Michoacan.

Evaluation of the disease

In an area of 100 m^2 (10×10 m) by commercial plot, incidence of wilt plants was recorded monthly from September to January during the crop growth and biweekly from February to May, during flowering and fructification. A plant was considered diseased if it exhibited wilting and gradual death. The spatial location of the plants was recorded using field maps.

Temporal analysis

Epidemics in each of the three regions were characterized by the model of simplified Weibull distribution with two parameters (b and c) (Pennypacker et al., 1980; Thal et al., 1984): $Y = 1\text{-}e[-(t/b)^c]$, $t>0$; where Y = incidence ratio, t = time in days after planting, b = parameter estimator of the epidemic rate in its inverse form, and c = parameter of the curve shape. Additionally, the intensity of epidemics was estimated by calculating the absolute area under the disease progress curve (AUDPCa) by the trapezoidal integration method: AUDPCa = $\Sigma_1^{n\text{-}i}$ $[(Y_i + Y_{i+1}) / 2] (t_{i+1} - t_i)$, where: Y_i = proportion of disease in the i-th evaluation, t_i = time at the *i*-th observation, n = number of evaluations (Campbell and Madden, 1990; Jeger and Viljanen-Rollinson, 2001). The parameter AUDPCa) was standardized (AUDPEe) by dividing its value between the time of duration of the epidemics. The relative reduction of area (AUDPEr) in percentage was calculated in relation to AUDPCa of plantations T (AUDPEaT) by location and crop cycle (100-[AUDPCa /AUDPEaT] [100]). Confidence intervals with p = 0.05 and t test were applied for comparison of intensity parameters of epidemics. All data were analyzed with the Statistical Analysis System (SAS) ver. 6.10 (SAS Institute, Cary, NC) (Jesus Junior et al., 2004).

Spatial analysis

The optimum quadrant sizes of (OQS) to calculate indices of aggregation were obtained by the Greig-Smith method (Campbell and Madden, 1990) in blocks 1, 2, 4, 8, 16, 32, 128 and 256 plants with software, MorLloyd version 1.0® MS EXCEL (Rivas and Mora-Aguilera, 2011. Unpublished). The spatial pattern of strawberry dry wilt was determined with Lloyd´s Index of Patchiness (LIP) and Morisita Index (Iδ) (Campbell and Noe, 1985). LIP = m + [(V / m) -1] / m, where m = average number of diseased plants per quadrant, and V = variance. $I\delta = n[\sum(y)^2 - \sum y]/(\sum y)^2 - \sum y$; where: n= total number of quadrants, and y = number of diseased plants per quadrant. Criteria to determine the spatial pattern with these indexes were: 1 = at random, >1 = aggregate and <1 = uniform. The patterns of proximity and spatial dependency of diseased plants were defined by autocorrelation analysis, with the LCOR2® program (Gottwald et al., 1992), and the geostatistical GEO-EAS 1.2.1 software; to determine the spatial dependency in a row (isotropy) and in any direction (anisotropy).

RESULTS AND DISCUSSION

Temporal analysis

Disease onset was delayed until 45 days after planting with an initial incidence less than 1% (Y_0) regardless the agronomic management (Figure 1A to F). However, management significantly influenced the further progress of the disease (Tables 1 and 2). The incidence of the dry wilt increased between 213 and 228 days after planting, being higher in the gravity irrigation system without mulch (T). The flexibility of the Weibull model (Thal et al., 1984) allowed describing the epidemic progress of the strawberry dry wilt with determination coefficients of 0.92 to 0.99 and from 0.97 to 0.99 in the first and second production cycles, respectively. With the exception of Tamándaro and Ario de Rayón in the 2004-05 cycles, the estimator of the epidemic rate (1/b) was lower in A+G. However, only statistical differences (p=0.05) were found in Tamándaro and between this with the T values of Villafuerte and Ario de Rayón (2003-04 cycle) and between Villafuerte (A+G) and Tamándaro (T) (2004-05 cycle) (Table 1). The mechanism of dispersal of the primary inoculum in soil can have large impacts on disease onset, progress, and final incidence (Sujkowski et al., 2000), in this study the shape of the epidemic curve (c) in all cases was sigmoidal asymptotic, typical of

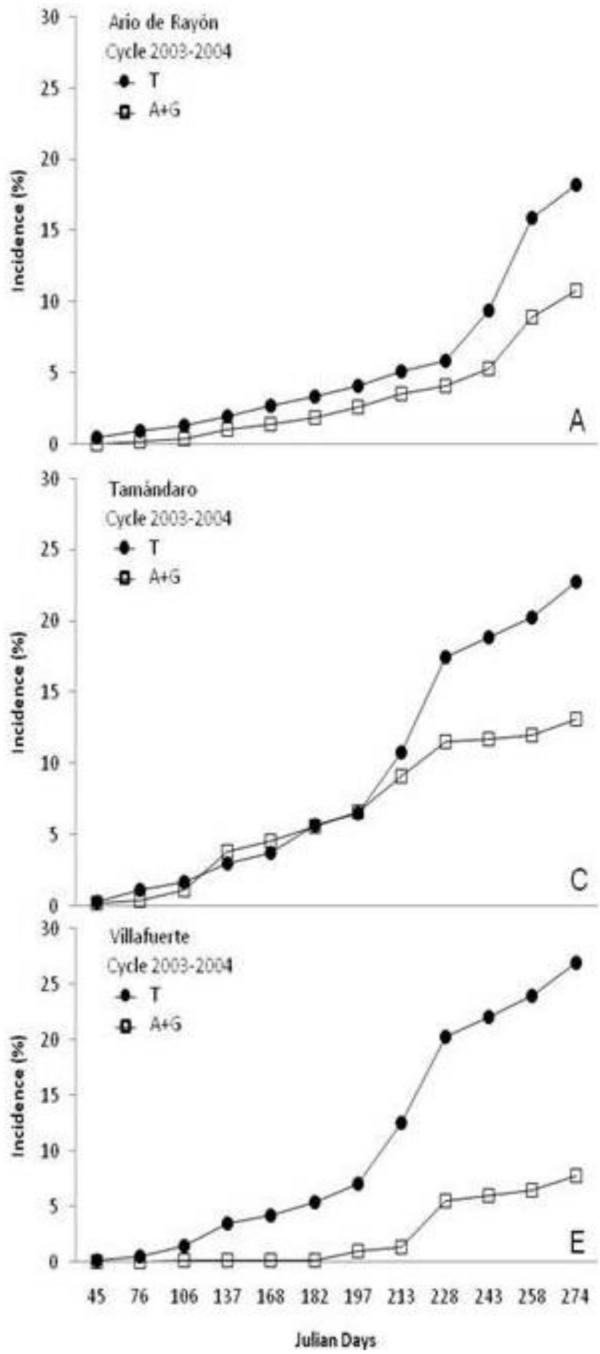

Figure 1. Curves of the temporal progress accumulated of strawberry dry wilt in plantations with plastic mulch and drip irrigation (A+G) and non-mulch and gravity irrigation (T), in the Valley of Zamora, Michoacan, Mexico (A, C and E 2003-04 cycle; B, D and F 2004-05 cycle).

low-level epidemics with limited inoculum dispersal. In this case, statistical differences were also detected between the curve shapes being clearer between Tamándaro (A+G) and the other localities in both cycles. Although the intensity of disease was generally lower in A+G, Weibull analytical results were not fully consistent with the graphical inspection of the curves where less epidemic intensity was detected in A+G (Figure 1B to F). Final incidence (Y_f) in the 2003-04 cycle ranged from 7.8 to 14.5% (11.8 ± 3.5%) in plantations A+G and 13.1 to 27% (21.7 ± 7.1%) in T, and in the 2004-05 cycle, 10.8 to 16.4% (13.8 ± 2.8) and from 18.2 to 28.2% (24.1 ± 5.3%) in the same order (Table 2). The significance of mean differences in the first and second cycles was 11.6 and

Table 1. Description of 12 epidemics of strawberry dry wilt by the Weilbull model for the 2003-04 and 2004-05 cycles in the Valley of Zamora, Michoacan, Mexico.

Locality (management)	Model $Y= 1-e[-(t/b)^c]$	R^2	Interval of confidence (95%)*	
			b	c
2003-2004 cycle				
Ario de Rayón (A+G)	$Y= 1-e[(t/426.6)^{3.99}]$	0.97	381-472[a]	3.22-4.77[a]
Ario de Rayón (T)	$Y= 1-e[(t/410.4)^{4.84}]$	0.99	395-426[a]	4.47-5.21[a]
Tamándaro (A+G)	$Y= 1-e[(t/653.0)^{2.16}]$	0.97	516-790[b]	1.72-2.60[b]
Tamándaro (T)	$Y= 1-e[(t/397.0)^{3.41}]$	0.96	352-442[a]	2.64-4.19[a]
Villafuerte(A+G)	$Y= 1-e[(t/459.0)^{4.69}]$	0.92	361-558[ab]	3.01-6.37[a]
Villafuerte (T)	$Y= 1-e[(t/366.1)^{3.67}]$	0.96	331-402[a]	2.82-4.51[a]
2004-2005 cycle				
Ario de Rayón (A+G)	$Y= 1-e[(t/374.6)^{5.26}]$	0.98	348-401[ab]	4.31-6.21[bc]
Ario de Rayón (T)	$Y= 1-e[(t/386.9)^{3.24}]$	0.99	305-409[ab]	2.86-3.61[a]
Tamándaro (A+G)	$Y= 1-e[(t/355.0)^{6.99}]$	0.97	323-378[a]	5.59-8.39[c]
Tamándaro (T)	$Y= 1-e[(t/362.6)^{4.07}]$	0.98	338-387[a]	3.38-4.75[ab]
Villafuerte(A+G)	$Y= 1-e[(t/435.8)^{4.68}]$	0.98	397-475[b]	3.92-5.44[b]
Villafuerte (T)	$Y= 1-e[(t/375.1)^{5.06}]$	0.97	341-409[ab]	3.91-6.20[bc]

b, Estimator of the apparent infection rate in its inverse form (1/b); c, estimator of the curve shape. *Values of same parameter for each crop cycle, with different letter are statistically different (p = 0.05%).

Table 2. Absolute, relative and standardized AUDPE, Y_f y b^{-1} (Weibull) of 12 cumulative progress curves of strawberry dry wilt for the 2003-04 and 2004-05 cycles in the Valley of Zamora, Michoacan, Mexico.

Locality	Management	AUDPEa	AUDPEr	AUDPEe	Y_f	b^{-1}
2003-2004 cycle						
Ario de Rayón	A+G	7.58	-30.69	0.033	14.5	0.0023
Ario de Rayón	T	5.80	0.00	0.025	13.1	0.0024
Tamándaro	A+G	11.35	22.21	0.050	13.1	0.0015
Tamándaro	T	14.59	0.00	0.064	22.8	0.0025
Villafuerte	A+G	3.84	76.63	0.028	7.8	0.0022
Villafuerte	T	16.43	0.00	0.072	27.0	0.0027
2004-2005 cycle						
Ario de Rayón	A+G	8.59	57.14	0.038	16.4	0.0027
Ario de Rayón	T	20.04	0.00	0.088	28.2	0.0026
Tamándaro	A+G	5.89	64.96	0.026	14.3	0.0028
Tamándaro	T	16.81	0.00	0.073	26.0	0.0028
Villafuerte	A+G	5.74	42.94	0.025	10.8	0.0023
Villafuerte	T	10.06	0.00	0.044	18.2	0.0027

A+G, Plastic mulch with drip irrigation; T, non-mulch and gravity irrigation.

0.05%, respectively. Weibull failure to reflect these trends could be due to the extension of the lower asymptote which was due to the delay that A+G caused in the increase of the epidemics. Another alternative analytical unaffected by the asymptotic factor was AUDPE. Lower values of AUDPEa and AUDPEe were obtained with the operation A+G except in the 2003-04 cycle in Ario de Rayón which was consistent with the values of Y_f (Table 2). The Weibull rate parameter (b^{-1}) was significantly correlated with AUDPEa but with low accuracy (r^2=0.58) confirming its limited ability to describe in the context of this work. The reduction range of epidemic intensity of A+G on T was 22.1 to 76.6% and 42.1 to 64.9% in 2003-04 and 2004-05 cycles, respectively, which demonstrates the strong suppressive effect of the combination of plastic mulch with drip irrigation (Table 2). There were no

Table 3. Spatial pattern of strawberry dry wilt with different crop management for the 2003-04 and 2004-05 cycles in the Valley of Zamora, Michoacan, Mexico.

Locality (management)	Optimum quadrant size	LIP	Morisita Index	Spatial pattern
2003-04 cycle				
Ario de Rayón (A+G)	128	1.09	1.07	Aggregate
Ario de Rayón (T)	128	1.02	1.01	Aggregate
Tamándaro (A+G)	128	1.02	1.02	Aggregate
Tamándaro (T)	128	0.99	0.99	Uniform
Villafuerte (A+G)	128	1.14	1.12	Aggregate
Villafuerte (T)	128	1.07	1.05	Aggregate
2004-05 cycle				
Ario de Rayón (A+G)	32	0.94	0.96	Uniform
Ario de Rayón (T)	32	1.09	1.06	Aggregate
Tamándaro (A+G)	32	1.17	1.10	Aggregate
Tamándaro (T)	32	0.98	0.98	Uniform
Villafuerte (A+G)	16	1.01	1.01	Aggregate
Villafuerte (T)	32	1.05	1.04	Aggregate

differences between cycles with no parameters (p = 0.05) suggesting that the increase of inoculum required longer periods of time, typical of soil organisms (Michreff et al., 2005).

The temporal analysis of dry wilt showed that the A+G management affected the efficiency of fungal and pseudofungi inoculum associated with the disease, leading to a lower intensity of epidemics, but not total elimination capacity. The increase in incidence in all cases coincided generally with fructification and average room temperature during this period between 19 and 25.5°C, range biasing the expression of pathogens associated with diseases of root diseases in part by the increase of the transpiration rate combined with the productive stress of the plants (Michereff et al., 2005). In Australia *Fusarium oxysporum* and binucleate *Rhizoctonia* particulary AG-A, caused severe disease on root and crowns, resulting in the eventual death of plants, still severely retarded the growth and development at 27°C, but *Macrophomina phaseolina* was most virulent and caused most severe disease at 32°C (Xiangling et al., 2011), this last pathogen has not been reported in Mexico. Although soil temperature was not measured, plastic mulch can increase temperature from 3 to 7°C and drastically change the soil moisture (Mbagwu, 1991; Schmidt and Worthington, 1998), which could explain the reduced efficiency of inoculum in the initial phase of the epidemics, as evidenced by the prolongation of the initial asymptote A+G. It is shown that these factors differentially affect the pathogenicity of microorganisms (Pinkerton et al., 2002; Michereff et al., 2005). For example, *Phytophthora capsici* was more aggressive in *Capsicum annuum* and caused a higher incidence of wilting than *Rhizoctonia solani* due to soil temperature (20 to 22°C) and humidity at field capacity not optimal for this fungus (Vázquez-López et al., 2009). Previous studies with strawberry dry wilt indicate that this disease increases in poorly drained clay soils (Castro and Dávalos, 1990; Mendoza, 1992), which was characteristic of plots under study. In a subsequent regional study it was confirmed that the distribution and prevalence of fungi and pseudofungi causing of strawberry dry wilt was influenced by soil texture and other factors such as the level of organic matter (Ceja-Torres et al., 2008).

Spatial analysis

Optimum quadrant size (TOC) for use in the calculation of Lloyd's Index of Patchiness (LIP) and Morisita Index (Iδ) was 120 plants in 2003-04 and 32 plants in 2004-05 from a matrix of data of 40 × 20 in the first crop cycle and 20 × 20 in the second crop cycle (Table 3). Exploratory maps were obtained using SURFER 4.0® (Figure 2) and the LIP indices of 1.01 to 1.17 and Iδ of 1.01 to 1.12, indicated that dry wilt of strawberry had a pattern of slightly aggregated dispersion in 75% of the plantations studied predominantly at A+G (83.3%) (Table 3). This is because the values of these indices were slightly higher than one, indicating weak aggregation, which verifies previous observations regarding the distribution of the disease in patches (Téliz et al., 1986). Only two plantations with gravity irrigation and one with drip irrigation, showed a trend toward a uniform pattern of disease (LIP and Iδ of 0.94 to 0.99) (Table 3). These results suggest that the distribution pattern of the inoculum is influenced by the plantations management in addition to biological attributes inherent in the aggregate behavior of some pathogens due to the effect of rhizosphere (Mora-Aguilera et al., 1990) and to

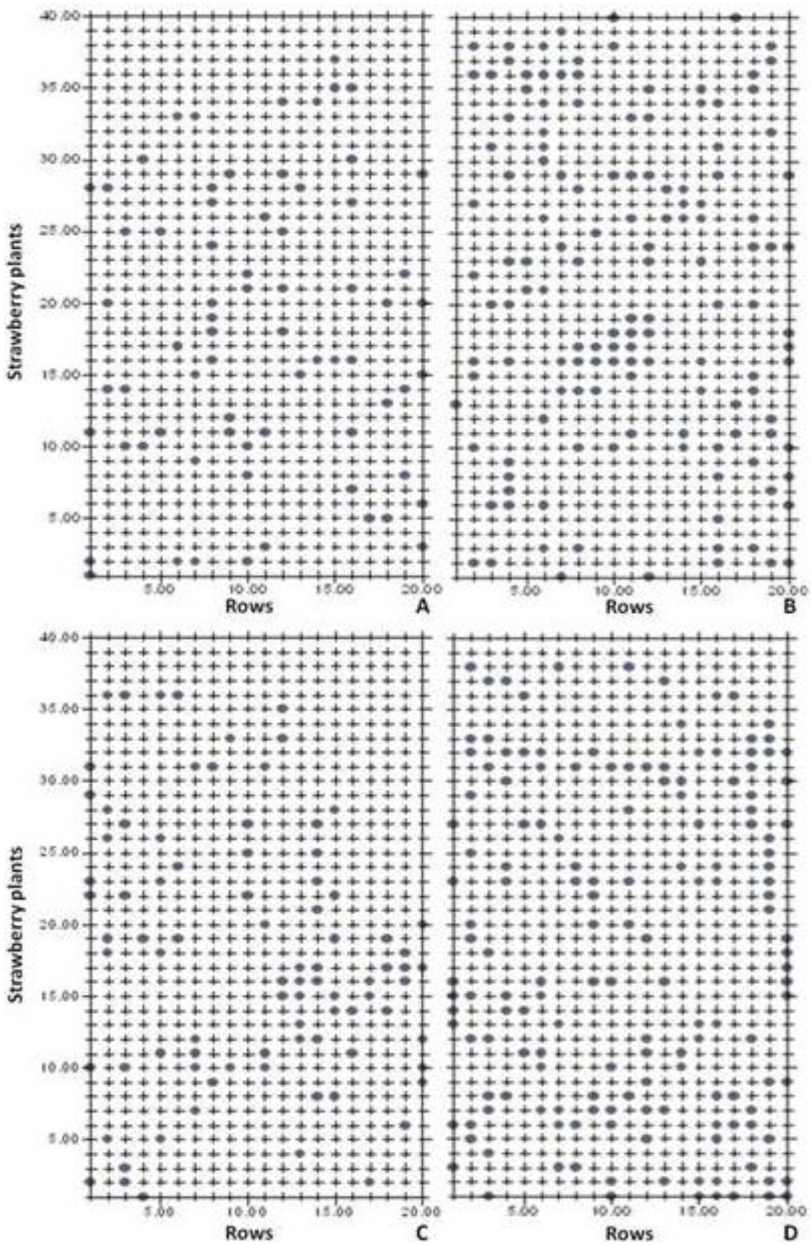

Figure 2. Spatial behavior of strawberry dry wilt into quadrants of 100 m^2 (10 × 10 m) in Tamándaro (A and B 2003-04 cycle, C and D 2004-05 cycle). Plantations with plastic mulch and drip irrigation (A+G) left, and non-mulch and gravity irrigation (T) right.

competition for sites of infection between individuals which can result in consistent patterns of damage (Ludwig and Reynolds, 1988).

Autocorrelation analysis confirmed the spatial effect of management and allowed to estimate attributes of distance and directionality of dispersal. The greatest dispersion in aggregates (continuous dependence) and sub-aggregates (discontinuous dependence), it is generally found in Ario de Rayón and Tamándaro in both crop cycles. In Villafuerte only sub-aggregates were found (Table 4).

Confirmation of aggregates within and between rows was of order 1, with exception of Tamándaro with order 2, which implied small patches of diseased plants. The sub-aggregates were in the range of order 2 to 20 with higher dominance within the row. The epidemic intensity level was not associated with a specific spatial pattern possibly by Y_f less than 23% (Table 2 and Figure 1). With regard to the management, higher aggregation was found in T plantations; with patterns of 1 to 5 diseased plants, by

Table 4. Spatial self-correlation to determine spatial dependence of strawberry dry wilt with two irrigation technologies and three locations of Valle de Zamora, Michoacan, Mexico.

Location (management)	Spatial dependence		
	Within rows	Between rows	GG
2003-04 cycle			
Ario de Rayón (A+G)	C1**, D5*, 11** and 20*	C1**	+
Ario de Rayón (T)	C1*, D7** and 15**	-	+
Tamandaro (A+G)	D8* and 14**	D6*	+
Tamandaro (T)	C2*, D19*	C1**, D13* and 16*	+
Villafuerte (A+G)	D3*	D10**	+
Villafuerte (T)	D2*, 7* and 15**	D2*	-
2004-05 cycle			
Ario de Rayón (A+G)	C1*, D7* and 27*	-	+
Ario de Rayón (T)	D2*, 3**, 4**, 5** and 14*	C1**, D7*, 8**, 15** and 17**	+
Tamandaro (A+G)	C1**, D16**	D2**, 4*	-
Tamandaro (T)	C1**	C1**, D3**, 7** and 10*	+
Villafuerte (A+G)	D8** and 18*	D2* and 12*	-
Villafuerte (T)	D12**	D2* and 7**	+

GG, Continuous general gradient (aggregated); C, continuous dependence; D, discontinuous dependence. The number indicates the “order” of the dependence.

autocorrelation and 1 to 8 plants with geostatistics while in A+G were 1 to 3 and 1 to 5 plants by the respective analysis, which made it possible to visualize patterns of spatial dependence in different directions (Nelson et al., 1999). The dominant sub-aggregates were formed up to 4.5 and 6.5 m in plots T and A+G, respectively. Remarkably, A+G had the smallest aggregates, but the restrictive space could explain the highest displacement of the inoculum within the row. This level of dispersal is consistent with root pathogens, because it depends on the spatial pattern of host population, especially when disease transmission requires contact between healthy and susceptible tissues, that is, root to root (Sujkowski et al., 2000; Willocquet et al., 2000) and the airborne pathogens have a less restrictive dispersion. Since 2006, *Fusarium* wilt of strawberry has increased in incidence and severity in California, USA. Initial problems in 2006 consisted of multiple small patches (2 to 4 beds wide × 3 to 10 m long) of diseased plants; in these patches disease incidence could range from 80 to 100%. By 2009, in some fields, the disease affected large sections that ran the length of the field (Koike et al., 2009). Moreover, the existence of spatial dependencies presupposes the action of a common inoculum source (primary source of infection). This may be valid for a single pathogen-host association. Strawberry dry wilt is caused by a complex of pathogens which involves various sources of inoculum and roles of primary and secondary infection, originating from soil-borne inoculum and diseased plants, respectively (Willocquet et al., 2007; Ellis, 2008; Xiangling et al., 2011). Hence the interpretation of spatial dependencies cannot be via a conventional method without considering other effects such as competition and aggression.

Conclusions

The commercial applications of plastic mulch and drip irrigation (A+G) for the purpose of productivity of strawberry crops in the Zamora Valley were effective in reducing inoculum potential of organisms associated with dry wilt, although these did not induce a total suppressive action. Spatiotemporal studies in two production cycles and three localities selected for high inductance to the disease confirmed in all parameters used (yf, AUDPCa, AUDPEr, AUDPEe, b^{-1}) the lowest epidemic intensity in plantations A+G with reductions in the range of 22.2 to 76.6%. Similarly, the indexes and spatial statistical analysis showed lower aggregates in A+G (up to 5 plants) but found an apparent increased dispersiveness along the row, possibly as a result of its plastic enclosure (up to 6.5 m). The plastic mulch and drip irrigation should be adopted in a program of integrated management of dry wilt. Future studies may be aimed to optimize these technologies with respect to the suppressive ability of the organisms associated with the disease.

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