Factors Associated with *Fusarium* Crown and Root Rot of Asparagus Outbreaks in Quebec

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ABSTRACT

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The *Fusarium* spp. causing *Fusarium* crown and root rot (FCRR) are ubiquitous and abundant in soils, but in contrast, disease expression is localized and sporadic. Previous studies have related FCRR infection to phenolic acids released by asparagus, to the repression of Mn-reducers in soil, and to various soil physicochemical conditions. Fifty commercial asparagus plantations were surveyed using an exploratory approach in order to pinpoint the ecological conditions associated with FCRR development. Twenty-eight variables were used to describe the soil environments of the asparagus crops as well as the influence of crop management practices used locally. The data set was analyzed both as a whole and parsed by main cultivars (Jersey Giant and Guelph Millennium). Both field

conditions and percentage of field area affected by FCRR varied widely between asparagus plantations. Planting depth was positively correlated with percentage of field area affected by FCRR and, hence, deep planting may favor FCRR infection. Plantation age was positively correlated with percentage of field area affected by FCRR, while soil available Mn was inversely correlated. Most importantly, soil Mn availability decreased with increasing plantation age, supporting the hypothesis of an asparagus-mediated negative impact on Mn-reducing bacteria and of the involvement of reduced Mn availability in FCRR development. Improving the availability of Mn could provide a solution to the problem of FCRR in asparagus plantations.

Additional keywords: *Asparagus officinalis*, crop management factor, ecology of disease expression, soil available manganese, soil quality.

Asparagus (*Asparagus officinalis* L.) plantations can be utilized for 15 years and sometimes longer, depending on the resilience of the plantation to *Fusarium* crown and root rot (FCRR). The presence of this disease in plantations markedly reduces the profitability of the crop. *Fusarium* spp. are ubiquitous in soils where they cause damping-off of newly emerged seedlings, wilt, and crown and root rots in different crops (18). In asparagus, the disease is caused by a complex of *Fusarium* spp. including *F. proliferatum* (T. Matsush.) Nirenberg, *F. culmorum* (W. G. Sm.), *F. oxysporum* (Schlenchtend):Fr. f. sp. *asparagi* Cohen & Heald (8,9), and *F. redolens* Wollenw. f. sp. *asparagi* Baayen (1). Reddish lesions develop at the base of stems followed by yellowing and death of the ferns. Depopulated areas in asparagus fields are the typical expression of FCRR occurrence (10).

In contrast to the ubiquity of *Fusarium* spp. in soils, the incidence of FCRR is localized and sporadic suggesting that a set of ecological conditions influences disease development. Information on the ecological conditions associated with the expression of FCRR is sparse. Nonnecke (19) reported that low soil pH and fine-textured soil, or inadequate drainage favor the disease. In a survey of the ecological conditions associated with healthy and infested asparagus plantations, high soil organic matter, high soil water holding capacity, and in contrast to Nonnecke (19), high

clay content were associated with low disease incidence except when drainage was poor (11). In the same study, magnesium (Mg) appeared to be the only deficient nutrient in infested fields and this condition was associated with high soil potassium (K) to Mg ratio. In agreement with Nonnecke (19), conducive sites had a low soil pH. Gehlker and Scholl (11) concluded that FCRR disease in asparagus was provoked by several soil-related factors with negative impact on plant growth, which could be acting either independently or in combination. Asparagus plants weakened by stresses related to nutrients, water, harvest, or other factors may be more susceptible to infection, but certain ecological conditions may also be conducive to increased aggressiveness of indigenous *Fusarium* populations, which would lead to disease outbreaks. Sodium chloride (NaCl) is known to reduce the severity of FCRR (7,21), and it was proposed that the positive effect of NaCl was due to a plant-mediated stimulation of manganese (Mn) reducing bacteria, which would, in turn, increase soil Mn availability in asparagus plantation soils (21). Application of NaCl resulted in increased Na and decreased Mg and calcium (Ca) concentrations in asparagus tissues (27) suggesting that the effect of NaCl could also be physiological. However, in a split root experiment, disease suppression was stronger where NaCl was applied (10). This suggests that the impact of NaCl is not, or is not only plant-mediated, but is also soil-mediated, although the amount of NaCl used was apparently too low to have fungicidal activity.

Resolving the complexity of the soil-*Fusarium*-asparagus interactions may promote the development of methods to control FCRR. Therefore, a multidisciplinary study was undertaken to improve our understanding of FCRR in the province of Quebec, Canada. An improved selective medium for the isolation of

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Fusarium species from asparagus fields (25) and a culture-independent molecular fingerprinting approach to assess *Fusarium* diversity (29) were developed. The distribution in soil and plant parts of 16 *Fusarium* taxa isolated during the survey, and the diversity of their populations, as affected by geographic localization is reported elsewhere (26). The analysis of the relation between FCRR and soil-microbiological variables revealed a microbial community structure reorganization occurring concurrently with the development of FCRR (12). The objective of the present paper was to identify the soil physicochemical conditions and management variables that may favor or suppress FCRR expression.

MATERIALS AND METHODS

Experimental conditions. Fifty commercial asparagus fields were sampled during the last 2 weeks of June 2001, which corresponded to the end of the asparagus-harvesting season in Quebec, Canada. The fields were located in four regions of Southern Quebec, namely Quebec City, Trois-Rivières, L'Assomption, and St-Hyacinthe. Information on crop management practices (Table 1) was obtained from growers via a survey completed with the assistance of local agronomists. Physicochemical characteristics of soil from the asparagus fields were determined (Tables 1 and 2). The cultivars planted in the fields studied were Guelph Millennium (13 fields), Jersey Giant (15 fields), Viking KB3 (4 fields), Lucullus (2 fields), Jersey Knight (4 fields), Mary Washington (1 field), and SYN-456 (5 fields). Cultivar identity was uncertain in seven fields. Plantation age spanned from 1 to 21 years.

TABLE 1. Characteristics of the asparagus plantations surveyed

Parameters	Median	Mean	Min.	Max.
Area affected (%)	10.0	12.3	<0.1	65.0
N-fertilization rate (kg N ha ⁻¹) ^a	95	83.1	0.0	147.5
P-fertilization rate (kg P ₂ O ₅ ha ⁻¹) ^a	55.0	57.0	0.0	125.0
K-fertilization rate (kg K ₂ O ha ⁻¹) ^a	75.0	82.9	0.0	186.0
Harvest length (day)	36.0	33.2	0.0	60.0
Yield (kg fresh weight ha ⁻¹)	1,500	1,720	0	5,000
Length of spears harvested (cm)	21.5	21.5	20	26
Row spacing (m)	1.5	1.4	0.3	1.8
Planting depth (cm)	20.0	21.8	15.0	31.0
Tillage depth (cm)	5.0	4.6	0.0	15.0
Area in cultivation (ha)	1.2	3.3	<0.1	23.0
Plantation age (year)	5.0	7.5	1.0	21.0

^a Forty-three asparagus plantations were surveyed. Sixteen fields receiving organic fertilization were excluded from this group because the amount of nutrients provided by the organic amendments was unknown.

Sampling and analysis. The Quebec asparagus industry occupies the local market of fresh consumption and asparagus is typically one of many crops grown on mix production farms having less than 0.1 to 23 ha in cultivation (Table 1). Quebec asparagus fields are typically a fraction of a hectare in size. The proportion of the field area occupied by depopulated zones bordered with plants harboring lesions and yellow ferns typical of FCRR was visually estimated and rated as a percentage of field area affected. Two microsites with at least three asymptomatic or three symptomatic asparagus plants were chosen at each plantation site. At each microsite, a composite sample made of three 100-g soil samples of each plant environment taken 20 cm from the plant (approximately 10 m apart) was collected from the top 0 to 15 cm of the soil with a probe. The proportion of sand, silt, and clay in the soil substrate was determined according to Sheldrick and Wang (24); soil nitrate (NO₃⁻) and ammonium (NH₄⁺) were determined by the method of Maynard and Karla (17); and soil available P, K, Ca, Mg, Mn, Cu, Zn, and Na were extracted with Mehlich-3 solution (23). Soil pH was determined in water (13), and soil organic matter was determined by the method of weight loss-on-ignition (22).

Data analysis. Microenvironments with symptomatic plants and microenvironments with asymptomatic plants were compared using paired *t* tests and found to be similar. Data from the microenvironments of symptomatic and asymptomatic plants were combined into a complete data set. Principal component analyses (PCAs) were conducted to explore the complete data set and to identify the variables potentially correlated with the percentage of field area affected by FCRR (14).

The PCAs for correlation matrices were sought, as variables had different units (14). One was set as the minimum Eigenvalue for factor consideration, as per Kaiser's rule. In the exploratory phase of data analysis, multiple PCA models were created successively using various sets of variables related to soil physicochemical characteristics, or crop management practices. This grouping was done to avoid placing excessive weight on soil nutrient influence by including soil fertility and fertilization-related variables in the same model. Numerical variables were selected for their level of influence on the plant-soil systems, as assessed by PCAs. The models best explaining the variance found in the data were used as indicators of relationships or absence of relationship between the percentages of field area affected by FCRR and soil characteristics or management-related variables. Soil organic matter was considered to be a management-related variable since, in light textured soils, organic matter level is largely influenced by site preparation, tillage intensity, and organic fertili-

TABLE 2. Soil physicochemical characteristics of symptomatic and asymptomatic microsites

	Min.	Max.	Microenvironment with			
			Symptomatic plants		Asymptomatic plants	
			Mean	95% CI ^a	Mean	95% CI ^a
Clay (%)	0.0	29.3	4.8	1.5	5.3	1.7
Silt (%)	3.8	66.8	22.0	4.5	23.0	4.5
Sand (%)	19.6	95.0	73.2	5.7	71.8	5.8
Soil organic matter (%)	1.55	11.66	4.77	0.59	5.00	0.49
pH	4.35	7.41	5.90	0.19	5.80	0.18
NO ₃ -N (µg g ⁻¹)	6.3	225.5	32.8	9.4	33.9	10.2
NH ₄ -N (µg g ⁻¹)	0.00	9.88	0.54	0.51	0.30	0.36
P (µg g ⁻¹)	57	844	284	49	284	54
Na (µg g ⁻¹)	0.00	22.49	5.57	1.41	5.70	1.64
Mg (µg g ⁻¹)	17	261	83	14	85	16
K (µg g ⁻¹)	51	591	244	38	264	37
Ca (µg g ⁻¹)	103	5,521	1,781	298	1,594	270
Mn (µg g ⁻¹)	1.4	59.6	14.8	3.18	14.8	2.8
Fe (µg g ⁻¹)	81	476	219	30	213	27
Cu (µg g ⁻¹)	0.10	10.00	1.87	0.43	1.99	0.50
Zn (µg g ⁻¹)	1.2	41.0	6.3	2.1	6.1	1.7

^a 95% confidence interval of the mean.

zation, which was practiced to different degrees in 38% of the fields. The variables included with “percentage of field area affected by FCRR” in a model were unrelated as verified by correlation analysis when collinearity was suspected; when correlation occurred, the variable contributing best to model performance was retained and the other was rejected.

In a second step, the potential relationships between variables and percentage of area affected by FCRR, as revealed by PCA, were further examined by regression analyses. While PCA efficiently indicates where relationships could be found, this analysis gives little information on the nature of these potential relationships. Regression analysis was used to confirm relationships and give information on their nature and magnitude. Statistical analy-

ses were conducted on the entire data set and on two subsets of the data that were related either to cv. Guelph Millenium or cv. Jersey Giant, the cultivars encountered most frequently in our study. All statistical analyses were conducted using the software Systat (version 9.1, Systat Software Inc., Richmond, CA).

RESULTS

The characteristics of soil microenvironments with symptomatic and asymptomatic plants were similar (Table 2). The soil environment varied extensively between fields, however, and potential association of variables with FCRR was investigated using PCA.

PCA of the six most significant crop management variables produced a two-factor model explaining 66.3% of the variance (Fig. 1). This model revealed positive relationships that were confirmed by regression analysis. There was no relationship between percentage of field affected by FCRR and row spacing, but percentage of field area affected by FCRR was correlated with both depth of planting ($P = 0.004$) (Fig. 2) and plantation age ($P < 0.001$) (Fig. 3A). Although these relationships were highly significant, their low correlation coefficient ($R^2 = 0.2333$ for plantation age and $R^2 = 0.1112$ for planting depth) indicated that other factors also influenced the percentage of field area affected by FCRR.

Since different cultivars express different susceptibilities to FCRR (12), the data set also was analyzed by cultivar to avoid confounding effects of cultivar within plantation age (Fig. 3). The frequency of ‘Guelph Millenium’ (13 plantations) and ‘Jersey Giant’ (14 plantations) in our data set made it possible to search for the factors affecting FCRR expression within each of these cultivars. Furthermore, ‘Guelph Millenium’ and ‘Jersey Giant’ are particularly important because they are the cultivars currently used in new plantations. The use of the ‘Guelph Millenium’ and ‘Jersey Giant’ subsets, therefore, enabled the development of PCA models that explained more exactly the variation present in the data, justifying the parsing of the complete data set into cultivar-related subsets. A PCA solution obtained from the analysis of the crop management variables of the data for ‘Jersey Giant’ revealed a relationship between plantation age and percentage of field area affected (Fig. 4A). This positive relationship was strong, supported by the high R^2 value of the polynomial relationship between these two variables (Fig. 3B) ($R^2 = 0.6806$).

Fig. 1. Factor loading plot of the best solution of a principal component analysis conducted on the all-cultivar data, using crop management-related variables and the percentage of field area affected by Fusarium crown and root rot. Factor 1 explains 41.14% of the variance and factor 2 explains 25.16%; cumulative variance explained 66.30%. Eigenvalues are 2.47 for factor 1 and 1.51 for factor 2.

Fig. 2. Relationship between asparagus planting depth and percentage of area affected by Fusarium crown and root rot. Regression $R^2 = 0.1112$, $P = 0.004$; coefficient $P < 0.001$.

Fig. 3. Relationship between asparagus plantation age and percentage of area affected by *Fusarium* crown and root rot. **A**, Regression $R^2 = 0.2333$, $P < 0.001$; coefficient x^1 , $P = 0.002$; coefficient x^2 , $P = 0.08$. **B**, Regression $R^2 = 0.6806$, $P < 0.001$; coefficient x^1 , $P < 0.001$; coefficient x^2 , $P = 0.001$. **C**, Regression nonsignificant.

The relationship suggests that FCRR progression is slow during the first few years after planting and progresses at an increasing rate as plantations age. It is important to note, however, that this relationship depended heavily on the observation associated with one highly affected 21-year-old field (Fig. 3B). Furthermore, several highly affected fields were likely destroyed as time went by, and hence, since older fields were obviously under-represented, the relationships presented in Figure 3A and B probably underestimate FCRR development rate in the later part of the curve. On the contrary, the percentage of field area affected by FCRR was not related to plantation age in fields of 'Guelph Millenium' (Fig. 3C) probably because there was no 'Guelph Millenium' plantation older than 7 years; the 'Guelph Millenium' cultivar was released only recently.

PCA of the 'Jersey Giant' data subset pertaining to soil characteristic variables suggested an inverse relationship between the percentage of field area affected and the level of available soil N and Mn, in a two-factor model involving eight variables, which explained 71.2% of the variance (Fig. 4B). Regression analysis did not support the negative relationship between nitrogen level and percentage of field area affected ($P = 0.10$), but confirmed the occurrence of a logarithmic relationship ($P < 0.002$) between field area affected and the level of available soil Mn (Fig. 5B). A relationship between field area affected by FCRR and soil available Mn was also found ($P < 0.001$) when the complete data set was submitted to regression analysis (Fig. 5A), but this relationship was not found in 'Guelph Millenium' fields (Fig. 5C), perhaps because the range of FCRR expression levels in 'Guelph Millenium' plantations was too small for this relationship to be revealed. Importantly, Mn availability was observed to decrease with plantation age as indicated by regression analysis. Again, the correlation existed when the whole data set ($P = 0.002$) (Fig. 6A) or only the 'Jersey Giant'-related data ($P = 0.02$) (Fig. 6B) were considered, but not when the 'Guelph Millenium' data were examined (Fig. 6C).

A three-factor PCA model involving seven crop management-related variables of the 'Guelph Millenium'-related data subset explained 86.8% of the variance (Fig. 7A). However, the factor-loading plots of this best PCA solution did not indicate any clear relationship between FCRR and crop management-related variables. The absence of a significant relationship also was confirmed

Fig. 4. Factor loading plots of the best solutions of principal component analyses conducted on the 'Jersey Giant' data. **A**, Relationship between crop management-related variables and the percentage of asparagus field area affected by *Fusarium* crown and root rot. Factor 1 explains 45.20% of the variance and factor 2 explains 29.97%; cumulative variance explained 75.18%. Eigenvalues are 2.26 for factor 1 and 1.50 for factor 2. **B**, Relationship between variables related to soil characteristics and the percentage of asparagus field area affected by *Fusarium* crown and root rot. Factor 1 explains 50.71% of the variance and factor 2 explains 20.51%; cumulative variance explained 71.23%. Eigenvalues are 4.06 for factor 1 and 1.64 for factor 2.

by regression analysis. Eight variables pertaining to soil characteristic in the 'Guelph Millenium'-related data subset produced a best PCA solution explaining 76.1% of the variance in a three-factor model (Fig. 7B). The latter model did not indicate any clear relationship between FCRR and soil characteristic variables. As for crop management-related variables, no significant relationship between the percentage of field area affected by FCRR and any soil characteristic variable could be found using regression analysis.

DISCUSSION

This unique study on association of cultural and edaphic factors with FCRR of asparagus in Quebec plantations revealed a number of significant although weak relationships, expressing the complexity of the asparagus field system and that of FCRR. It probably also reflects noise created by important influences on the systems, such as climate, which cannot be controlled. Investigations conducted in situ typically yield imprecise, but highly relevant, results. Soil Mn availability was negatively correlated with the percentage of field area affected by FCRR, suggesting an association between FCRR development and scarcity of soil available Mn. The absence of differences in soil Mn levels between

microplots with asymptomatic and with symptomatic plants, within fields, suggests that low Mn availability is conducive to FCRR outbreaks without being the cause of the disease. The negative correlation between available Mn and FCRR concurs with the conclusion of other studies (6,10). In previous reports, the beneficial impact of NaCl application was associated with increased Mn availability. The reduction of FCRR severity with NaCl application was concurrent with an increase in the number of Mn-reducers in soil, mostly fluorescent pseudomonads but also some *Serratia* spp. In our study, we found that higher levels of available Mn were correlated with low percentages of field area affected by FCRR. We also have reported elsewhere (12) that a profound reorganization of microbial community structure occurred concurrently with the development of FCRR, in the soil of the asparagus plantations surveyed. This change in microbial community structure was associated with FCRR development. We did not quantify Mn-reducers in soil, but a shift in soil microbial community involving a decrease in the Mn-reducer population, as reported previously in fields of the United States (6), or conversely, an increase in Mn-oxidizers may have occurred. This would be in agreement with our observations of bacterial biodiversity shifts (12). Reports on the negative effects of phenolic acids produced by asparagus on soil microorganisms (4,20) along with the appearance in this study of reduced soil Mn availability

Fig. 5. Relationship between percentage of area affected by Fusarium crown and root rot and soil Mn availability in soil. **A**, Regression $R^2 = 0.1285$, $P < 0.001$; coefficient x^1 , $P = 0.001$; coefficient x^2 , $P = 0.04$. **B**, Regression $R^2 = 0.2910$, $P = 0.002$; coefficient x^1 , $P = 0.005$; coefficient x^2 , $P = 0.01$. **C**, Regression nonsignificant.

Fig. 6. Relationship between plantation age and soil available manganese. **A**, Regression $R^2 = 0.1044$, $P = 0.002$; coefficient x^1 , $P < 0.001$. **B**, Regression $R^2 = 0.1843$, $P < 0.02$; coefficient x^1 , $P < 0.001$. **C**, Regression nonsignificant.

with increasing plantation age would rather suggest that asparagus exudates suppress Mn-reducers. Many Mn-reducing bacteria have disease suppressive characteristics in addition to increasing Mn availability in soil (6,10). Thus, asparagus exudates might increase the conduciveness of soil toward FCRR.

The problem of FCRR would be alleviated by Mn fertilization if FCRR is simply related to the scarcity of soil available Mn. Application of Mn fertilizer to soil can have two effects. First, it can improve the Mn nutrition of plants and other soil inhabitants. Improved asparagus Mn nutrition may be important as suggested by the fact that reduced disease severity following NaCl application was associated with increased Mn in asparagus roots (10). Second, applications of Mn to soil could also reduce the negative impact of phenolic acids produced by asparagus as Mn was shown to be involved in the abiotic oxidation of phenolic compounds (16). Soil available Mn may enhance the destruction of toxic phenolics accumulating in soil under asparagus and thus increase the length of the productive period of asparagus plantations. The production of autotoxic compounds by asparagus roots is well documented (2,15,30,31).

The causal agents of FCRR of asparagus are widespread in plantations, yet asparagus does not always express FCRR symptoms. Blok and Bollen (3) found *F. oxysporum* f. sp. *asparagi* in 69% of fields surveyed in asparagus growing regions of the Netherlands although these fields were never planted with asparagus. Only slight root rot symptoms could be seen, although most 1-year-old asparagus crowns from nurseries were infected with *F. oxysporum* f. sp. *asparagi*. In Northeastern United States, Elmer (8) surveyed asparagus sprouts sold in 22 markets of New Haven, CT, and found *F. oxysporum* and *F. proliferatum*, causal agents of FCRR, on the basal parts of 16.5% of the 1,776 spears examined. In our survey, pathogenic *Fusarium* spp., in particular *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*, were found in all plantations on both asymptomatic and symptomatic plants (26). This suggests that FCRR expression requires the combined influence of pathogenic *Fusarium* strains and other factors, such as reduced Mn availability.

Outbreaks of FCRR may depend not only on inoculum distribution in the plantation soils but also on the susceptibility of individual plants. This susceptibility might vary from plant to plant of one cultivar as a consequence of the accumulation of

autotoxic materials in soil or of stress from differential pressure of other pests (e.g., rust, which is also common in Quebec plantations). Overlapping multiple influences, each with low spatial heterogeneity, could result in enhanced overall spatial heterogeneity of conduciveness to FCRR; thus, the requirement of multiple factors for FCRR outbreaks would explain the patchiness of disease expression in the field.

Planting depth was also positively correlated with FCRR, but only when the whole data set was analyzed. This relationship had a low coefficient of determination ($R^2 = 0.1112$), and hence, it is difficult to conclude with certainty that planting depth influenced the percentage of field area affected by FCRR. It is interesting to note, however, that a negative relationship between resistance to crown rot and depth of crown formation was reported in wheat (28). A similar effect of crown placement may also influence the success of pathogenic *Fusarium* in asparagus. The possibility of a planting depth effect on FCRR should be further investigated.

Soil pH, texture, and drainage were not identified as factors affecting FCRR development in this study. This contrasts with a previous report indicating increased disease incidence in fine-textured soils (19). Fine-textured soils are relatively nutrient rich, whereas coarse-textured soils are typically nutrient poor. Micro-nutrient deficiencies are more likely to occur in these latter soils (5). Hence, it may be worthwhile to revise the nutrient requirements of asparagus. Our observations indicate that, in particular, Mn fertilization and Mn uptake by asparagus should be examined in relation to disease severity. In low pH soils, FCRR is supposedly more severe (19). We did not observe any effect of pH, although values ranged from 4.35 to 7.41 (Table 2). Thus, it appears that in Quebec asparagus fields, pH is not a factor determining the percentage of field area affected by FCRR.

Based on our results, a solution to FCRR may be found in the management of Mn availability in soil. Further investigation is needed to determine if Mn effect on FCRR proceeds through enhanced plant Mn nutrition or through an Mn-mediated enhancement of the degradation of phenolic acids in the soil under asparagus. It is also possible that the observed relation between Mn and FCRR simply reflects the importance of the negative impact of Mn-reducers on *Fusarium* in soil. In any case, planting depth should be kept shallow to prevent possible negative effects of deep planting.

Fig. 7. Factor loading plots of the best solutions of principal component analyses conducted on the 'Guelph Millennium' data. **A**, Relationship between crop management-related variables and the percentage of field area affected by *Fusarium* crown and root rot. Factor 1 explains 39.18% of the variance, factor 2, 25.90%, and factor 3, 21.69%; cumulative variance explained 86.77%. Eigenvalues are 2.63 for factor 1, 1.61 for factor 2, and 1.48 for factor 3. **B**, Relationship between variables related to soil characteristics and the percentage of asparagus field area affected by *Fusarium* crown and root rot. Factor 1 explains 41.90% of the variance, factor 2, 20.12%, and factor 3, 14.08%; cumulative variance explained 76.10%. Eigenvalues are 3.35 for factor 1, 1.61 for factor 2, and 1.13 for factor 3. Factors 1 and 2 give a realistic picture of what the data look like. Thus, for clarity, two-dimension plots rather than three-dimension plots are presented (14).

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