European Journal of Plant Pathology (2006) 116:187–198
DOI 10.1007/s10658-006-9046-z

Spatial pattern of Cercospora leaf spot of sugar beet in fields in long- and recently-established areas

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Accepted 6 July 2006

Key words: Beta vulgaris, semi-variogram analysis

Abstract

Spatial disease pattern of *Cercospora beticola* was characterised during natural epidemics of Cercospora leaf spot (CLS) in sugar beet. We applied linear regression and geostatistical analyses to characterise CLS spatial patterns in three field trials, in long-established and recently-established CLS-areas, during two consecutive years. Linear regression showed a positive influence of average disease severity of within-row neighbouring plants ($0.38 < \hat{\beta}_1 < 0.88$). Semi-variograms modelled the spatial dependence of disease severity for two directions per week in both years. Disease severity displayed strong spatial dependence over time. The within-row spatial dependence was the largest, but across-row dependence was irregular and weaker. Both long- and recently established areas showed strong spatial dependence of disease severity within row, decrease in variability between years and within the second trial year and a relation between $\hat{\beta}_1$ and the relative nugget. Observed differences were more field than area specific. These spatial and temporal analyses indicated that disease severities of adjacent plants were dependent; hence, we concluded that *C. beticola* is dispersed mainly over short distances from plant to plant.

Introduction

Cercospora beticola causes Cercospora leaf spot (CLS) on sugar beet (*Beta vulgaris*). First symptoms in The Netherlands normally occur from mid-June after row closure. The incubation period (time from infection to leaf symptoms) of *C. beticola* is approximately 8–10 days in the field, depending upon temperature and humidity (Shane and Teng, 1982). The pathogen is dispersed over short distances by conidia during the growing season, with rain considered the principal agent and wind playing a secondary role in inoculum dispersal (McKay and Pool, 1918; Carlson, 1967). The leaf spots coalesce to form large necrotic areas. Under favourable weather conditions, i.e. high temperature and high relative humidity at night, the necrotic areas increase in size and finally all leaves become necrotic. In this phase, flushes of new growth of leaves occur, which reduce sugar content and weight of the roots. At harvest the leaves are cut and left in the field in most beet-growing areas. Before a new crop is grown, the arable layer is tilled and infected leaf material is buried into this layer. Pseudostromata of CLS surviving in the soil are capable of infecting sugar beet plants in later years (Nagel, 1938). First infections may occur on the lower leaves by splash dispersal of inoculum from the soil (Nagel, 1938), or through the roots (Vereijssen et al., 2004, 2005). Later, infections take place on higher (younger) leaves both by autoinfection, e.g. re-infection of the same plant, and by

alloinfection, i.e., inoculum spreads to adjacent plants. In fields with no history of sugar beets, however, CLS also can be severe, thus making the occurrence of disease unpredictable.

In The Netherlands, a relevant distinction can be made between long-established CLS areas with historically high disease severities and recently-established CLS areas with low disease severities. High disease severity areas, located mainly in the south-eastern part of the country, have a long history of CLS (>25 years), whereas low disease severity areas in the other areas are characterised by more recent outbreaks of CLS. Because CLS is becoming more severe in The Netherlands, a better understanding of its epidemiology is needed for effective disease control. This study was motivated by preliminary observations in 1999, when differences in spatial variation of CLS between long- and recently-established disease areas were noticed. In the long-established areas (Cercospora outbreaks since 1977) a uniform CLS pattern occurred, with almost all plants having a similar level of disease according to the Agronomica diagram (Battilani et al., 1990; Vereijssen et al., 2003). In the recently-established areas (Cercospora outbreaks since 1991), healthy plants occurred next to heavily diseased plants, resulting in a patchy occurrence of CLS. A wide range of soil-borne and aerial pathogens and their resulting diseases are characterised by spatial patchiness (Campbell and Noe, 1985). The spatial pattern of a disease may change over time, such as described for the pathogens *C. arachidicola* in groundnut (Lannou and Savary, 1991), and *Leptosphaerulina briosiana* in alfalfa (Thal and Campbell, 1986).

Spatial patterns of CLS on sugar beet have not been documented. For other diseases much work has been done on spatial patterns of incidence (Nelson and Campbell, 1993; Stein et al., 1994; Rekah et al., 1999) and severity (Thal and Campbell, 1986; Lannou and Savary, 1991). Severity data often reflect the importance of environmental conditions for pathogen reproduction and spread. For CLS, disease incidence data cannot be used to assist directly in disease management as incidence increases rapidly, but severity data can readily be used to predict disease development.

The objective of this study was to model spatial patterns of CLS of sugar beet using (geo)statistical methods. In particular, we focused on assessing differences in patterns between long-established and recently-established CLS areas in successive years. First-order spatial dependence was modelled using a linear regression model. Geostatistics was then applied to quantify second-order spatial dependence. Geostatistics has been used to quantitatively characterise changes in spatial disease patterns over time and to improve understanding of epidemiology and ecology (Chellemi et al., 1988; Lecoustre et al., 1989; Todd and Tisserat, 1990; Lannou and Savary, 1991; Munkvold et al., 1993; Stein et al., 1994; Larkin et al., 1995).

Materials and methods

Field trial establishment

CLS epidemics in sugar beet in the Netherlands were studied during two consecutive growing seasons in the long-established area in fields near Vlodrop and Koningsbosch (province of Limburg) and in the recently established area in fields near Toldijk and Wehl (province of Gelderland) (Table 1). The two areas are separated by approximately 85 km. In 2000, sugar beet fields at Vlodrop (V_0), Koningsbosch (K_0), and Toldijk (T_0) were selected for determining the spatial pattern of CLS. The field at Wehl (W_0) was selected because it differed from the other fields by not having grown sugar beet for over 20 years. Data from this field will only be presented when they differ from the other fields. The fields, except for W_0, were replanted to sugar beet in 2001, and renamed V_1, K_1 and T_1, respectively. The design of the trial was a rectangular block of gross 30 rows $\times$ 7.5 min length; on average 30 plants were assessed per row in 2000, and all plants (varying from 35 to 41 per trial) assessed per row in 2001. Width between rows was 0.5 m, but spacing between plants was slightly different for each trial due to mechanical drilling. The three rows closest to the edge of a field were omitted when establishing the trial in both years, to eliminate field edge effects. Individual sugar beet plants served as sampling units. Farmers' fields were sown with different cultivars equally susceptible to CLS at each location (Table 1). No fungicides were applied, thus allowing a natural CLS epidemic to develop. Drilling, fertilisation and herbicide application were performed according to the farmers' practices at the three sites.

The field at Wehl is situated 15 km (straight line) from Toldijk. In October 2000, metal plates

Table 1. Descriptions of trial fields used in experiments for investigating Cercospora leaf spot (CLS) in 2000 and 2001

Location and year[a]	Province[b]	Sowing dist. within-row (cm)	Cultivar	Rotation[c]	Precrop	Disease assessment (from–until)[d]
V_0	Limburg[L]	16	Lenora	1:4	Maize	Week 30–40
K_0	Limburg	16.5	Winsor	1:5	Maize	Week 29–40
T_0	Gelderland[R]	19	Aristo	1:3	Maize	Week 31–40
W_0	Gelderland	18	Hector	No beets for over 20 years	Grass	Week 34–40
V_1	Limburg	18	Savannah	1:1	Sugar beet	Week 28–39
K_1	Limburg	16.5	Winsor	1:1	Sugar beet	Week 29–43
T_1	Gelderland	19	Savannah	1:1	Sugar beet	Week 32–47

[a]V_0 = Vlodrop in 2000, V_1 = Vlodrop in 2001, K_0 = Koningsbosch in 2000, K_1 = Koningsbosch in 2001, T_0 = Toldijk in 2000, T_1 = Toldijk in 2001, and W_0 = Wehl in 2000.
[b]L: CLS long-established area; R: CLS recently-established area.
[c]Rotation: 1:4 = 1 year of sugar beet after 3 years of other crops.
[d]In calendar weeks, weekly disease assessment started after row closure except for W_0.

(0.10×0.10 m) were dug into the soil at the three sites at 0.30–0.35 m depth, to mark the corners of the field trials. The marked coordinates were recovered using a metal detector. In 2001, sugar beet was sown within the marked coordinates at V_1 (May 14) and T_1 (May 23), which were surrounded by maize crops. At K_1, the field trial was situated in a sugar beet field (May 10), which was not treated with fungicides. Herbicide applications in maize at V_1 caused the loss of three rows of sugar beet, which was taken into account when analysing the data. At K_1, the neighbouring sugar beet field was replaced by wheat in 2001.

Disease assessment

Disease assessment started in different weeks at first appearance of CLS and thereafter weekly until the harvest, pre-set by the Sugar Industry (Table 1). Cercospora leaf spot was assessed visually on individual plants using the pictorial Agronomica disease severity index (Vereijssen et al., 2003, modified after Battilani et al., 1990). This index covers a scale from 0 (healthy) to 5 (totally destroyed foliage), with increments of 0.5. At disease severity = 1, circa 50% of the outer leaves (fully grown or old) show up to 20 spots per leaf. At disease severity = 2, all outer leaves are affected by numerous, but still isolated spots. At disease severity = 3, fully and almost fully grown leaves show several coalesced necrotic areas of 1–2 cm diam, that do not lead to large necrotic areas. Disease severity = 4 represents 80–100% severity of some leaves (2–8). At disease severity = 5 the original foliage is completely destroyed. After reaching 5, an increment of 0.5 was added to the disease index, thus compensating for newly formed leaves becoming diseased. Plants suffering from rhizoctonia disease often died and were marked as missing. At Vlodrop, Koningsbosch, Toldijk and Wehl, rhizoctonia disease was limited to only a few plants (<6 plants) in 2000. At Koningsbosch, rhizoctonia disease was slightly higher (20–25 plants) in 2001, whereas for Vlodrop and Toldijk rhizoctonia disease remained low (<6 plants).

Data analysis

Disease development

Disease incidence (number of plants diseased) and mean disease severity were calculated per field for each week. Maps of disease severities of individual plants were constructed to visualise the spatial pattern for each assessment in each field. Significant differences between disease severities were determined using least significant differences using residual maximum likelihood (REML) analysis.

First order relations: linear regression

We hypothesised that disease severity of an arbitrary plant depends upon the disease severity of its immediate neighbouring plants. As notation, we use the capital Z to denote a variable and a lower case z to denote an observed or measured value. Greek characters denote population parameters;

roman characters denote parameters estimated from the sample. A simple linear regression model was constructed to relate the disease severity of every plant with that of its immediate neighbours within and across. At first a model including upper, lower, left and right neighbouring plants was analysed with upper and lower referring to 'within row' plants, and left and right plants to 'across row' plants. Of the 24 datasets analysed, arbitrarily chosen over field, week and year, the upper plant was statistically significant 18 times, the lower plant 19 times, the left plant five times, and the right plant eight times. Including all four neighbouring plants in the analysis gave a significant regression only 12 times, whereas the regression was significant for all datasets when left and right neighbours were omitted from the analysis (data not shown). Therefore across-row neighbours contributed less to observed CLS severities than within-row neighbours. Let $Z_t(x)$ be the disease severity of an arbitrary plant at location x in week t, and $Z_t(x+1)$ and $Z_t(x-1)$ that of its upper and lower neighbours, respectively, then $Z_t(x)$ is regressed on the mean disease severity of its within-row neighbours [$\tilde{Z}_t(x) = {}^1\!/_2(Z_t(x+1) + Z_t(x-1))$]:

$$\hat{Z}_t(x) = \beta_0 + \beta_1 \tilde{Z}_t(x) \qquad (1)$$

with regression parameters β_0 a constant indicating external factors and β_1, indicating the contribution of average disease severity of the two neighbouring plants to the disease severity of $Z_t(x)$. When the spatial pattern was random, then β_1 equals 0. When the pattern is patchy, then β_1 takes positive values, i.e. the disease severity of a plant depends on the disease severity of its within-row neighbours. For inverse patterns, i.e. showing alterations of high and low values, the β_1 takes negative values. The estimator of β_1 is denoted as $\hat{\beta}_1$.

Second order relations: geostatistical analysis

For each field, each assessment week, and for both years, a semi-variogram was calculated for the disease severities. The semi-variogram, $\gamma_{Z,t}(h)$, is defined as a function of the distance h (lag) between plants as $\gamma_{Z,t}(h) = {}^1\!/_2 E(Z_t(x) - Z_t(x+h))^2$, with $Z_t(x)$ and $Z_t(x+h)$ the disease severities on plants at a location x and a location $x + h$ at a distance h from x, and $E(\cdot)$ the expectation (Chilès and Delfiner, 1999). The semi-variogram is used to measure autocorrelation between plants at various lag-distances (h), lag 1 equals distance to the first neighbouring plant. It increases from a relatively low value (c_0, the nugget) until a sill value (c_1) is reached at distance a, the range. For distances smaller than a, the disease severity is spatially dependent, and beyond a, no spatial autocorrelation exists. The nugget variance is caused by non-spatial variability, such as measurement errors and microvariability less than the shortest sampling distance. The relative nugget, defined as $c_0/(c_0 + c_1)$ measures the fraction of non-spatial variation in the total spatial variability (Stein et al., 1994). Spatial variability is isotropic when there are no directional effects. When a variogram displays different variability in different directions (e.g. within and across rows), spatial variability is anisotropic.

In this study, spatial variability of the diseased plants was studied with omnidirectional and directional variograms, within rows (90°) and across rows (180°), using a directional tolerance of 45° (angles subtended by the segments [degrees] over which averaging is to be done) of 180°, 45°, and 90°, respectively, to ensure sufficient data points for modelling. Linear, spherical and exponential models were fitted to the empirical variograms (Chilès and Delfiner, 1999). Highest R^2 and lowest nugget variance (data not shown) were used as criteria for model selection and only one type of model was used per field trial, unless stated otherwise. The analysis was only done with disease severity data because preliminary field trials indicated a rapid increase in incidence, reaching 100% early in the season. The use of class variables, like the Agronomica disease severity index, in geostatistical analysis is well-founded (Journel, 1983). When non-stationarity of the mean was observed, e.g. a bounded linear model for the omnidirectional variogram, the trend was removed from the original values on the basis of the fitted trend model (Burrough, 1987). The directives FVARIOGRAM and MVARIOGRAM of Genstat 5, release 4.2 (Genstat 4.2, Rothamsted, UK) were used for this analysis.

Relating linear regression with the variogram

We hypothesised that first- and second-order statistics are related, i.e. that a relation exists between estimated parameters of linear regression and the variogram. If so, spatial dependency, observed in

variograms, should then correspond to a term indicating dependence in linear regression. In the linear regression model it is assumed that for each pair of points the parameter β_1 expresses the spatial dependence at distance $h = 1$ (lag 1) for the whole field. In a variogram, the relative nugget indicates the amount of spatial dependence. A natural choice is then the relation between relative nugget and estimated $\hat{\beta}_1$ parameter.

Results

Disease development

At V_0 and V_1 the epidemic started on the east-side of the field, bordering a sandy road, and severities were higher on the east-side in both years. The mean disease severity (MDS) increased from 0.0 ± 0.5 in week 32 to 2.4 ± 0.9 in week 40 for V_0 and from 0.1 ± 0.2 in week 31 to 3.3 ± 0.6 in week 39 for V_1. At T_1 a disease gradient that led to non-stationarity of the mean was observed. Disease severities on the west-side of the plot, adjacent to a maize field, were higher than on the east-side. MDS increased from 0.1 ± 0.3 in week 32 to 3.3 ± 0.9 in week 40 for T_0 and from 0.6 ± 0.5 in week 34 to 4.7 ± 1.3 in week 47 for T_1. The spatial distribution of CLS over time for V_0, V_1, T_0, and T_1 is illustrated in Figure 1. Disease at K_0 and K_1 increased gradually in time. MDS for K_0 increased from 0.0 ± 0.2 (week 31) to 3.7 ± 0.9 (week 40) and for K_1 from 0.0 ± 0.2 (week 31) to 3.9 ± 0.7 (week 43). CLS had a patchy distribution in both years. Weekly MDS at each field increased significantly ($p = 0.05$).

At Vlodrop, Koningsbosch and Toldijk incidence first increased (Figure 2a) and followed by an increase in severity (Figure 2b) when incidence was ca. 60% in both years. The relationship between incidence and severity were similar for Vlodrop, Koningsbosch and Toldijk in both years (Figure 2c). The length of time when 60% incidence was reached differed between the fields. In the second year, 60% incidence was reached two weeks earlier at V_1 and K_1 than in the first year. In the recently established area at T_1, this was one week earlier (Figure 2a).

At K_0 the increase in severity was highest, followed by T_0 and V_0 over the same period (Figure 2b). A faster increase of disease severity was observed at V_1 than at T_1 in 2001 and the increase in severity in the second year in the recently-established area at T_1 was higher than in the first year in the long-established area at K_0 (Figure 2b). For all three field trials, the incidence and severity increased more rapidly in 2001 than in 2000. At W_0 disease increased gradually, but statistically significantly ($p = 0.05$), in time, with a MDS of 0.2 ± 0.5 in week 34 to 2.2 ± 1.0 in week 40 (data not shown). The disease occurred in patches. Disease incidence and severity increased more slowly than in the other fields (Figure 2a and b).

Linear regression

The estimated parameter $\hat{\beta}_1$ fluctuated between weeks within each field and season but was consistently positive, indicating positive within-row dependence except at the beginning of the epidemic. In 2000, estimated $\hat{\beta}_1$ values were between 0.58 ± 0.046 ($R^2 = 20.7\%$) and 0.85 ± 0.035 ($R^2 = 48.2\%$) at V_0, between 0.66 ± 0.039 ($R^2 = 30.6\%$) and 0.86 ± 0.030 ($R^2 = 56.7\%$) at K_0, between 0.64 ± 0.041 ($R^2 = 26.3\%$) and 0.81 ± 0.032 ($R^2 = 49.4\%$) at T_0, and between 0.48 ± 0.050 ($R^2 = 11.4\%$) and 0.80 ± 0.034 ($R^2 = 44.0\%$) at W_0 (data not shown). Thus, the disease severity of a plant depends on its within-row neighbours. In 2001, $\hat{\beta}_1$ values were between 0.38 ± 0.052 ($R^2 = 8.5\%$) and 0.76 ± 0.039 ($R^2 = 41.3\%$) at V_1, between 0.49 ± 0.049 ($R^2 = 12.7\%$) and 0.88 ± 0.029 ($R^2 = 58.6\%$) at K_1, and between 0.46 ± 0.048 ($R^2 = 12.7\%$) and 0.85 ± 0.029 ($R^2 = 57.3\%$) at T_1. Variation in β_1 in 2001 was larger than in 2000, but β_1 had more small values in 2001, indicating less influences of within-row neighbours in disease severities in 2001 than in 2000.

Geostatistical analysis

The disease severity of a given plant was spatially dependent on the disease severities of its within-row neighbours at Vlodrop, Koningsbosch and Toldijk in both years, as indicated by the spherical and exponential within-row semi-variograms (Table 2). In both the long- and recently-established areas, the same behaviour of the semi-variograms was observed (Figure 3). The direction of maximum spatial variation is within-rows (Figure 3); but only

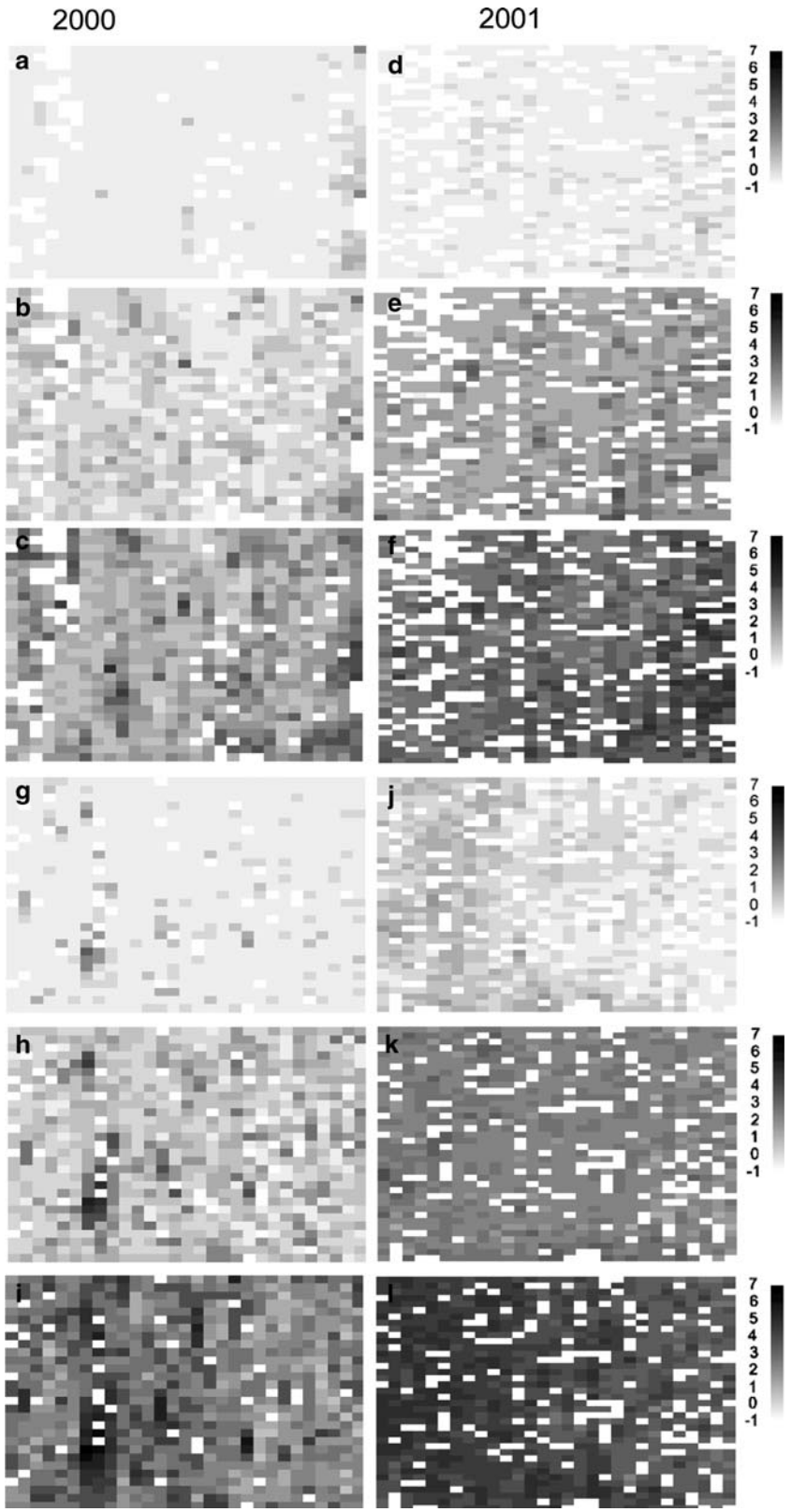
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Figure 1. Spatial patterns of Cercospora leaf spot severity in sugar beet at Vlodrop 2000 (a) week 32, (b) week 35, (c) week 38; at Vlodrop 2001 (d) week 31, (e) week 35, (f) week 39; at Toldijk 2000 (g) week 33, (h) week 37, (i) week 40; and at Toldijk 2001 (j) week 34, (k) week 38, and (l) week 42. Each pixel represents one plant and a blank (white) pixel represents a missing plant (as indicated by −1 in the legend).

irregular semi-variograms could be fitted for the across-row direction (Table 2). At the beginning of the epidemic, disease severity of a plant was not spatially related to disease severities of its neighbouring plants. This is indicated by essentially flat semi-variograms at the early stages of the epidemic (Figure 3). For all three fields in both years, the nugget variance increased when spatial dependence and disease severity increased as the season progressed. The relative nugget, however, remained small (<0.5), which indicates that a large proportion of the variation is explained by spatial variation and there is a large spatial dependence of disease severities within-row.

In the first year, the extent of the within-row dependence in disease severity tended to increase gradually over time. Both the slope and sill of this spatial dependence increased over time (Table 2; Figure 3). At the end of the season, however, there was less dependence in disease severities among neighbouring plants at Koningsbosch and Toldijk, indicating a more uniform infestation of the field (Table 2). At Vlodrop slope and sill increased till the last assessment. In 2001, all three field trials were more uniformly diseased, which resulted in less within-row dependence in disease severities. The semi-variogram sill was lower and the slopes less steep than in the first year. For all three trials disease severities within-row became less dependent on their neighbouring plants, as indicated by a lower sill and less steep slope. At T_1 non-stationarity of the mean was observed, which was a result of the west-side of the trial having higher disease severities than the east-side. At W_0, within-row dependence of disease severities was observed (Table 2). Similar to V_0, the slope and sill of the variograms at W_0 increased until the last assessment in week 40 (Table 2).

For V_0 and V_1, $\hat{\beta}_1$ increased as the relative nugget decreased. For K_0, K_1, T_0, T_1 and W_0 the same trend was observed (data not shown). The correlation coefficient between relative nugget and $\hat{\beta}_1$ was −0.92, −0.77, −0.75, −0.81 and −0.61 for V_0, V_1, K_0, K_1 and W_0, respectively. Thus, disease severity of a plant in the present study was largely determined by the average disease severity of its upper and lower neighbour and CLS was characterised by spatial patchiness rather than by spatial randomness.

Discussion

Using linear regression and geostatistics, we found that in both long- and recently- established CLS areas, the disease severity of a given plant was dependent on the disease severity of its within-row neighbours. Similar spatial dependence has also been shown for the root pathogen *Phymatotrichum omnivorum* in cotton (Stein et al., 1998) and *Phytophthora capsici* in bell pepper (Larkin et al., 1995). Within-row dependence for CLS is expected as conidia are spread over short distances by leaf to leaf contact, rain splash, and/or dew drops (McKay and Pool, 1918; Carlson, 1967). Within-row leaves touch with each other more frequently and overlap earlier in time than with across-row leaves, as the distance between within-row plants (16–19 cm) is smaller than between across-row plants (50 cm). Contrary to other studies where the degree of patchiness increases in time for other leaf diseases (Thal and Campbell, 1986; Lannou and Savary, 1991; Larkin et al., 1995), we observed for Toldijk and Koningsbosch a more uniform infestation at the end of the season (September–October). This was irrespective of the geographical area (long- or recently-established), even when fields were in close vicinity. Microclimates may be affected by cultivar differences in foliage quantity and structure and by the number of missing plants. No relation between decrease of variability and sowing distance or plant population in our trials was found.

Also in the second year, where beets were sown after beets, a more uniform infestation of the field was observed in both long- and recently-established CLS areas. Both linear regression and geostatistical analysis showed that disease severities in the second year were less spatially dependent, which would be expected, where a more uniform build-up of inoculum in soil is likely to account for a more uniform initial infection. This was shown previously for the soil-borne pathogens *Cylindrocladium crotalariae* (Taylor et al., 1981) and

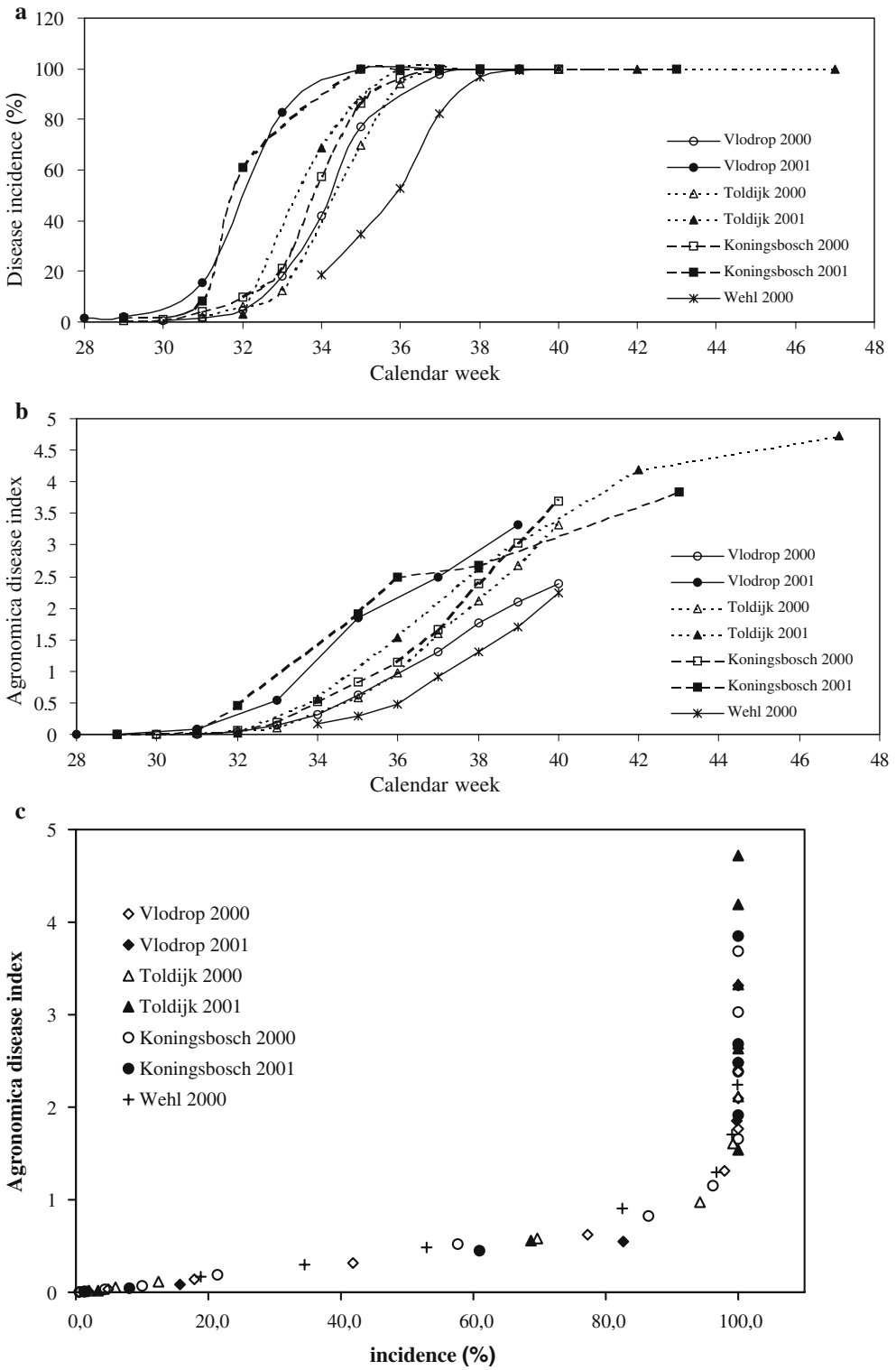

Figure 2. The incidence (a) and average disease severity (b) of Cercospora leaf spot in sugar beet fields in the long established area at Vlodrop and Koningsbosch and the recently established area at Toldijk and Wehl in 2000 and 2001, and the relationship (c) between the Agromica disease index and disease incidence.

Table 2. Variogram parameters, estimated by exponential, linear and spherical models, at each assessment week for Cercospora leaf spot in sugar beet at Vlodrop, Koningsbosch, and Toldijk in 2000 and 2001

Field[a]	Week[b]	Omnidirectional					Across rows					Within rows					
		Model[c]	Nugget[d]	Sill[e]	Range[f]	R^2 [g](%)	Model[c]	Nugget[d]	Sill[e]	Range[f]	R^2(%)	Model[c]	Nugget[d]	Sill[e]	Range[f]	Rel. nugget[h]	R^2(%)
V_0	32	E	0.01	0.02	5.79	96.1	–	–	–	–		S	0.01	0.02	3.42	0.30	45.6
	34	E	0.14	0.20	1.37	67.7	–	–	–	–		S	0.12	0.19	4.89	0.39	89.6
	35	E	0.21	0.27	2.93	66.7	–	–	–	–		S	0.17	0.25	6.80	0.41	85.2
	37	–	–	–	–		–	–	–	–		S	0.14	0.46	4.02	0.23	89.0
	38	–	–	–	–		–	–	–	–		S	0.20	0.59	5.65	0.25	89.2
	40	–	–	–	–		–	–	–	–		S	0.23	0.79	4.64	0.23	94.1
V_1	31	–	–	–	–		–	–	–	–		E	0.03	0.04	3.27	0.46	44.5
	33	L	0.08	PN		94.1	–	–	–	–		S	0.05	0.09	7.74	0.34	92.1
	37	L	0.32	PN		68.7	–	–	–	–		S	0.15	0.34	6.13	0.30	93.7
	39	L	0.26	PN		94.0	–	–	–	–		S	0.17	0.30	7.28	0.36	84.0
K_0	33	E	0.14	0.20	9.54	32.3	L	0.16	PN		56.7	E	0.10	0.21	8.12	0.32	95.5
	34	E	0.25	0.38	6.54	96.5	E	0.28	0.41	9.11	78.0	E	0.13	0.39	4.28	0.26	96.0
	36	E	0.38	0.63	5.66	97.0	E	0.41	0.64	4.60	81.8	E	0.20	0.62	4.14	0.24	97.0
	38	E	0.60	1.14	6.79	97.1	–	–	–	–		E	0.22	1.04	4.25	0.18	99.0
	40	E	0.51	0.92	14.10	84.3	–	–	–	–		E	0.17	0.74	4.05	0.18	96.8
K_1	31	E	0.02	0.03	6.56	75.2	E	0.01	0.03	3.24	56.8	–	–	–	–		
	32	E	0.14	0.27	8.51	95.6	E	0.11	0.27	3.92	77.9	E	0.08	0.20	4.17	0.28	88.3
	35	E	0.25	0.36	8.34	74.2	E	0.22	0.33	2.18	17.3	E	0.05	0.32	3.08	0.14	96.0
	38	E	0.33	0.52	6.31	90.7	E	0.29	0.53	3.46	82.0	E	0.07	0.46	3.02	0.14	96.8
	43	E	0.34	0.50	4.84	90.3	E	0.33	0.53	3.84	79.2	E	0.15	0.44	2.65	0.26	85.8
T_0	33	–	–	–	–		–	–	–	–		–	–	–	–		
	36	–	–	–	–		–	–	–	–		E	0.07	0.42	1.60	0.14	92.9
	38	–	–	–	–		–	–	–	–		E	0.25	0.65	3.07	0.28	92.9
	40	–	–	–	–		–	–	–	–		E	0.04	0.60	2.02	0.06	88.5
T_1	34	E	0.07	0.17	1.56	85.6	E	0.08	0.17	1.33	48.4	E	0.06	0.17	2.47	0.27	93.2
	38	–	–	–	–	–	L	0.11	PN		35.3	E	0.06	0.12	1.52	0.34	79.5
	42	E	0.14	0.17	8.12	95.1	E	0.13	0.17	3.74	73.9	E	0.07	0.17	3.21	0.30	93.7
	47	E	0.06	0.09	3.91	84.6	E	0.06	0.09	11.27	45.8	E	0.06	0.09	2.13	0.59	95.0
W_0	34	–	–	–	–		–	–	–			E	0.02	0.21	0.81	0.10	11.5
	35	E	0.17	0.33	0.71	11.1	–	–	–			E	0.07	0.34	1.43	0.17	89.4
	36	E	0.31	0.44	1.03	44.2	L	0.41	PN		16.4	E	0.04	0.45	1.33	0.09	88.6
	38	L	0.59	PN		67.8	–	–	–	–		E	0.06	0.65	1.44	0.09	90.9

Table 2. (Continued)

Field[a]	Week[b]	Omnidirectional					Across rows					Within rows					
		Model[c]	Nugget[d]	Sill[e]	Range[f]	R^2 [g](%)	Model[c]	Nugget[d]	Sill[e]	Range[f]	R^2(%)	Model[c]	Nugget[d]	Sill[e]	Range[f]	Rel. nugget[h]	R^2(%)
	39	L	0.64	PN		73.9	L	0.59	PN		68.1	E	0.03	0.72	1.47	0.04	92.4
	40	L	0.83	PN		80.2	L	0.80	PN		65.3	E	0.04	0.92	1.79	0.04	96.7

[a]Cercospora leaf spot in sugar beet at V_0: Vlodrop in 2000; V_1: Vlodrop in 2001; K_0: Koningsbosch in 2000; K_1: Koningsbosch in 2001; T_0: Toldijk in 2000 and T_1: Toldijk in 2001.
[b]Calendar week in which disease assessment took place.
[c]Best fitting model for the experimental variogram, based on % variance accounted for by the model and minimal nugget variance. L: linear model ($c_0 + c \cdot h$, which is a special case of the power function with exponent 1); S: spherical model ($\gamma_S = c_0 + c\,\{1.5h/a–0.5(h/a)^3\}$); E: exponential model ($\gamma_e = c_0 + c * \{1\text{-EXP}(-h/a)\}$); and-: no model could be fitted. (c_0 = nugget variance, c = sill, h = distance between plants, and a = range). Variogram model at T_0 and T_1 obtained after pre-whitening.
[d]The value of the variogram near the origin (c_0).
[e]The limiting value of the variogram (c) for large distances, h. For linear variograms: PN = pure nugget.
[f]The range for the variogram (a).
[g]R^2 = percentage variance accounted for by the model.
[h]Relative nugget is the ratio between nugget and sill ($c_0/c + c_0$).

Aspergillus flavus (Griffin et al., 1981). *Cercospora beticola* survives in soil on leaf debris for several years (Nagel, 1938), which is of great importance for crop rotation. Since 2001 CLS has spread over all sugar beet growing areas in only 2 years; most fields are infested now, and all Dutch beet growers must control CLS in the near future.

At Wehl (W_0), where sugar beet had not been grown for over 20 years, or in neighbouring fields, conidia or soil with leaf debris must have been blown in by wind from distant fields. Conidia are not very likely as the nearest sugar beet field was at least 2 km away in a recently-established CLS area, and wind plays a secondary role in conidial dispersal (Carlson, 1967). Wind dispersal of sugar beet crop debris was not described, but is not likely to account for the infestation. Other dispersal agents could be machinery of contractors, humans, insects and seed but these aspects of the epidemiology are not documented.

The patchy occurrence of diseased plants does not mean inoculum had a patchy distribution as symptom development depends on local temperature and humidity (microclimate). The inoculum distribution in soil was not studied. The effect of root infection by *C. beticola* (Vereijssen et al., 2004, 2005) on the spatial distribution is not known. Possibly different tillage techniques and soil biology (antagonists and leaf decaying organisms) have an influence on the distribution or decay of infected leaf material.

For each field, a linear relation was found between β_1, the first-order dependency of disease severity among within-row neighbours, and the relative nugget, the second-order dependency, indicating spatial dependence at lag 1 in both years. With this analysis we have shown that when spatial variation exceeds non-spatial variation (low relative nugget), the upper and lower neighbours become more important in influencing the disease severity of a plant. The lower β_1 in 2001 indicates more autoinfection in the second year. Also β_0 increases more in 2001 than in 2000, suggesting increasing influence of external factors. These factors may be inoculum coming from neighbours > lag 1, a higher inoculum density or another distribution pattern in soil. The increase of β_1 at W_0 suggested more alloinfection with increasing time. This is in agreement with the observed range of spatial dependence (< lag 2) at W_0 and also suggests increasing secondary leaf-to-leaf

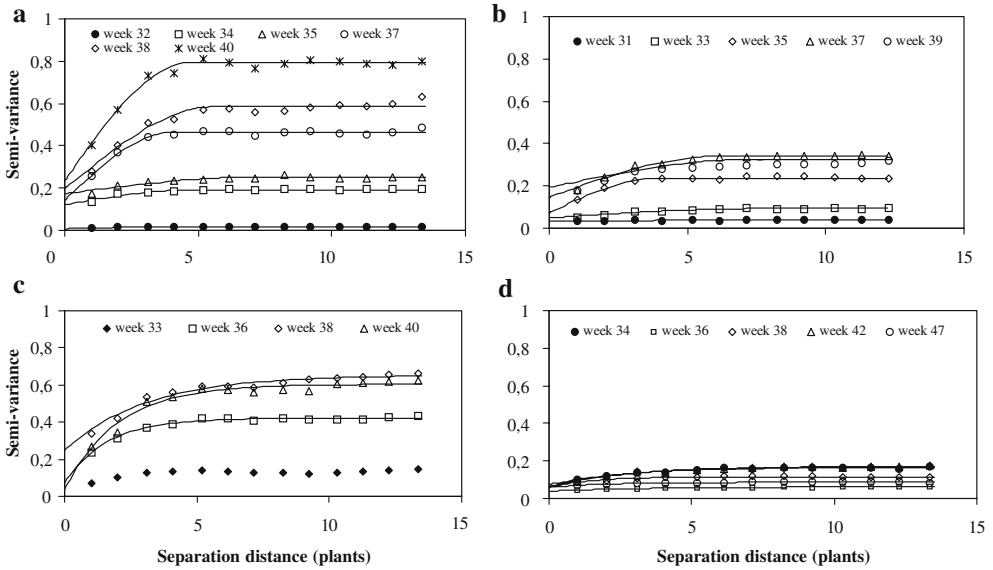

Figure 3. Selected within-row variograms for Cercospora leaf spot in sugar beet at (a) Vlodrop 2000, (b) Vlodrop 2001, (c) Toldijk 2000 and (d) Toldijk 2001. Table 2 has presented the complete data.

spread in time. The amount of alloinfection compared to autoinfection cannot be extracted from our data. In a subsequent paper, within-plant increase in disease will be described and compared with that occurring between plants.

Geostatistics is a useful tool to study spatial dependence. This study showed a change of disease pattern after 1 year, suggesting a relatively fast build-up of inoculum of CLS. As a consequence, inoculum build-up is likely to increase for the more recently-established CLS areas, especially when the crop rotation is narrowed. This is a current problem for Dutch farmers where the area of arable land has decreased by 3.5% over the last 20 years (CBS, 2001). This information may assist in the development of appropriate sampling and control strategies for both long- and recently-established CLS areas. Disease prediction models work reasonably well (Rossi et al., 1994; Windels et al., 1998), but can be improved with an increased understanding of CLS epidemiology, including inoculum build-up and distribution. This study has provided additional insight into the epidemiology of CLS, which can be used in the development of a supervised control system (Vereijssen et al., 2006).

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