A Model for Infestation Foci of Potato Cyst Nematodes *Globodera rostochiensis* and *G. pallida*

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ABSTRACT

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Infestation foci of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) in the provinces of Zeeland, Friesland, Groningen, and Drente of the Netherlands were sampled to validate a model describing infestation foci based on data from Flevoland. Eighty-two fields were presampled to locate infestation foci using a coarse sampling grid (8 × 3 m). Parts of 37 fields containing one or more foci were sampled intensively by extracting at least 1.5 kg of soil per square meter (1.33 × 0.75 m). Forty foci were analyzed for spatial distribution characteristics of cysts using multiple regression with generalized linear models and classical linear models. The results showed that the data from all investigated cropping areas fit well to an exponential model with two parameters, the length and width gradient parameters. Significant differences in these parameter values between cropping areas could not be demonstrated. As both parameters follow a normal distribution, the probability of any combination can be described by a bivariate normal distribution. Gradient parameters were correlated, but significant correlations between these parameters and certain variables such as the nematode species involved, the time interval between sampling and the last potato crop, soil type, cropping frequency, and cyst density in the focus center could not be demonstrated.

Additional keywords: distribution patterns, monitoring, negative binomial distribution, parameter estimation, statistical inference.

Since 1968, the use of nematicides (mostly 1,3-dichloropropene and methyl isothiocyanate) was promoted in the Netherlands as a major solution to all problems caused by plant-parasitic nematodes, especially potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*). Soil fumigation on light, sandy soils under optimum conditions could, presumably, reduce populations of plant-parasitic nematodes by an average of 80%, thus enabling short rotations (once in 3 years or less) while maintaining high yields of economically important crops, especially potatoes (4). At the time, the input of nematicides was thought to be a temporary one, as the availability of other control measures such as biological control and potato cultivars resistant to the newly discovered pathotypes 2 and 3 of *G. rostochiensis* would make the use of nematicides obsolete. However, the expectations of effective biological control agents were not fulfilled. Potato cultivars resistant to the new pathotypes were introduced, but new virulent pathotypes of potato cyst nematodes emerged (pathotypes 2 and 3 of *G. pallida*) and the use of nematicides increased to $12{,}535 \times 10^3$ kg in 1986, 60% of the total pesticide use in the Netherlands (1).

Since 1968, nematicide applications after each potato crop have become common practice in the starch potato production area of the provinces Groningen and Drente, as they were strongly recommended by the provincial research stations and extension services. In the late 1970s, nematicides were also applied, mostly as a precaution, in the province of Flevoland, where seed and table potatoes were grown on a much heavier soil type. Farmers had two reasons for applying fumigants. First, at the time, potato cyst nematodes were quarantine organisms and government legislation did not allow farmers to grow potatoes on infested fields. Second, it was almost impossible for farmers to find out whether their fields were infested or not, as neither the accuracy nor the precision, as defined by Campbell and Madden (8), of the current sampling methods were known. Therefore, farmers tended to apply nematicides as a precaution.

Only in 1985 was research directed towards the efficiency of nematicides on marine clay soils, where most of the Dutch seed and table potatoes are grown. Research into the effectiveness of these nematicides revealed an accelerated breakdown of the active component by microorganisms (17,18), not only in fields that had been fumigated in previous years but also in fields treated for the first time. Mortality was about 50% in fields where accelerated breakdown was observed and did not exceed 70% in fields with a normal breakdown of the active component (5). These figures applied to the first 30 cm of the tilth, but cysts in these and other fields were often found to be evenly distributed down to a 40-cm depth and to occur, in decreasing numbers, in soil layers down to a 60-cm depth. Therefore, the percentage of mortality achieved throughout the root zone, down to 1.2 m on these soils (19), was insufficient to compensate for the density-dependent multiplication rate of potato cyst nematodes on potatoes. Moreover, all infestations mapped during these nematicide trials were patchy and very similar to the medium-scale distribution patterns (further referred to as infestation foci) described by Seinhorst (15). As the infested area was small compared with the area commonly treated with a soil disinfectant, mostly an agricultural unit of 1 to 10 ha, a large proportion of the nematicide was wasted on uninfested soil; the rest did not reach all infested soil strata (5). Therefore, the benefit/cost ratio of soil fumigation was poor.

To reduce the use of nematicides, a sampling method was needed that detects a predefined infestation focus with predefined high probability and minimizes the area of a soil fumigation if one is needed, thus providing the means to make intelligent decisions on the nature and extent of control measures. To develop such a sampling method, a general model was needed that describes the size and shape of an infestation focus. Using data available from the nematicide trials and a few intensively sampled fields in Flevoland, Schomaker and Been (12) developed a compound model for

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infestation foci in Flevoland, further referred to as the Flevoland model, and a simulation model for sampling methods to detect infestation foci in that area. An evaluation by De Groene Vlieg Ltd. and the Ministry of Housing, Physical Planning, and the Environment of the effect of the prototype sampling method in Flevoland concluded that a decrease of 86% or more of the volume of nematicide use could be realized in the tested area (2,12). At the same time, the Dutch government proclaimed a drastic, mandatory reduction of nematicides in the near future (1). As preliminary results with the new sampling method indicated a potential to reduce the use of nematicides substantially, the Flevoland prototype was used throughout the country.

To establish whether the Flevoland model for infestation foci (12) also applies to other cropping areas with different soil types, cropping histories, and cropping frequencies and to estimate the distribution functions of the gradient parameters, it was necessary to map and analyze infestation foci in other parts of the Netherlands. Therefore, in 1990, a research program was initiated to investigate the shape of infestation foci in Zeeland, Friesland, Groningen, and Drente. To this purpose, fields infested with potato cyst nematodes were identified, and the small- and medium-scale distribution patterns of the nematodes were described mathematically using two models for multiple regression, namely classical linear models and generalized linear models (GLMs). The data of Schomaker and Been (12) from foci in Flevoland, suitable for multiple regression analysis, were added to this data set.

MATERIALS AND METHODS

First sampling: Location of the foci. Infested fields, 82 in number, were selected in the cropping areas of Groningen, Drente, Friesland, Zeeland, and Flevoland using sampling results provided by the Dutch Plant Protection Service (PD) originating from their statutory soil-sampling survey of potato fields. The survey gives the dimensions of the field sampled and the number of cysts found in bulk soil samples of 200 ml collected from a sampling area of 0.33 ha (11). Sampling areas were always strips covering the full length of a field in the direction of cultivation with a width varying between 8 and 18 m to obtain the 0.33-ha area needed. For the first sampling, infested strips of fields were divided into rectangular plots of 8 × 3 m (length × width). These plot dimensions were chosen because changes in nematode densities per unit distance are smaller in the length than in the width direction as potato cyst nematodes are mostly dispersed by farmers' practices (i.e., harvesting, tillage, and planting) and, therefore, primarily in the direction of cultivation (15). From the central square meter (0.75 m wide × 1.33 m long) of these 8 × 3-m plots, a sample of approximately 1 kg of soil, consisting of 40 cores of 25 g, was collected with a 25-cm-long, 1-cm-diameter auger. In the Netherlands, it is common practice to grow potatoes in rows with a distance between rows of 0.75 m and an average distance between plants in a row of 0.66 m. Immediately after harvest, without further disturbance of the tilth, the majority of cysts are in the ridges (16). Therefore, the dimensions of the central square meter were chosen so that the relative amount of soil retrieved from ridges and furrows was approximately the same. Soil samples were transferred to special paper bags (Harmonika; Burgers BV, Apeldoorn, the Netherlands) that allow slow evaporation of water from moist soil and do not rupture during this process. Samples in the bags were dried for 1 week at temperatures not exceeding 20°C to keep the nematodes alive. Cysts were extracted from the soil (elutriated) at a station of the Dutch General Inspection Service for Agricultural Seeds and Seed Potatoes in the region where the sample was taken. For clay soils, a carousel (Pollähne, Hannover, Germany) was used; for sandy and peaty soils, the Schuiling centrifuge was used (Folkerts, Pancreas, the Netherlands). Elutriation residues of the sandy and peaty soils were cleaned with acetone, using the improved Seinhorst device (13,15). Finally, cysts were removed from the remaining organic debris and counted. However, in 9 of the 82 sampled fields, no infestations were found. The positive results given by the PD were probably due to cross-contamination of samples in the laboratory.

Second sampling: Mapping of the focus. Lack of capacity prevented a second sampling of the 73 sampled fields in which infestations were confirmed. Thirty-seven fields, at least five fields per cropping area, were chosen for the second sampling. Apart from the criterion that gradients in foci should be large enough to make regression analysis possible, this choice was random. The requirement of sufficiently large gradients implied that very small foci and one infestation caused by filling of a ditch with infested soil were excluded. No bias in the results was expected from the restriction on the size of foci for two reasons. First, the locomotion of potato cysts nematodes is limited to a few centimeters per year and the dispersal mechanisms, which consist mainly of agricultural machinery, are the same for small and large infestations; and second, in results from earlier investigations, the decrease in cyst numbers per unit distance was independent of location in the focus and, therefore, of cyst numbers (12,15). Depending on the size of the focus, which was estimated from the first sampling, every square meter was sampled with a 1.33 × 0.75-m grid or a wider grid ranging up to 4 × 3 m. Samples of 1.5 to 2.5 kg of soil were collected from each square meter (1.33 × 0.75 m; length × width) by taking 80 cores with the above-described auger in a stratified plan (as defined by Campbell and Madden [8]). Sample size was chosen so that cyst counts in most samples would be sufficient to keep sampling errors, expressed as coefficients of variation, below 17% (15). Samples were divided into two or three subsamples and elutriated separately because of capacity limitations of the equipment. After extraction, all cysts were counted and foci were mapped.

Screening of data sets. Some cyst count data had to be excluded from the statistical analysis. Some fields contained several foci. In most cases, the secondary foci in the conglomerates could be analyzed separately. When foci in a conglomerate overlapped, the cyst counts in the overlapping parts (often a small plateau of identical cyst densities) were excluded from regression analysis, but the remaining data were sufficient to estimate all (four) gradient parameters. Extremely small or high cyst densities, caused by elutriation errors reported by the operators, were also excluded. On field FL10B, belonging to a school farm, the farm manager had changed the direction of cultivation recently. As such routines have consequences for the shape of an infestation focus and, moreover, are not common practice in normal potato agriculture, this field was excluded from regression analysis. The infestations on fields Z02B, Z29B, and D04B were not fully relocated during the second sampling by the sampling team due to insufficient documentation of landmarks or because the farmer changed the position of characteristics used as landmarks, such as fences or borderlines. Therefore, the foci in these three fields were only partly mapped or even completely missed and had to be excluded. In most samples from fields D03B, F01B, G15B, L02B, L05B, Z09B, and Z34B, few or no cysts were found, which resulted in exclusion of these fields. The remaining 26 fields contained 46 foci of which 6 had to be excluded because there were too few data points available for regression analysis. Thus, data sets from 40 foci were suitable for analysis. These data sets included four data sets from Flevoland, analyzed earlier by Schomaker and Been (12). The other, earlier data sets from Flevoland consisted only of two or three length or width transects through foci or results from the first sampling (locating the focus) and were omitted as they were only suitable for single regression analysis to the ^{10}log-transformed cyst density. From a statistical point of view, however, single linear regression is not an appropriate tool to evaluate a three-dimensional model.

A computer program, FOCUS V1.0 (7), was written to manipulate, display (in two and three dimensions), and analyze the chosen data sets. FOCUS also performs single and multiple regression analysis with classical linear models.

Two models for medium-scale distribution. All foci analyzed by Schomaker and Been (12) appeared to be approximately lozenge-shaped and cyst densities decreased exponentially away from the focus center, but more slowly in the length than in the width direction. Consequently, the relation between ^{10}log cyst numbers and distance from the center was linear. The model for the general shape of a focus, described by Schomaker and Been (12), is a symmetrical one for which the expected cyst density $E[p(x,y)]$ in all directions is

$$E\left[p(x,y)\right] = p(0,0) \cdot l^{|x|} \cdot w^{|y|} \tag{1}$$

with variables and parameters as previously defined (Table 1).

However, if foci are mirrored in a plane through the focus center in the length or width direction, it appears that they are often not symmetrical. In most foci, one side is steeper than the other in both length and width directions. This is probably caused by one-directional harvest and cultivation practices of farmers. Because of this phenomenon, a second model, an extension of equation 1 with four parameters, w_{st}, w_{sh}, l_{st}, and l_{sh}, was also fitted:

$$\begin{aligned}
E\left[p(x,y)\right] &= p(0,0) \cdot l_{sh}^{|x(sh)|} \cdot w_{sh}^{|y(sh)|} && \text{for } x > 0 \text{ and } y > 0 \\
E\left[p(x,y)\right] &= p(0,0) \cdot l_{st}^{|x(st)|} \cdot w_{st}^{|y(st)|} && \text{for } x < 0 \text{ and } y < 0 \\
E\left[p(x,y)\right] &= p(0,0) \cdot l_{sh}^{|x(sh)|} \cdot w_{st}^{|y(st)|} && \text{for } x > 0 \text{ and } y < 0 \\
E\left[p(x,y)\right] &= p(0,0) \cdot l_{st}^{|x(st)|} \cdot w_{sh}^{|y(sh)|} && \text{for } x < 0 \text{ and } y > 0
\end{aligned} \tag{2}$$

The gradients in a focus with the smallest values of l or w were defined as steep gradients; those with the largest values as shallow gradients. The subscripts st and sh relate parameters and variables to the steep side and the shallow side of the focus, respectively. Both models 1 and 2 (equations 1 and 2, respectively) were fitted to each focus. By means of an F test, it was decided whether model 2 indeed explained the sampling results better than model 1.

A model for small-scale distribution. Cyst counts, estimated by $p(x,y)$, in samples from a small area (grid quadrat) of about 1 m^2 in a focus with coordinates x and y were assumed to follow a negative binomial distribution (14,15). This distribution is often used in situations when organisms are clumped or aggregated, resulting in a sample variance larger than the mean. The negative binomial distribution and its fitting to biological data has been extensively described (6). The negative binomial distribution function is given by

$$\Pr\left[p(x,y) = \alpha\right] = \binom{\alpha + k - 1}{k - 1} \left\{\frac{E\left[p(x,y)\right]}{E\left[p(x,y)\right] + k}\right\}^{\alpha} \left\{\frac{k}{E\left[p(x,y)\right] + k}\right\}^{k} \tag{3}$$

with terms as previously defined (Table 1). Both models 1 and 2 enabled calculation of the expected cyst density, $E[p(x,y)]$, at any location in the focus if the cyst density of the focus center $p(0,0)$ was given. Model 3 (equation 3) calculated the probability of any cyst count in a sample from a given grid quadrat in the focus with coordinates x and y if the coefficient of aggregation, k, and $E[p(x,y)]$ were known.

Conversion of cyst counts. Because of inevitable variation in the sample size (weight) when 80 cores are collected per square meter, each data set had to be recalculated for a standard unit of soil. Conversion of actual counts (cysts per actual weight of soil) for considerably smaller units of soil would decrease the estimated variance and increase the estimated k, whereas conversion for larger units of soil would have the opposite effect and the more so, as coefficients of variation (standard error/mean) are smaller. Of course, conversion would not affect the coefficient of variation itself. Thus, cyst counts were converted to number of cysts per mean sample weight per data set (as close as possible to the actual counts) to avoid distortion of k and its estimates.

Multiple regression analysis with classical linear models. Cyst numbers were ^{10}log-transformed to satisfy the assumptions for linear regression: constancy of variance, approximate normality of errors, and additivity of systematic effects (9,10). Using this transformation, equation 1 becomes

$$E\left\{\log\left[p(x,y)\right]\right\} = p(0,0) + |x| \cdot \log(l) + |y| \cdot \log(w) \tag{4}$$

A potential problem with this method of analysis was that the ^{10}log transformation of cyst density may not be the most appropriate transformation to meet all three assumptions for linear regression entirely. Before regression analysis could be done to estimate the gradient parameters of the focus, the exact position of the center of the focus had to be estimated, which was not known a priori. The center cannot simply be derived from the two-dimensional map of the focus. Not every square meter of all foci was sampled, and the grid quadrat with the highest cyst density might not represent the true maximum density in the focus. Even when every square meter of the focus had been sampled, it would be unlikely that any quadrat of the sampling grid over the focus fully coincided with the position of the focus center. Thus, a series of regression analyses were performed at variable positions of the putative focus center to estimate the location of the true focus center by an iterative procedure. The position of the focus center was estimated as the position with the best-fitting result (i.e., the smallest sum of squares).

TABLE 1. Definition of terms used

Term	Definition
Infestation focus	Patchy infestation originating from an infected point with a small numbers of cysts transmitted by seed potatoes or agricultural machinery. These numbers are increased by multiplication on hosts and spread by farmer's practices.
Length direction	The direction of cultivation.
Width direction	The direction perpendicular to the direction of cultivation.
Cyst density	Cyst counts from samples of 1.5 to 2.5 kg of soil converted into numbers of cysts per unit dry weight of soil. For mathematical analysis, mean sample dry weight was used; for tabulating, graphs, and mapping, 1 kg was used.
Grid quadrat	Area in the focus of length (1.33 m) by width (0.75 m) (≈1 m^2), with (assumed) average cyst density $E[p(x,y)]$.
Focus center	Position in the focus with the highest cyst density.
l	Gradient parameter, the ratio of the cyst density at a position with length coordinate x and the density at a position with length coordinate $x - 1$.
w	Gradient parameter, the ratio of cyst density at a position with width coordinate y and the density at a position with width coordinate $y - 1$.
x	Length coordinate of a given position in the focus: the distance in meters between the focus center and that position in the length direction.
y	Width coordinate of a given position in the focus: the distance in meters between the focus center and that position in the width direction.
Pr	Probability
α	Integer ≥0.
$p(0,0)$	Cyst density in a sample from the focus center. This position has the predefined length and width coordinates $x = 0$ and $y = 0$, respectively.
$p(x,y)$	Cyst density in a sample from a grid quadrat in the focus with length coordinate x and width coordinate y.
$E[p(x,y)]$	Expected value of $p(x,y)$. Parameter of the negative binomial distribution.
st	Subscript relating a parameter or variable to the steep side of the focus.
sh	Subscript relating a parameter or variable to the shallow side of the focus.
k	Coefficient of aggregation. Parameter of the negative binomial distribution.
k'	Estimate for k. $k' \approx k$ if variation due to errors in laboratory procedures and random disturbances of $E[p(x,y)]$ from the gradient described by equation 1 or 2 is negligible.

The iteration proceeded as follows. (i) The area with the expected center was identified. (ii) This area was subdivided, usually in 5 × 5-m squares, resulting in 25 possible center positions. (iii) For each position, multiple regression analysis was done. (iv) The position with the best fit was again subdivided into 25 areas. Steps iii and iv are repeated as often as necessary until the best fit was obtained. The number of iterations was determined by examining the distance between center positions in the last two iterations. If this distance was smaller than a predefined value (down to 1 mm was possible), the iteration was ended. The minimum number of iterations depended upon the size of the chosen area and the dimensions of the grid quadrats.

Multiple regression analysis with GLMs. McCullagh and Nelder (10) point out that, in most cases, no single scale satisfies

0	0	0	1	2	1	0	2	0	1	1	6	6	7	11	7	5	3	0	6	1	1	1	0	0	1	1	2	1	1
0	1	1	0	0	1	2	1	1	3	7	7	12	4	11	19	6	4	10	0	1	3	2	1	1	2	0	0	1	1
1	1	1	0	0	1	3	2	5	3	8	6	9	27	19	103	13	13	4	6	9	4	1	2	2	1	1	4	2	1
1	2	1	1	1	1	2	5	12	7	8	12	35	34	27	35	26	28	26	10	12	8	15	5	4	7	2	0	4	1
1	1	1	2	7	4	5	6	21	12	33	62	142	59	70	89	56	106	70	31	9	18	9	15	6	7	4	4	2	4
1	5	3	2	10	6	7	19	36	48	102	173	285	181	157	98	198	113	86	44	25	25	28	13	10	5	3	5	4	4
5	2	6	8	3	7	15	21	31	13	68	117	235	242	223	212	148	147	100	62	59	18	18	9	12	11	2	8	3	5
7	13	3	10	10	22	22	44	59	132	147	175	296	288	386	325	168	145	63	43	42	27	39	23	16	14	11	8	4	4
8	13	9	7	15	15	54	63	96	116	139	171	239	216	380	250	163	170	84	59	47	44	23	20	8	6	3	8	4	3
3	10	14	8	21	13	30	53	61	140	130	127	259	349	327	281	269	107	92	53	19	23	13	13	7	14	5	3	5	4
3	6	5	10	12	28	32	29	54	94	59	78	154	309	224	134	174	50	48	16	12	10	13	6	0	2	3	2	1	1
6	1	5	4	7	8	13	15	37	28	22	39	41	99	114	96	61	42	30	10	15	13	5	1	3	1	1	0	0	1
2	1	2	3	3	4	5	8	10	16	13	15	32	39	30	39	49	34	12	9	8	4	3	3	1	2	1	0	0	1
1	1	1	1	3	6	2	6	8	9	11	12	13	23	21	9	11	8	3	2	2	4	5	1	1	0	0	1	0	0
0	0	1	5	2	1	1	2	4	6	3	8	15	11	13	18	7	3	1	2	3	1	2	2	1	1	1	1	1	0
0	1	1	0	0	1	3	2	1	6	1	5	4	8	6	5	3	1	2	0	1	0	0	1	0	0	0	1	0	0

1.33 by 0.75 m

Fig. 1. Cyst densities (cysts per kg) in every square meter within a part of field F27B resulting from the second sampling.

TABLE 2. Fields sampled to map infestation foci

Field	Province	Crop rotation of potato	Year sampled	Grid[a] (m × m)	Number of[b] Rows	Columns	Samples	Size of sample (kg)	Last potato crop	Maximum cyst density (kg^{-1})	Used for analysis[c]
FL01B	Flevoland	1 : 4	1987	1.33 × 0.75	4	37	148	2.5	1984	296	Yes
FL02B	Flevoland	1 : 4	1989	1.33 × 0.75	21	27	47[d]	2.5	1988	481	No
FL04B	Flevoland	1 : 4	1987	1.33 × 0.75	16	37	592	2.0	1984	85	Yes
FL05B	Flevoland	1 : 4	1987	4.00 × 3.00	4	8	20	1.5–10.0	1986	1,423	No
FL09B	Flevoland	1 : 3	1987	1.33 × 0.75	16	31	496	2.5	1985	527	Yes
FL09C	Flevoland	1 : 3	1988	4.00 × 2.25	8	13	104	2.5	1985	330	Yes
FL10B	Flevoland	1 : 4	1988	2.66 × 2.25	9	16	128	2.5	1988	184	No
F01B	Friesland	1 : 3	1991	1.33 × 0.75	9	24	216	1.5	1988	21	No
F11B	Friesland	1 : 2.5	1991	2.66 × 1.50	14	27	378	1.5	1989	468	Yes
F26B	Friesland	1 : 3.5	1991	2.66 × 1.50	16	49	784	1.5	1989	1,217	Yes
F27B	Friesland	1 : 2	1991	1.33 × 0.75	16	30	480	1.5	1989	386	Yes
F28B	Friesland	1 : 3	1991	2.66 × 1.50	20	34	680	1.5	1989	808	Yes
G10B	Groningen	1 : 4	1990	2.66 × 1.50	15	50	750	1.5	1989	561	Yes
G14B	Groningen	1 : 4	1990	2.66 × 1.50	17	34	578	1.5	1989	284	Yes
G15B	Groningen	1 : 3	1990	2.66 × 1.50	11	15	165	1.5	1989	34	No
G18B	Groningen	1 : 3.5	1990	2.66 × 1.50	15	19	285	1.5	1989	235	Yes
G19B	Groningen	1 : 3	1990	2.66 × 1.50	13	13	169	1.5	1989	150	Yes
Z01B	Zeeland	1 : 4	1992	2.66 × 0.75	24	18	429	1.5	1990	113	Yes
Z02B	Zeeland	1 : 4	1992	1.33 × 0.75	21	18	378	1.5	1990	2	No
Z04B	Zeeland	1 : 4	1992	1.33 × 0.75	20	30	600	1.5	1990	189	Yes
Z09B	Zeeland	1 : 2	1991	2.66 × 1.50	15	23	345	1.5	1989	55	No
Z10B	Zeeland	1 : 4	1991	2.66 × 1.50	20	21	420	1.5	1989	565	Yes
Z17B	Zeeland	1 : 4	1991	1.50 × 2.66	14	20	280	1.5	1989	1,209	Yes
Z21B	Zeeland	1 : 6	1991	2.66 × 0.75	24	14	336	1.5	1989	237	Yes
Z22B	Zeeland	1 : 4	1991	2.66 × 1.50	16	37	592	1.5	1989	295	Yes
Z29B	Zeeland	1 : 4	1991	2.66 × 1.50	14	18	252	1.5	1989	55	No
Z34B	Zeeland	1 : 8	1991	2.66 × 1.50	14	12	168	1.5	1989	53	No
D02B	Drente	1 : 2.25	1991	2.66 × 1.50	14	28	392	1.5	1989	532	Yes
D02C	Drente	1 : 2.25	1993	1.33 × 0.75	16	18	288	1.5	1989	307	Yes
D03B	Drente	1 : 5	1993	1.33 × 0.75	12	24	288	1.5	1987	164	No
D04B	Drente	1 : 3	1993	2.66 × 0.75	18	22	324	1.5	1992	12	No
D06B	Drente	1 : 3	1991	1.33 × 0.75	20	18	360	1.5	1991	571	Yes
D07B	Drente	1 : 2.67	1991	2.66 × 1.50	14	21	294	1.5	1991	1,164	Yes
D11B	Drente	1 : 3	1991	2.66 × 1.50	14	19	266	1.5	1991	845	Yes
D12B	Drente	1 : 3.33	1991	1.33 × 0.75	14	27	342	1.5	1991	576	Yes
D13B	Drente	1 : 3	1993	1.33 × 0.75	20	24	480	1.5	1992	627	Yes
D17B	Drente	1 : 3	1993	2.66 × 0.75	20	21	420	1.5	1992	810	Yes

[a] Grid indicates the distance between the sampled units (e.g., a 1.33 × 0.75 grid implies that every square meter of the infestation focus was sampled).
[b] The sample unit is 1 m^2 (1.33 × 0.75 m). Eighty cores of approximately 20 g were collected in a stratified way (as defined by Campbell and Madden [8]), resulting in 1.5-kg bulk samples. In Flevoland, even more cores were collected and bulk sample size was larger (described in text).
[c] Forty foci were present in the 26 fields suitable for analysis.
[d] The sampled area of focus FL02B consisted of one length and one width transect.

all the conditions for regression analysis. In our data set, the ^{10}log scale satisfied the condition of additivity, but possibly did not meet the requirements of normality and constancy of variance because of the negative binomial distribution of cysts in samples. With GLMs, the conditions of normality and constancy of variance are no longer required for valid application of the analytical technique, so that any distribution from the exponential family can be used as an error distribution function, whereas the function that satisfies the condition of additivity need not be the same as the distribution for error (10). Because of the constraints of multiple regression analysis with classical linear models, the data set was also analyzed using GLMs. To this purpose, the position of the focus center as estimated with classical linear models was used. The negative binomial distribution was chosen as the error distribution; the log transformation was used as a link function to linearize the models. Goodness-of-fit of the models was assessed by the mean deviance (D), which should be close to unity. D is defined as twice the log-likely ratio of the saturated and the fitted model, divided by the number of degrees of freedom. In this study, two models were fitted.

If k' is fixed, the negative binomial distribution can be considered as a member of the exponential family. Earlier observations (12,14,15) support the assumption that the error distribution of cysts in samples from small plots in foci was well described by the negative binomial distribution with variable $E[p(x,y)]$ between plots of 1 m^2 but a common k for all plots of the focus (and k dependent on sample size and not, with a certain minimum, on the number of cores). So it was safe to assume that k' was a constant.

As maximum likelihood estimation was not possible, k' was estimated iteratively. An estimate for k' that produced a value of D closest to unity was considered the best (10). The value of k' obtained for the extended model (equation 2) was regarded as the best estimator and, therefore, also used as input parameter for the simple model (equation 1). The GLM analyses were done with GENSTAT, release 5.3 (GENSTAT 5 release 3; Numerical Algorithms Group, Ltd., Oxford).

TABLE 3. Parameter estimation in two models for foci using multiple regression with classical linear models and generalized linear models

			Classical linear models[c]								Generalized linear models[c]							
			Extended model					Simple model			Extended model					Simple model		
Field	Data points[a]	Months between[b]	l_{st}	l_{sh}	w_{st}	w_{sh}	R^2	l	w	R^2	l_{st}	l_{sh}	w_{st}	w_{sh}	D	l	w	D
FL01B	222	33	0.85	0.86	0.60	…[d]	0.92	0.85	0.60	0.91	0.844	0.86	0.57	…	1.00	0.85	0.57	1.02
FL04B	105	33	0.67	0.83*	0.55	0.60	0.87	0.75	0.58	0.81	0.65	0.83*	0.55	0.60	1.00	0.75	0.58	1.36
FL04B/2	66	33	0.74	0.86*	0.51	0.66	0.76	0.78	0.61	0.70	0.72	0.85*	0.50	0.68	1.00	0.77	0.62	1.14
FL09B	285	21	0.69	0.82*	0.46	0.58*	0.92	0.80	0.54	0.86	0.68	0.81*	0.48	0.57*	1.00	0.78	0.54	1.66
FL09C	42	34	0.78	0.82	0.44	0.65	0.83	0.81	0.57	0.82	0.78	0.83	0.44	0.66*	0.96	0.80	0.57	1.20
F11B	160	21	0.83	0.87*	0.59	0.64*	0.90	0.86	0.62	0.88	0.83	0.87*	0.59	0.64*	1.01	0.85	0.62	1.16
F26B	259	22	0.89	0.91*	0.48	0.68*	0.90	0.90	0.64	0.87	0.89	0.91*	0.48	0.68*	0.94	0.90	0.63	1.21
F26B/2	118	22	0.67	0.86*	0.72	0.77	0.85	0.78	0.73	0.79	0.68	0.86*	0.73	0.79	1.06	0.78	0.74	1.42
F26B/3	46	22	0.79	0.86*	0.51	0.69*	0.93	0.82	0.60	0.89	0.77	0.86*	0.52	0.67*	1.00	0.81	0.62	2.03
F27B	258	21	0.79	0.79	0.49	0.50	0.92	0.79	0.49	0.91	0.74	0.77	0.36	0.49	1.02	0.76	0.45	1.04
F28B	197	21	0.86	0.89*	0.66	0.75*	0.88	0.88	0.71	0.86	0.85	0.90*	0.69	0.72*	1.00	0.87	0.71	1.06
F28B/2	80	21	0.84	0.89*	0.49	0.81*	0.89	0.86	0.79	0.73	0.83	0.88*	0.45	0.81*	0.99	0.86	0.77	2.48
G10B/4	148	15	0.88	0.91	0.68	0.75	0.78	0.90	0.72	0.78	0.93	0.94	0.69	0.78*	1.03	0.92	0.74	1.06
G10B/5	65	15	0.79	0.84	0.49	0.79*	0.72	0.81	0.70	0.64	0.73	0.94*	0.47	0.78*	1.06	0.84	0.69	1.42
G10B/7	113	15	0.90	0.91	0.66	0.70*	0.85	0.90	0.68	0.84	0.90	0.91	0.66	0.70*	0.97	0.90	0.68	1.00
G10B/11	51	15	0.75	0.83	0.62	0.68	0.80	0.83	0.65	0.79	0.75	0.84	0.63	0.67	1.00	0.84	0.65	1.01
G14B	139	27	0.88	0.89	0.60	0.67*	0.87	0.89	0.64	0.86	0.90	0.90	0.60	0.68*	1.04	0.89	0.64	1.18
G14B/2	59	27	0.84	0.93*	0.65	0.78	0.86	0.86	0.67	0.85	0.83	0.92	0.64	0.75*	1.02	0.85	0.66	1.14
G14B/4	71	27	0.79	0.81	0.46	0.78*	0.90	0.80	0.60	0.87	0.79	0.81	0.45	0.77*	0.97	0.80	0.61	1.16
G18B	47	15	0.76	0.77	0.51	0.54	0.83	0.77	0.53	0.83	0.76	0.77	0.50	0.55	0.95	0.76	0.54	0.94
G19B	50	15	0.73	0.75	0.69	0.72	0.81	0.74	0.71	0.81	0.72	0.74	0.69	0.72	1.01	0.73	0.70	0.98
Z01B	79	27	0.76	0.81	0.57	0.63	0.79	0.79	0.61	0.77	0.76	0.81	0.56	0.63	0.98	0.79	0.60	1.01
Z04B	135	27	0.77	0.82*	…	0.46	0.87	0.78	0.46	0.86	0.77	0.78*	…	0.48	0.97	0.78	0.48	1.03
Z10B	153	28	0.80	0.88*	0.70	0.71	0.81	0.84	0.71	0.78	0.79	0.88*	0.70	0.71	1.01	0.84	0.71	1.18
Z17B	186	28	0.87	…	0.67	0.81*	0.93	0.87	0.75	0.87	0.88	…	0.68	0.81*	1.05	0.87	0.75	1.88
Z21B	183	8	0.82	0.92*	0.74	0.76	0.71	0.88	0.74	0.69	0.82	0.93*	0.73	0.78	1.01	0.88	0.74	1.09
Z22B	156	28	0.84	0.85	0.64	0.77*	0.89	0.84	0.73	0.85	0.83	0.85*	0.64	0.76*	0.99	0.84	0.73	1.18
Z22B/1	57	28	0.85	0.86	0.58	0.62	0.82	0.86	0.61	0.82	0.85	0.86	0.57	0.61	1.00	0.85	0.60	1.01
D02B	77	27	0.77	0.85*	0.60	0.67	0.82	0.82	0.62	0.80	0.76	0.84*	0.59	0.65	0.95	0.80	0.61	1.07
D02B/1	60	27	0.80	0.83	0.55	0.59	0.89	0.81	0.59	0.88	0.79	0.82	0.52	0.57	0.99	0.80	0.57	1.02
D02C	131	45	0.80	0.86*	0.56	0.67*	0.88	0.81	0.59	0.87	0.80	0.86*	0.57	0.67*	1.00	0.81	0.60	1.07
D06B	169	3	0.75	0.78	0.50	0.65*	0.87	0.76	0.62	0.86	0.75	0.78	0.50	0.65*	0.95	0.76	0.61	1.03
D07B	140	3	0.81	0.88*	0.65	0.85*	0.92	0.85	0.71	0.86	0.82	0.88*	0.65	0.85*	0.95	0.84	0.72	1.54
D07B/2	79	3	0.86	0.94*	0.61	0.75*	0.92	0.90	0.67	0.89	0.87	0.95*	0.62	0.76*	0.97	0.90	0.68	1.18
D11B	73	4	0.80	0.84*	0.52	0.65	0.87	0.82	0.63	0.86	0.84	…	0.54	0.64*	0.94	0.84	0.64	1.12
D11B/2	58	4	…	…	…	…	…	…	…	…	…	0.77	0.59	0.73*	0.92	0.77	0.61	1.12
D12B	200	21	0.83	0.88*	0.41	0.76*	0.91	0.86	0.59	0.80	0.84	0.88*	0.33	0.82*	1.01	0.86	0.58	2.48
D13B	116	10	0.74	0.84*	0.55	0.75*	0.90	0.82	0.73	0.89	0.78	0.84*	…	0.74	1.00	0.84	0.74	1.25
D13B/3	57	10	0.82	0.83	0.61	0.81*	0.85	0.82	0.65	0.84	0.83	0.83	0.62	0.82	1.01	0.83	0.66	1.05
D17B	163	15	0.85	0.90*	0.65	0.65	0.86	0.88	0.65	0.84	0.85	0.90*	0.65	0.65	1.05	0.88	0.65	1.16

[a] Number of data points per focus.

[b] Time in months between harvest of last potato crop and second sampling.

[c] Parameter estimates of the extended model (equation 2) and the simple model (equation 1) for each focus. l = Gradient parameter in the simple model, the ratio of the cyst density at a position with length coordinate x and the density at a position with length coordinate $x - 1$; w = gradient parameter in the simple model, the ratio of cyst density at a position with width coordinate y and the density at a position with width coordinate $y - 1$. l_{sh}, l_{st}, w_{sh}, and w_{st} are gradient parameters in the extended model, 'st' and 'sh' referring to the steep and the shallow side of the focus, respectively. Goodness-of-fit was estimated by the regression coefficient, R^2, in classical linear model analysis and by the mean deviance, D, for generalized linear models. Significant differences between the steep and the shallow gradient parameters l_{st}, w_{st}, l_{sh}, and w_{sh}, calculated by either method of analysis, are indicated by an asterisks (*).

[d] Parameter estimation was not possible.

RESULTS

Goodness-of-fit and parameter estimation. For each focus, cyst numbers per grid quadrat were mapped (Fig. 1). Data sets of 40 foci, sampled with different grids and maximum densities ranging from 85 up to 1,217 cysts per kg of soil, were analyzed (Table 2). Some of these fields contained single foci, others conglomerates of foci (Fig. 2).

Both models, the simple two-parameter model (equation 1) and the extended four-parameter model (equation 2), fit well to the available data sets (Table 3). For the simple model (equation 1), the average values of R^2 (analysis with classical linear models) and D (analysis with GLMs) were 0.83 and 1.26, and for the extended model (equation 2), 0.86 and 1, respectively. These differences in goodness-of-fit were small but significant ($P < 0.05$) with either method of analysis. Twenty-seven of thirty-seven foci were better described by the extended model (equation 2) than by the simple model (equation 1), meaning that 73% of the foci were asymmetric. Data sets of 3 of the 40 foci did not allow fitting of the four-parameter model.

There was little difference in parameter estimates of the gradient parameters between the regression analyses with classical linear models and with GLMs. An asterisk (Table 3) indicates that a gradient parameter on the shallow side of a focus differed significantly from its equivalent on the steep side. In regression analysis with classical linear models, such a difference occurred in 55% of the foci in parameter *l*, in 51% of the foci in parameter *w*, and in 31% of the foci in both gradient parameters *l* and *w*. In 25% of the foci, only gradient parameters l_{sh} and l_{st}, and in 19%, only the gradients parameters w_{sh} and w_{st} differed significantly, while the other gradient parameters did not. In 25% of the foci, neither *l* nor *w* gradient parameters differed significantly. These percentages were almost the same for the GLM analysis: 58, 60, 35, 21, 21, and 23%, respectively.

For the simple model (equation 1), the average parameter estimates were calculated for every cropping area: Drente, Flevoland, Friesland, Groningen, and Zeeland. One-way analysis of variance was used to investigate whether there were differences in gradient parameters between cropping areas (Fig. 3, Table 4). As prob-

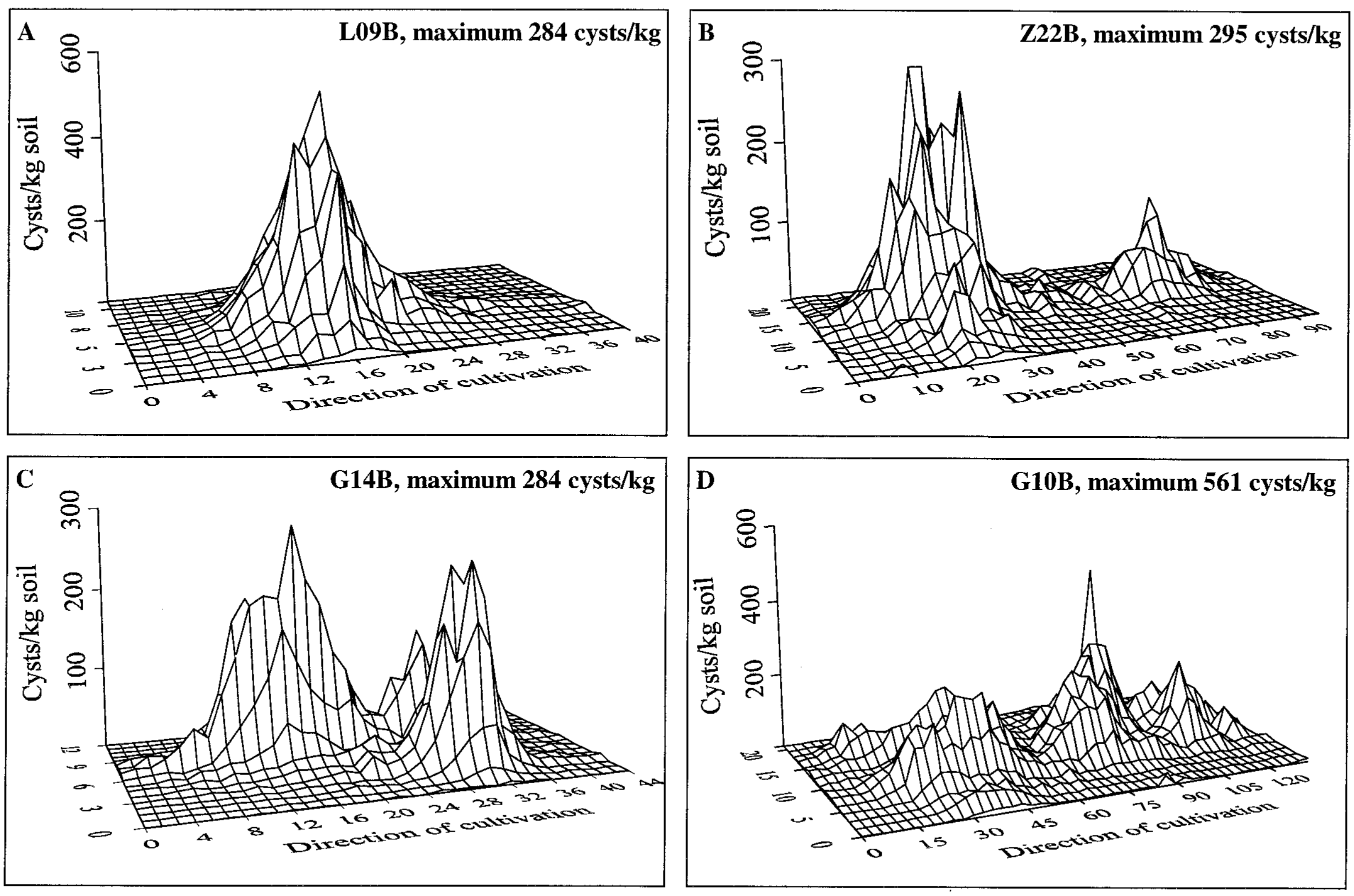

Fig. 2. Several infestation foci ranging from **A,** a single infestation focus to **B,** an emerging secondary focus; and **C,** two confluent foci to **D,** a conglomerate of foci. Densities (cysts per kg of soil) are maximum densities of the primary focus in each sampled field from the second sampling.

TABLE 4. Parameter estimates for each cropping area using generalized linear models

	l[a]		w[a]		Confidence limits			
Province	Mean	SD[b]	Mean	SD	$l_{95\%}$	$l_{5\%}$	$w_{95\%}$	$w_{5\%}$
Drente	0.83	0.042	0.64	0.053	0.90	0.76	0.73	0.55
Flevoland	0.80	0.045	0.58	0.075	0.87	0.73	0.70	0.46
Friesland	0.84	0.035	0.65	0.065	0.90	0.78	0.76	0.54
Groningen	0.83	0.032	0.65	0.055	0.88	0.78	0.74	0.56
Zeeland	0.84	0.038	0.66	0.065	0.90	0.78	0.77	0.55
All data	0.83	0.048	0.64	0.075	0.91	0.75	0.76	0.52

[a] *l* and *w* are the length gradient and width gradient in the simple model of the focus. The additions ‘5%’ and ‘95%’ to the gradient parameters *l* and *w* refer to the 5% lower and the 95% upper limit, respectively.
[b] Standard deviation.

abilities of significant differences between cropping areas were 0.32 (for *w*) and 0.48 (for *l*), the answer to this question was negative. Mean values of parameters *l* and *w* for equation 1 were virtually the same for multiple regression analyses with the classical linear model or with the GLM (Table 5).

Probability distributions of parameters and correlations. As no differences in parameter values between cropping areas were found, the parameter estimates of all investigated foci were averaged. The length and width gradient parameters, *l* and *w*, respectively, of all foci appeared to be normally distributed, *N*(0.83, 0.0023) and *N*(0.64, 0.0056), respectively (Fig. 4). Therefore, the probability of any combination of *l* and *w* in any investigated focus could be described by a bivariate normal distribution (13) with parameters $l = 0.83$, $w = 0.64$, $\sigma_l^2 = 0.0023$, $\sigma_w^2 = 0.0056$, and correlation coefficient $\varphi = 0.5$ (Fig. 5).

Several variables related to the sampled fields were collected and correlated pair-wise, such as cropping frequency, time interval (months) between last potato crop and the second sampling, cyst density of the focus center, nematode species, and cropping frequency. Apart from the correlation already mentioned between *l* and *w*, no other correlations were found.

DISCUSSION

Both multiple regression analyses with classical and generalized linear models provided good fits of the data to both models,

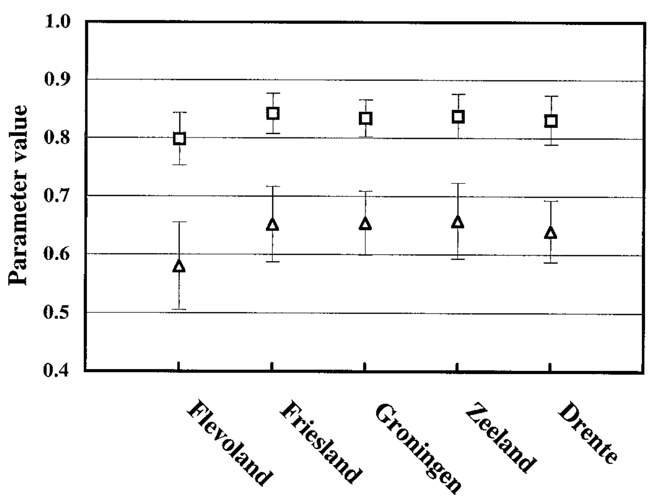

Fig. 3. Averages of the gradient parameters *l* (□) and *w* (△) for foci in five cropping areas in the Netherlands with their standard deviations (bar represents ±σ). Soils of the first four cropping areas are mainly (heavy) marine clays, those of the last, sand and peat.

TABLE 5. Summary statistics for two methods of analysis for the simple model pooled over all data for 40 foci

	Classical linear models[a]		Generalized linear models[a]	
Feature	*w*	*l*	*w*	*l*
Number of values	39	39	40	40
Mean	0.642	0.832	0.640	0.831
Minimum value	0.459	0.739	0.447	0.731
Maximum value	0.791	0.904	0.773	0.926
Standard deviation	0.073	0.044	0.075	0.048
Upper 5% confidence limit	0.76	0.73	0.76	0.91
Lower 5% confidence limit	0.52	0.76	0.52	0.75
R^2/deviance (averaged)		0.83		1.26

[a] *w* = Gradient parameter in the simple model, the ratio of cyst density at a position with width coordinate *y* and the density at a position with width coordinate *y* – 1; *l* = gradient parameter in the simple model, the ratio of the cyst density at a position with length coordinate *x* and the density at a position with length coordinate *x* – 1.

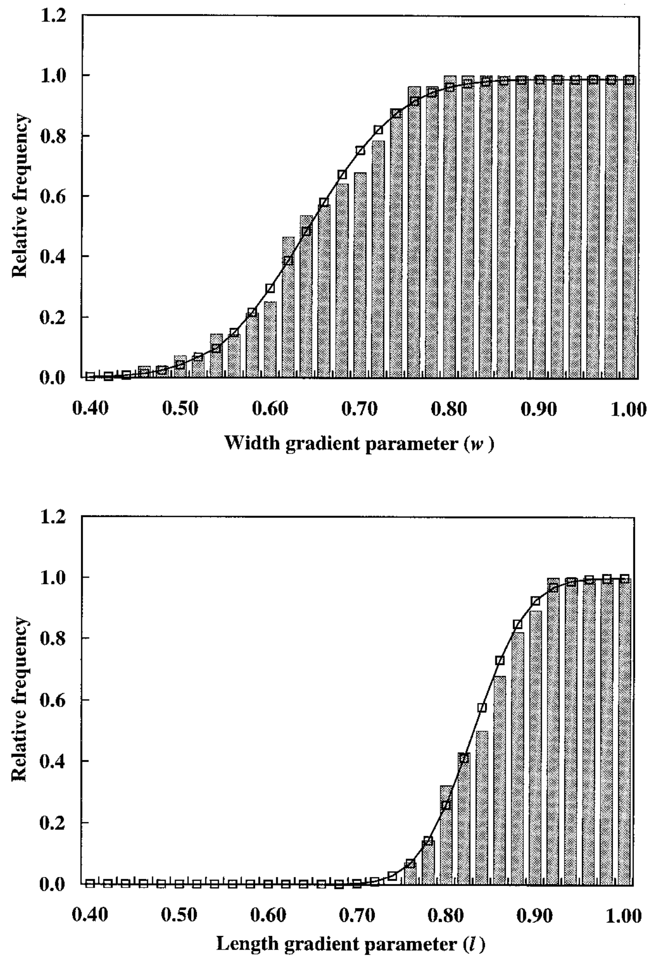

Fig. 4. Evaluation of normality of the two gradient parameters *l* and *w*, describing an infestation focus. Bars = relative frequencies of the estimates of *l* and *w*; and line = relative frequencies of *l* and *w* according to a normal distribution.

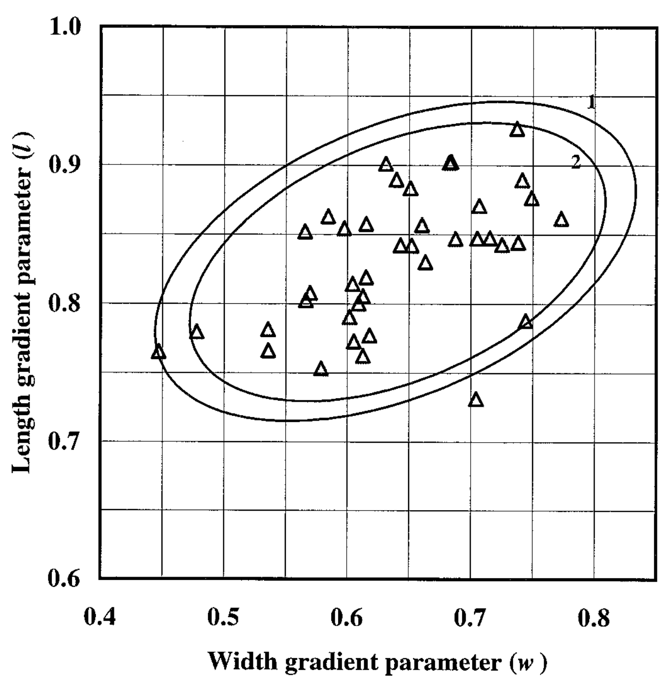

Fig. 5. Two-dimensional display of the bivariate normal distribution of the gradient parameters *l* and *w*. Triangles = actual estimates of *l* and *w* per focus; and curves 1 and 2 = 95 and 90% probability contours, respectively.

equations 1 and 2, with almost identical results (Table 3). The requirements for multiple regression analysis with classical linear models (i.e., normality of residuals and constancy of variance) were sufficiently met. The latter requirement, constancy of variance, was satisfied because of the large cyst counts (obtained by taking large soil samples), estimated by $p(x,y)$, relative to the value of k' (3). The parameter k' cannot be considered without any reservations as an estimate for the coefficient of aggregation, k, of cysts. Seinhorst (15) distinguishes three causes of plot-to-plot variation of cyst density: (i) exponential density gradients, (ii) (random) disturbances of these gradients, and (iii) sampling error due to a negative binomial distribution. The distribution of deviations from the mean log cyst density, caused by factors ii and iii, approximate a normal distribution well enough, except that at a log cyst density of 1.3 to 2 the upper tail of the normal frequency distribution contained fewer observations than the lower tail and at a log cyst density of 2 to 3 there were too many observations in both tails (15). From this normal distribution, an estimate (k') of k can be derived (15) that is smaller than or equal to k. The value of k' equals k only if the second cause of variation, random disturbances of gradients, is small. Another condition for k' to approximate k is that the variation connected with laboratory procedures must be a negligible component of the total variance.

The good description of the shape and size of the investigated infestation foci by equations 1 and 2 implies that the rate change of cyst density per meter is the same at any locus within these foci and, therefore, independent of place and cyst density. It is not unreasonable to then assume that both models apply to all foci in the Netherlands, including those with smaller or larger cyst densities than those investigated. There are two restrictions. First, fields should have one main direction of cultivation, and second, patchy infestations should not be caused by large amounts of recently applied infested soil. These requirements were satisfied in 80 of 82 investigated fields.

The extended model (equation 2) explained the observations best. Considered from a statistical point of view only, the extended model should be preferred over the simple model, represented by equation 1. For scientific purposes, such as the disentangling of sources of variance in cyst counts or studying the processes contributing to the shape and expansion of a focus, equation 2 indeed is the best model. For practical purposes, however, such as the development of detection methods, simplicity is required as long as there are no large deviations from reality. The R^2 values of the multiple regression analysis with classical linear models of the simple model (equation 1) are only slightly reduced compared with those of the extended model (equation 2), and D from the regression analyses with GLMs never moves dramatically from unity. Both the goodness-of-fit and the robustness of the simple two-parameter model (equation 1) make it preeminently suitable for extension purposes.

Notwithstanding the large number of 40 foci available for analysis, no correlation was found between the focus size and the two gradient parameters of the model or the time interval between sampling and the last potato harvest. This implies that the shape and form of an infestation focus was not influenced by these variables. For instance, the detection probability will not be influenced by the time interval between harvest and sampling. Of course, this interval will be restricted by the natural decline of the population density if no host plants are grown. A safe margin for the time interval between harvest of a host and sampling would be 3 to 4 years at most.

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