Comparison of Soil Receptivity to *Thielaviopsis basicola*, *Aphanomyces euteiches*, and *Fusarium solani* f. sp. *pisi* Causing Root Rot in Pea

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ABSTRACT

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Soil receptivity as a quantifiable characteristic ranging from conduciveness to suppressiveness to soilborne pea pathogens *Thielaviopsis basicola* and *Aphanomyces euteiches* was determined by analysis of differences in disease response curves obtained by artificial introduction of inoculum into natural field soil samples. Several parameters, including maximum root rot severity, the area under the health index curve, scores on the first axis of a principal component analysis (PCA) on dose responses, and Weibull model fitting were used to describe the disease responses. In all cases, the Weibull model gave satisfactory fits. PCA yielded a first axis that comprised 86% of the variance found when using Weibull predicted responses for *T. basicola* and 74% of the variance found for *A. euteiches*. This PCA axis essentially represented the average increase in disease severity due to the addition of increasing doses of inoculum to the soil. The Weibull scale parameter B, which represents the amount of inoculum necessary to increase root rot severity by 63% with respect to the level caused by pathogens naturally present in the soil, is another means of quantifying the receptivity of soils to these plant pathogens. Weibull parameter B, maximum root rot severity, the area

under the health index curve, and the scores on the first PCA axis were strongly correlated for each of the pathogens tested individually. To compare the extent and behavior of soil receptivity responses to different pathogens, Weibull parameters B and C (slope at dose B) were chosen because of their universal definition, in contrast to PCA scores. Comparison of the average levels of Weibull parameters B and C indicated significant differences between the pathogens. Yet, no significant similarity in the ranking of the soils was found for the three pathogens, demonstrating that individual soils may interact with different pathogens in totally different ways. In general, soils were suppressive to *T. basicola* but conducive to *A. euteiches*, whereas their response to *Fusarium solani* f. sp. *pisi* ranged from conducive to suppressive. Therefore, risk assessment of soils prior to planting may require different strategies for each pathogen. Bioassays with soil samples taken before the last pea crop in 1987 and 1991 revealed a significant increase in the natural inoculum potential of soils that mainly was accounted for by *A. euteiches* and *Pythium* spp. These results strongly indicate that *A. euteiches* must be considered one of the most threatening pathogens to pea crops in the Netherlands.

Additional keywords: black root rot, common root rot, disease suppressiveness, dry root rot, risk assessment strategy, soft root rot.

Soil receptivity, as defined by Alabouvette et al. (1), describes the effect of natural soils on the ability of pathogen inoculum to cause disease in susceptible host plants. Soil receptivity is a quantifiable characteristic used to describe a continuum of effects ranging from disease suppressiveness to conduciveness. A previous report hypothesized that the observed variability of root rot disease between pea fields could be caused, in part, by differences in soil receptivity to soilborne pea pathogens (17). Significant differences in the receptivity of field soils to pea root rot caused by highly virulent isolates of *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (F.R. Jones) W.C. Snyder & H.N. Hansen have been reported (15). *Thielaviopsis basicola* Berk. & Broome and *Aphanomyces euteiches* Drechs., which cause black root and soft root rots of pea, respectively, also are found in Dutch arable soils (14). These pathogens can attack pea plants soon after sowing and can cause serious disease throughout the period of crop development (16). Studies on soil receptivity to *T. basicola* have been reported for diseases of tobacco (28) and bean (9), but not in pea. No evaluations of soil receptivity re-

search have been reported on *A. euteiches*, one of the most serious pathogens of pea (23).

The first aim of this study was to characterize and quantify soil receptivity to two major soilborne pathogens (*T. basicola* and *A. euteiches*) of the root rot disease complex of pea in the Netherlands and to compare these with previous findings on *F. solani* f. sp. *pisi* (15). A second objective was to determine changes in the inoculum potential of the soils in the field over a 4-year period. Implications for risk analysis are discussed.

MATERIALS AND METHODS

Selection of fields. In 1991, 50 arable fields were selected in which root rot of pea had not been observed or in which disease development at flowering was moderately low (1.8 on a disease severity scale of 0 to 5) during 1986 or 1987 (25). Sampling procedures were described previously (15). The aim of the current study was to compare samples, not fields. Characteristics of most of the field soil samples ("soils") were described previously (15), and those not previously reported are provided in Table 1. For each pathogen, a selection was made from these 50 soils on the basis of the criterion that for the description of soil receptivity by dose response curves, soils are required that have a low inoculum potential of the soil. Soil receptivity tests for *T. basicola* were per-

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formed with 40 soils, including 32 soils previously tested for *F. solani* f. sp. *pisi* (15). Soil receptivity tests for *A. euteiches* were conducted with 27 soils, including 23 soils previously tested for both *F. solani* f. sp. *pisi* and *T. basicola*.

***T. basicola* and *A. euteiches* in natural soils.** The occurrence of *T. basicola* in rhizosphere soils (not artificially inoculated) was examined by dilution plate assays using a selective medium (20), by carrot disks (10), and in roots of 21-day-old pea bait plants. The presence of chlamydospores of *T. basicola*, conidia of *Fusarium*, and oospores of *A. euteiches* in roots was examined microscopically. The presence of *A. euteiches* was determined using pea seedlings in petri-dish tests (19) and confirmed by isolation of the fungus on diluted corn meal agar (CMA) (22).

Soil receptivity tests. *Inocula.* Monospore cultures of *T. basicola* highly virulent to pea (isolates Tb21 and Tb36) were grown separately on malt agar in the dark at 24°C for 21 days. Suspensions of endoconidia (almost without chlamydospores) were obtained from 21-day-old cultures by washing endoconidia from the agar surface with sterile tap water. The suspension with endoconidia from both isolates was filtered through two layers of cheese-cloth, after which the inoculum concentration was determined by counting 10 haemocytometer units of 3.2 mm³. The suspension was kept on melting ice until inoculum was diluted and atomized into soils under continuous shaking. The suspension was diluted to obtain densities of 100, 1,000, 5,000, 10,000, and 50,000 spores per g of dry soil (D1 to D5). For the control treatments (D0), only sterile water was added to the soil.

Two isolates of *A. euteiches* (Ae1 and Rst1) first were grown on diluted CMA at 24°C. Zoospore suspensions were obtained by incubating 7-day-old CMA disks with the fungus for 5 days at 26°C in liquid maltose-peptone medium, aerated for 24 h, and then washed and incubated in water for 48 h. Zoospore density was determined microscopically with a haemocytometer. The suspension was kept at 0°C until use. Dilutions of the suspension were atomized into soil as described above to provide densities of 50, 100, 500, 1,000, and 5,000 zoospores per g of dry soil (D1 to D5). For control treatments (D0), only sterile water was added to the soil.

Check soils. As a check on the quality of the artificial inoculum and experimental conditions, partially heat-sterilized soils were used in each experiment. The soils were kept at 104°C for at least 5 h, eliminating pea root rot pathogens and other microflora. The check soil for *T. basicola* consisted of an alkaline (pH-KCl 7.8), lime-rich (8% carbonate), moderately humic (3.4% organic matter) sandy loam with a high natural disease level of this pathogen (Table 1, soil code re, disease severity index [DI] = 4.0). For *A. euteiches*, a light marine clay soil obtained from a plot mono-

cropped with pea for 10 seasons was used. This reference soil was the same as that described (15) for *F. solani* f. sp. *pisi* (Table 1, code R).

Test plants. Pea (*Pisum sativum* L. cv. Finale) seed was surface-disinfested in 5% NaClO for 10 min and then thoroughly rinsed in sterile demineralized water.

Experimental conditions. Black plastic minipots, 4 × 4 × 12 cm (width × length × height), were filled with test soil, and four pea seeds were sown at a depth of 2 cm in each pot. Water was added gradually to the pots up to approximate field capacity (10 kPa, pF = 2.0; Table 1). During germination, temperature was kept at 20°C, and pots were covered to avoid water loss. One day after emergence, the minipots were placed on top of a block of florist's foam (Smithers-Oasis, Agrimedia, Eisenberg, Germany) in 4 × 24 × 32-cm (width × length × height) test tanks. Each test tank contained six minipots representing six treatments of one soil sample. The test tanks were part of a computerized system in a phytotron that automatically regulated soil water potential (18). During the first, second, and third week after germination, soil water potential was adjusted to pF 1.0, 1.5, and 2.0, respectively. Light intensity, relative humidity (RH), and temperature were adjusted automatically to 400 µmol m⁻² s⁻¹ for 12 h day⁻¹, 80% RH, and 22/18°C day/night for *T. basicola* and 24/18°C for *A. euteiches*, respectively.

Experimental design. The experiments had a split-plot design. Seeded pots were placed in a block with soil samples as the main plot (tanks) and inoculum densities (minipots) as subplots. Blocks were replicated four times. Per pathogen, in successive runs, responses of 10 different soils were tested, together with the check soil.

Disease assessment. Four weeks after sowing, plants were uprooted, roots were rinsed with tap water, and root rot disease severity was scored. Disease severity of epicotyl, cotyledons, and roots was scored separately on a 0 to 5 disease scale: 0 = healthy and 5 = 100% diseased. A disease severity index (DI) per plant was calculated as a weighted mean of these three scores (14).

Analysis of data. Prior to fitting the dose response data, DI was converted into a health index (HI = 5 – DI). Because of its flexibility compared to other models (15), a Weibull model (24) was fitted to these data to obtain parameters characterizing disease development in different soils and to calculate areas under the health index curves (AU) and the same corrected for the inoculum potential of the soil (AUC). Three Weibull parameters were estimated (equation 1): A (=position) is the indexed value of the root health in naturally infested soil (D0), therefore (5 – A) is an estimator of the inoculum potential of the soil; B (=scale) represents the logarithm of the number of inoculated spores needed to reduce A to (e⁻¹) of

TABLE 1. Characteristics of soils tested for soil receptivity to *Thielaviopsis basicola* and *Aphanomyces euteiches*: silt fraction, organic matter, gravimetric water content, soil density, root rot severity, and occurrence in field soil of *T. basicola*, *A. euteiches*, and *Fusarium solani* f. sp. *pisi*^a

Soil code	Soil type	Humus class	% H ₂ O ^v	S.D. ^w	DI ^x	<i>T. basicola</i> ^y	<i>A. euteiches</i> ^y	<i>F. solani</i> f. sp. <i>pisi</i> ^y
bu	Light loam	Lightly humic	23	1.2	1.5	–	+	+
el	Heavy loam	Lightly humic	27	1.1	0.5	–	+	+
st	Heavy clay	Moderately humic	45	0.9	1.5	–	+	+
th	Heavy clay	Moderately humic	44	0.9	0.6	–	–	+
sm	Heavy clay	Lightly humic	35	1.0	0.5	–	+	+
le	Peat	Strongly humic	60	0.9	0.2	–	–	+
aa	Heavy clay	Moderately humic	40	1.0	0.7	–	–	+
ad	Heavy clay	Moderately humic	40	1.0	0.9	–	–	+
mt	Heavy loam	Lightly humic	25	1.1	0.7	–	–	+
hg	Heavy loam	Lightly humic	26	1.1	1.1	–	+	+
er	Light loam	Lightly humic	24	1.2	1.2	–	–	+
re ^z	Heavy loam	Moderately humic	24	1.2	4.0	+	–	+

^a Soils previously reported (18) are not included.

^v Gravimetric water content of soil sample at pF = 2 (10 kPa).

^w Soil density used in the bioassay (grams per cubic centimeter).

^x Disease index: root rot severity at flowering in the last pea crop: 0 = no necrosis, 5 = 100% necrotic roots.

^y Presence (+) or absence (–) of *T. basicola*, *A. euteiches*, or *F. solani* f. sp. *pisi* in soil.

^z *T. basicola* check soil before sterilization.

its value; and C is the value determining the shape (slope at dose B) of the health index curve:

$$HI = A e^{-[\log(\text{dose} + 1)/B]^C} \quad (1)$$

To summarize the curve characteristics in another way, principal component analysis (PCA) was carried out on a variance-covariance matrix of the average disease response per soil*inoculum dose combination or the Weibull fitted values (disease units). The different characteristics obtained by Weibull curve fitting and PCA, including the inoculum potential of the soil (IPS), AU and AUC, and maximum root rot severity (Ymax) were compared by Spearman rank correlation for each of the two pathogens in the current study (*T. basicola* and *A. euteiches*) and, ad-

ditionally, for *F. solani* f. sp. *pisi* (15). The significance of differences between Weibull parameters B and C of soils for *T. basicola* and *A. euteiches* was determined by analysis of variance. Because single replicates sometimes gave unsatisfactory Weibull fitting, mean health indices of two replicates were used to obtain two better Weibull curve fits per soil (29 degrees of freedom were left).

Comparing soil receptivity to the three pathogens. Weibull parameters B and C of the HI curves for *T. basicola*, *A. euteiches*, and *F. solani* f. sp. *pisi* (15) were compared by the Wilcoxon signed ranks test and Spearman rank correlation within a restricted subset of 23 soils tested for all three pathogens and an average value of the check soils (72 combinations). Statistical analyses

A

B

Fig. 1. Symptoms of black root rot in pea in response to doses of added inoculum (left to right: 0, 100, 1,000, 5,000, 10,000, or 50,000 spores per g of dry soil [D0 to D5]) of *Thielaviopsis basicola* in **A**, sterilized soil and **B**, field soil in the bioassay.

were performed using procedures in Statistix 4.0 (*An Interactive Statistical Analysis Program for Microcomputers*. J. Siegel, ed. 1985. NH Analytical Software. St. Paul, MN), Statistical Ecology (J. A. Ludwig and J. F. Reynolds, 1988. Wiley-Interscience Publications, New York), GENSTAT 5. (P. Digby, N. Galwey, and P. Lane. 1988. Oxford Science Publication, Oxford), and CANOCO (*CANOCO—A FORTRAN Program for Canonical Community Ordination by [Partial] [Detrended] [Canonical] Correspondence Analysis, Principal Component Analysis and Redundancy Analysis*. Verion 2.1. C. J. F. Braak. 1987. Agriculture Mathematics Group, Wageningen, the Netherlands).

Determination of temporal changes in inoculum potential of soil. For 50 soils sampled in 1991, the inoculum potential of the soil was calculated as the average root rot DI obtained from the noninoculated treatment (D0) in soil receptivity tests. The results were compared with those obtained in 1986 or 1987 before the last pea crop was grown (14). Analyses were carried out using the sign test (27).

RESULTS

***T. basicola* and *A. euteiches* in natural soils.** Detection of *T. basicola* in soil dilution plates and carrot disks was unsuccessful, but baiting with pea seedlings followed by microscopic observation enabled us to detect the presence of *T. basicola* in 8 of the 50 soils tested. In the soil receptivity tests, however, black root rot did not develop in the D0 treatment (noninoculated) of any of these eight soils. *A. euteiches* was detected in 20 of the 50 soils tested. Severe soft root rot occurred in 14 of the soils in which *A. euteiches* was found, and soft rot due to *Pythium* spp. was detected in 2 soils. These soils had to be excluded from the soil receptivity tests.

Soil receptivity tests. Of the 40 soils tested for soil receptivity to *T. basicola*, 7 were discarded because of the high inoculum potential of the soil (DI > 2). Due to technical problems, one of the sterilized check soils with *A. euteiches* (R4) had to be discarded. In all experiments, the interaction of dose and soil was highly significant ($P \leq 0.001$). In the density ranges used, black root rot (*T. basicola*) increased at a slower rate with increasing dose than soft root rot (*A. euteiches*). For *A. euteiches*, the highest dose caused maximum disease severity in all but one soil. For *T. basicola*, this was the case in only 30% of the soils.

In the sterilized soil, the lowest doses of *T. basicola* produced moderate to severe black root rot (Fig. 1A), but in most of the natural soils, root rot severity was moderate, even at D5 (Fig. 1B). Soft root rot development by artificial infestations with *A. euteiches* was similar or even faster in natural soil than in sterilized soil.

Weibull curve fitting. For both pathogens, the Weibull model described responses adequately (Table 2). Weak responses to inoculation were expressed by a relatively large value of scale parameter B. For *T. basicola*, 90% of the soils showed suppressive responses ($B > 4$). For *A. euteiches*, 90% of the soils showed conducive responses ($B < 3$). Large values of shape parameter C indicate a sudden increase in dose response. For *T. basicola*, a sudden increase occurred only at high inoculum doses, but for *A. euteiches*, a sudden increase occurred only at low inoculum doses. Extremely large values of shape parameter C ($C > 10$) were found mainly for *A. euteiches*. Figure 2 represents plots of shape parameter C against scale parameter B. The subplots drawn at the top of Figure 2 illustrate the range of response types for extreme values of B and C, showing the suitability of these parameters to quantify the relative extent and behavior of soil receptivity, respectively.

PCA ordination of disease responses. PCA of average disease responses (four replicates of soil*dose) gave a first PCA axis that accounted for 86% of the variation when using Weibull predicted responses for *T. basicola* and 74% of the variation for *A. euteiches*. When nontransformed data were used, only a few percent

TABLE 2. Mean^w Weibull parameter (A, B, and C) values and percentage of variance (% var) accounted for by fitting health indexes^x obtained as responses to soil infestation with *Thielaviopsis basicola* or *Aphanomyces euteiches*

Soil code	A	B ^y	C	% Var
<i>T. basicola</i>				
lf	4.5	9.1 f	2.0	62
le	4.7	6.5 e	2.3	73
tr	3.8	5.6 de	6.3	72
aa	4.6	5.2 cd	5.0	82
jg	3.4	5.2 cd	4.9	77
zu	4.1	5.2 cd	6.9	69
hm	3.9	5.1 b-d	9.0	63
jr	3.9	5.1 b-d	7.2	69
cm	4.3	5.1 b-d	4.5	73
rg	4.4	5.0 b-d	7.3	91
to	4.1	4.9 b-d	5.6	85
cf	4.6	4.9 b-d	8.4	93
cv	3.0	4.8 b-d	2.4	63
jn	4.4	4.8 b-d	5.9	66
bl	4.3	4.8 b-d	14.4	89
wa	4.1	4.8 b-d	7.6	85
co	4.2	4.7 b-d	4.6	64
ro	4.1	4.7 b-d	7.4	83
as	4.1	4.7 b-d	4.5	90
wi	4.4	4.7 b-d	4.4	89
ad	4.8	4.7 b-d	5.7	90
kh	4.2	4.6 b-d	6.9	95
bk	3.3	4.5 b-d	4.6	86
ja	4.0	4.5 b-d	9.4	89
ls	4.3	4.5 b-d	6.5	94
cb	4.2	4.5 b-d	6.4	92
rc	4.3	4.4 b-d	6.5	84
ve	4.4	4.4 b-d	6.0	93
dm	3.7	4.2 bc	3.5	77
rh	3.2	4.1 bc	7.0	74
gv	3.4	4.1 bc	2.8	76
r1 ^z	4.2	4.1 bc	2.3	86
er	3.8	4.1 bc	5.9	92
lu	4.5	4.0 b	4.4	94
r2 ^z	5.0	2.6 a	2.1	96
r4 ^z	5.0	2.3 a	1.7	96
r3 ^z	4.9	1.9 a	1.2	98
<i>A. euteiches</i>				
mu	4.1	5.0 o	3.8	50
rg	3.7	3.6 n	17.5	93
ve	3.9	3.2 m	13.8	93
r2 ^z	4.9	2.7 l	1.8	91
bl	4.2	2.6 kl	7.3	98
cf	4.1	2.6 j-l	5.3	93
tr	4.2	2.6 j-l	5.3	96
bk	2.2	2.6 i-l	3.2	73
as	3.9	2.6 i-l	7.0	99
rc	4.0	2.5 i-k	16.0	99
cm	4.3	2.5 i-k	3.9	88
wa	4.2	2.5 i-k	3.8	96
le	4.4	2.4 h-j	4.1	94
ja	3.8	2.3 g-i	3.9	94
cv	3.2	2.2 gh	3.5	89
r3 ^z	5.0	2.1 fg	3.9	99
to	3.9	1.9 ef	8.8	91
wi	4.2	1.9 ef	8.2	99
ad	4.2	1.9 e	7.7	93
jn	3.8	1.9 e	5.0	95
ls	3.9	1.9 de	13.2	98
lf	4.1	1.8 c-e	7.1	95
lu	4.0	1.8 c-e	11.5	98
r1 ^z	5.0	1.7 c-e	3.5	100
co	3.8	1.6 b-d	6.3	94
ro	3.7	1.6 bc	4.6	98
gv	3.1	1.6 bc	10.2	99
kh	3.9	1.6 bc	7.7	99
cb	3.6	1.5 ab	7.5	99
aa	4.5	1.3 a	1.9	97

^w Mean of two Weibull fitted curves.

^x Health index = 5 - DI (disease index); DI: 0 = no necrosis, 5 = 100% necrotic roots.

^y Means followed by the same letter are not significantly different; LSD(0.05) = 1.2 for *T. basicola* and 0.2 for *A. euteiches*.

^z Sterilized check soil.

less of the variance was accounted for. The first PCA axis essentially represents the weighted average increase in disease severity (represented by DI) due to the artificial introduction of inoculum in the soil.

Comparing soil receptivity criteria per pathogen. A comparison of all available curve characteristics by Spearman rank correlation shows the strong correlation between scale parameter B, Ymax, AU and AUC, and the scores on the first PCA axis (Table 3). For *T. basicola* and *F. solani* f. sp. *pisi*, shape parameter C was correlated with the same curve characteristics as B, except for Ymax. For *A. euteiches*, C was not correlated with any of the other curve characteristics. Parameter A was strongly correlated with the inoculum potential of the soil for all three pathogens.

Strong correlations of curve characteristics with scores on the first PCA axis indicate their potential to describe differences in soil receptivity. For both pathogens, significant differences in Weibull parameter B were found between soils: 6 levels for *T. basicola* and 15 levels for *A. euteiches* (Table 2). Within most groups of soils in which scale parameter B was not significantly different, different levels ($P = 0.05$) of shape parameter C could be distinguished.

Comparison of soil receptivity among the pathogens. Weibull parameters B and C were chosen to compare soil receptivity responses of soils to different pathogens (Fig. 2) because of their universal definition, in contrast to PCA scores, AUC, and Ymax. Pairwise comparisons of the average levels of Weibull parameters B and C by Wilcoxon's signed ranks test showed significant differences among pathogens, except in comparisons of *F. solani* f. sp. *pisi* versus *T. basicola* regarding B and *A. euteiches* versus *T. basicola* regarding C (Table 4). However, when comparing responses to the different pathogens by Spearman rank correlation, no significant similarity in the ranking of soils was found ($P = 0.05$).

Temporal changes in the inoculum potential of the soil. In 1991, a significant increase in the inoculum potential of the soil for pea root rot was found ($P \leq 0.001$, $n = 50$) with respect to the inoculum potential of the soil determined prior to growing peas in 1986 or 1987 (14) (Sign Test). A higher inoculum potential of the soil was found in 35 soils in 1991, with a strong increase in 19 of them. In the latter category, 16 cases of soft root rot (a.o., *A. euteiches* and *Pythium* spp.) and 3 cases of dry rot (a.o., *T. basicola* and *F. solani* f. sp. *pisi*) occurred. The increase in inoculum potential of the soil during the rainy year, 1987, was almost entirely due to soft root rot pathogens (9 of 10 soils). If soils with soft root rot were excluded from the data set, no change in the inoculum potential of the soil was found over the years.

DISCUSSION

Inoculum densities and disease expression in natural soils. The results of this study emphasize once again (15) that estimating inoculum density in soil can be meaningless in risk assessment of soilborne diseases. Under natural conditions, root rot of pea is caused by a complex of pathogens that not only includes different species but also forms with different degrees of pathogenicity (16). Moreover, in soil, fungal populations can persist in both active and passive states. In natural soils, a realistic determination of the inoculum density of these pathogens is impossible. Instead of inoculum densities, an indexed inoculum potential of the soil often has been used to assess densities of infective units of pathogenic species in soils. In this paper, the term inoculum potential of soil is used as an equivalent to the natural pea root rot potential of the field soil (25), which refers to an indexed estimation of the potential disease activity that combines both the propagule density and infectivity of all pathogens present in a soil as well as soil factors affecting the relationship between a susceptible host and pathogens under optimum conditions for infection.

In our study, the presence of *T. basicola* was not associated with the inoculum potential of the soil for black root rot in pea. In eight soils in which *T. basicola* was detected, no black root rot developed at D0 (noninoculated), and in soils in which initially no *T. basicola* was detected, some black root rot developed at D0. Similarly, previous investigations on pea root rot caused by *F. solani* f. sp. *pisi* (15) demonstrated a lack of correlation between the number of propagules of the species in soil, the inoculum potential of the soil, and the degree of soil receptivity. Apparently, the detection of these pathogens is not a reliable criterion for forecasting black and dry root rot outbreaks in natural soils. This finding agrees with results previously published (3–8,12). However, this may not hold for *A. euteiches* according to our results (discussed below) and a previous report (11).

Soil receptivity tests. For comparison of soil receptivity among pathogens, care was taken to include densities of virulent inoculum as high or higher than those occurring in highly infested natural soil (2,4,9,13,21,23). For *T. basicola*, *A. euteiches*, and *F. solani* f. sp. *pisi*, different dose ranges were used in testing soil receptivity to obtain the full range of disease severity. In natural soils, inoculation with *T. basicola* below 100 spores per g of dry soil (D1) did not produce obvious disease symptoms. With *F. solani* f. sp. *pisi*, disease could be induced at a 10-fold lower density

Fig. 2. Plot of Weibull shape parameter C against scale parameter B of health index curves of the soils, illustrating their pathogen-specific ranges and relationships for *Thielaviopsis basicola* (Tb), *Aphanomyces euteiches* (Ae), and *Fusarium solani* f. sp. *pisi* (Fs). In the subplots at the top, examples are presented of extreme disease index curves that correspond to soils (LS, JA, R3, and JR) in the graph, illustrating the meaning of the parameters.

(15). In experiments with sterile soil, however, *F. solani* f. sp. *pisi* and *T. basicola* caused equal disease severity at 10 spores per g of dry soil (data not shown). The higher initial level of infestation (D1) required in natural soil for *T. basicola* compared to *F. solani* f. sp. *pisi* might be explained by *T. basicola*'s greater sensitivity to biological factors in soil (9,26,28).

For *T. basicola*, endoconidia were chosen as inoculum because they are single quantifiable units abundantly produced during an epidemic and they serve as a primary source of infection. Endoconidia also may transform into chlamydospores for survival (9). These macroconidia are formed to survive and produce hyphae as a primary source of infection as well, but their infectivity would be difficult to predict because of their multicellular structure. For *A. euteiches*, zoospores are a quantifiable artificial inoculum. Oospores act as a primary source of infection but also can produce infective zoospores. Therefore, oospores do not represent a constant number of infective units. Moreover, they are difficult to obtain in a pure form.

Description of soil receptivity. Our results confirmed the drastic effect of soil on the inoculum effectiveness of the pathogens

studied and emphasized the contrast in receptivity of the same soil to different pathogens. Several parameters appeared suitable for describing the response curves, c.q., soil receptivity per pathogen. In all cases, the Weibull model gave satisfactory fits (Table 2). In a previous study (15), this was not the case for some curves of sterilized soils tested with *F. solani* f. sp. *pisi* that were too steep for adequate fitting (value of B between doses 0 and 1). With only a few exceptions, the value of parameter A (HI at D0) was higher than 3.2 (5 to 1.8) as was intended. Soils with a low inoculum potential were selected for this study, in the first place, to retain an acceptable number of potentially suppressive soils and, secondly, to leave space for independent behavior of the scale B and shape C parameters. Weibull scale parameter B, which represents the amount of inoculum necessary to increase the root rot severity by 63% with respect to the level caused by pathogens naturally present in the soil, by definition is a quantification of soil receptivity (health units/log(amount of inoculum added)). Different levels of soil receptivity to *T. basicola* and *A. euteiches* could be distinguished within the sets of soils on the basis of parameter B (Table 2). Variation in C (slope at dose B) appeared to be of addi-

TABLE 3. Spearman rank correlation coefficients for the relation between parameters and other criteria characterizing pea root rot disease response curves, caused by *Thielaviopsis basicola*^w, *Aphanomyces euteiches*^x, and *Fusarium solani* f. sp. *pisi*^y, in different field soils^z

Pathogen	IPS	Ymax	A	B	C	AU	AUC	PCA
<i>T. basicola</i>								
Ymax	0.08							
A	-0.95	-0.10						
B	0.01	-0.95	-0.03					
C	0.21	-0.26	-0.25	0.29				
AU	-0.41	-0.76	0.42	0.69	0.45			
AUC	0.20	-0.76	-0.23	0.83	0.69	0.58		
PCA	0.24	0.89	-0.24	-0.86	-0.51	-0.92	-0.92	
PCAC	-0.21	0.78	-0.24	-0.86	-0.62	-0.56	-0.99	0.80
<i>A. euteiches</i>								
Ymax	-0.07							
A	-0.98	0.14						
B	-0.26	-0.52	0.19					
C	0.26	-0.18	-0.32	-0.04				
AU	-0.55	-0.52	0.48	0.90	0.01			
AUC	0.24	-0.52	-0.31	0.82	-0.03	0.58		
PCA	0.42	0.52	-0.34	-0.94	-0.01	-0.96	-0.98	
PCAC	0.15	0.52	+0.21	-0.94	-0.10	-0.67	-0.98	0.80
<i>F. solani</i> f. sp. <i>pisi</i>								
YMAX	0.16							
A	-0.94	-0.12						
B	0.15	-0.85	-0.21					
C	0.24	-0.30	-0.26	0.22				
AU	-0.37	-0.91	0.29	0.69	0.43			
AUC	0.34	-0.73	-0.42	0.74	0.64	0.64		
PCA	0.24	0.93	-0.19	-0.73	-0.48	-0.99	-0.73	
PCAC	-0.34	0.74	-0.42	-0.75	-0.62	-0.64	-1.00	0.73

^w n = 37; critical values $P < 0.05$: 0.33, $P < 0.01$: 0.42.

^x n = 30; critical values $P < 0.05$: 0.36, $P < 0.01$: 0.46.

^y n = 33; critical values $P < 0.05$: 0.35, $P < 0.01$: 0.44.

^z IPS = inoculum potential of soil, i.e., severity of infection caused by indigenous pathogens present in soils; Ymax = maximum root rot severity; A (position), B (scale), and C (shape) = Weibull parameters; AU = area under (Weibull) health index curve; AUC = AU calculated by correcting for the inoculum potential of the soil; PCA = score on first principal component axis; and PCAC = PCA on data corrected for the inoculum potential of the soil.

TABLE 4. Pairwise comparison of levels and soil ranks of the Weibull scale B and shape C parameters for *Aphanomyces euteiches*, *Thielaviopsis basicola*, and *Fusarium solani* f. sp. *pisi* by Wilcoxon's signed ranks test and Spearman rank correlation, respectively, on a set of 24 soils

Comparison	Scale B			Shape C		
	<i>A. euteiches</i> / <i>T. basicola</i>	<i>A. euteiches</i> / <i>F. solani</i> f. sp. <i>pisi</i>	<i>F. solani</i> f. sp. <i>pisi</i> / <i>T. basicola</i>	<i>A. euteiches</i> / <i>T. basicola</i>	<i>A. euteiches</i> / <i>F. solani</i> f. sp. <i>pisi</i>	<i>F. solani</i> f. sp. <i>pisi</i> / <i>T. basicola</i>
Negative	-300	-294	-167	-98	-21	-268
Positive	0	6	133	202	279	32
Wilcoxon P^y	***	***	ns	ns	***	***
Spearman R^z	0.37	0.19	0.07	0.10	0.40	0.09

^y *** = $P < 0.001$; ns = $P > 0.05$

^z Critical values for R : 0.42 ($P = 0.05$) and 0.54 ($P = 0.01$)

tional importance in differentiating between shapes of disease response curves, which may imply different mechanisms of disease suppression. For all three pathogens, Weibull scale parameter B, which accounted for much of the variation in disease responses of soils, was strongly correlated with Ymax, AU, and scores on the first PCA axis (Table 3).

AU is based on the sum of dose responses and, therefore, is biased by the doses used. The same holds for Ymax. The scores on the first PCA axis (dimensionless) also reflect an integration of dose responses, but each dose response obtains a different weight that is dependent on the doses used and the range and slopes of soil receptivity responses within the set of soils tested.

Comparing soil receptivity among the pathogens. As proposed in a previous paper (15), the scores on the first PCA axis offer a possibility of differentiating among soils in their reaction to a single pathogen. However, PCA scores as well as AU are not universally defined and, therefore, as such, are not suitable to compare soil receptivity to different pathogens. This is illustrated particularly by the absence of correlation between these two variables and Weibull parameter C in the case of *A. euteiches*. Hence, parameters B and C were chosen to compare soil receptivity responses between pathogens, of which B is considered of prime importance.

The differences found in the average values of Weibull parameter B indicate that the level of soil receptivity to *A. euteiches* was definitely higher than to *T. basicola* and *F. solani* f. sp. *pisi*, as demonstrated by Wilcoxon's signed ranks test (Table 4) and Figure 2. Soils suppressive to *A. euteiches* were not prevalent. For *F. solani* f. sp. *pisi*, strongly concave curves (high C) were rare compared to *A. euteiches* and *T. basicola*, indicating a dose-independent suppressiveness. The absence of similarity in ranking of soils for the three pathogens (Table 4, Spearman rank correlation) demonstrates that individual soils may react to different pathogens in a totally different way. A highly conducive soil for *A. euteiches* may be moderately suppressive to *T. basicola* and *F. solani* f. sp. *pisi* (e.g., soil kh). Such differences in soil receptivity to individual pathogens suggest the existence of multiple mechanisms selectively affecting the pathogens or the existence of mechanisms affecting the pathogens in different ways. Soil factors associated with soil receptivity to these pathogens will be explored in a subsequent paper.

Temporal changes in the inoculum potential of the soil. Information on the importance of soil receptivity can be obtained by studying the change in inoculum potential of soil over time. In our 1991 sample set, a period of 3 to 4 years had elapsed since the last pea crop and, thus, the inoculum potential of the soil was expected to have reached an ecologically stabilized state. Mainly due to *A. euteiches*, the inoculum potential of the soil was significantly higher in 1991 than before the last pea crop in 1986 or 1987. This result is in line with the high receptivity of the soils to this pea pathogen. If soils with soft root rot (a.o., *A. euteiches* and *Pythium* spp.) were excluded from the set, no temporal change in the inoculum potential of the soil was found, indicating the importance of soil receptivity to dry root rot (*T. basicola* and *F. solani* f. sp. *pisi*). The large proportion of soil samples from 1987 (a very rainy year) severely infested by *A. euteiches* in 1991 confirms the well-known association of this pathogen with very wet soil conditions (11).

The occurrence of *A. euteiches* in a large number of fields, the increase of the inoculum potential in these soils after a pea crop had been grown, as well as the conducive response of test soils that were practically free from this pathogen (Table 2; A > 3 and low B) indicate that this pathogen represents the greatest risk relative to the other pathogens in the pea root rot complex in the Netherlands.

Our results explain the limited effect of pea cropping frequency on root rot disease in pea crops reported earlier (17). Pea root rot inoculum potential of the soil is not only a result of cropping frequency but also of pathogen species composition in the soil and the receptivity of the soil to these pathogens.

Final remarks. The results obtained from laboratory experiments with a limited number of pea root rot pathogens and a relatively small number of soils need to be validated in field situations. In the current study on disease severity, effects of soil receptivity on disease incidence as an aspect of risk assessment was not taken into account. Nevertheless, the different disease responses of soils may imply that different disease management strategies need to be employed. Because most soils are relatively suppressive to *T. basicola*, this pathogen might be neglected in root rot risk assessment in the Netherlands. The differences in soil receptivity to *F. solani* f. sp. *pisi*, however, suggest that standard soil receptivity testing of soils for this pathogen may be useful in this respect, whereas the general conduciveness of soils to *A. euteiches* suggests that checking for the presence of this pathogen is more informative than measuring soil receptivity to it. To enable rational management of soil receptivity, the causative mechanisms must be clarified.

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LITERATURE CITED

1. Alabouvette, C., Couteaudier, Y., and Louvet, J. 1982. Comparaison de la réceptivité de différents sols et substrats de culture aux fusarioses vasculaires. *Agronomie* 2:1-6.
2. Blume, M. C., and Harman, G. E. 1979. *Thielaviopsis basicola*: A component of the pea root rot complex in New York state. *Phytopathology* 69:785-788.
3. Burke, D. W. 1965. Fusarium root rot of beans and behavior of the pathogen in different soils. *Phytopathology* 55:1122-1126.
4. Burke, D. W., Hagedorn, D. J., and Mitchell, J. E. 1970. Soil conditions and distribution of pathogens in relation to pea root rot in Wisconsin soils. *Phytopathology* 60:403-406.
5. Chellemi, D. O., Rohrbach, K. G., Yost, R. S., and Sonoda, R. M. 1988. Analysis of spatial pattern of plant pathogens and diseased plants using geostatistics. *Phytopathology* 78:221-226.
6. Campbell, C. L., Madden, L. V., and Pennypacker, S. P. 1980. Structural characterization of bean root rot epidemics. *Phytopathology* 70:152-155.
7. Campbell, C. L., and Noe, J. P. 1985. The spatial analysis of soil-borne pathogens and root diseases. *Annu. Rev. Phytopathol.* 23:129-148.
8. Kraft, J. M., and Wilkins, D. E. 1989. The effects of pathogen number and tillage on root rot disease severity, root length, and seed yield in green peas. *Plant Dis.* 73:884-887.
9. Lechappe, J. 1986. Place et évolution des équilibres microbiennes dans l'écosystème cultural: Cas du complexe parasitaire du haricot associant *Fusarium solani* f. sp. *phaseoli*, *Thielaviopsis basicola* et *Fusarium oxysporum*. Ph.D. thesis. Université de Rennes I, France.
10. Lloyd, A. B., and Lockwood, J. L. 1962. Precautions in isolating *Thielaviopsis basicola* with carrot disks. *Phytopathology* 52:1314-1315.
11. Long, E. 1985. An early warning test to cut out pea foot rot. *Grower* November:40-42.
12. McFadden, W., Hall, R., and Phillips, L. G. 1989. Relation of initial inoculum density to severity of Fusarium root rot of white bean in commercial fields. *Can. J. Plant Pathol.* 11:122-126.
13. Nash, S. M., and Snyder, W. C. 1965. Quantitative and qualitative comparisons of the *Fusarium* populations in cultivated fields and noncultivated parent soil. *Can. J. Bot.* 43:939-945.
14. Oyarzun, P. J. 1993. Bioassay to assess root rot in pea and effect of root rot on yield. *Neth. J. Plant Pathol.* 99:61-75.
15. Oyarzun, P. J., Dijst, G., and Maas P. W. Th. 1994. Determination and analysis of soil receptivity to *Fusarium solani* f. sp. *pisi* causing dry root rot of pea. *Phytopathology* 84:834-842.
16. Oyarzun, P. J., Gerlagh, M., and Hoogland, A. E. 1993. Pathogenic fungi involved in root rot of peas in the Netherlands and their physiological specialization. *Neth. J. Plant Pathol.* 99:23-33.
17. Oyarzun, P. J., Gerlagh, M., and Hoogland, A. E. 1993. Relation between cropping frequency of pea and other legumes and foot and root rot in peas. *Neth. J. Plant Pathol.* 99:35-44.
18. Oyarzun, P. J., Van der Zalm, A., Roelofs, H., and Dijst, G. 1994. Computerized equipment to control soil water potential and soil temperature

- in inoculum potential assays of soil-borne pathogens. *Plant Soil* 166: 125-135.
19. Oyarzun, P. J., and Van Loon, J. 1989. *Aphanomyces euteiches* as a component of the complex of foot and root rot pathogens of peas in Dutch soils. *Neth. J. Plant Pathol.* 95:259-264.
 20. Papavizas, G. C. 1964. New medium for the isolation of *Thielaviopsis basicola* on dilution plates from soil and rhizosphere. *Phytopathology* 54:1475-1481.
 21. Papavizas, G. C. 1968. Survival of root-infecting fungi in soil: VI. Effect of amendments on bean root rot caused by *Thielaviopsis basicola* and inoculum density of the causal organism. *Phytopathology* 58:421-428.
 22. Pfender, W. F. 1984. *Aphanomyces* root rot. Pages 25-28 in: Compendium of Pea Diseases. D. J. Hagedorn, ed. The American Phytopathological Society, St. Paul, MN.
 23. Pfender, W. F., and Hagedorn, D. J. 1983. Disease progress and yield loss in *Aphanomyces* root rot of peas. *Phytopathology* 73:1109-1113.
 24. Rawlings, J. O., Lesser, V. M., and Dassel, K. A. 1988. Statistical approaches to assessing crop losses. Pages 389-416 in: Assessment of Crop Loss from Air Pollutant. W. W. Heck, O. C. Taylor, and D. T. Tingey, eds. Elsevier Applied Science, New York.
 25. Sherwood, R. T., and Hagedorn, D. J. 1958. Determining common root rot potential of pea fields. *Wis. Agric. Exp. Stn. Bull.* 531.
 26. Sneh, B., Holdaway, B. F., Hooper, G. R., and Lockwood, J. 1976. Germination-lysis as mechanism for biological control of *Thielaviopsis basicola* pathogenic on soybean. *Can. J. Bot.* 54:1499-1508.
 27. Steel, R. G. D., and Torrie, J. H. 1985. Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed. MacGraw-Hill, Mexico City, Mexico.
 28. Stutz, E. W., Défago, G., Hantke, R., and Kern, H. 1985. Effect of parent material derived from different geological strata on suppressiveness of soils to black root rot of tobacco. Pages 215-217 in: Ecology and Management of Soil-borne Plant Pathogens. C. A. Parker, D. J. Moore, P. T. W. Wong, A. D. Rovira, and J. F. Kollmorgen, eds. The American Phytopathological Society, St. Paul, MN.