Oecologia (Berlin) (1988) 75:535–538

Oecologia

Variation in host susceptibility among and within populations of *Plantago lanceolata* L. infected by the fungus *Phomopsis subordinaria* (Desm.) Trav.*

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Summary. Susceptibility to *Phomopsis* stalk disease of *Plantago lanceolata* genotypes, sampled in three different populations with a variable degree of infection by the fungus *Phomopsis subordinaria*, was determined under greenhouse conditions. Susceptibility of the host varied within, but not among populations. No relationship between the intensity of the disease in the field and the mean susceptibility of the host genotypes sampled at those locations could be established. Host susceptibility appeared to be composed of different (uncorrelated) plant characteristics. Determining whether host genotypes are highly or slightly susceptible can only be achieved by field trials, where the plants are exposed to the whole set of disease inducing factors. The relevance of host susceptibility to the intensity of disease in the field is discussed in relation to the variation in pathogenicity of the fungus and the variation in environmental factors prevailing in *P. lanceolata* populations under *P. subordinaria* pathogen pressure.

Key words: *Plantago lanceolata* – *Phomopsis subordinaria* – Intensity of disease – Susceptibility – Biotic interaction

It has been hypothesised that infectious diseases play a role in determining the genetic variability within plant populations (Wills 1981). Several authors, studying intraspecific diversity in plant populations, have demonstrated the existence of high levels of variation within as well as between populations (e.g. Schmid 1985; Van Groenendael 1985). The characteristics used to quantify the genetic variation in these studies were very diverse. In the present study characteristics involved in interactions with a fungal pathogen are used for determining the intraspecific structure of populations of *Plantago lanceolata* L. (cf. Burdon 1980).

The accumulation of disease resistance in plants is generally supposed to be the result of a continuous process of natural selection in which populations at a greater risk of infection attain higher levels of resistance than those which only rarely suffer significant damage (Harlan 1976). However, genes for resistance can probably only accumulate at the cost of overall fitness. For example in *Avena* species, an increased resistance has been shown to be accompanied by a reduction of reproductive output of up to 10% (Simons 1979). Burdon and Müller (1987) found differences in germination and relative fecundity between lines of *Avena fatua* L. being susceptible or resistant to *Puccinia coronata* CDA. But it was not possible to draw conclusions from these data with respect to the cost of resistance. Selection is expected to maintain a relatively high level of resistance within the infected plant population in situations where host and pathogen have interacted for a long time. But when pathogen pressure is low or absent, host resistance will also be low (Burdon 1980). The interaction between hosts and pathogens is characterized by a great disparity in genome size, generation time, and speed of adaptability. Pathogens would (through faster evolution) leave their hosts defenseless, were it not for the counter strategies of the host. The basic feature of the counter strategies is variability. This implies polymorphism at loci that are involved in resistance against the pathogen (Clarke 1976; Bremermann 1980).

Variation in the intensity of disease within and among populations may be due to a number of causes: a) genetic variation among plants for traits influencing their susceptibility to attack, b) local variation in density or in genotype of pathogens, and c) local variation in the abiotic and biotic environment affecting the incidence of disease. In the present paper the relevance of the first factor will be considered with regard to the *Plantago lanceolata* – *Phomopsis subordinaria* pathosystem. In this system, described by De Nooij and Van der Aa (1987), the fungus *P. subordinaria* causes a stalk disease in its host which results in a decreased seed production. The relevance of the second factor mentioned above has been considered by De Nooij (1987), who studied the variation in pathogenicity of the fungus among and within populations of *P. subordinaria* infecting *P. lanceolata*. The pathogen needs a wound to enter the plant tissue. Weevils – among them *Ceutorhynchidius troglodytes* F. – were proved to be able to transfer the fungus into the host. The third factor is investigated by De Nooij (1988) who studied the role of these weevils in the infection process of *P. subordinaria* on *P. lanceolata*.

In the *Plantago-Phomopsis* pathosystem the host may have several lines of defence or disease escape: 1. The pathogen may be prevented from entering the plant tissue, e.g. when the plant is not attractive to the disease spreading insects. 2. The growth of the fungus in the stalk may be inhibited by chemical or physical barriers in the host, e.g. preformed fungitoxic compounds including phytoalexins, lignification of infected tissue. 3. The fungus may be prevented from spreading from the initially infected stalk into other plant parts, in particular into other rosettes, so that the host can survive by means of healthy side rosettes. The first line of defence has been considered by De Nooij (1988), the other two are the subject of the present paper.

* Grassland Species Research Group Number 123

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The aim of this paper is to examine to what extent variation in susceptibility within the host exists, and in particular whether these differences can explain the observed differences in the intensity of disease in the field. To this end two *P. lanceolata* populations with a high intensity of disease caused by *P. subordinaria* were compared to a population with a low intensity of disease. The lesion sizes of a particular pathogen isolate in the stalks of different plant genotypes from each population were determined. Lesion size was correlated with a decreased seed production (De Nooij and Van der Aa 1987). The pathogen commonly kills the rosette when it has reached the base of the stalk and spreads into the leaves and the other scapes. The ability of host genotypes to prevent the pathogen from growing into other rosettes of the same plant was studied. To obtain an impression of the physiological mechanism of plant resistance, germination of conidia and germ tube growth were studied in plant extracts of two host genotypes differing in their level of susceptibility.

Materials and methods

Ten host genotypes were sampled along a transect at relative distances of 5 m in each of three different *P. lanceolata* populations:

- Baarn: road-side, ruderal vegetation type, percentage of plants with at least one diseased stalk amounted to 80%.
- Papendal: verges of playing fields, ruderal vegetation type, percentage of plants with at least one diseased stalk amounted to 60%.
- Westduinen: dune grassland, extensively grazed by cattle, percentage of plants with at least one diseased stalk amounted to 1–2%.

Each genotype was cloned into three individual rosettes. To this end the mother plant, growing in a pot with a perforated bottom, was placed on a poth with perlite. After a period of seven weeks the motherplant was cut from the roots it had developed in the pot of perlite. Roots in the perlite yielded new rosettes which were planted in pots with soil (Van der Toorn and Ten Hove 1982). The pots were placed in the greenhouse under a regime of 16 h of light and a temperature of 21° C. The pathogen (isolate 3B, sampled at Papendal) was grown on oatmeal agar slants under Black Light at a temperature of 22° C. A suspension of 10^6 conidia/ml sterile water was used as inoculum. Five scapes per plant were inoculated by applying a drop of suspension of conidia just under the ear [flowering stage: second phase of male flowering (De Nooij and Van der Aa 1987)] and subsequently the stalk was wounded through the drop with a sterilized needle (diam. 0.2 mm). A total number of 15 stalks per genotype were inoculated. Four weeks after inoculation the length of the lesion in the stalk was determined as well as the total length of the stalk and its diameter.

To study the behaviour of the pathogen after it had reached the basis of the scape and grown into other parts of the plant, 14 out of the 30 *P. lanceolata* genotypes mentioned above were chosen. The motherplants were cloned into maximally five plants per genotype, using the method described above, each plant consisting of at least two connected rosettes. After flowering started, the third scape of one of the rosettes was inoculated with *P. subordinaria* (isolate 71A6, sampled at Papendal). The length of the lesion in each stalk was measured four weeks after inoculation. The plants were observed weekly during a period of 4 months in order to determine: a) the time the fungus needed to become visible in the first rosette, b) the time until the first rosette wilted, c) the time necessary to reach the second rosette and d) the time until the second rosette wilted. As the genotypes differed in the number of side rosettes they developed in the pots, the death of the whole plant was also recorded.

To obtain an impression of the physiological mechanism of resistance and susceptibility, behaviour of the pathogen was studied in extracts of plant material of two host genotypes differing in level of susceptibility, both originating from population Baarn. Six grams of fresh leaves were ground in 5 ml water and filtered through a paper filter and a bacterium filter (0.2 μm) to remove micro-organisms. Dilutions of 10^0, 10^{-1} and 10^{-2} of this raw extract were used to prepare suspensions of 10^6 conidia/ml. Germination of conidia was determined as were the lengths of the germtubes after 16 hours of incubation at room temperature. Per dilution two slides were used for counts, per slide 5 × 100 conidia for germination and 25 conidia for germtube length.

Results

The size of the lesions, induced by the pathogen in the plant after a period of four weeks, varied from genotype to genotype (Table 1). Significant variation, however, was restricted to the within population level. There was no difference in mean size of the lesions between the three populations.

As the individual plants were grown, with genotypes randomized with respect to the environment in the greenhouse, the variance component between the clones was mainly due to differences of genotype and can be regarded as an estimate of the total genotypic variance component V_g (Falconer 1981). The ratio V_g/V_p (where V_p is the phenotypic variance component) or the "clonal repeatability" estimates the relative importance of heredity versus environment for the characteristic measured. The V_g/V_p ratio appeared to be 0.68 for the development of lesions in the stalks, i.e. a maximum of 68% of the total variation could be attributed to genetic differences. As some part of the environmentally induced differences between individuals may be transmitted to all their clonal descendants, the V_g/V_p ratio should be regarded as an upper limit of the degree of genetic determination. There was no significant correlation between the development of lesions and the length of the stalks ($r=0.21$, $P>0.10$, $N=23$), and also the correlation between development of necrosis and the diameter of the stalk was not significant ($r=0.38$, $P>0.05$, $N=23$).

Fungal growth into and death of side rosettes occurred in all genotypes tested (Table 2). Only genotype B14 succeeded in surviving by means of healthy side rosettes, and also some of the plants of the genotypes B4, B11, P35, W3 and W12 survived. The time necessary for death of rosette 1 (i.e. the rosette from which the inoculated scape had budded) and fungal spread into and death of rosette 2 (i.e. the first side rosette that showed symptoms) varied significantly among genotypes. The time of arrival of the fungus in rosette 1 and death of rosette 2 varied among populations. None of the four parameters determined (Table 2) were correlated significantly with the size of the le-

Table 1. Variation in size of lesions caused by the fungus *Phomopsis subordinaria* (isolate 3B), among populations and among genotypes within populations of *Plantago lanceolata*, expressed as mm necrosis 4 weeks after inoculation. Each entry is the mean of 15 replications. C.V. = coefficient of variation. For statistical analysis data were transformed with log (x + 1). * $P < 0.001$

Population Baarn		Population Papendal		Population Westduinen	
Genotype	Necrosis	Genotype	Necrosis	Genotype	Necrosis
B11	193	P6	194	W15	203
B15	197	P1	198	W2	206
B14	207	P12	208	W4	206
B17	211	P9	210	W1	211
B4	221	P62	211	W7	215
B20	228	P63	212	W9	219
B19	238	P66	214	W8	226
B2	242	P39	216	W11	230
B16	245	P35	223	W12	230
B13	246	P64	227	W3	237
Population					
Mean	222		211		218
C.V.	0.11		0.11		0.10

	MS	df	F	P
Among populations	0.00824	2/27	0.75	> 0.25 NS
Among genotypes within populations	0.01102	27/55	7.25	< 0.001 *
Among slips within genotypes	0.00152	55/266	0.62	> 0.75 NS
Error	0.00245			

sions of *P. subordinaria* isolate 71A6 in the stalks of the host genotypes. There was no significant correlation between the percentage of surviving plants per genotype and the mean number of rosettes in the plants of each genotype ($r = 0.17$, $P > 0.10$, $N = 14$).

Germination of *P. subordinaria* conidia in extracts of fresh plant material was not related to the mean size of the lesions in the host genotype from which the extract was prepared (Table 3). The growth of germtubes was somewhat faster in all three dilutions of the extracts of genotype B16, the host with the largest lesions. The differences between B11 and B16 were not significant.

Discussion

Qualitative resistance is characterized by the failure of the disease to develop beyond a visible symptom stage, while quantitative resistance is expressed by a range of responses to the disease by different host individuals (Van der Plank 1963). Susceptibility in the *Plantago* – *Phomopsis* interaction was proved to be quantitative of character. None of the genotypes tested appeared to be totally resistant.

Host susceptibility in the *P. lanceolata* – *P. subordinaria* pathosystem appeared to be a complex plant feature. One aspect is susceptibility with respect to the formation of a lesion in the stalk. The development of *Phomopsis* lesions in *P. lanceolata* varied within but not among populations. Lesion size is correlated with reduction in seed production (De Nooij and Van der Aa 1987). The correlation coefficients (r) were significant but not higher than 0.48. So the predictive value of lesion size in the stalk for a decrease of host fitness in terms of seed production is not more than 23% ($= r^2$). With regard to the physiological mechanism of differences in lesion development, only non-signifi-

Table 2. Time in weeks necessary for *Phomopsis subordinaria* isolate 71A6 (sampled from Papendal) to grow into rosettes and to cause death of rosettes of 14 *Plantago lanceolata* genotypes. Rosette 1 = the one from which the inoculated scape had budded, rosette 2 = the first side rosette that showed symptoms. F-values were computed from nested analyses of variance; r expresses the correlation between a parameter and the length in mm of the lesion which isolate 71A6 had caused at 4 weeks after inoculation. * $P < 0.05$, ** $P < 0.01$

Host genotype[a]	Time till arrival in rosette 1	Time till death of rosette 1	Time till arrival in rosette 2[b]	Time till death of rosette 2[c]	Percentage surviving plants[d]
Baarn					
B4 (5)	6.4	7.6	7.6 (5)	8.0 (4)	20%
B11 (5)	6.4	8.2	9.0 (3)	9.3 (3)	40%
B13 (4)	5.6	6.6	7.3 (4)	7.8 (4)	0%
B14 (4)	5.2	6.2	9.5 (4)	10.0 (1)	100%
B16 (3)	7.0	7.5	8.5 (3)	8.7 (3)	0%
Mean	6.1	7.2	8.4	8.8	32%
Papendal					
P8 (3)	7.0	7.6	8.1 (3)	9.0 (3)	0%
P35 (5)	6.0	7.0	7.0 (4)	8.3 (4)	20%
P62 (5)	7.0	8.0	8.0 (5)	9.3 (5)	0%
P63 (5)	6.6	7.8	7.8 (5)	8.4 (5)	0%
Mean	6.7	7.6	7.7	8.8	5%
Westduinen					
W2 (4)	6.8	8.2	8.8 (4)	9.0 (4)	0%
W3 (5)	6.4	7.4	7.8 (5)	8.2 (2)	60%
W4 (5)	6.0	7.6	7.8 (5)	8.3 (5)	0%
W7 (4)	5.4	6.4	7.0 (4)	7.2 (4)	0%
W12 (5)	6.0	7.0	7.8 (5)	8.5 (4)	20%
Mean	6.1	7.3	7.8	8.2	16%
$F_{population}$	6.54*	0.61 NS	0.44 NS	5.51*	1.05 NS
$F_{genotype}$	0.46 NS	3.22 NS	2.53**	2.57*	–
r	0.27 NS	−0.03 NS	−0.21 NS	−0.20 NS	−0.35 NS

[a] Between brackets: the number of pots per genotype
[b] Between brackets: the number of plants in which the fungus had reached rosette 2
[c] Between brackets: the number of plants in which rosette 2 died
[d] Data have been transformed with arcsin $\sqrt{x}$ before analysis

Table 3. Percentage of germination and length of germ tubes (μm) after 16 hours of incubation of *Phomopsis subordinaria* conidia in extracts of fresh plant material from two genotypes of *Plantago lanceolata* (B16 and B11) which differed in size of lesions formed by the pathogen. * $P < 0.001$

	Dilution			$F_{genotype}$	$F_{dilution}$	$F_{interaction}$	df error
	10^0	10^{-1}	10^{-2}				
% Germination							
B11	94.0	88.0	63.8	0.68 NS	134.66*	2.18 NS	54
B16	95.4	83.4	62.8				
Germ tube length (μ)							
B11	20.1	15.2	9.0	1.70 NS	14.25*	0.14 NS	294
B16	21.6	16.5	12.2				

cant differences in fungal growth in extracts of plant material of host genotypes, differing in level of susceptibility, could be observed.

Another aspect of host fitness under pathogen pressure is the survival of an infected individual by means of side rosettes. Although some of the parameters determined (Table 2) varied among genotypes or among populations, the differences in time necessary for the fungus to reach or to kill a rosette were so small that it is questionable whether these are ecologically relevant. Qualitative differences, on the other hand, are of importance, for instance the 100% survival of genotype B14. With respect to the size of the lesions in the stalks genotype B14 should be regarded as one of the more susceptible genotypes. Development of lesions and survival of the host through side rosettes were not correlated among host genotypes. This suggests that the genetics of these components of susceptibility may be determined independently.

The set of characteristics determining host susceptibility consists of more aspects than has been studied in this paper. The occurrence and intensity of disease in a certain host genotype is also determined by its attractiveness to the weevils which transmit the disease. The fact that susceptibility to the disease is determined by several characteristics implies that establishing which host genotype will have a higher or lower fitness under pathogen pressure can only be achieved by means of field trials where the plants are exposed to the whole set of disease inducing factors.

One might argue that a low intensity of disease in a population is the result of natural selection for low levels of susceptibility in the host. As a consequence one would expect a population with a low intensity of disease to be composed of less susceptible plants than a population with a high intensity of disease. Alternatively, however, when low susceptibility carries a cost relative to high susceptibility, one would expect high susceptibility to be accumulated in populations which have little pathogen pressure. Neither of these two expectations were confirmed in the present study, since plants from Westduinen (a location with a low intensity of disease) appeared to have the same mean susceptibility as the plants from the other two locations where the intensity of disease was much higher. Possibly fungus and host have not interacted long enough to let resistance develop in the populations under high pathogen pressure. The *P. lanceolata* populations at Baarn and Papendal are no older than 20 years. Westduinen has been in use as a common for several centuries, but no records on *P. lanceolata – P. subordinaria* interaction are available from the past. Another explanation is that variation in susceptibility among the three populations under study is too subtle to detect or that the available genetic variation in susceptibility may not be large enough to cause a decrease in the intensity of disease. The V_g/V_p ratio was rather high, but this yields no information about the additive genetic variation.

De Nooij (1987) found differences in mean pathogenicity between the pathogen populations at the same three locations, the isolates from Baarn being more pathogenic than those from the other two populations. This variation in pathogenicity could partly explain the observed differences in the intensity of disease in the field. In the literature variation in host resistance and pathogenicity of the fungus, in space or in time, have been proposed as major causes of patterns in disease intensity in natural ecosystems (e.g. Heather and Chandrashekar 1982). However, variation in certain environmental factors may also be significant in the stability of the host-pathogen relationship. Host and pathogen interact not only with each other, but also with the environment and the latter can contribute significantly to the total variation (Zadoks and Van Leur 1983). An example of this type of interaction is given by Paul and Ayres (1986) who studied the effects of infection by *Puccinia lagenophorae* on the growth of *Senecio vulgaris* cultivated under a range of nutrient concentrations. An important environmental factor in the *P. lanceolata – P. subordinaria* interaction is the transmission of the pathogen into the host by weevils, which has been studied by De Nooij (1988).

It can be concluded that, although variation in host susceptibility was demonstrated, the contribution of this trait to the actual intensity of disease in the field is not very large, so that variation in pathogenicity of the fungus and variation in the environment remain as factors that determine the intensity of disease in the field.

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Received July 17, 1987