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Observations on the host range of an isolate of Septoria nodorum from wheat

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Abstract
An isolate of *S. nodorum* from wheat was inoculated onto grasses in the field. Re-isolates from these grasses were tested in a cross-inoculation experiment, performed in a growth chamber. The wheat isolate was pathogenic to each element of a set consisting of *Elytrigia repens*, *Hordeum vulgare*, *Lolium perenne*, *Poa annua*, and *Triticum aestivum*. Re-isolates from any of the elements of this set were pathogenic to all other elements. The effects of hosts and inoculum-density treatments were statistically significant. A significant isolate × host interaction suggests a form of specialization, which is possibly due to a passage effect. These observations may contribute to a better understanding of the epidemiology of *S. nodorum* in the Netherlands.

Introduction

Septoria nodorum Berk., the imperfect stage of *Leptosphaeria nodorum* Müller, is found on a wide range of graminaceous hosts (Sprague, 1950). Limited cross-inoculation studies have been reported by Weber (1922), Becker (1956), and Holmes and Colhoun (1970).

In the present study, grasses were infected outdoors under natural conditions, using an isolate of *S. nodorum* from wheat. The fungus was re-isolated from infected grasses, and the re-isolates were inoculated on all hosts in the growth chamber. The experiment resulted in a host – isolate response matrix, suitable for statistical analysis.

Materials and methods

Experimental design. The experiment was a 2 × 5 × 5 factorial design. The independent variables were 2 inoculum densities, 5 isolates, and 5 hosts. The dependent variable was host response, read in 5 replications. The total number of readings amounted to 250.

Fungus, inoculum density. An isolate of *S. nodorum* from wheat was obtained from the Institute of Phytopathological Research (IPO), Wageningen, by courtesy of Ir E. Ubels. In May, 1969, inoculum consisting of heat-killed wheat seeds carrying pycnidia was broadcast in a field of barley, in a field of wheat cv. 'Felix', and among grasses growing on a ditch side. At the end of August, leaf samples were taken from infected grasses and cereals, and the fungus was re-isolated. Infected leaves were soaked in 1 ml

of sterile deionized water for 15 minutes, and the resulting pycnidiospore suspension was streaked onto wheatmeal agar (2% wheatmeal + 2% agar; Shearer, 1967). Plates were incubated at about 15°C, and 22,000 lux irradiation from fluorescent tubes (Philips TLM 40W/33RS) during 16 hours.day^{-1}. Pycnidia appeared after one week. Re-isolates were purified by one transfer on wheatmeal agar. Of each isolate, one for every host, a spore suspension was made by scraping the pycnidiospores from the surface of a sporulating plate, and suspending them in 0.5% gelatin solution (Rosielle, 1968). Spore suspensions were standardized to inoculum densities of 5×10^5 and 5×10^3 spores.ml^{-1}.

Hosts. The hosts used were *Elytrigia* (*Agropyrum*) *repens*, *Hordeum vulgare* 'Cambrinus', *Lolium perenne*, *Poa annua* and *Triticum aestivum* 'Felix'. Plants for inoculation were grown in sterilized sandy peat soil in 7 cm ⌀ plastic pots. Grasses were lifted from the field one month prior to inoculation, and kept in the greenhouse at approx. 20°C. *Poa annua* was heading, the other grasses were in the vegetative phase at the time of inoculation; all grasses were healthy. *T. aestivum* and *H. vulgare* were sown at the rate of 10 plants per pot, and were at the 2 to 3 leaf stage at the time of inoculation.

Inoculation. The spore suspensions were sprayed onto the plants until run-off, using a De Vilbiss No. 15 adjustable tip atomizer (one for each isolate) and compressed air. After inoculation, the plants were enclosed in clear polythene bags during 72 hours, to provide a water-saturated atmosphere conducive to infection. Inoculated plants were incubated at about 18°C and 11,500 lux irradiation from fluorescent tubes during 16 hours.day^{-1}.

Host response. Ten days after inoculation, 5 leaves were sampled at random from each pot (host-re-isolate combination). Symptoms were assessed using a scale of 10 classes (Table 1). For the analysis of variance, scale values were transformed into percentages of leaf area showing *S. nodorum* symptoms (Table 1, column 3).

Table 1. Scale for the assessment of symptoms on leaves due to *Septoria nodorum*.

Scale value	Percentage leaf area showing symptoms (x)	
	class limits	central values of classes
0	0	0
1	$0 < x < 1$	0.5
2	$1 \leqslant x < 10$	5.5
3	$10 \leqslant x < 25$	17.5
4	$25 \leqslant x < 50$	37.5
5	$50 \leqslant x < 75$	62.5
6	$75 \leqslant x < 90$	82.5
7	$90 \leqslant x < 99$	94.5
8	$99 \leqslant x < 100$	99.5
9	100	100

Tabel 1. Waarnemingsschaal voor het bepalen van symptomen op het blad veroorzaakt door S. nodorum.

Results

Symptoms in the field. Pycnidia of *S. nodorum* were found in leaves of *E. repens*, *H. vulgare*, *L. perenne*, *P. annua* and *T. aestivum*. *E. repens* showed flecks and streaks, which at coalescence formed dead areas. Pycnidia could most readily be found in dead areas at the tips or edges of the leaves. Symptoms observed on the other grasses were: *H. vulgare*, yellowish brown, indistinct lesions; *L. perenne*, distinct rectangular lesions of approx. 4 mm^2 with dark brown edges and often with off-white dead centres; *P. annua*, indistinct, yellowish brown lesions irregular in size; *T. aestivum*, as usual.

Symptoms in the growth chamber after inoculation. E. repens showed brown flecks and streaks which were conspicuous along the veins. Depending upon isolate, the flecks merged to form lesions with dead centres. Lesion development on *H. vulgare* tended to be restricted to small brown spots, independent of isolate. *L. perenne* showed indistinct pale brown spots. Infected leaves of *P. annua* were irregular yellowed or mottled, whereas floral bracts exhibited a distinct brown discoloration. *T. aestivum* showed dark brown lesions which coalesced gradually to cause a general necrosis of the leaf; non-inoculated checks of *T. aestivum* remained healthy.

Response matrix. Host response is tabulated in Table 2. The analysis of variance is given in Table 3. Host response at the high inoculum density is significantly greater than at the low inoculum density. Differences between hosts were significant, and the hosts could be ranked in order of decreasing response: *T. aestivum* > *H. vulgare* > *E. repens* > *L. perenne* > *P. annua*. Differences between isolates were not significant.

Table 2. Response matrix of *Septoria nodorum*, re-isolates versus hosts, at two inoculum densities. Entries are host leaf responses expressed as percentage of leaf area showing symptoms. Each entry is the average of five replications.

Inoculum density	Host	Re-isolate from:				
		Elytrigia repens	*Hordeum vulgare*	*Lolium perenne*	*Poa annua*	*Triticum aestivum*
5×10^5 pycnidiospores ml^{-1}	*Elytrigia repens*	52	18	28	18	10
	Hordeum vulgare[1]	17	58	48	52	55
	Lolium perenne	<1	38	22	26	18
	Poa annua	1	8	6	26	1
	Triticum aestivum[2]	85	78	86	96	84
5×10^3 pycnidiospores ml^{-1}	*Elytrigia repens*	2	2	4	2	23
	Hordeum vulgare	2	<1	38	18	1
	Lolium perenne	0	0	1	2	2
	Poa annua	0	<1	0	<1	<1
	Triticum aestivum	69	59	52	52	66

[1] cv. 'Cambrinus'
[2] cv. 'Felix'

Tabel 2. Matrix van reacties op S. nodorum, her-isolaten tegenover waardplanten, bij twee inoculumdichtheden. Iedere waarde is het gemiddelde van vijf herhalingen. De getallen geven het percentage van het bladoppervlak, dat symptomen vertoont.

Table 3. Analysis of variance of the response matrix of *Septoria nodorum*, re-isolates versus hosts, at two inoculum densities.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Leaves	4	1908.38	477.09	1.9
Inoculum density	1	28783.22	28783.22	113.2*
Host	4	137079.21	34269.80	134.8*
Isolate	4	1271.61	317.90	1.2
Inoculum density × host	4	1601.59	400.39	1.6
Inoculum density × isolate	4	1926.17	481.54	1.9
Host × isolate	16	23109.57	1444.34	5.7*
Inoculum density × host × isolate	16	15245.19	952.82	3.7*
Error	196	49848.22	254.32	
Total	249	260773.16		

* Significant at $P < 0.001$.

Tabel 3. Variantieanalyse van de matrix van reacties op S. nodorum, her-isolaten tegenover waardplanten, bij twee inoculumdichtheden.

Interestingly, isolate × host interaction and inoculum density × isolate × host interactions were significant. The host × isolate interaction is evident only at the high inoculum density, and indicates that each host is most susceptible to its corresponding isolate. This host × isolate interaction could be interpreted as a form of specialization, induced by a 'host passage' effect.

Discussion

The response of a host to infection by a fungal pathogen is influenced by the environment, the inoculum density, and host characteristics such as growth stage or maturity (Zadoks, 1961), leaf size and leaf texture (hairiness, etc.). Except for *P. annua*, the plants were in the vegetative phase. The effect of leaf size on response assessment was reduced by reading the percentage of leaf area showing symptoms instead of counting flecks. The effect of leaf texture was minimized by suspending the spores in 0.5% gelatine rather than in water (Rosielle, 1968).

The effect of inoculum density level is quite marked, and it is an important determinant of the significance of the host × isolate interaction, see *L. perenne* and *P. annua* in Table 2. The data support Becker's contention that higher inoculum loads are needed for the infection of *P. annua* than for *T. aestivum* (Becker, 1956). The differences between the two inoculum densities is a hundredfold, or 20 decibels (the difference between two treatments expressed in decibels is $10 \times {}^{10}\log T_1/T_2 : T_1$ and T_2 representing the high and low inoculum density treatments respectively). The expected difference in the percentages of leaf area showing symptoms should also be 20 decibels, unless infections overlap or compete with each other. The observed values (Table 4), always less than 20, indicate that competition between or overlap of infections has taken place, the most in *T. aestivum* and the least in *L. perenne*.

Table 4. Percentages of leaf area covered by lesions of *Septoria nodorum* 10 days after inoculation calculated as the average of the 5 re-isolates per host and per inoculum density, and the differences between these percentages, expressed in decibels per host, due to a difference of 20 decibels in inoculum density.

Hosts	Percentage leaf area covered by lesions (average per host)		Difference in this percentage expressed in decibels per host
	Inoculum density		
	high	low	
Elytrigia repens	25	7	5.5
Hordeum vulgare	46	14	5.2
Lolium perenne	23	1	13.6
Poa annua	8	1	9.0
Triticum aestivum	90	60	1.8

Tabel 4. *Percentages van het bladoppervlak bedekt met symptomen van S. nodorum, berekend als gemiddelden van de* 5 *her-isolaten per waard en per inoculumdichtheid, en de verschillen tussen deze percentages in decibels tengevolge van een* 20 *db verschil in inoculumdichtheid.*

Note that all data pertain to the amount of leaf flecking 10 days after inoculation. The choice of the value 10 is arbitrary, and may have introduced bias, because the period from inoculation to the appearance of flecks (incubation period) for the more resistant grasses may have been longer than that of wheat.

The effect of environment has been minimized firstly by infecting grasses in the natural environment, and secondly by performing the inoculation experiment in the growth-chamber at near-optimal conditions for infection and symptom development. However, the low response of *L. perenne* when compared to its response in the field may be due to the absence of post-infection leaf wetness periods in the growth chamber.

The host-passage effect merits special attention. An isolate from wheat was sprinkled in the field and left alone for three months. Natural infection in the field cannot be excluded, but inspection at the time of inoculation did not reveal any *S. nodorum* symptoms. The isolate passed at least once, and maybe up to six times, through its respective grass hosts before re-isolation. The possibility of the isolate passing from one grass host to another can, however, not be excluded. After two passages over agar, some re-isolates exhibited an increased specificity for their field hosts, at the high inoculum density. Apparently, the passage over a host induces some kind of specialization.

Examination of the results of the cross inoculation experiment by Weber (1922) supports the preceding conclusion. In table 5, Weber's data have been recalculated and presented as a response matrix. Weber obtained inoculum from naturally infected plants, and he made no attempt to control inoculum density. As he did not take precautions to maintain a watersaturated atmosphere during a post-inoculation period, his results must be used with caution. They may have been an estimate of disease escape rather than of susceptibility. Nevertheless, a marked specificity of an isolate towards its field host, and the reversibility of this specialization on *T. aestivum* is evident. Specificity of isolates of *S. nodorum* for their field hosts was also found by Holmes and Colhoun (1970).

The role of grass hosts in the overwintering and oversummering of *S. nodorum* of

Table 5. Re-interpretation of a cross-inoculation experiment by Weber (1922). Data for successful interaction of three host species by three isolates of *Septoria nodorum*, expressed as percentages of leaves infected, and rearranged to form a response matrix.

Host	Isolate		
	Poa pratensis	*Secale cereale*	*Triticum aestivum*
Poa pratensis	67	42	37
Secale cereale	54	75	67
Triticum aestivum	70	94	90

Tabel 5. Herinterpretatie van een kruisinoculatieproef door Weber (1922) *met drie herkomsten van S. nodorum en drie waardplantsoorten. Het aantal zieke bladeren is uitgedrukt als percentage van het aantal geïnoculeerde bladeren; de gegevens zijn in matrixvorm gerangschikt.*

wheat is not yet known. This study indicates that grass hosts may play a part in the epidemiology of *S. nodorum* in the Netherlands. This idea is supported by an observation in a field experiment with winter wheat cv. 'Felix', where a focus of *S. nodorum* was found in a non-inoculated plot. In this focus, plants of *E. repens* carrying *S. nodorum* were present. Becker (1956) found *S. nodorum* on *P. annua* in a severely infected wheat field.

Samenvatting

Waarnemingen over de waardplantenreeks van een herkomst van Septoria nodorum van tarwe

Grassen en granen te velde werden geïnoculeerd met een *S. nodorum* isolaat van tarwe. Symptomen van aantasting door de schimmel werden gevonden op *Elytrigia repens, Hordeum vulgare, Lolium perenne, Poa annua* en *Triticum aestivum*. Van deze vijf waardplanten werden her-isolaten gewonnen, die vervolgens in een klimaatkamer geïnoculeerd werden op ieder van de vijf waardplanten. Aldus ontstond een kruisinoculatiematrix van bladaantastingspercentages (Tabel 2), die aan een variantieanalyse (Tabel 3) kon worden onderworpen. De volgende effecten waren significant: waardplanten, inoculumdichtheden, interactie herisolaat × waardplant, en interactie herisolaat × waardplant × inoculumdichtheid. De interacties suggereren beïnvloeding door de waardplant, dus een passage-effect. Bij passage over een waardplant zou enige mate van fysiologische specialisatie kunnen optreden. Dit wordt bevestigd door gegevens van Weber (Tabel 5). Deze waarnemingen over de waardplantreeks kunnen van belang zijn voor een beter begrip van de epidemiologie van *S. nodorum* in Nederland.

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References

Becker, G. J. F., 1956. Verslag over de werkzaamheden betreffende de afrijpingsziekten van granen in 1956. Stichting Nederlands Graan-Centrum, Werkgroep Graanziekten – Commissie Afrijpingsziekten, 14 pp.

Holmes, S. J. I. & Colhoun, J., 1970. *Septoria nodorum* as a pathogen of barley. Trans. Br. mycol. Soc. 55: 321–325.

Rosielle, A. A., 1968. Studies in the breeding of wheat for resistance to *Septoria nodorum* Berk. Honours thesis, University of Western Australia, 171 pp.

Shearer, B. L., 1967. Epidemiology of *Septoria nodorum* Berk. Honours thesis, University of Western Australia, 142 pp.

Sprague, R., 1950. Diseases of cereals and grasses in North America. Ronald Press Co., New York, xvi + 538 pp.

Weber, G. F., 1922. II. *Septoria* diseases of wheat. Phytopathology 12: 537–585.

Zadoks, J. C., 1961. Yellow rust on wheat, studies in epidemiology and physiologic specialization. Tijdschr. PlZiekten (Neth. J. Pl. Path.) 67: 69–256.

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