Neth. J. Pl. Path. 83 (1977) 205–215

Contamination of ryegrass seed with Drechslera species and its effect on disease incidence in the ensuing crop

R. E. Labruyère

Research Institute for Plant Protection (IPO), Wageningen

Accepted 27 April 1977

Abstract

Infection of five ryegrass cultivars grown from seed with different initial contamination of *Drechslera siccans* and *D. catenaria/dictyoides* was examined four times after emergence. Susceptibility or resistance of the cultivars to the fungi proved to have a greater effect on disease indidence than the level of seed contamination. Seed disinfection with imazalil proved unsatisfactory. No yield differences resulting from different levels of seed contamination were found.

Introduction

Transmission of *Drechslera* species by seed is generally acknowledged. Contamination of seed lots may be caused by external contamination of the seed coats but is mainly due to infection of the seeds by these fungi. Therefore a certain percentage of contaminated seeds (contaminated in its broadest sense) may act as an important source of crop infection.

In the Netherlands three *Drechslera* spp. are considered important on ryegrass seed. Mäkelä (1971) considers *D. siccans* (Drechs.) Shoem. as the main seed-borne pathogen causing both foot rot and leaf spot on *Lolium multiflorum* and *L. perenne*. Andersen (1955) also found it on seeds of *Dactylis glomerata*, *Festuca pratensis*, *F. rubra* and *Poa trivialis*.

Drechslera dictyoides (Drechs.) Shoem. f. sp. *perennis* (Braverman & Graham) Shoem., common on seeds of *Lolium* spp., may cause net blotch (Chidambaram et al., 1973) and *D. catenaria* (Drechs.) Ito attacks *Lolium* spp. and other grasses (Andersen, 1959). As with infection by *D. dictyoides*, the lesions on *Lolium* leaves caused by *D. catenaria* show a reticulate necrosis (net blotch) and are associated with withering and yellowing of the leaf tips (Wilkins, 1973). Although differences in conidial morphology do exist between these two fungi, they are not easily distinguished under a low power stereomicroscope used in routine inspection. When *Lolium* seed is tested at the Dutch Government Seed Testing Station, therefore only *D. siccans* is distinguished from the less important *D. catenaria* and *D. dictyoides*. However, in my experience these last two fungi are often responsible for much leaf damage late in the growing season as found elsewhere (Latch, 1966, Mäkelä, 1972).

It was still insufficiently known whether seed contamination with these *Drechslera* spp. had any influence on emergence, disease incidence in the field and crop productivity. This was investigated in the Netherlands during the 1975 season.

Materials and methods

Seed samples from three Italian and two perenniao ryegrass cultivars, contaminated with *D. siccans* and *D. catenaria/dictyoides* (percentages in Table 1,A) were obtained from the Dutch Government Seed Testing Station, where contamination rates had been determined with the blotter test with deep-freezing (De Tempe, 1968). In this study also no distinction has been made between *D. catenaria* and *D. dictyoides*.

As it was not possible to obtain samples of the same cultivar with different levels of natural contamination, attemps were made to produce them artificially by 'mixing' distinfected and infected seed. Of each cultivar part of the seed was disinfected with imazalil (1-[β-(allyloxy)-2,4-dichlorophenylethyl]-imidazol) as a 10% powder at 6 g/kg seed. This disinfected seed was used to adjust the contamination level to 0, 5, 10 or 20% (Table 1, B. C. D), on the assumption that the fungicide was 100% effective. The treated and untreated seeds were kept and sown separately to avoid contamination of the untreated seed with the fungicide.

Two types of field trial were carried out with the seed mixtures indicated in Table 1, one to monitor emergence and disease development, and the other to examine the effect of disease on crop yield.

In the first series of experiments situated on a heavy river clay each cultivar formed one block. Each block consisted of small plots (50 × 50 cm) with in the centre a control sampling area (20 × 20 cm) in which 40 seeds were sown at a density of 1 seed/10 cm^2; the surrounding part of the plot was sown with a weighed quantity of seed. Planting was done with the aid of an aluminium frame. One corner of each plot was marked by a wooden label so that with the frame in position the exact position of the sampling area could be located (Fig. 1).

The number of plots in each block was determined by the number of sampling dates (4), the number of seed mixtures (two for cv. 1, three for cv. 2, and four for the cvs. 3, 4 and 5) and the number of replicates (10). Plots were separated by bare soil as indicated in Fig. 2. Between blocks 2 m remained bare. The emergence of plants on all sampling plots was recorded on May 23, about three weeks after sowing. Observations on disease development began two weeks later when plants were dug from the appropriate sampling area of plots. Most of the rootlets and, when plants grew bigger, the apparently healthy leaves were cut off and discarded. Plants were washed, placed on wet filter paper in boxes, which were then wrapped in polyethylene bags

Table 1. Original (A) and fungicide-adjusted (B, C, D) percentages of contamination with *D. siccans* (1) and *D. catenaria/dictyoides* (2) of seed of ryegrass cultivars.

	Lolium multiflorum						*Lolium perenne*			
cv.	1. Tetila		2. unknown		3. Barmultra		4. Reveille		5. Splendor	
fungus	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
A original	0	0.25	11	0	12	36	0	47.5	0.5	52.5
B (A + D)			5	0	5	15	0	20	0	20
C (A + D)					2.5	7.5	0	10	0	10
D disinfected	0	0	0	0	0	0	0	0	0	0

Tabel 1. Oorspronkelijke (A) en door gedeeltelijke ontsmetting aangebrachte (B, C, D) percentages besmetting met D. siccans (1) en D. catenaria/dictyoides (2) bij zaad van raaigrascultivars.

Fig. 1. Aluminium frame used for sampling. For sowing the 40 seeds the inner square was used, the remaining area was sown with the same seed mixture at approximately the same density.

Fig. 1. Aluminium frame in gebruik tijdens het monsteren. Bij het zaaien werden de 40 zaden in het binnenste vierkant gezaaid, het overblijvende gedeelte werd gezaaid met hetzelfde zaad in dezelfde dichtheid.

Fig. 2. One of the three blocks of 160 plots. Sampling areas are the small squares inside the bigger ones as shown in the detail below. The other two blocks comprised 120 and 80 plots, respectively.

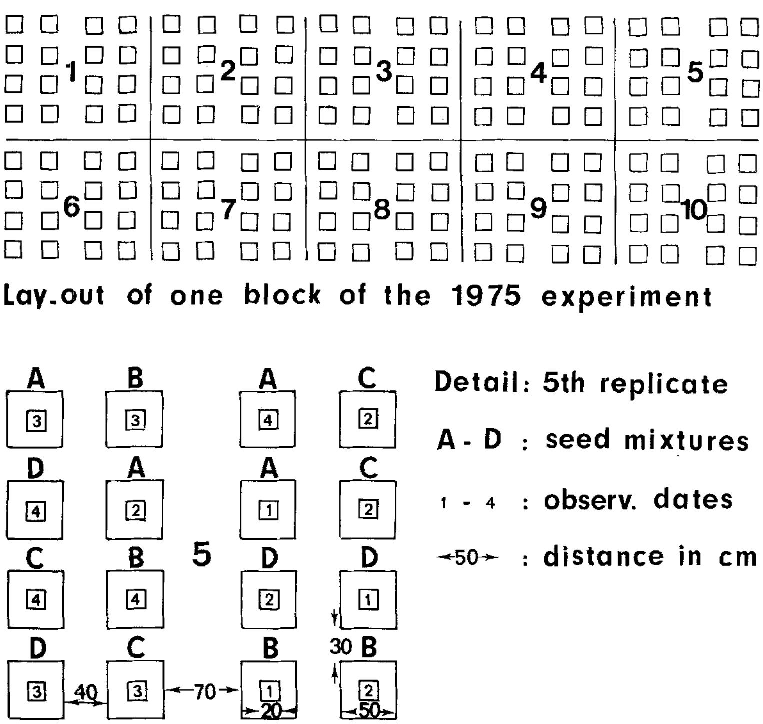


Fig. 2. Een van de drie blokken van 160 veldjes. De monsterplekken zijn de kleine vierkanten binnen de grotere, zoals getoond in de detailtekening. De andere twee blokken bestonden uit respectievelijk 120 en 80 veldjes.

and kept at room temperature in normal daylight for about a week. Individual plants were examined with a stereomicroscope and, if sporulation of *D. siccans* or *D. catenaria/dictyoides* was seen, that plant was recorded as diseased. Observation dates were different for each cultivar because only 20 plots could be dealt with in one day. However, the time interval between successive observations was about four weeks for all cultivars. The whole experiment (including plots of which the sampling area had already been removed) was mowed and subsequently fertilized just after the second and after the third sampling period.

In the second series of experiments productivity of the seed mixtures of Table 1 was tested both on a heavy river clay and a sandy soil in Wageningen. The cultivars were sown in five replicates on 1×1 m plots of which on mowing 0.7×0.7 m control area was sampled, dried at 80°C and weighed.

Results

Emergence of different seed mixtures of the same cultivar (Table 2) did not differ significantly and a high percentage of seed contamination was not correlated with poor emergence. At each successive sampling date the number of plants within the area was noted but these numbers did not differ significantly from the original emergence counts.

Infection with D. catenaria/dictyoides. The percentage plants infected with *D. catenaria/dictyoides* on the four observation dates is given in Table 3. An analysis of variance with transformed values ($y = 2 \arcsin \sqrt{x}$) showed highly significant differences between observation dates for all cultivars. Only with the unknown cultivar was the disease index less on the fourth date than on the third. However, this cultivar proved very susceptible to *D. siccans* and some infection by *D. catenaria/dictyoides* may not have been noticed because of the abundance of *D. siccans* conidia.

Disinfection of seed with imazalil was ineffective against *D. catenaria/dictyoides* except with cv. Barmultra, where small but significant effect between mixtures was found.

Table 2. Percentage of emergence of five ryegrass cultivars from seeds with different *Drechslera* contamination.

	Lolium multiflorum			*Lolium perenne*	
cv.	1. Tetila	2. unknown	3. Barmultra	4. Reveille	5. Splendor
A	59.6	76.4	63.2	71.2	78.1
B		72.9	60.2	72.8	74.6
C			57.4	74.1	74.4
D	56.3	70.8	58.7	70.6	70.1

Tabel 2. Percentage opkomst van zaad met verschillende besmetting met Drechslera-schimmels bij vijf raaigrascultivars.

Table 3. Percentage of ryegrass plants infected with *D. catenaria/dictyoides* on four observation dates.

Cultivar	Mixture	June	July	August	Sept.	Mean
L. multiflorum						
1. Tetila	A	2	9	33	31	18.5
	D	0	9	30	29	16.8
	mean	0.9	8.8	31.3	29.6	17.6
2. unknown	A	0	11	30	17	14.4
	C	1	9	34	24	16.6
	D	0	9	27	28	16.1
	mean	0.2	9.6	30.3	22.7	15.7
3. Barmultra	A	8	6	26	30	17.5
	B	3	8	25	30	16.5
	C	3	2	25	26	13.9
	D	4	4	16	31	13.8
	mean	4.6	5.1	22.9	29.3	15.4
L. perenne						
4. Reveille	A	3	20	77	97	49.1
	B	6	14	66	98	46.1
	C	4	14	64	98	45.3
	D	5	15	58	97	43.9
	mean	4.5	16.0	66.2	97.6	46.1
5. Splendor	A	10	23	48	89	42.4
	B	6	20	41	87	38.4
	C	8	27	46	78	39.8
	D	4	26	42	86	39.6
	mean	6.9	24.0	44.4	84.9	40.0

Tabel 3. Percentage raaigrasplanten besmet met D. catenaria/dictyoides op vier waarnemingsdata.

Infection with D. siccans. Little disease was recorded in cvs. Tetila, Reveille, and Splendor (Table 4), presumably because the initial seed contamination was too low and therefore no statistical analysis was possible. 'Barmultra' and the unknown cultivar had a higher initial seed contamination (Table 1). However, the disease developed only slowly on 'Barmultra', and although the proportion of diseased plants had significantly increased on the fourth observation date no diseased plants were recorded in 13 out of 40 plots.

Disease development in the unknown cultivar started early and increased considerably between the third and fourth observation dates. This was the only cultivar to show plants killed by *D. siccans* on the fourth date. No analysis of variance has been carried out with the data of the first three observation dates because of the many *D. siccans*-free plots. Transformed figures for the fourth date when analysed showed highly significant differences between seed mixtures.

Table 4. Percentage of ryegrass plants infected with *D. siccans* on four observation dates.

Cultivar	Mixture	June	July	August	Sept.	Mean
L. multiflorum						
1. Tetila	A	0	0.4	2.4	5.2	2.1
	D	0	0.4	0.4	3.0	1.0
	mean	0	0.4	1.4	4.2	1.6
2. unknown	A	4.0	5.1	10.3	68.5	23.1
	C	6.1	0.7	10.1	60.6	20.0
	D	1.1	1.4	5.4	30.3	10.0
	mean	3.8	2.4	8.7	53.5	17.8
3. Barmultra	A	3.2	0	1.9	15.2	4.9
	B	0.8	0.4	1.2	6.7	2.3
	C	0.4	0	1.2	4.8	1.6
	D	0.4	0.4	0.4	7.0	2.2
	mean	1.3	0.2	1.2	8.3	2.8
L. perenne						
4. Reveille	A	0	0.7	1.1	0.4	0.5
	B	0.3	0.4	0.7	0.4	0.4
	C	0	0	2.2	0.4	0.6
	D	0	0	0	1.1	0.3
	mean	0.1	0.3	1.0	0.5	0.5
5. Splendor	A	0	0.7	3.5	5.7	2.5
	B	0	0.7	2.5	4.8	2.0
	C	0	0.7	2.7	2.3	1.5
	D	0	0.4	2.7	1.2	1.1
	mean	0	0.6	2.9	3.5	1.8

Tabel 4. Percentage raaigrasplanten besmet met D. siccans op vier waarnemingsdata.

Yield. The grass yields after two cuts from the clay and three from the sandy sites are given in Table 5. No significant differences between seed mixtures with adjusted contamination were found either for single cuts or for total yields. Differences between cultivars and between soils did exist but were due to differences in time of sowing and harvesting.

Table 5. Yield in dry matter in kg per 2.45 m² of ryegrasses infected with *Drechslera* spp.

		Lolium multiflorum						*Lolium perenne*			
	cv.	1. Tetila		2. unknown		3. Barmultra		4. Reveille		5. Splendor	
	soil	clay	sand	clay	sand	clay	sand	clay	sand	clay	sand
A		2.6	3.9	3.0	3.7	2.8	3.7	2.3	3.8	2.8	3.7
B				3.0	3.6	2.8	3.4	2.3	3.7	2.6	4.0
C						2.9	3.5	2.3	3.9	2.8	3.9
D		2.9	4.1	2.8	3.7	2.9	3.7	2.3	3.8	2.6	3.6

Tabel 5. Opbrengst in droge stof in kg per 2,45 m² van raaigrassen besmet met Drechslera-schimmels.

Discussion and conclusions

It was expected that disinfection and subsequent mixing at planting could achieve different levels of contamination in seed stocks. Although imazalil had not been used before on grass seed, this new fungicide was chosen because of prominent results against leaf stripe of barley (*Pyrenophora graminea*, personal communication Ir. J. Kuipers, Philips-Duphar), and because of its systemic action. When tested at the Dutch Government Seed Testing Station, *Drechslera* spp. proved to be killed for almost 100% on imazalil-treated ryegrass seed lots used in the experiments. However, seed treatment with imazalil did not improve emergence or the health of emerged plants in my experiments, although it did retard the development of *D. siccans* significantly (Fig. 3-IV compared to Fig. 3-III) but not *D. catenaria/dictyoides* (Fig. 3-II compared to Fig. 3-I) (Fig. 3 graphically represents the data of Table 3 and Table 4 for seed mixtures A and D). The fungicide may have eliminated the *Drechslera* spp. externally but not internally, as is supported by the absence of sporulation on seed coats of treated seed still present on plants of the first observation date.

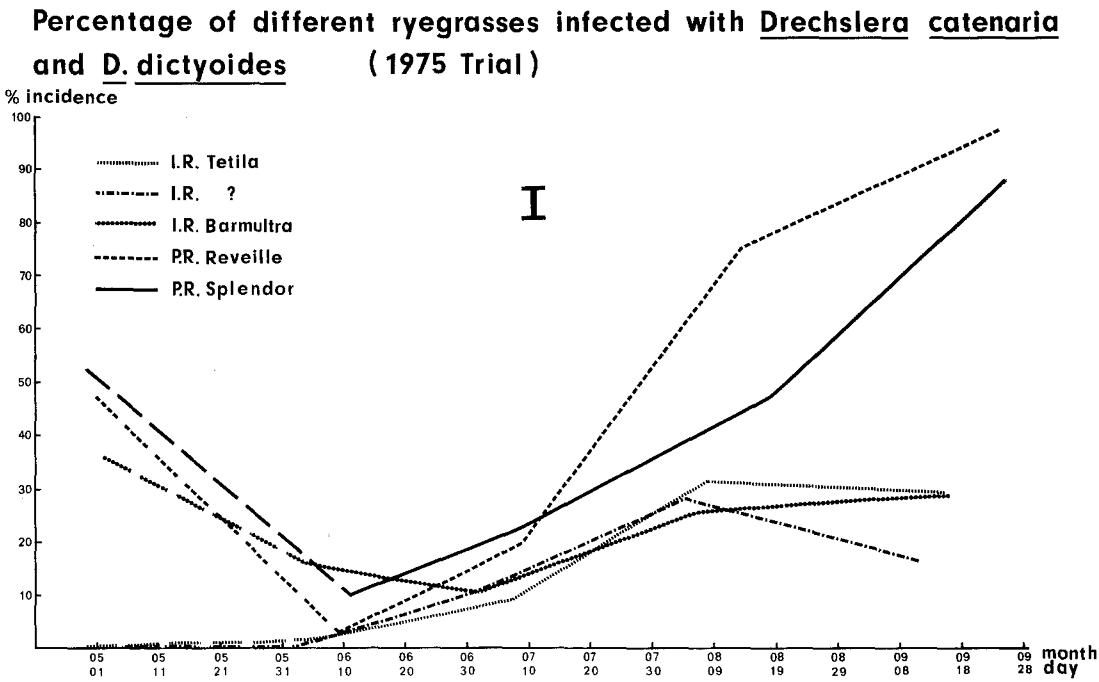
The initial level of *Lolium* seed contamination with *D. catenaria/dictyoides* has little influence on subsequent disease development (Fig. 3-I and 3-II). The three Italian ryegrass cultivars with initially 0.25, 0, and 36% contaminated seed ended up with more or less the same percentage of infected plants (30, 23, and 29%, respectively). The perennial ryegrasses with a high initial seed contamination showed disease symptoms on the green leaves at the end of the experiment but on the Italian ryegrasses the pathogen could only be found on senescent and dead leaves. Although initial seed contamination has little to do with ultimate disease levels, differences in susceptibility between cultivars do exist and can drastically curtail disease development. In plants inoculated with *D. catenaria* in the glasshouse, Wilkins (1973) found both intervarietal and intravarietal variation in leaf spot development which depended on restriction of hyphal growth following infection.

Susceptibility of the different cultivars to *D. siccans* differed in my experiment. The unknown cultivar and 'Barmultra' had almost the same initial seed contamination but produced quite different numbers of infected plants, showing that 'Barmultra' was much more resistant than the unknown cultivar (Fig. 3-III and 3-IV). No information on the susceptibility of the other cultivars was obtained because of absence or low level of contamination on seed.

The spread of *D. siccans* in the crop, even with a susceptible cultivar, was slow till August in contrast to *D. catenaria/dictyoides*. After August there was a steep rise in number of diseased plants indicating dramatic change in the infection conditions. The meteorological data shown in Fig. 4, however, show little change in August. Only the relative humidity is higher than 75% for most of the days after the first decade in August indicating many nights with dew. Wilkins (1973) found 48 h of high moisture sufficient for initial infection of *D. siccans*. In the prolonged periods of high humidity prevalent after mid August the crop was full-grown. In June and July it was still open so that occasional moist periods were less effective in establishing *D. siccans*.

Whilst *D. catenaria* requires 72 h of high moisture for infection (Wilkins, 1973). I found that *D. catenaria/dictyoides* became already established soon after emergence but predominantly on senescent and dead leaves. As 72 h of high moisture seldom

Fig. 3. Graphs of the results of Table 3 and Table 4 for original (I, III) and disinfected (II, IV) seed.
N.B. All graphs start at the time of sowing with the initial percentage of seed contamination. I.R. = Italian ryegrass, P.R. = perennial ryegrass.



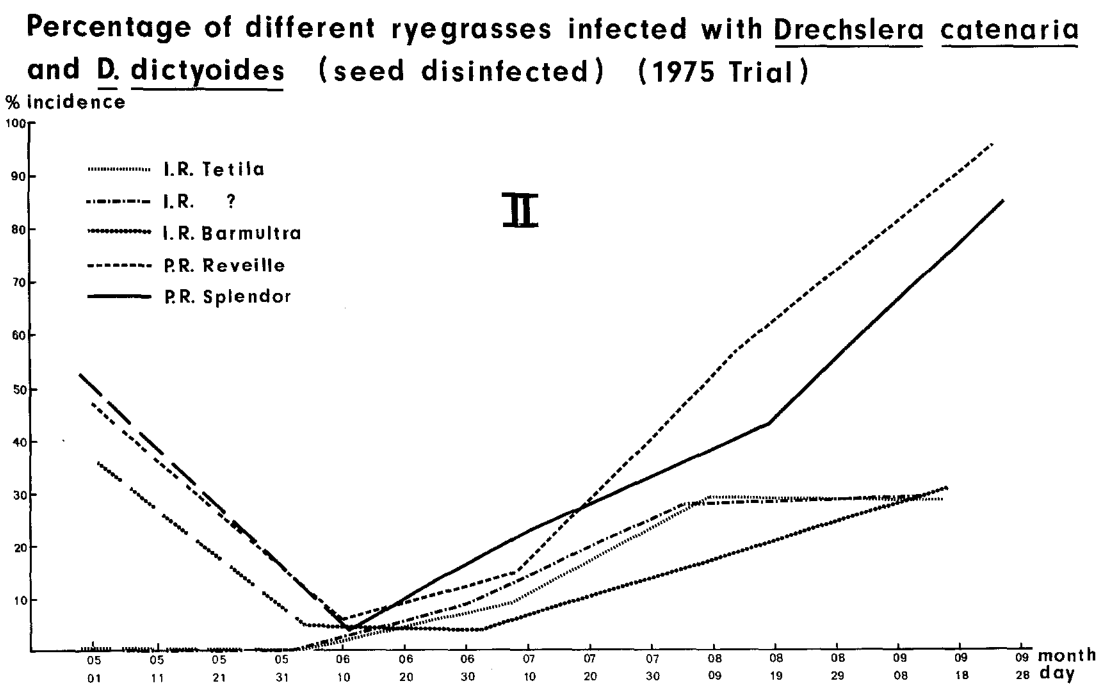
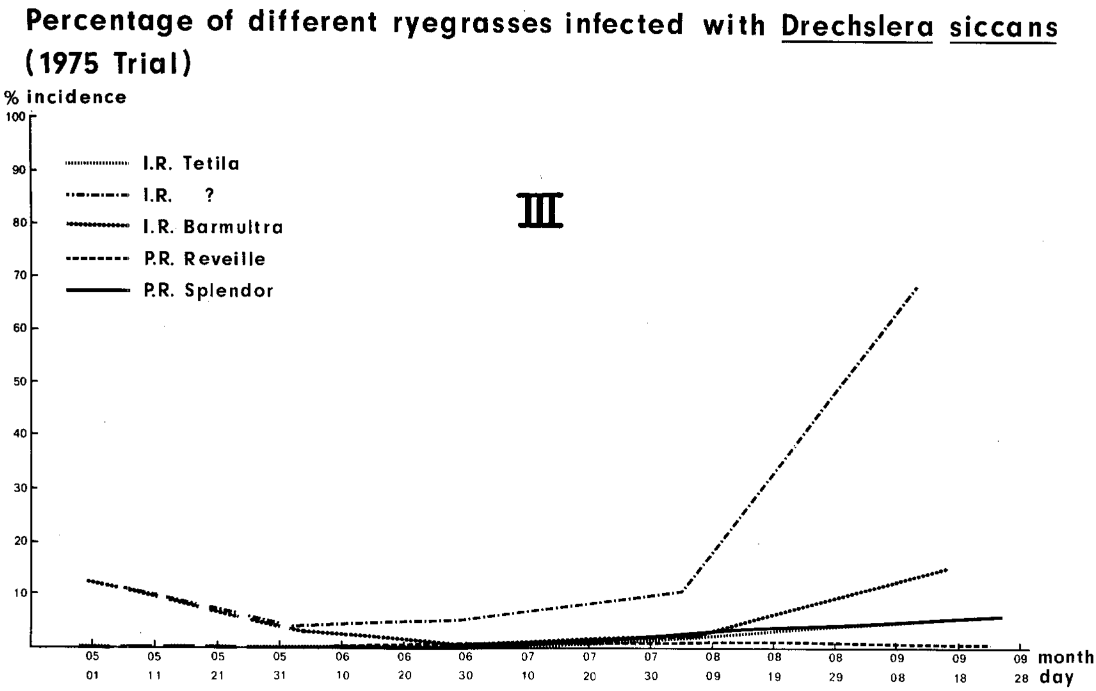


Fig. 3. Grafische weergave van de resultaten van Tabel 3 en Tabel 4 voor het oorspronkelijke (I, III) en voor het ontsmette (II, IV) zaad.
N.B. Alle grafieken beginnen met het oorspronkelijke percentage zaadbesmetting op de datum van zaaien. I.R. = Italiaans raaigras, P.R. = Engels raaigras.



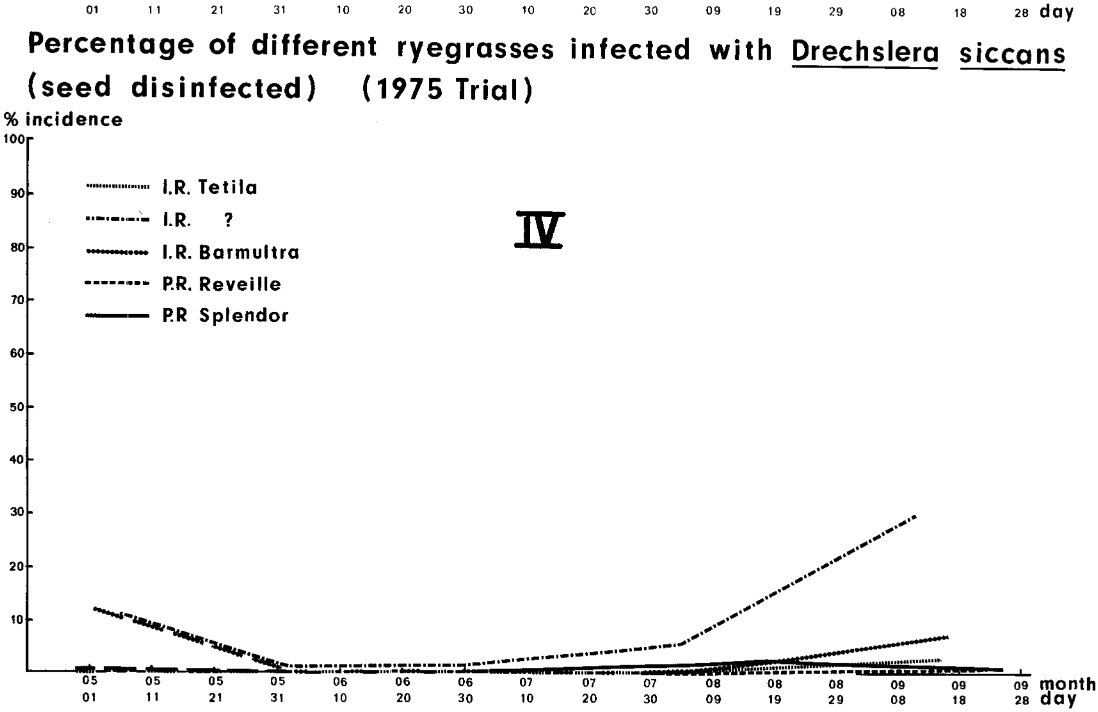


Fig. 4. Rainfall, daily hours of sunshine and relative humidity as measured at a distance of 1 km from the experiment.

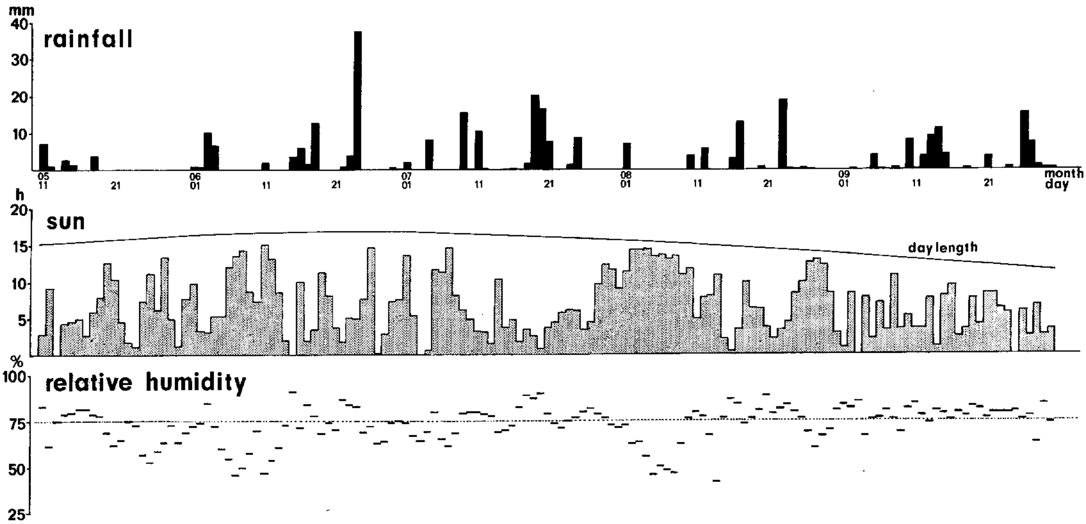


Fig. 4. Regen, dagelijkse zonneschijn en de relatieve luchtvochtigheid zoals gemeten op 1 km afstand van de veldproef.

occurred before mid August, senescent and dead leaves have a higher chance of being infected than healthy leaves which Wilkins probably used in his experiment. *D. catenaria/dictyoides* may also have a greater competitive ability than *D. siccans* over other, mostly saprophytic fungi that are found in quantity on these leaves.

Furthermore, another mechanism of conidium transport that is effective over larger distances than splashing with rain drops can be postulated. Both the unknown cultivar and 'Tetila' showed more infected plants than 'Barmultra' on the second observation date, although plots were free of infection at the first observation date. Air transport of conidia over larger distances is most likely but needs further experimental confirmation.

Yield reduction caused by *D. siccans* after inoculation in pot experiments has been reported by Cook (1975). In my experiments yield losses may have occurred but were not detected because of insignificant differences in disease incidence in the same cultivar.

Samenvatting

De besmetting van raaigraszaad met Drechslera-soorten en het effect op de aantasting van het eruit gegroeide gewas

Op raaigraszaad worden door het Rijksproefstation voor Zaadcontrole ondermeer het voorkomen van de schimmel *Drechslera siccans* en van *D. catenaria* in combinatie met *D. dictyoides* bepaald. De invloed van de mate van zaadbesmetting met deze schimmels op de aantasting en op het ziekteverloop in een daaruit groeiend gewas is nagegaan bij verschillende raaigrascultivars. Omdat van elke cultivar slechts één monster met een bepaalde besmettingsgraad voorhanden was, is door ontsmetting met imazalil getracht verschillende besmettingspercentages te verwezenlijken. Het verloop van de aantasting werd nagegaan door vaststelling van het percentage, dat sporulatie van de betreffende schimmel vertoonde. Dit gebeurde vier maal met tussenruimten van ongeveer vier weken aan de hand van geoogste planten, die vochtig werden weggezet. De resultaten daarvan zijn vastgelegd in Tabel 3 en 4 en in Fig. 3. De invloed op de opkomst is weergegeven in Tabel 2, en de opbrengst aan droge stof, zowel op zandgrond als op klei, in Tabel 5.

Opkomst- noch opbrengstverschillen waren gecorreleerd met de mate van zaadbesmetting. Wel was er verschil in de snelheid van verspreiding van de schimmels in het gewas en in de vatbaarheid van de cultivars. Vooral voor *D. catenaria/dictyoides* is de vatbaarheid van de cultivar van meer belang dan de aanvankelijke zaadbesmetting, mede omdat klaarblijkelijk aanvoer van conidiën van elders mogelijk is. Zaadontsmetting met imazalil gaf volgens de laboratoriumtoets vrijwel 100% resultaat, maar had in de veldproeven onvoldoende effect.

Acknowledgments

The author is greatly indebted to Dr J. de Tempe for advice and for providing the seed samples, to Ir J. Th. N. M. Thissen and Ir R. F. van de Veer for statistical analysis, to Mr J. Scheele for laying out and attending one of the experiments, to Mrs M. Spaargaren-v. d. Veen, Miss E. Otma and Mr G. v. d. Top for technical assistance, and to Dr D. H. Lapwood for critically reading and correcting the English text.

References

Andersen, H., 1955. Species of *Helminthosporium* on cereals and grasses in Denmark. Friesia 5: 80–89.

Andersen, H., 1959. *Helminthosporium catenarium* Drechs. på graesser i Danmark. T. Planteavl 63: 710–736.

Chidambaram, P., S. B. Mathur & P. Neergaard, 1973. Identification of seed-borne *Drechslera* species. Friesia 10: 165–207.

Cook, F. G., 1975. Production loss estimation in *Drechslera* infection of ryegrass. Ann. appl. Biol. 81: 251–256.

Latch, G. C. M., 1966. Fungous diseases of ryegrasses in New Zealand. I. Foliage diseases. N.Z. Jl agric. Res. 9: 394–409.

Mäkelä, K., 1971. Some graminicolous species of *Helminthosporium* in Finland. Karstenia 12: 5–35.

Mäkelä, K., 1972. Disease damage to the foliage of cultivated grasses in Finland. Acta Agric. Fennica 124, 1: 1–56.

Tempe, J. de, 1968. The detection of *Helminthosporium* and *Fusarium* spp. in ryegrass and meadow fescue seed samples. Proc. int. Seed Test. Ass. 33: 541–545.

Wilkins, P. W., 1973. Infection of *Lolium* and *Festuca* spp. by *Drechslera siocans* and *D. catenaria*. Euphytica 22: 106–113.

Address

Instituut voor Plantenziektenkundig Onderzoek, Binnenhaven 12, Wageningen, the Netherlands.