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Effects of induced resistance on infection efficiency and sporulation of *Puccinia striiformis* on seedlings in varietal mixtures and on field epidemics in pure stands

Agnès Calonnec, Henriette Goyeau and Claude de Vallavieille-Pope
Laboratoire de Pathologie Végétale, Institut National de la Recherche Agronomique, 78 850 Thiverval Grignon, France (Fax: (33 1) 30 81 53 06)

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Abstract

An inducer race of *Puccinia striiformis* inoculated two days before a challenger race on wheat seedlings of cv. Clement, reduced infection efficiency by 44% and lesion expansion by 7.7%; there was no effect on sporulation rate. In field epidemics, induced resistance restricted the disease intensity on cvs. Clement and Austerlitz in pure stands by 44% and 57% respectively. In the glasshouse at the seedling stage, disease intensity in a varietal mixture was reduced by 82% compared to the susceptible pure stands; one third of the total disease reduction was attributable to induced resistance.

Introduction

Varietal mixtures are efficient in controlling epidemics caused by biotrophic airborne pathogens such as barley powdery mildew (*Erysiphe graminis* DC. f. sp. *hordei* Marchal), wheat brown rust (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Eriks. & Henn.), and wheat yellow rust (*Puccinia striiformis* West). (Chin and Wolfe, 1984; Brophy and Mundt, 1991; de Vallavieille-Pope et al., 1991). The main factor influencing disease reduction is the low density of susceptible plants (Burdon and Chilvers, 1976; Wolfe, 1985). However, induced resistance could explain part of mixture efficacy in field barley varietal mixtures attacked by powdery mildew (Chin and Wolfe, 1984). Results from controlled environment studies show the effect of induced resistance increases with the quantity of avirulent spores (Littlefield, 1969). The minimum time required for the establishment of induced resistance after deposition of avirulent spores, depends on the host and the pathogen: no delay for wheat yellow rust (Johnson and Allen, 1975), 6 h for barley powdery mildew (Ouchi et al., 1976) and 2 days for oat crown rust (Kochman and Brown, 1975).

Induced resistance reduced spore germination, spore penetration, and number of pustules of crown rust and stem rust on oats after inoculation by a non-pathogenic species (*P. recondita* f. sp. *tritici* and *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks & E. Henn.) (Kochman and Brown, 1975). Spore penetration and hyphal growth of *E. graminis* f. sp. *hordei* were reduced after previous inoculation by the non-pathogen *E. graminis* f. sp. *tritici* (Ouchi et al., 1976). The number of pustules of *Melampsora lini* (Ehrenb) Desm. (Littlefield, 1969), *P. recondita* f. sp. *tritici* (van Asch et al., 1992) or number of colonies of *E. graminis* f. sp. *hordei* (Martinelli et al., 1993) were also reduced by an avirulent race of the same species. Induced resistance seems to have an effect mainly localised close to the chloroses caused by the inducer. For wheat brown rust, pustules were of a smaller size at the proximity of chloroses due to a non-pathogenic species than pustules developed some distance away, or pustules developed on non-induced plants (Johnston and Huffman, 1958). For barley powdery mildew, the number of sporulating colonies decreased when the extension of chloroses due to an avirulent race increased (Martinelli et al., 1993).

Table 1. Interactions between the four wheat cultivars and three races of *Puccinia striiformis* used in the experiments

Cultivars	Resistance genes[b]	Infection types[a] produced by races		
		45E140	106E139	232E137
Clement	Yr9, (Yr2),+[c]	1	1	7
Austerlitz	Yr6, +[c]	7	2	1–2[d]
Récital	Yr6, +[c]	7	2	2/6[e]
Talent	Yr7	3	7	4

[a] A variety is considered as resistant for infection types from 0 to 6 and susceptible for infection types from 7 to 9 on the scale of McNeal et al. (1971), at the seedling stage.
[b] *Yr9* was identified in Clement by Priestley et al. (1984) and *Yr2* was suggested by Stubbs (1985); *Yr6* was identified in Austerlitz and Récital and *Yr7* in Talent by de Vallavieille-Pope et al. (1990).
[c] + means that at least another unknown gene in present (in Clement: Johnson, 1992, in Austerlitz and Récital: de Vallavieille-Pope et al., 1990).
[d] – magnitude of the range of variation.
[e] / indicates different infection types on first and second leaves.

The aim of our study was to determine to what extent induced resistance could be a factor in controlling yellow rust epidemics in wheat varietal mixtures. Resistance was induced by avirulent spores against infection by virulent spores of the same pathogen, *P. striiformis*. Effect of induced resistance was analysed at the monocyclic level in seedlings on spore infection efficiency, sporulation rate and lesion expansion and at the polycyclic level in microepidemics in the glasshouse to compare pure stands and varietal mixtures and in field epidemics in pure stands.

Materials and methods

Wheat cultivars. Winter wheat cvs clement, Austerlitz Récital and Talent were used (Table 1).

Isolates of P. striiformis. French isolates of races 45E140 (isolate J89101), 232E137 (isolate J89108) and 106E139 (isolate J9008) from a monospore culture were used. The isolates were multiplied on seedlings of the susceptible cv. Michigan Amber inoculated with a uredospore suspension in mineral oil (Soltrol®, Philips Petroleum, Paris); these were placed at 100% RH at 8 °C for 24 h and then transferred to the glasshouse maintained at 15–20 °C. For the monocyclic experiments, the inoculum consisted of spores kept for 4 days in a desiccator after being collected. Sporulating seedlings of wheat cv. Michigan Amber were used to initiate epidemics in the glasshouse and the field.

Effect of induced resistance on infection efficiency, spore production and lesion expansion

Experimental design. Seedlings were grown in commercial compost in 8 × 8 cm pots (6 seedlings per pot) in the glasshouse from sowing to scoring; they were inoculated in a spore-settling tower (Eyal et al., 1968), first with the inducer race (In-race) when the primary leaves were fully expanded, and second with the challenger race (Ch-race), 2 days later. The leaves were attached horizontally with plasticine, adaxial side up, on a plexiglass sheet. For the inoculation, the tower was divided into five blocks (i.e. 5 replications.) One replicate (one pot) of treatment Pr (plants protected by an avirulent race before inoculation with the Ch-race) and one replicate (one pot) of treatment Ch (plants inoculated with the Ch-race only) were placed in each block. An analysis of variance (ANOVA) was performed after three independent inoculations, to verify the homogeneity of inoculum deposition inside one block. The expected number of chloroses due to the virulent race was calculated by counting the number of chloroses in each replicate and each treatment divided by the total number of chloroses for the five replicates of the same treatment. There was no variation inside one block ($P = 0.99$), allowing the comparison between Pr and Ch treatments.

Infection efficiency of the challenger race. Seedlings of cv. Clement were inoculated either with a Ch-race (treatment Ch) or with both races (treatment Pr) (Table 2). The In-race inoculation (race 45E140) was made with 350 spores cm^{-2} and the Ch-race inoculation (race 232E137) with 150 spores cm^{-2}. Seven days after the Ch-race inoculation (after this delay the coalescence of chloroses prevented further counting), infection efficiency was estimated by counting the number of chloroses due to the penetration of germ tubes. Plants of Clement had different resistance reactions with the In-race, from no symptom, to flecks, or extended chloroses. Chloroses of the Ch-race were distinguishable from those of the In-race by their round aspect and diffuse contour (de Vallavieille-Pope et al., 1995b); all leaves were assessed. The germination rate of spores was determined microscopically on slides covered with water agar (0.5%) placed in the centre of the tower and kept at 8 °C for 18 h in a Petri dish wrapped in wet paper. The percentage of germination *in vitro* was 85% for the In-race and 39% for the Ch-race.

Table 2. Statistical analyses used for the different experiments performed to measure the effects of induced resistance on infection efficiency, lesion enlargement, sporulation rate and mixture efficiency in the glasshouse and on disease intensity in pure stands in the field

Experiment	No. of leaves or tillers/treatment	No. of replicates/ treatment	Analysis	Variable	Covariate	Factors or cofactors
Infection efficiency	30 (Ch[a]) 29 (Pr[a])	5	ANOVA	No. of chloroses.cm^{-2}	–	Pr/Ch, blocks
Lesion expansion	55 (Ch) 76 (pr)	19 (Ch) 20 (Pr)	Repeated measures ANOVA[b]	Lesion surface (cm^2)[c]	–	Pr/Ch, time, blocks
Sporulation rate	55 (Ch) 76 (Pr)	19 (Ch) 20 (Pr)	ANOVA	Spores.cm^{-2} of sporulating lesion.day^{-1}	–	Pr/Ch
Mixture efficiency	197 (T1[d]) 155 (T2[d]) 154 (T3[d])	4	Analysis of covariance	Total length of sporulating lesions (cm)	Distance from the source	T1/T2/T3, replication
			Analysis of covariance	No. of diseased leaves	Distance from the source	T1/T2/T3, replication
Disease intensity in pure stands (field)	270 to 675[e]	3	ANOVA for each scoring date and each variety	Percentage of diseased area	–	Pr/Ch, plots, distance from source

[a] Treatments, Pr for seedlings protected by the inducer race before inoculation of the challenger race, Ch for seedlings inoculated with the challenger race only.
[b] Described in Madden, 1986.
[c] The lesion surface was measured at 15 and 18 days after inoculation of the challenger race; the lesion enlargement between day 15 and day 18 was assessed by the interaction treatment*time.
[d] Treatments: T1 susceptible cultivar in pure stands, T2 protected mixture, and T3 non-protected mixture.
[e] Depending on the scoring date (degrees of freedom in Table 4).

Lesion expansion and sporulation of the challenger race. The In-race was inoculated as previously described on first leaves and the Ch-race was inoculated locally in order to observe lesion expansion from one inoculation site. The leaves were covered with a plastic band with a hole of 4 mm diameter. Based on our calculations this should result in one lesion per leaf, for an infection efficiency of 5% for yellow rust (Sache and de Vallavieille-Pope, 1993). Twenty pots (120 plants) were inoculated per treatment, and only plants with a single sporulating lesion were scored (Table 2). Only 10 pots could be inoculated at the same time. Therefore, two independent inoculations of the In-race and four of the Ch-race were made. Pots were identified by their position under the settling tower (block). For the lesion surface area, there was no interaction between inoculation and block ($P = 0.85$) for the two treatments. At 15 and 18 days after inoculation of the Ch-race, the lesion surface area was assessed by measuring the lesion length and width. Spore production during this period was measured as follows: at the 15th day, spores were collected and discarded, and pots were laid on glass Petri dishes. On the 18th day, spores were collected with a miniaturized cyclone collector (Mehta and Zadoks, 1970). All spores from one pot were collected together. Spores, dissociated in a drop of Tween 20® (polyoxyethylen sorbitan monolaurate, Merck-Schuchardt, Hohenbrunn, Germany) and then in 20 ml of a buffered electrolyte solution (Isoton® II, Coultronics France S.A., Margency), were counted using a Coulter® Counter (McGregor and Manners, 1985).

Effect of induced resistance on varietal mixture in microepidemics

Microepidemics were generated on seedlings planted in pure stands or mixtures, in 35 × 52 cm trays, with a density of 330 seedlings per tray (Lannou et al., 1994b). Seedlings were grown in the glasshouse from sowing to scoring (40 days) under an alternating 16 h light, 8 h dark regime, daylight being complemented by high pressure Na-lamps at 6 to 27 °C. For inoculation, trays were placed in a metal-framed cabinet covered with PVC plastic. A uniform air flow (constant velocity 1.6 m.s^{-1}) was passed over the seedling foliage from the cabinet opening. Microepidemics were generated through two infection cycles. The first infection cycle was initiated with seedlings of cv. Michigan Amber, with primary leaves entirely covered with sporulating lesions, placed in the air flow in front of each tray (six pots of 10 seedlings per tray). Spores were dispersed during 2 h from source plants onto one-leaf

stage seedlings in the trays. Fifteen days later, sporulating lesions from the first infection cycle appeared and trays were placed again the metal-framed cabinet for a second dispersal event generated from the sporulating seedlings inside the trays.

Three treatments with four replicates were used: (T1) pure stand of Clement, (T2) mixture of Clement (25%) and Austerlitz (75%), (T3) mixture of Clement (25%) and Talent (75%). In each treatment, Clement was the susceptible cultivar on which disease was measured, and was recognizable by the colour of its seeds stained with a film coating of Seripet®, (Seppi, France). In treatment T2, the In-race was multiplied on cv. Austerlitz. In treatment T3, Talent was fully resistant to all races used. Treatment T2 was firstly inoculated with the In-race 45E140. Two days later, treatments T1, T2, T3 were inoculated with the Ch-race 232E137. At the end of the In-race latent period, a second In-race dispersal event was carried out on treatment T2 to disseminate In-race spores, and two days later a second Ch-race dispersal event was performed on each treatment to disseminate the Ch-race inoculum. For the first Ch-race dispersal event, trays of the different treatments were randomised in each of the three cabinets, and for the second Ch-race dispersal event, trays of each treatment were grouped in the same cabinet.

Overlap of some of the sporulating lesions made counting of individual lesions impossible. A global measure of severity was generated by measuring the total length of sporulating lesions from the second infection cycle (leaves 3 to 6 of the main tiller) on seedlings of the susceptible cultivar. To separate the effect of induced resistance on infection efficiency from its effect on lesion expansion, the number of diseased leaves per plant was also counted. This second measure was based on the hypothesis that the number of infected leaves should be correlated with the infection efficiency. Susceptible plants were sampled five times every 10 cm along the trays starting at 5 cm distance. A sample of about 50% of the Clement plants were observed for treatments T2 and T3 (40 plants per tray) and 15% for treatment T1 (50 plants per tray). The mixture efficacy (ME) was calculated as the relative difference between the average length of lesions (lol) on the susceptible component in the mixture and in the pure stand:

$$ME = \frac{lol(\text{pure stand}) - lol(\text{mixture})}{lol(\text{pure stand})}$$

The difference between MEs in T2 and T3 estimated the induced resistance effect in the varietal mixture.

Induced resistance during epidemics in pure stands in the field

The field plots (1 × 5 m) were arranged in a randomised design with three blocks, with 5 to 20 m between the plots planted with rye. Two cultivars were tested for the effect of induced resistance. Each plot consisted of a pure stand of a cultivar A (7 rows) edged on both long sides with a cultivar B (2 × 4 rows). The disease was measured on the cultivar A whereas the cultivar B multiplied the In-race protecting A from the Ch-race during the whole epidemic. Two cultivars were tested, Clement and Récital. Clement plots were edged by Récital and were inoculated by the In-race 45E140 on Récital and the Ch-race 232E137 on Clement. Récital plots were edged by Talent and were inoculated by the In-race 106E139 on Talent and the Ch-race 45E140 on Récital. For the protected treatment (Pr), the In-race was inoculated by planting 10 sporulating seedlings on the first inner row of each of the borders of the B cultivar. One and a half infection cycles later, the Ch-race was inoculated by planting two sporulating seedlings at one end of each plot in order to generate a disease gradient. The duration of infection cycles was determined by the equation of latent period function of the average temperature (Zadoks, 1971; Rapilly, 1976). Ch-treatments were inoculated with the Ch-race only. The percentage of diseased surface area was assessed every 2 weeks after the first lesions had appeared. A visual scale of 0 to 10 (Zadoks, 1961) was used to score 3 samples of 15 consecutive tillers every meter from 0 to 4 m from the source plants.

Statistical analyses

Statistical analyses performed for the different experiments are summarised in Table 2. In addition, a Tukey's studentised range test ($a = 0.05$) was used to compare treatments for mixture efficacy experiments. In the pure stand experiment in the field, distance from the source was analysed as a factor instead of the covariate because the disease percentage could not be expressed as a linear function of distance to the source, except for cv. Clement at the last scoring date where Log (disease % + 1) = f(Log(dist + 1)) with f standing for a linear function.

Results

Effect of induced resistance on primary stages of the infection cycle

Infection efficiency. A total of 1,070 chloroses on a total leaf surface of 101.1 cm^2 was counted for treatment Ch, and a total of 415 chloroses on a total surface of 74.45 cm^2 for treatment Pr. The number of chloroses.cm^{-2} of Ch-race was significantly lower for Pr than for Ch treatments (Table 3), with a decrease of about 44% of infection efficiency attributable to induced resistance.

Lesion expansion. At day 15, there was no effect of induced resistance on lesion surface; at day 18 the lesion surface area was significantly smaller for Pr than Ch plants. The lesion expansion differed between both treatments, as shown by a significant treatment*time interaction (Table 3). The lesion surface area increased on average between both dates from 1.11 to 1.8 cm^2 for Pr plants and from 1.10 to 1.95 cm^2 for Ch plants, the lesion of Pr plants being shorter by about 3 mm for a mean width of 4.7 mm.

Sporulation rate. Sporulation rates of Pr and Ch plants did not differ (Table 3).

Effect of induced resistance on varietal mixture in microepidemics

The disease gradient with the distance to the source of inoculum did not differ between cv. Clement in pure stand, and in the protected and the non-protected mixtures (Figure 1). Disease severity was significantly different between the three treatments. The mean lesion lengths for cv. Clement were 5.18 cm in the pure stand, 2.33 cm in the non-protected mixture and 0.93 cm in the protected mixture. The disease reduction relative to the pure stands was 55% for the non-protected mixture and 82% for the protected mixture. Therefore, 27% of the mixture efficacy could be attributed to induced resistance. The number of diseased leaves differed between the three treatments ($P = 0.0001$). The mean number of Clement diseased leaves was 1.09 for the pure stand, 0.83 for the non-protected mixture, and 0.64 for the protected mixture.

Effect of induced resistance on pure stands in the field

Disease severity. For Clement, the disease severity was higher in Ch than in Pr plots, starting at the end of the third infection cycle (i.e. scoring date 2) until the end of the epidemic (i.e. scoring date 4) (Table 4). At the end of the epidemic, the mean severity was 19.2% for Ch plots and 10.8% for Pr plots, i.e. a disease reduction of 43.7%. For Récital, the difference between Pr and Ch plots was significant from scoring date 3 to the end of the epidemic. At scoring date 4 the mean severity was 48.2% and 20.7% for Ch and Pr plots, respectively, i.e. a disease reduction of 57%.

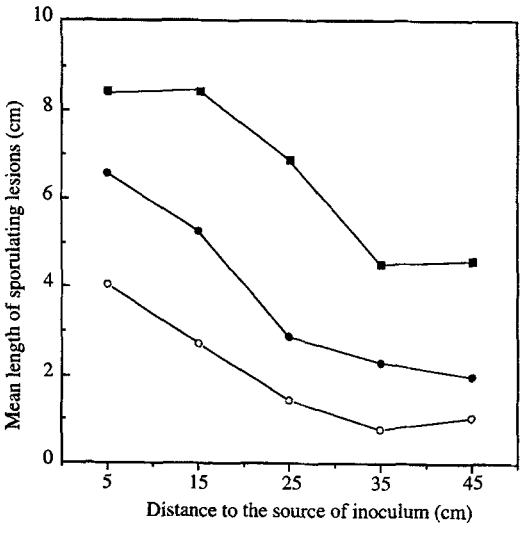

Figure 1. Mean length of sporulating lesions of *Puccinia striiformis* per wheat seedling, with the distance to the source of inoculum, in Clement in pure stand (■), Clement in non protected mixtures (25% Clement: 75% Talent) (●), and Clement in protected mixtures (25% Clement : 75% Austerlitz) (○), in a glasshouse after 2 infection cycles. (Distance effect, $P = 0.0001$; treatment*distance interaction, $P = 0.7$, $F2,491 = 0.36$; treatment effect $P = 0.0001$, $F2,491 = 64.22$).

Disease gradient. For Clement, a gradient in disease severity was shown at the end of the epidemic (scoring date 4) with an interaction between Pr and Ch treatments and the covariate distance to the inoculum source. The disease gradient was steeper for Pr plots than for Ch plots with slopes of −2.10 and −1.78, respectively. The epidemic progressed more slowly in protected than in non-protected plots (Figure 2). For Récital, heterogeneity among replications and a high level of disease on all the plots at the end of the epidemic prevented detection of a disease gradient.

Discussion

For the first time, the effect of induced resistance in restricting yellow rust epidemic development was quantified; the magnitude of the disease reduction was about 50%. Induced resistance slowed down the focus extension which was detectable when the disease level

Table 3. Effect of induced resistance on infection efficiency, lesion growth, and sporulation rate of wheat seedlings of cultivar Clement inoculated with uredospores of *Puccinia striiformis*

Infection efficiency			Lesion expansion[a]		Sporulation rate		
Chloroses.cm^{-2}			Lesion surface	Lesion enlargement	Spores.cm^{-2}.day^{-1}		
Pr[b]	Ch[b]	Pr/Ch	Pr/Ch	(Pr, Ch)*time[c]	Pr	Ch	Pr/Ch
6.02	10.7	$F^{d}_{1,58} = 25.4$ $P^{e} = 0.0001$	$F_{1,121} = 5.98$ $P = 0.0159$	$F_{1,121} = 16.48$ $P = 0.0001$	13,964	14,022	$F_{1,8} = 0.72$ $P = 0.42$

[a] Lesion growth measured 18 days after inoculation with the challenger race.
[b] Pr: Clement protected by the inducer race 45E140 against the challenger race 232E137; Ch: Clement inoculated with the challenger race only.
[c] Interaction treatment*time.
[d] Fi,j represents the F of Fischer with i degrees of freedom for the factor and j degrees of freedom for the error.
[e] Probability of a more extreme variance ratio occurring by chance (F-test)

Table 4. Analyses of variance on the percentage of disease severity in field plots in pure stands of wheat cultivars protected or not by an avirulent race against *Puccinia striiformis*

		Clement				Récital			
Scoring date	Source of variation[a]	Degrees of freedom	Mean square	F value	P[b]	Degrees of freedom	Mean square	F value	P
1[c]	Error	28	0.0016			27	0.0014		
	Treatment	1	0.0008	0.51	0.4816	1	<0.0001	0.06	0.8100
	Plots	2	0.0002	0.12	0.8909	2	0.0009	1.00	0.3824
	Distance	1[d]	0.1822	113.76	0.0001	1[d]	0.0246	17.56	0.0003
	Treatment*plots	2	0.0008	0.48	0.6266	2	0.0016	1.12	0.3412
2	Error	80	0.0012			80	0.0019		
	Treatment	1	0.0114	9.53	0.0028	1	0.0065	3.47	0.0662
	Plots	2	0.0109	9.11	0.0003	2	0.0107	5.67	0.0050
	Distance	4	0.1510	125.71	0.0001	4	0.1087	57.42	0.0001
	Treatment*plots	2	0.0166	13.81	0.0001	2	0.0063	3.32	0.0411
3	Error	63	0.0009			63	0.0029		
	Treatment	1	0.0745	77.45	0.0001	1	0.3117	104.60	0.0001
	Plots	2	0.0077	8.01	0.0008	2	0.0408	13.71	0.0001
	Distance	3[e]	0.0330	34.35	0.0001	3[e]	0.0067	2.26	0.0900
	Treatment*plots	2	0.0083	8.66	0.0005	2	0.0653	21.92	0.0001
4	Error	80	0.0051			79	0.0262		
	Treatment	1	0.4745	91.88	0.0001	1	2.2991	87.66	0.0001
	Plots	2	0.0345	6.70	0.0020	2	0.2556	9.75	0.0002
	Distance	4	1.3713	265.50	0.0001	4	0.2784	10.62	0.0001
	Treatment*plots	2	0.0727	14.07	0.0001	2	0.02867	10.93	0.0001

[a] Factors as follows: treatment: variety protected by a inducer race (Pr) or non protected (Ch); plots: three plots per treatment; distance: from 0 to 4 m from the source of inoculum; interaction between treatments and plots.
[b] Probability of a more extreme variance ratio occurring by chance (F-test).
[c] Date 1 corresponded to the end of the second infection cycle of the virulent race, three weeks after its inoculation. The avirulent race was inoculated 1.5 infection cycles before the virulent race. The dates 2, 3 and 4 corresponded to 8, 10 and 11 weeks after inoculation respectively.
[d] Disease present only at the distances 0 and 1 m.
[e] Disease severity at the distance 0 m not measured.

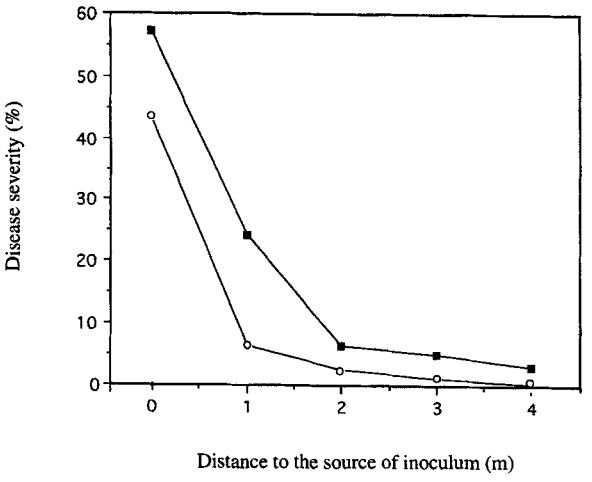

Figure 2. Mean disease severity with the distance from the source of inoculum, 11 weeks after inoculation (scoring date 4) of *Puccinia striiformis*, on non-protected (■) and protected (○) plots of pure stands of wheat cultivar Clement in the field. (Treatment*covariate distance interaction, $P = 0.0222$.)

was not too high in relation to the plot size, as it was in the case for Clement. In microepidemics conducted once at the seedling stage, about one third of the mixture effect was attributable to induced resistance. This level of efficiency obtained after only two infection cycles is similar to the maximum level of protection observed in field experiments (70%) after 5 to 8 infection cycles without induced resistance (de Vallavieille-Pope et al., 1988). Glasshouse conditions would represent optimum environmental conditions for development of both races of the pathogen and the expression of induced resistance. Glasshouse results corroborate those observed for 2-year field experiments on a mixture of cultivars Clement/Arcane or Slejpner/Arcane. In protected plots, the disease reduction was 63% and 83% respectively from which about one third was attributable to induced resistance (de Vallavieille-Pope et al., 1995a); these results are similar in magnitude to the 24% restriction of barley powdery mildew measured on a protected varietal mixture (Chin and Wolfe, 1984). Induced resistance was also suggested as a mechanism for disease reduction in a wheat mixture against yellow rust (Finckh and Mundt, 1992).

On isolated seedlings, induced resistance restricted the infection efficiency by about 44%, and was responsible for delaying lesion expansion. By comparison, a reduction in the number of pustules of *M. lini* on flax could reach 55 to 71% (Littlefield, 1969), and a reduction of colony number of *E. graminis* f. sp. *hordei* 71% to 75% on different lines of barley (Martinelli et al., 1993).

In the present study, no direct effect was shown on sporulation rate per sporulating lesion on seedlings, which could mean either that the sporulation was not affected, or that it was too variable to identify slight effects. *P. striiformis* sporulation rates on the same cultivar can vary from 20,000 to 31,000 spores.cm^{-2}.day^{-1} (Young, 1978). Johnson and Allen (1975) found an effect of induced resistance by weighing spores accumulated in the tube in which the leaves were inserted, but this technique was not adapted to analyse a large number of plants. Martinelli et al. (1993) showed a positive correlation between the effect of induced resistance on infection efficiency and on sporulation rate for *E. graminis* f. sp. *hordei*. Lesion growth of yellow rust could explain the absence of an effect on sporulation rate in spite of an effect on infection efficiency. However, reduced infection efficiency and lesion growth should decrease the spore production over time under field conditions, these effects being cumulative over infection cycles.

In the microepidemics, the reduction in the severity of disease in the susceptible cultivar in the protected mixture compared to the non-protected mixture could be explained by a decrease in the infection efficiency and/or lesion size. The significant decrease in the number of diseased leaves between the protected and the non-protected mixtures again suggests a decrease in the quantity of efficient spores in protected treatments agreeing with the observations on isolated seedlings.

Our results must, however, be treated with caution because the reduced infection efficiency was not constant on isolated seedlings in the glasshouse. In one case an increase in the infection efficiency was observed on isolated seedlings with identical races/cultivar; this possible variation means that under uncontrolled conditions (temperature, light, physiological stage of the seedling) the development of the Ch-race could be stimulated by a previous inoculation of an avirulent race. Induced susceptibility through increased conidial production of a virulent race by prior inoculation with an avirulent race was described for *E. graminis* f. sp. *hordei* on barley cultivars (Chin et al., 1984) and could be due to the alteration by the avirulent race of the metabolites available for the virulent race by a 'sink' effect; this implies that the avirulent race is delayed compared to the induced resistance effect. It is also possible that the expression of induced resistance is dependent on the In-race of the pathogen (van Asch et al., 1992) or the non-pathogen (Kochman and

Brown, 1975; Ouchi et al., 1976), or on the interaction between both races and the ability of the host to react. The rapidity and degree of expression of the resistance gene may also be a limiting factor for inducing resistance.

It could also be hypothesised that induced resistance should require a plant to be near to stress when its defense mechanisms would be partially active. Frequent changes in light intensity and temperature such as in the field or glasshouse could be necessary to obtain an induced protection for barley, wheat and beans against *E. graminis* f. sp. *hordei, P. hordei, E. graminis* f. sp. *tritici*, and *Uromyces appendiculatus* (Pers.) Unger (Falkhof et al., 1988). Many experiments of successful induced resistance have been carried out in growth cabinets using detached leaves (Littlefield, 1969; Chaudhary et al., 1983; McRae and Brown, 1983) or with seedlings inoculated with a mineral oil (Cheung and Barber, 1972; van Asch et al., 1992). Such conditions involving damaged leaves and inoculation with an abiotic agent such as mineral oil may also enhance the ability of plants to react to pathogens. The inconsistency of the results reported in the literature and in the present study could be due to varying conditions favourable or not favourable host for a partial activation of host defense mechanisms.

The observations of several authors suggest that induced resistance is effective provided that the reaction to the avirulent race is not limited to the infection point. Lannou et al. (1995) indicated by computerised simulated epidemics that induced resistance has an effect in varietal mixtures even if the protection is localised, provided that the protected area is not limited to the infection site of avirulent spores; this would be particularly important for pathogens which cause lesions of a limited size, such as *P. recondita* f. sp. *tritici*.

Conclusion

Induced resistance slowed down a polycyclic epidemic of wheat yellow rust in the field in pure stands and in the glasshouse in varietal mixtures. Induced resistance was due to reduced infection efficiency and slower lesion growth. The effect of induced resistance on lesion expansion is important because firstly, sporulating lesions of *P. striiformis* grow throughout the infectious period and thus increase the diseased leaf area without any new infections, so effecting the rate of spread of the epidemic (Emge et al., 1975) and secondly, in computerised epidemics, lesion growth has a negative effect on mixture efficacy (Lannou et al., 1994a). It would therefore be useful to quantify induced resistance components on adult plants in the context of using varietal mixtures to restrain epidemics of yellow rust.

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