

Resistance Induced in Wheat by an Avirulent Race of *Puccinia recondita* f. sp. *tritici*

M. A. J. VAN ASCH, F. H. J. RIJKENBERG, and T. A. COUTINHO, Department of Microbiology and Plant Pathology, University of Natal, P.O. Box 375, Pietermaritzburg, 3200, Republic of South Africa

ABSTRACT

Van Asch, M. A. J., Rijkenberg, F. H. J., and Coutinho, T. A. 1992. Resistance induced in wheat by an avirulent race of *Puccinia recondita* f. sp. *tritici*. Plant Dis. 76:412-415.

Seventeen-day-old seedlings of the spring wheat cultivar SST25 were inoculated with an avirulent race of *Puccinia recondita* f. sp. *tritici*. After various time intervals, the plants were reinoculated with a virulent race. No change in latent period or infection type was observed. However, the infection frequency was reduced by approximately 60%.

The present research was conducted to determine the level of resistance to a virulent wheat leaf rust race, *P. r. tritici* race 3SA86, induced when wheat plants had been previously infected by an avirulent race (3SA126) of the same pathogen. Upon infection with the virulent race, the parameters used to assess possible induced resistance were latent period (LP) and infection frequency (IF), whereas the infection type (IT) was recorded to determine whether there was an effect on uredosorus size. Induced resistance was to be characterized by an increase in LP, a decrease in IF, and a lower IT when challenged plants were subsequently infected with a virulent race.

MATERIALS AND METHODS

The spring wheat cultivars Morocco and SST25, obtained from the Small Grain Centre, Bethlehem, South Africa, were selected for their specific reaction to the races used (3SA86 and 3SA126). Morocco is susceptible to both races (reaction type 4), and SST25 gives a hypersensitive reaction (reaction type 0; 1⁻) with race 3SA126 and a reaction type 4 infection with race 3SA86. This differ-

Since the work of Yarwood (13), in which induced resistance by rust fungi was reported for the first time, it has become widely accepted that all pathogens, including rusts, are capable of inducing resistance. But, although the cereal-rust interaction is one of the most studied fields in plant pathology, relatively little work has been done on the induction of resistance by cereal rusts. Kochman and Brown (6) used *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* Eriks. & Henn. (cause of wheat leaf rust) and *P. graminis* Pers.:Pers. f. sp. *tritici* Eriks. & Henn.

(cause of wheat stem rust), both non-pathogens, to induce resistance in oats (*Avena sativa* L.) to the oat rust pathogens, *P. coronata* Corda f. sp. *avenae* W. P. Fraser & Ledingham and *P. g.* f. sp. *avenae* Eriks. & Henn. Results obtained by Johnson and Allen (4) showed that resistance induced in wheat (*Triticum aestivum* L.) by application of an avirulent race of *P. striiformis* Westend. can delay and reduce sporulation resulting from infection with a virulent race of the same rust. McRae and Brown (9) found that resistance in wheat leaf segments to leaf and stem rust could be induced by previous inoculation with avirulent races of these fungi. A later study by Bahamish and Wood (2) dealt with the induction of susceptibility to an avirulent race of *P. r. tritici* by a virulent race of the same rust in wheat.

Accepted for publication 17 July 1991.

© 1992 The American Phytopathological Society

ence in resistance reaction is controlled by the *Lr24* gene. The races were increased on Morocco plants under controlled greenhouse conditions.

Plants were grown in trays (26.5 × 18.5 × 6.5 cm), and each tray contained at least five Morocco and 15 SST25 plants. One replication consisted of eight trays with plants consecutively inoculated with races 3SA126 and 3SA86, eight trays with plants inoculated with 3SA86 only, and, as a control for infection with the first race, four trays with plants inoculated with 3SA126 only. Because the SST25 plants do not show any symptoms after inoculation with 3SA126, infection was recorded on the Morocco plants.

Secondary leaves of 17-day-old seedlings (growth stage 13 on the scale of Zadoks et al [13]) were inoculated on the upper leaf surface with an Andres and Wilcoxson (1) inoculator modified (C. A. Crookes, unpublished) to permit more accurate regulation and replication. Leaves to be inoculated were affixed to a screen set at 20 cm from the inoculator orifice, and the horizontal speed was set at 9.0 (15 cm/sec) and spray volume at 5.0 (0.1 ml/sec). Approximately 2.5 mg of rust spores was suspended in 0.8 ml of Soltrol 170 oil and applied to a single tray, which caused about 360 uredospores to be deposited per square centimeter of leaf.

After inoculation, the plants were incubated for 12 hr in a mist chamber at 20 C where 100% relative humidity and darkness favored germination and penetration. After infection, the plants were transferred to a greenhouse where the temperature ranged from 12 to 15 C during the night and from 20 to 24 C during the day.

For each experiment, the viability of the spores applied was checked by spraying spores onto four water agar (2%) plates, of which two were incubated in a 20-C incubator, while the remaining two were placed in the mist chamber with the inoculated trays. The percentage of germinated spores on the plates was microscopically determined by observing approximately 200 spores.

The time intervals between inoculation with the avirulent race (3SA126) and the virulent race (3SA86) were 1, 4, 7, and 10 days. The 1- and 7-day intervals were repeated, while the other tests were done once. All results obtained were analyzed using ANOVA followed by LSD ($P < 0.05$).

The LP was determined by counting daily the number of uredosori visible in a marked area on each second leaf (using a ×10 pocket lens) until the number of primary uredosori no longer increased. The number of leaves counted was 15 for SST25 and five for Morocco, however, results of leaves with 10 or less pustules in the marked areas were discarded to obtain a more reliable set of data. The time at which 50% of the

terminal number of uredosori had appeared was estimated by interpolation. The LP was taken as the time period from the beginning of incubation in the mist chamber to the time at which 50% of the uredosori had appeared.

The number of uredosori on the leaf surface was measured using an aluminum sheet with a 2 × 0.5 cm window (10). The metal sheet was randomly placed on the leaf over the inoculated area. For the single inoculation, four leaves per tray were used, whereas for the double inoculation, six leaves were counted. The number of the uredosori within the window was divided by the number of rust spores applied per square centimeter, corrected with a factor for germination percent (as determined from water agar plates in the mist chamber), to give the IF. ITs (11) were recorded 10 days postinoculation.

To establish whether adult plants react similarly to seedlings, three plants were grown in pots (12 cm diameter) to the flowering stage (stage 49–51 on the Zadoks scale). Eight pots with Morocco plants and 16 with plants of SST25 were used for a single experiment. The plants were inoculated on the adaxial surface of the flag leaf, employing a 4-day interval between inoculations. Procedures similar to those for seedlings were used, and induced resistance criteria were assessed similarly.

RESULTS

Latent period. The LP of 50% for infection with 3SA86 was 210.6 ± 4.1 hr on SST25. No statistical differences ($P < 0.05$) between the LPs at different time intervals were found for this single inoculation (Table 1). The average latent period of 3SA86 on leaves that had been inoculated previously with 3SA126 was 212.4 ± 6.9 hr (Table 1). Statistical, but very small, differences were found between the 1-day interval and both the 4- and 7-day intervals of the double (both

3SA126 and 3SA86) inoculation (Table 1). The difference between the single and the double inoculation was found to be 1.7 ± 3.1 hr on average. However, the differences between the single and double inoculation were significantly different ($P < 0.05$) at four treatments: once with a 1-day interval between inoculations, at both 4-day intervals, and once at a 7-day interval (Table 1).

When the results are expressed as a percentage of the LP for 3SA86 only (Table 1), the low standard deviation indicates that no major differences existed among the time intervals.

Infection frequency. The average IF of inoculation with 3SA86 only was 21.7 ± 13.2 pustules per square centimeter, for inoculation with 3SA126 was only 20.5 ± 10.8 pustules per square centimeter, and for double inoculation (3SA86 after inoculation with 3SA126) was 11.7 ± 6.1 pustules per square centimeter (Table 2). No statistical differences ($P < 0.05$) of the IF between different time intervals were found for both inoculation with 3SA86 only and the double inoculation. The IF of the inoculation with 3SA126 only showed significant differences ($P < 0.05$) between the 1- and the 4-day intervals (Table 2).

The significant differences ($P < 0.05$) in the IF of the single inoculation (Table 2) were not reflected in the IF of the double inoculation (Table 2). The correlation coefficient between the IFs of these inoculations was not significant ($r = -0.38$).

Compared with the inoculation with 3SA86 only, a decrease of the IF was found for the double inoculation at all time intervals, except for one experiment with a 1-day interval, where the IF increased (Table 2). All differences between inoculation with 3SA86 only and the double inoculation were statistically significant ($P < 0.05$) at each time interval.

The data of the average IF values,

Table 1. Average latent period (LP) values (in hours postinfection) at various time intervals between inoculation with an avirulent race (3SA126) and a virulent race (3SA86) of *Puccinia recondita* f. sp. *tritici* on leaves of spring wheat cultivar SST25

Time interval ^w (days)	Leaf	Inoculation with				Difference (hr)	LP values ^x
		3SA126 and 3SA86	No. of samples	3SA86	No. of samples		
1	Second	202.4 a ^y	28	203.1 a	21	-0.7	99.7
1	Second	205.0 a	87	208.4 a	118	-3.4** ^z	98.4
4	Second	218.7 b	86	214.6 a	85	4.1**	101.9
4	Flag	215.4 b	20	212.0 a	34	3.4**	101.6
7	Second	213.3 b	41	211.8 a	91	1.5	100.7
7	Second	221.2 b	49	215.2 a	112	6.0**	102.8
10	Second	210.7 ab	42	209.4 a	62	1.3	100.6
Average		212.4		210.6		1.7	100.8
SD		6.9		4.1		3.1	1.5

^wInterval between consecutive inoculations.

^xValues are percentage of the inoculation with 3SA86.

^yFigures in a column followed by the same letter do not differ significantly ($P < 0.05$) in a LSD test.

^z** = Significant ($P < 0.05$) differences.

Table 2. Average infection frequency (IF) values (in pustules per square centimeter) at various time intervals between inoculation with an avirulent race (3SA126) and a virulent race (3SA86) of *Puccinia recondita* f. sp. *tritici* on leaves of spring wheat cultivars SST25 and Morocco

Time interval [†] (days)	Leaf	Inoculation with						Difference [‡] (hr)	LP values [§]
		3SA126 on Morocco	No. of samples	3SA86 on SST25	No. of samples	3SA126 and 3SA86 on SST25	No. of samples		
1	Second	0.5 a [†]	16	2.3	48	2.5	48	0.2** [‡]	108.7
1	Second	14.0 a	16	17.9	48	15.6	48	-2.3** [‡]	87.2
4	Second	33.9 b	16	26.6	48	16.5	48	-10.1** [‡]	62.0
4	Flag	38.2 b	16	15.7	48	9.5	48	-6.2** [‡]	60.5
7	Second	19.1 ab	8	22.5	48	8.8	48	-13.7** [‡]	39.1
7	Second	15.9 ab	12	37.6	48	20.7	48	-16.9** [‡]	55.1
10	Second	30.6 ab	8	21.2	36	8.6	48	-12.6** [‡]	40.6
Average		21.7		20.5		11.7		-8.8	64.7
SD		13.2		10.8		6.1		6.3	25.2

[†] Interval between consecutive inoculations.

[‡] Difference between inoculation with 3SA126 and 3SA86 and 3SA86 only on SST25.

[§] Values are percentage of the inoculation with 3SA86.

[¶] Figures in a column followed by the same letter do not differ significantly ($P < 0.05$) in a LSD test.

^{**} = Significant ($P < 0.05$) differences.

expressed as a percentage of the IF for 3SA86 only, indicate that the IF decreases as the time interval between inoculations increases (Table 2); however, this was not supported statistically.

Infection type. No differences in the ITs of the different treatments were observed (reaction type 4), nor was any zone of fungal inhibition noticed. Occasionally, pustules were found on the edge of necrotic flecks caused by the inoculation with the avirulent race 3SA126.

DISCUSSION

Latent period. No major increase in LP could be demonstrated after a virulent race of *P. r. tritici* was inoculated at various time periods after prior inoculation with an avirulent race. Although significant increases in LP were found, they were very small and may, for all intents, be of little biological significance.

Littlefield (8) reported that preinoculation of flax, *Linum usitatissimum* L., with an avirulent race of *Melampsora lini* (Ehrenb.) Desmaz. caused a reduction in the number, size, and rate of development of virulent races of the fungus, and Johnson and Allen (4) found that the onset of sporulation was delayed by 7 days on seedlings that had been inoculated with one avirulent race of *P. striiformis* 6 days before inoculation with a virulent race. However, our data are in general agreement with those reported by both Cheung and Barber (3), who used an avirulent race of *P. g. tritici* before inoculation with a virulent race on wheat leaf pieces, and those of Kochman and Brown (6), who used wheat rust, both *P. g. tritici* and *P. r. tritici*, as preinoculation before both *P. c. avenae* and *P. g. avenae* on oats. These authors did not find any difference in the size or the rate of pustule development, but they did find a reduction in the number of pustules per square centimeter of leaf area.

Infection frequency. In the present study, a decrease in the IF was found

at almost every time interval between the consecutive inoculations when compared with an inoculation with the avirulent race only. With both a 7- and a 10-day interval between the inoculations, the IF was reduced by approximately 60%. In one of the tests with a 1-day interval between the inoculations, a slight increase in IF was found, but this might have been an effect of the low spore density in this particular experiment.

Cheung and Barber (3) found a reduction of 80% in the number of pustules per square centimeter leaf area using two different races (inoculation with the avirulent before the virulent) of *P. g. tritici* on wheat. In their research, the time between the inoculations was 3 or 6 days. Such a reduction also was noticed by Bahamish and Wood (2), who conducted research on induced susceptibility in wheat to *P. r. tritici*, initially inoculated with a virulent race, followed by an avirulent race 4 days later. In the research of Kochman and Brown (6), the challenge inoculation had no significant effect during the first 2 days after infection. The maximum effect was found 4 days after inoculation, and the effect remained the same until the longest time interval (7 days) of their study. Johnson and Allen (4) found a 70% reduction in total spore mass produced with a 6-day time interval between the successive inoculations.

The low negative correlation coefficient between the IF of the avirulent race (3SA126) and that of the double inoculation shows that the IF only partially reflects the effects of such a preinoculation. Therefore, it is concluded that IFs indicate induced resistance, but no predictions about the extent of it can be made using this parameter.

The cause of the reduction in IF may result from killing or plugging of many stomata by the avirulent fungus (5). But because the number of stomata on the wheat leaf surface is approximately 3,000

per square centimeter (C. A. Crookes, *personal communication*) and up to six appressoria can be found on one stoma (C. A. Crookes, *personal communication*), it is unlikely that plugging of infection sites is the reason for the reduction in IF.

Both Johnson and Allen (4) and McRae and Brown (9) found that the induced resistance was systemic in the sense that it was expressed on the opposite leaf surface to that on which the inducer strain was inoculated. However, the mechanism involved was not discussed in these articles.

Electron microscopy studies by C. A. Crookes (*personal communication*) indicated that *P. r. tritici* in a resistant wheat cultivar developed to the substomatal vesicle or haustorium mother cell stage before development stopped. It may, therefore, be postulated that after a certain number of propagules have reached the substomatal vesicle/haustorium mother cell stage, the resistance mechanism is activated to such an extent that development of subsequent infecting mycelia beyond the appressorium/infection peg stage is not possible.

On the basis of the work by Kuć (7) and others, preinoculation with an avirulent race renders the plant more resistant to subsequent challenge by a virulent race. It is noteworthy that in the experimental system used in the present study, such increased resistance is only manifested in a lower number of pustules per square centimeter of leaf area and not by an increase in latent period or a change in infection type. It will be interesting to establish if this lack of response is encountered more widely in monocotyledonous taxa.

ACKNOWLEDGMENTS

We would like to thank Z. A. Pretorius and J. Le Roux, Small Grain Centre, Bethlehem, for supplying the races and wheat cultivars used in this study.

LITERATURE CITED

1. Andres, M. W., and Wilcoxson, R. D. 1984. A device for uniform deposition of liquid-suspended urediospores on seedling and adult cereal plants. *Phytopathology* 74:550-552.
2. Bahamish, H. S., and Wood, R. K. S. 1985. Induction of susceptibility and resistance of wheat leaves by preinoculation with high and low virulence races of *Puccinia recondita* f. sp. *tritici*. *Phytopathol. Z.* 113:97-112.
3. Cheung, D. S. M., and Barber, H. N. 1972. Activation of resistance of wheat to stem rust. *Trans. Br. Mycol. Soc.* 58:333-336.
4. Johnson, R., and Allen, D. J. 1975. Induced resistance to rust diseases and its possible role in the resistance of multiline varieties. *Ann. Appl. Biol.* 80:359-363.
5. Johnston, C. O., and Huffman, M. D. 1958. Evidence of local antagonism between two cereal rust fungi. *Phytopathology* 48:69-70.
6. Kochman, J. K., and Brown, J. F. 1975. Studies on the mechanism of cross-protection in cereal rusts. *Physiol. Plant Pathol.* 6:19-27.
7. Kuć, J. 1983. Induced systemic resistance in plants to disease caused by fungi and bacteria. Pages 191-221 in: *The Dynamics of Host Defence*. J. A. Bailey and B. J. Deverall, eds. Academic Press, Sydney.
8. Littlefield, L. J. 1969. Flax rust resistance induced by prior inoculation with an avirulent race of *Melampsora lini*. *Phytopathology* 59:1323-1328.
9. McRae, C. F., and Brown, J. F. 1983. Induced resistance in wheat to rust infection. *Australas. Plant Pathol.* 12:44-47.
10. Parlevliet, J. E., and Kuiper, H. J. 1977. Partial resistance of barley to leaf rust, *Puccinia hordei*. IV. Effect of cultivar and development stage on infection frequency. *Euphytica* 26:249-255.
11. Stakman, E. C., Stewart, D. M., and Loegering, W. O. 1962. Identification of physiological races of *Puccinia graminis* var. *tritici*. U.S. Dep. Agric. Agric. Res. Serv. E617. 53 pp.
12. Yarwood, C. 1954. Mechanism of acquired immunity to a plant rust. *Proc. Natl. Acad. Sci. U.S.A.* 40:374-377.
13. Zadoks, J. C., Chang, T., and Konzak, C. 1974. A decimal code for the growth stages of cereals. *Weed Res.* 14:415-421.