

Responses of Detached Tissues of Adult Wheat Plants to *Puccinia graminis tritici*

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ABSTRACT

Stem rust (*Puccinia graminis* f. sp. *tritici*) was studied on detached flag leaves, leaf sheaths, and peduncles from adult plants of ten wheat cultivars in benzimidazole and kinetin solutions. The infection type, incubation period, and severity of rust varied with cultivars, addition of glucose to the kinetin and benzimidazole solutions, time of tissue detachment in relation to inoculation, and age of the plants. Temperature

did not influence infection type or stem rust severity, but did alter the incubation period. The interactions of cultivars with benzimidazole treatments, and with the time of tissue detachment in relation to inoculation, were significant. Stem rust development was excellent on all the detached plant parts, but it was less on older tissues than on younger ones.

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The study of stem rust on adult cereal plants may be difficult because greenhouse space may not be available long enough to test adult plants, because large plants are difficult to inoculate uniformly, and because the environment varies from one part of the plant to another due to plant size. These difficulties have been overcome with seedlings by using detached-leaf techniques to study life cycles (4), race identification (2, 3, 8), genetics (8), host-parasite relationships (10, 11), and routine culture maintenance (1, 7). This study was made to learn whether detached-leaf techniques might be applied to studies on the stem rust infection of leaves, leaf sheaths, and peduncles from adult wheat plants.

MATERIALS AND METHODS.—Experiments were made with *Triticum aestivum* L. 'Baart', 'Chris', 'Lee', 'Marquis' and 'Thatcher'; *T. durum* Desf. 'Langdon', 'Mindum', 'Ramsey' and 'Sentry'; and *T. compactum* Host. 'Little Club'. The cultivars were inoculated with race 15B2 of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. Seedlings of all the cultivars were susceptible except Chris, which was also resistant in the adult plant stage. Langdon, Lee, Thatcher, Ramsey, and Sentry had some adult plant resistance. Adult plants of Marquis, Baart, Little Club, and Mindum were susceptible.

The wheats were grown in 25.4-cm diameter pots in a greenhouse at 17 to 27 C and illuminated with 8,600 to 10,760 lux of light (incident daylight supplemented by fluorescent and incandescent lamps). The plants were kept thrifty in appearance by fumigation once per week to control insects, and by routine watering and fertilizing. Seeds were treated with Milstem to control powdery mildew.

When experiments were made, one of the two upper leaves, flag-leaf sheaths, and peduncles were removed, fastened to a plate, sprayed with distilled water and exposed to urediospore showers for 2 minutes in a settling tower. About 20 spores per low-power microscope field were thus deposited on the leaves. After inoculation, the plant parts were again sprayed with water and individually dropped, cut-end down, into test tubes containing solutions of kinetin (40 µg/ml of water) or benzimidazole (25 µg/ml of water) and placed in a dark moist chamber for 24 hours. Then the plant parts were returned to the greenhouse for the duration of the experiment. Occasionally it was necessary to add more kinetin or benzimidazole solution to the test tubes to maintain the liquid level. Clean, but not aseptic, technique was used.

Leaves were always about 15 cm long and inoculated on the lower surface. In the settling tower, six to eight leaves of leaf sheaths could be inoculated at one time.

Urediospores of stem rust race 15B2, produced on Marquis wheat seedlings, were stored in test tubes at about 5 C until used, not more than 15 days after collection.

Data taken were: (i) rust severity using the modified Cobb scale (6), (ii) infection type (12) and (iii) incubation period (the time from inoculation to the appearance of flecks). Tests were repeated, usually several times; analysis of variance and Duncan's multiple range test was used to assist in evaluation of data (5).

Effect of benzimidazole and kinetin.—In three trials, four leaves from 28-day-old plants of each variety were inoculated and placed in benzimidazole in water or 1% or

2% glucose. Similar tests were made with kinetin in water and glucose.

Effect of time of leaf detachment in relation to inoculation.—Two trials were made with leaves from 35-day-old plants. Flag leaves attached to plants were inoculated in the settling tower and kept in the greenhouse until flecks appeared. They were then detached and placed in benzimidazole solution for the remainder of the experiment. Other flag leaves were detached, inoculated, and placed in benzimidazole solution. There were four replicate leaves per treatment in each trial.

The experiment was also made with leaves sprayed with urediospores suspended in light mineral oil (9). All other treatments were as stated above.

Effect of temperature.—Flag leaves of 35-day-old plants grown in the greenhouse were detached and inoculated in the settling tower, placed in benzimidazole solution, kept at about 18 C in a dark moist chamber, and then in the greenhouse at 20, 25, and 27 C. There were two trials with four replicate leaves per treatment in each trial.

Effect of plant age.—Plants were grown in the greenhouse for 55, 65, and 85 days. Then flag leaves, leaf sheaths, and peduncles were detached, inoculated in the settling tower, and placed in benzimidazole solution. Not all of the tests were made at the same time, but when the plants had reached the desired age. Plants 55-days old had just begun to head, those 65-days old were in the soft dough stage of development, and those 85-days old were in the hard dough stage. Two tests were made, each with four replicate plants per treatment.

RESULTS.—*Effect of benzimidazole and kinetin.*—The two chemicals appeared to have had similar effects on stem rust development, but the plant parts in kinetin remained green a day or two longer than those in benzimidazole. Only the data for benzimidazole will be presented (Table 1).

The infection types on each cultivar were similar to those usually seen on seedlings inoculated with race 15B2, except that on Langdon, Ramsey, and Sentry they were smaller than usual. When glucose was added to the benzimidazole solution, the infection types often lowered.

Average stem-rust severity was about the same in benzimidazole alone and in benzimidazole containing 1% glucose, but it was reduced in benzimidazole containing 2% glucose. Varietal differences were statistically significant in each chemical treatment. The interaction of chemical treatments and wheat cultivars was statistically significant, with the stem rust being more severe in benzimidazole plus 1% glucose than in benzimidazole alone on all cultivars except Baart, Marquis, Thatcher, Ramsey and Sentry.

Effect of time of leaf detachment in relation to inoculation.—Because results of inoculation in the settling tower and by spraying the spores in oil onto the plants were similar, only data from tower inoculation are presented (Table 2).

The time when leaves were detached in relation to inoculation had little effect on the infection types that developed on the several wheats.

The incubation period was significantly longer on the leaves detached after inoculation than on those detached before inoculation; on Thatcher and Little Club the period was increased 6-7 days, and on the other cultivars, 2-3 days.

The severity of stem rust on all cultivars was significantly greater on leaves detached before inoculation, than afterwards. The difference on Thatcher and Ramsey was much greater than on the other cultivars.

Effect of temperature.—The infection types varied among cultivars, but differences were not attributable to the temperatures used in the study (Table 3). The length of the incubation period varied significantly with cultivars and temperature; the interaction of the two was not

TABLE 1. The effect of benzimidazole and glucose on infection types and severity of stem rust on leaves detached from 28-day-old wheat (*Triticum* spp.) plants

Wheat species and cultivars	Benzimidazole alone ^a		Benzimidazole in 1% glucose		Benzimidazole in 2% glucose	
	Infection type ^b	Severity (%)	Infection type	Severity (%)	Infection type	Severity (%)
<i>T. aestivum</i>						
Baart	4	50 A	4	50 A	4	30 A
Chris	1	5 E	2	7 E	0	0 D
Lee	3	12 D	2	20 D	1	2 D
Marquis	3	37 B	2	37 B	3	20 B
Thatcher	3	25 C	2	28 C	2	2 D
<i>T. compactum</i>						
Little Club	4	37 B	3	47 A	4	20 B
<i>T. durum</i>						
Langdon	2	37 B	2	50 A	2	5 D
Mindum	4	30 B	3	43 B	2	12 C
Ramsey	2	27 C	2	30 C	1	1 D
Sentry	2	47 A	3	37 D	2	10 C

^aTwenty-five micrograms benzimidazole per ml water. Data based on four leaves in each of three trials. Data in the columns followed by the same letter were not significantly different statistically, $P = 0.01$ (5). The entire experiment was repeated with kinetin and glucose, but data are not shown because the differences resembled those shown above.

^bInfection types according to Stakman et al. (12), and severity according to Peterson et al. (6).

TABLE 2. The effect of time of leaf detachment in relation to inoculation on development of stem rust on wheat cultivars in benzimidazole solution^a

Wheat species and cultivars	Stem rust on leaves detached before or after inoculation					
	Infection type		Incubation period (days)		Severity ^b (%)	
	Pre-inoculation	Post	Pre-inoculation	Post	Pre-inoculation	Post
<i>T. aestivum</i>						
Baart	4	4	8 C	10 C	40 A	10 B
Chris	1	1	15 A	18 A	5 C	2 C
Lee	4	3	7 D	10 D	40 A	15 B
Marquis	4	4	6 E	9 E	45 A	2 C
Thatcher	3	2	9 B	15 B	45 A	2 C
<i>T. compactum</i>						
Little Club	4	4	6 E	13 C	35 B	10 B
<i>T. durum</i>						
Langdon	2	2	7 D	10 D	40 A	15 B
Mindum	3	3	6 E	9 E	45 A	15 B
Ramsey	2	3	6 E	9 E	40 A	2 C
Sentry	2	3	6 E	9 E	40 A	15 B

^aTwenty-five micrograms benzimidazole per milliliter of water. Data based on four flag leaves per treatment in two trials at $P = 0.01$. Data followed by the same letter were not significantly different.

^bIncubation period was the days from inoculation to fleck formation.

significant. Thus, the average incubation period for Chris and Thatcher was longer than for the other cultivars, and the period was longer at 20 C than at 27 C for all cultivars.

Severity of stem rust on individual cultivars did not vary with the temperatures used in the study. The differences among the cultivars were significant largely because of the small amounts of rust that developed on Chris.

Effect of plant age.—The tests were with flag leaves, leaf sheaths, and peduncles from plants that were 55, 65,

and 85 days old. Stem rust developed on each of the parts from 55- and 65-day-old plants, but only traces of rust formed on the 85-day-old plants, if it formed at all. Data obtained in these experiments will not be presented in tables because similar information may be seen in other tables of the paper.

The severity of stem rust on flag leaves, leaf sheaths, and peduncles from 55-day-old plants of each cultivar was similar to that shown in Table 1 for leaves in benzimidazole solution. Differences in severity among the plant parts were not significant. The severity of the rust on the parts from 65-day-old plants was about 50% below that on the parts from 55-day-old plants.

Infection types on flag leaves were somewhat lower than those shown in Table 1 for leaves in benzimidazole. On flag leaves from 55-day-old plants the infection types were: 3 on Baart, 1 on Chris, 3 on Lee, 1 on Marquis and Thatcher, 3 on Little Club, 2 on Langdon and Mindum, and 1 on Ramsey and Sentry. On leaf sheaths and peduncles, the infection types tended to be somewhat larger than on flag leaves. They were respectively for leaf sheaths and peduncles, 3 and 3 for Baart, 1 and 2 for Chris, 3 and 3 for Lee, 3 and 2 for Marquis, 3 and 3 for Thatcher, 2 and 3 for Little Club, 2 and 2 for Langdon, 3 and 2 for Mindum, 1 and 1 for Ramsey, and 2 and 2 for Sentry. On parts from 65-day-old plants, the infection types were lower than on parts from 55-day-old plants of each variety.

The incubation period (the time needed for flecks to form on inoculated leaves, leaf sheaths, and peduncles) of both 55- and 65-day-old plants was about like that shown at 20 C in Table 3 for each cultivar except Chris. On Chris, the incubation period on parts detached from 65-day-old plants was 4 days longer than in the 55-day-old plants. Other deviations were not greater than about one day. Differences among the several plant parts were not significant.

DISCUSSION.—Some variation in stem-rust development was observed due to the presence of glucose in the benzimidazole and kinetin solutions, to the time of

TABLE 3. Effect of temperature on stem rust development on detached flag leaves of wheat cultivars in benzimidazole^a

Wheat species and cultivars	Infection type			Incubation period (days)			Severity (%)		
	20 C	25 C	27 C	20 C	25 C	27 C	20 C	25 C	27 C
<i>T. aestivum</i>									
Baart	4	4	4	10	8	7	50	45	40
Chris	1	2	2	14	10	7	5	15	15
Lee	3	3	3	9	8	7	50	45	40
Marquis	4	3	3	10	9	7	45	40	40
Thatcher	3	4	3	12	10	8	45	50	45
<i>T. compactum</i>									
Little Club	4	4	3	10	8	7	45	30	30
<i>T. durum</i>									
Langdon	2	2	2	9	8	7	40	35	25
Mindum	3	3	2	9	7	7	50	55	45
Ramsey	1	2	2	10	9	7	35	45	25
Sentry	1	2	2	1	9	8	30	30	30

^aTwenty-five micrograms benzimidazole per ml water. Data based on four leaves/treatment in two trials. Incubation period: differences due to cultivar and temperature were statistically significant ($P = 0.01$), but their interaction was not. Severity: only differences due to cultivar were significant ($P = 0.01$).

tissue detachment in relation to inoculation, to temperature, and to the age of the plant tissues. Despite these effects we concluded that the techniques of detached-leaf culture of rust fungi were adaptable for the study of rust on adult plant tissues. Wheat cultivars evidently can be differentiated on the basis of infection type, incubation period, and stem rust severity, by means of these culture techniques. The studies were not comprehensive enough to establish normal values for these characters in the several wheat cultivars tested, but that could be done with additional work. If this were done, we recommend using more precisely controlled environments and inoculations. Under our conditions it was possible to evaluate adult plant resistance to stem rust on detached wheat tissues, at least in a preliminary way.

In our tests, more stem rust developed on the detached plant parts than in greenhouse trials with intact plants, as Browder (1) also observed. The reason for this is unknown, but there are several possible explanations: (i) better control of environment to favor the pathogen; (ii) plant tissues grow after inoculation of intact plants, but not when the plant parts are detached; (iii) more uniform and efficient inoculation of the detached parts; and (iv) most of the spores produced remain in the uredia on detached leaves, but many fall away from intact plants. The fact that the rust was more severe on detached tissues did not detract from the potential value of the technique, because the differences among cultivars were consistent.

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