Development of the Stem and Crown Rust Fungi on Leaves, Sheaths, and Peduncles of Oats

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This research was supported by the Wheat Industry Research Council of Australia, and by funds provided by the University of New England.

Accepted for publication 27 June 1975.

ABSTRACT

The development of *Puccinia coronata* f. sp. *avenae* and *Puccinia graminis* f. sp. *avenae* was studied quantitatively, using histological methods, on leaves, sheaths, and peduncles of two commercial oat cultivars (Algerian and Garry) and on the naturalized weed species *Avena sterilis*. No major differences were observed in the percentage germination of urediniospores of both rusts on the different hosts or on the various parts of the host. The frequency of appressorium formation by crown rust was less on sheaths than on leaves. Appressoria failed to develop on the peduncles of the hosts examined. Minor differences were observed in appressorium formation by stem rust on the various plant parts. Crown rust had a greater ability to produce appressoria on leaves than stem rust, whereas on sheaths and peduncles the reverse was true. The frequency of penetration from appressoria by crown rust was less on sheaths than on leaves. Penetration by stem rust was lower on sheaths and peduncles than on leaves of Algerian and *A. sterilis* but was similar on Garry. Penetration frequency of both rusts was lowest on Garry, intermediate on *A. sterilis* and highest on Algerian. It is suggested that differences in penetration frequency may be a component of the "slow rusting" character.

Phytopathology 65:1404-1408

Additional key words: slow rusting, horizontal resistance, resistance to infection, epidemiology.

There is considerable evidence to suggest that the susceptibility of cereals to rust infection varies with plant age. Mature plants of some cereal cultivars have been shown (1, 6, 8, 10, 15, 20) to produce a more resistant host response than seedling plants. The term "adult resistance" has been used to describe this phenomenon. Moreover, some isolates of *Puccinia graminis* Pers. f. sp. *tritici* Erikss. and Henn. on the wheat cultivar Hope produce susceptible infection types on the lowermost leaves and sheaths and more resistant infection types on the uppermost plant parts (8). It seems that adult resistance resulting from changes in infection type is race-specific in nature and operates during the postpenetration phase of the infection process (1).

Some forms of adult plant resistance to cereal rusts may be related to differences in development during the prepenetration and penetration phases as well as in the postpenetration phases of infection. Simons and Murphy (17) stated that crown rust of oats normally developed on leaves and less frequently on sheaths and peduncles. Similarly, Romig and Caldwell (13) found less penetration on wheat peduncles and sheaths than on leaves by *Puccinia recondita* Rob. ex. Desm. f. sp. *tritici* Erikss. and Henn. They concluded that an exclusion mechanism prevented the fungus from entering the sheaths and peduncles of wheat. The relative proportions of leaf tissue to sheath and peduncle varies between plants of different ages. Thus, some reports of field resistance in adult plants may be related to differences in the ability of fungi to infect different parts of the plant.

Plants of different ages and different parts of plants show variations in susceptibility to rust infection. Furthermore the frequency of penetration from appressoria varies among cereal cultivars (4). Brown (2) considered that differences in penetration frequency among wheat cultivar-stem rust combinations might be significant in the development of rust epidemics. Moreover, Clifford (7) found that the number of pustules produced per unit of inoculum of crown rust varied among oat cultivars. Cultivars on which fewer and smaller pustules are produced are often referred to as "slow rusters". Epidemiologically, any mechanism limiting the number of viable propagules produced per unit of inoculum and time is potentially useful in plant breeding programs.

The purpose of these experiments was twofold: first, to study those mechanisms of resistance to crown and stem rust which did not appear to be associated with the hypersensitive reaction; and second, to determine whether different parts of oat plants varied in susceptibility to rust infection. It was considered that such a study would lead to a better understanding of the epidemiology of oat rusts.

MATERIALS AND METHODS.—The rust inoculum used was collected in the field and was divided into four groups: (i) a field collection consisting of a mixture of physiologic races 68, 70, 79, and 81 of stem rust; (ii) an isolate of race 68 of stem rust; (iii) a field collection of crown rust consisting of a mixture of physiologic races 233, 236, and 237 and; (iv) an isolate of race 237 of crown rust. The stem rust races were identified on the differential cultivars proposed by Stewart and Roberts (19) while the crown rust races were identified on the differential cultivars described by Simons and Murphy (16). A field collection consisting of a mixture of races as well as single spore isolates was used to overcome the possibility that single spore isolates were atypical in behavior. The rust isolates were virulent on the three oat hosts studied.

Leaves, sheaths, and peduncles of seedling and adult plants of two commercial oat cultivars (Algerian and Garry) and a naturalized weed species (*Avena sterilis* L.) were inoculated with freshly collected urediniospores. The abaxial surfaces of leaves were inoculated in a spore settling tower (3) enabling urediniospores to be deposited uniformly onto the leaf surface (approximately 60 spores per cm^2). However, sheaths and peduncles could not be inoculated uniformly by this method. Consequently, further inoculations were made with a small camel hair brush so that a total of 50 to 93 urediniospores per cm^2 were deposited onto the surfaces of sheaths and peduncles. Inoculated plants were placed in darkened dew chambers kept at 20 ± 1 C for 16 hours. After incubation plants were placed in growth cabinets where those inoculated with stem rust were kept at 30 ± 1 C and those inoculated with crown rust at 20 ± 1 C. Light was supplied at 5,625 lux in 12 hour photoperiods.

At appropriate times after inoculation the first, third, fourth, and flag leaf; the sheaths of the third, fourth, and fifth internodes; and the peduncle were sampled. These plant parts were prepared for microscopic examination by a whole-leaf cleaning and staining method (14). Leaves and sheaths were examined directly, whereas the peduncles were cut longitudinally and the epidermis removed before it was mounted on microscope slides. Each treatment was replicated three times, and each experiment was conducted twice, one week apart. Four plants were examined in each replicate for each phase of the infection process.

The percentage germination of urediniospores was determined by examining 75 spores on each plant part at 4 hours after inoculation. Forty germ-tubes were measured on each plant part at 4 hours after inoculation. The percentage of germinated spores that produced appressoria over stomates was assessed at 12 hours after inoculation by examining 40 germinated spores on each plant part. The percentage of appressoria from which sub-stomatal vesicles had developed (penetration %) was determined by examining a total of 160 appressoria per replicate at 48 hours after inoculation. Colony areas at 120 hours after inoculation was determined by measuring the longest and shortest diameters of 24 colonies on each plant part in each replicate and applying the formula $\pi(ab/4)$ where a and b are the diameters for stem rust and $\pi(d^2/4)$ for crown rust. The proportion of infection foci that developed into uredinia was determined by examining 40 infection foci on each plant part per replicate at 10 days after inoculation. The number of foci that formed uredinia was calculated as a percentage of the number of infection foci observed.

Results were subjected to a computerized analysis of variance program and means were compared by the studentized range test. Percentage data was transformed to arcsine ($\sqrt{\text{percentage}}$) before analysis. Significant differences were based on $P = 0.05$.

TABLE 1. Percentage germination of urediniospores of *Puccinia coronata avenae* on various plant parts of three oat hosts, *Avena sativa* 'Algerian' and 'Garry' and *A. sterilis*, a naturalized weed species

Plant part	Germination on each host (%)[a]		
	Algerian	*A. sterilis*	Garry
Leaves	80.2 A	79.9 A	76.0 A
Sheaths	79.9 A	75.6 B	75.1 A
Peduncle	71.6 B	71.3 C	71.8 B

[a]Values followed by the same upper case letter within each cultivar did not differ significantly ($P = 0.05$).

TABLE 2. The percentage of appressoria per 100 germinated urediniospores formed over stomata on various plant parts of three oat hosts (*Avena sativa* 'Algerian' and 'Garry' and *A. sterilis*, a naturalized weed species) by the crown and stem rust fungi, *Puccinia coronata* f. sp. *avenae* and *Puccinia graminis* f. sp. *avenae*, respectively

Rust/oat host	Appressorium formation on the indicated plant parts of each host (%)[a]		
	Leaves	Sheaths	Peduncle
Crown rust			
Algerian	60.5 A	8.0 B	0 C
A. sterilis	61.8 A	6.1 B	0 C
Garry	59.1 A	4.7 B	0 C
Stem rust			
Algerian	29.7 A	28.5 A	26.8 A
A. sterilis	29.8 A	28.4 A	27.6 A
Garry	28.3 A	24.8 B	23.8 B

[a]Values followed by the same upper case letter within each cultivar did not differ significantly ($P = 0.05$).

RESULTS.—*Germination, germ-tube growth and appressorium formation.*—There was no significant difference in the percentage germination of stem rust on the various plant parts of the oat hosts examined (overall mean of 72.1%). Furthermore, there was no significant difference between the germination percentage of mixed and single race isolates. With crown rust, however, there were significant differences between germination on the peduncles and other plant parts of Algerian and Garry and between peduncles, sheaths and leaves of *A. sterilis* (Table 1). Germination was similar on the various leaves and on the different sheaths examined. The germination percentage of single races and mixed isolates of crown rust was similar.

The length of germ-tubes of the stem and crown rust fungus at 4 hours after inoculation were similar on the various plant parts of the three oat hosts (mean length 193 μm).

The number of appressoria formed over stomates per 100 germinated urediniospores of stem rust was similar on the different plant parts of Algerian and *A. sterilis*. There were significant differences, however, between the number of appressoria formed on the leaves and the other plant parts of Garry (Table 2). The percentage appressoria formed by crown rust was significantly less on sheaths than on leaves and less on peduncles than on sheaths. The frequency of appressorium formation by single and mixed strains of both rusts was similar.

Crown rust produced fewer appressoria over stomates on sheaths than on leaves. Appressoria were rarely formed over stomata on the peduncles in the oat hosts studied. Moreover, appressoria were less frequently formed on the external surfaces than on the internal surfaces of leaf sheaths. As the external and internal surfaces of leaf sheaths occur on the same portion of the plant, it is unlikely that the reduction in appressorium formation was due to the presence of inhibitory substances secreted by the host. A possible explanation for the phenomenon is related to the structural differences in the epidermal and guard cells of the inner and outer surfaces of sheaths that influenced appressorium formation. However, differences in the microenvironment between the internal and external surfaces of sheaths cannot be eliminated, whether it was due to physical factors or associated with the epiphytic microbial population on the two surfaces. Possibly a combination of structural and other factors was responsible for the differences in appressorium formation and penetration frequency on the inner and the outer surfaces of sheaths.

The frequency of penetration from appressoria by crown rust was lower on sheaths than on leaves of Algerian and *A. sterilis*. However, on Garry, the penetration frequency was low on all the plant parts examined. These results are similar to those reported for leaf rust of wheat (13) which suggested that an exclusion mechanism prevented the fungus from entering sheaths and peduncles of wheat. It would seem, therefore, that Algerian and *A. sterilis* possess a mechanism(s) which inhibits crown rust from penetrating sheaths and peduncles, whereas in Garry this mechanism(s) also operates in leaves.

Mechanisms other than those associated with hypersensitive necrosis (5, 11, 12, 18) may operate in rendering oats resistant to rusts. Our results indicate that resistance of sheaths and peduncles, particularly to crown rust infection, was related to mechanisms that reduced appressorium formation and penetration. These exclusion mechanisms may be important in reducing the number of lesions produced per unit of inoculum, and hence, the amount of inoculum produced in each generation. Moreover, as the proportion of sheath and peduncle tissue relative to leaf tissue changes with plant age, it is possible that some forms of field resistance are related to differences in prepenetration and penetration development of rusts on different plant parts. Moreover, the relative rates of increase of epidemics of stem and crown rust may be related to the differing abilities of each rust to infect different plant parts.

Resistance mechanisms that operate before and during the penetration phase of the infection process (exclusion mechanisms) may be a major component of the "slow rusting" characteristic exhibited by some cereal cultivars (9). We found that penetration frequency was least on Garry, intermediate on *A. sterilis* and most on Algerian. It should not be a difficult task to screen cultivars for the presence of exclusion mechanisms of resistance. However, it is premature to suggest that these types of resistance are nonspecific in their action. Therefore, one cannot with confidence classify them as horizontal or generalized resistance (21, 22).

Penetration frequency.—The number of penetrations per 100 appressoria formed over stomata on various parts of the host differed for both rusts. Penetration by stem rust on Algerian and *A. sterilis* was significantly lower on the sheaths and peduncles than on the stem; whereas on Garry, the penetration frequency was similar on all plant parts. Penetration from appressoria of crown rust was lower on sheaths than on leaves (Table 3). The percentage penetration by both rusts was lowest on Garry, intermediate on *A. sterilis*, and highest on Algerian. Penetration by single and mixed races of both rusts were similar.

TABLE 3. Number of penetrations per 100 appressoria of stem and crown rust fungi (*Puccinia graminis* f. sp. *avenae* and *Puccinia coronata* f. sp. *avenae*, respectively) on various plant parts of three oat hosts, *Avena sativa* 'Algerian' and 'Garry' and *A. sterilis*, a naturalized weed species

	Penetration on each host (%)[a]					
	Stem rust			Crown rust		
Plant part	Algerian	*A. sterilis*	Garry	Algerian	*A. sterilis*	Garry
Leaf						
First	58.7 A	49.3 A	29.8 A	79.7 A	48.0 A	6.5 A
Third	60.8 A	49.8 A	30.5 A	79.2 A	49.0 A	5.8 A
Fourth	59.4 A	49.2 A	30.3 A	78.1 A	46.6 A	5.9 A
Flag	59.8 A	48.6 A	30.4 A	78.5 A	47.0 A	4.6 B
Sheath						
Third internode	49.3 B	40.4 B	29.3 A	6.7 B	4.4 B	1.0 C
Fourth internode	49.4 B	40.0 B	29.9 A	6.4 B	3.7 B	0.0 D
Fifth internode	47.5 B	40.4 B	29.0 A	5.3 B	2.1 C	0.0 D
Peduncle	40.7 C	41.0 B	28.1 A	...[b]	...	...

[a]Values for each rust followed by the same upper case letter within each cultivar did not differ significantly ($P = 0.05$).
[b]Penetration could not be measured as appressoria failed to form on peduncles.

Appressorium formation and penetration by crown rust on the internal and external surfaces of sheaths.—The epidermal anatomy of the internal surface of oat sheaths was markedly different to that of the external surface. The thickness of the epidermal and guard cell walls was about 1 μm less on the internal surface than the external surface. Moreover, the thickness of the epidermal cell walls on the internal surface was similar to those of the leaf.

To determine whether differences in appressorium formation and penetration frequency by crown rust on leaves and sheaths was related to differences in epidermal anatomy, the third, fourth, and fifth leaf sheaths of Algerian, *A. sterilis*, and Garry were pulled away from leaves. The internal surface of leaf sheaths were inoculated with race 237 of crown rust. The external surface of sheaths were inoculated on an equal number of plants. After incubation (20 C and darkness) for 16 hours, the plants were placed in growth cabinets kept at 20 C with light supplied at 5,625 lux in 12-hour photoperiods. Sheaths were sampled at 12 and 48 hours after inoculation and the number of appressoria formed over stomata per 100 germinated spores and the percentage penetration from appressoria was determined. Each treatment was replicated three times and the experiment was conducted twice, one week apart.

The frequency of appressorium formation and penetration from appressoria by crown rust was higher on the internal surfaces of sheaths than on the external surfaces (Table 4) for all the hosts used. However, the percentage of appressorium formation and penetration on the internal surfaces of sheaths was about half that observed on leaves (Tables 2 and 3).

Postpenetration development.—In these experiments, race 68 of stem rust and race 237 of crown rust were used. Furthermore, because of the low penetration frequency by crown rust on the sheaths and peduncles of Algerian and *A. sterilis* and on all plant parts of Garry, only leaves of Algerian and *A. sterilis* were inoculated with crown rust.

TABLE 4. The proportion of appressoria formed per 100 germinated urediniospores and the percentage penetration from appressoria by the crown rust fungus (*Puccinia coronata* f. sp. *avenae*) on the internal and external surfaces of sheaths of three oat hosts, *Avena sativa* 'Algerian' and 'Garry' and *A. sterilis*, a naturalized weed species

	Internal surface[a]	External surface[a]
Appressoria (%)		
Algerian	27.7 A	6.2 B
A. sterilis	23.4 A	5.8 B
Garry	21.5 A	4.4 B
Penetration (%)		
Algerian	30.8 A	3.6 B
A. sterilis	27.6 A	1.7 B
Garry	4.5 A	0.0 B

[a]Values followed by the same upper case letter within each cultivar did not differ significantly ($P = 0.05$).

Since not all substomatal vesicles give rise to uredinia (2), we were interested if the proportion that developed into pustules varied on different plant parts or on different oat hosts. The percentage of infection foci of stem and crown rust that developed into pustules was 85.5% and 90.4%, respectively. There was no significant difference in the proportion of infection foci that developed into pustules on the different parts of the host or between the hosts examined.

Colony areas of stem rust were similar on the various plant parts of Garry (mean 157.2×10^3 μm^2) and *A. sterilis* (272.8×10^3 μm^2). However the colony area on the peduncle of Algerian (270.8×10^3 μm^2) was less than on the leaves and sheaths (299.1×10^3 μm^2). Colony areas of crown rust was measured on the third, fourth and flag leaves of Algerian and *A. sterilis*. No significant differences were found in colony areas on the different leaves on a given host. The colony areas of both rusts

varied on the different hosts. The area of crown rust on Algerian (306.4×10^3 μm^2) was greater than that on *A. sterilis* (288.5×10^3 μm^2). No evidence of hypersensitive necrosis was observed in any of the host-pathogen combinations.

DISCUSSION.—We found that the development of crown and stem rust at different stages of the infection process varied on the leaves, sheaths, and peduncles of three oat hosts. Moreover, development of two rusts on different parts of the plant varied among the three oat hosts. In general, the variations observed were more pronounced with crown rust than with stem rust.

The percentage germination of crown rust was less on the peduncles of Algerian and Garry than on the leaves and sheaths. On *A. sterilis,* germination was greatest on the leaves, intermediate on the sheaths, and lowest on the peduncle. Whether the small reduction in germination of crown rust was related to exudates on the peduncle or sheath surfaces, to physical factors such as poor moisture retention or to substances produced as a result of a host-pathogen interaction, is uncertain. The magnitude of differences in percentage germination, however, are unlikely to be of major importance in the development of rust epidemics.

Crown rust produced fewer appressoria over stomates on wheaths than on leaves. Appressoria were rarely formed over stomata on the peduncles in the oat hosts studied. Moreover, appressoria were less frequently formed on the external surfaces than on the internal surfaces of leaf sheaths. As the external and internal surfaces of leaf sheaths occur on the same portion of the plant, it is unlikely that the reduction in appressorium formation was due to the presence of inhibitory substances secreted by the host. A possible explanation for the phenomenon is related to the structural differences in the epidermal and guard cells of the inner and outer surfaces of sheaths that influenced appressorium formation. However, differences in the microenvironment between the internal and external surfaces of sheaths cannot be eliminated, whether it was due to physical factors or associated with the epiphytic microbial population on the two surfaces. Possibly a combination of structural and other factors was responsible for the differences in appressorium formation and penetration frequency on the inner and the outer surfaces of sheaths.

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