Interactions of *Puccinia striiformis* and *Mycosphaerella graminicola* on Wheat

RICARDO MADARIAGA B., Former Graduate Student, and A. L. SCHAREN, USDA, ARS, Department of Plant Pathology, Montana State University, Bozeman 59717

**ABSTRACT**


*Puccinia striiformis* and *Mycosphaerella graminicola* are frequently found attacking the same wheat leaf. The effect of one pathogen on another and the effects of interactions between pathogens on host-pathogen interactions were studied. Seedlings of four spring wheat cultivars were inoculated at different times with various combinations of *P. striiformis* and *M. graminicola*. The two pathogens could colonize the same leaf simultaneously, and the area diseased was similar or smaller than the area affected by each organism separately. A smaller amount of leaf tissue was colonized by *P. striiformis* when *M. graminicola* was present. *M. graminicola* acted as a hypostatic parasite toward the rust. Wheat seedling leaves infected by *P. striiformis* remained green longer and had greater dry weight than leaves infected by both pathogens. This may have been due to the sequestering effect known to be characteristic of rusts. It is possible that *M. graminicola* interfered with the redirection of translocation of assimilates that is a common effect of rusts.

Additional key words: Septoria tritici, stripe rust

Research plant pathologists tend to study the effect of one disease at a time; however, the occurrence of one disease in a crop is the exception rather than the rule (23). The interaction between pathogens is usually represented as a predisposition phenomenon in which previous infection can predispose plants to infection by secondary unrelated plant pathogens (22). When two or more plant pathogens are present in the same host, interactions could be expressed as antagonism, antibiosis, and/or predation (5). The term parasitic epistasis could express the physiological modifications occurring in the interacting parasites (18).

In wheat (*Triticum aestivum* L.), it has been reported that a previous attack of *Mycosphaerella graminicola* (Fuckel) Schroeter induced a decrease in the incidence of *Puccinia recondita* Rob. ex Desm. f. sp. tritici in susceptible cultivars

Present address of first author: Quilamapu Research Station, INIA, Casillia 425, Chillan, Chile.

Cooperative investigation of the USDA and the Montana Agricultural Experiment Station. Journal Series No. J-1752.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

Accepted for publication 3 February 1986 (submitted for electronic processing).

---

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

This article is in the public domain and not copy-rightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 1986.

---

(4). *M. graminicola* as a secondary pathogen increased when tissues were infected first by *Gaumannomyces graminis* (Sacc.) Arx & Oliv. var. tritici Walker (2). When *Erysiphe graminis* DC. f. sp. tritici E. Marchal infected the plant first, a higher incidence of *M. graminicola* was observed. Cultivars resistant to *M. graminicola* may show susceptible reactions or pycnidial production when tissues are colonized first by *E. graminis* (3).

*M. graminicola*, teleomorph stage of the causal organism of Septoria tritici blotch, and *P. striiformis* West., causal organism of stripe rust, are frequently found together on the same wheat plants (8; C. C. Mork, personal communication), confounding research and causing substantial losses in farmers’ crops. Multiple infection methods have been developed (11,12) and used (20) where interactions between pathogens have not been sufficiently considered. Physiological changes in plant tissues affected by a biotrophic organism such as *P. striiformis* and a necrotrophic organism such as *M. graminicola*, acting as single colonizers, have been reported (6,7,19). Disease progression and changes in dry leaf weight induced by *P. striiformis* and *M. graminicola* as single and simultaneous colonizers were studied in these experiments.

**MATERIALS AND METHODS**

Wheat cultivars with a suitable range of resistance and susceptibility to both *P. striiformis* and *M. graminicola* were selected for the interaction studies (Table 1). Cultivars Lemhi (CI 11415), Baart (CI 1697), Anza (CI 15284), and Lakhish (Israel) were used.

Plants were grown in the glasshouse maintained at 20 ± 3°C in a 1:1 mixture of sand and sterile clay loam soil in aluminum pans 20 × 20 × 5 cm. No additional fertilizer was used. The soil- sand mix was sufficiently nutritious to support completely healthy-appearing control plants. Supplemental light was provided by 400W metal halide lamps to ensure a 12-hr photoperiod. Each container was planted with 15 seeds of a cultivar along the border of the pan, using an equal area for each cultivar. Plants were grown for 6–12 days and inoculated when they reached growth stage 12 (21). After disease symptoms appeared, 21, 26, and 31 days after the initial inoculation, nine plants per cultivar per pan were evaluated by assessing total nongreen area present on the oldest leaf. The presence of pycnidia and uredinia also was recorded. Disease-free control plants were maintained in all experiments. Twenty-seven plants were evaluated for each disease treatment.

A culture of *M. graminicola* (ORG-82076-1) obtained from Hyslop Farm, Oregon Agricultural Experiment Station was used exclusively. The Bozeman isolate of *P. striiformis* used in all experiments was collected in 1979 from a field of the wheat cultivar Itana, and the spores were continuously maintained at 5°C in vacuum-sealed tubes.

*M. graminicola* was increased in liquid nutrient media and adjusted to a concentration of 10⁷ spores per milliliter of suspension. The liquid medium was prepared using 9 g of yeast extract + 9 g of sucrose and 900 ml of distilled water. After 5 days on a wrist-action shaker at ambient laboratory temperature, abundant sporulation of the fungus was obtained. Plants were inoculated using a diaphragm pump connected to a DeVilbiss atomizer (10).

*P. striiformis* storage tubes were opened 2 hr before inoculation, and the spores were placed on a slide in a humid chamber at 22°C to allow hydration. Inoculation was performed by discharging a CO₂ gun loaded with 40 mg of hydrated urediospores twice in a settling tower to induce a slow and uniform precipitation of the spores on the plants on the mobile surface below (9). After the first shot, this mobile surface was moved 180 degrees from its original position to ensure a more uniform dissemination of the urediospores. Disease incidence was assessed by visual estimation of symptoms and signs of the disease with the modified Cobb scale. Areas under disease progress curves (AUDPCs) were measured with an area meter (Lamba Instrument Corporation Model LI-3000).
AUDPC was calculated for each pathogen using the assessment of total non-green area minus the value for the control.

In inoculations performed at time 1, 2 and 3 (17 days after time 1) with both pathogens simultaneously.

R = resistant (absence of pycnidia or uredinia), MS = moderately susceptible (moderate abundance of pycnidia or uredinia), and S = susceptible (abundance of pycnidia or uredinia).

Table 2. Areas under disease progress curves (AUDPC) for plants inoculated with Puccinia striiformis (Ps) alone or in combination with Mycosphaerella graminicola (Mg)

<table>
<thead>
<tr>
<th>Inoculation (time)</th>
<th>Cultivar</th>
<th>Reaction to</th>
<th>Ps</th>
<th>Mg</th>
<th>Ps x Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lakhish</td>
<td>MS</td>
<td>6.1</td>
<td>1.5</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>Anza</td>
<td>R</td>
<td>13.0</td>
<td>12.7</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>Lemhi</td>
<td>S</td>
<td>20.0</td>
<td>17.2</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td>Baart</td>
<td>S</td>
<td>16.6</td>
<td>16.9</td>
<td>17.9</td>
</tr>
</tbody>
</table>

Results of the urediniospore germi-nation test conducted in the presence and absence of bud-sores of M. graminicola indicated that significantly fewer (P = 0.05) germinate when they are associated with M. graminicola. With inoculation of P. striiformis at time 2 (MO OP and OM OP), we observed that the rust colonized only unaffected tissues. If M. graminicola was already present, the rust only penetrated and colonized unaffected portions of the leaf near the axil.

M. graminicola infected a significantly greater area of leaf on the susceptible cultivars Lemhi and Baart when that pathogen was inoculated alone (MO OA) than when inoculated in combination with P. striiformis (Table 3). M. graminicola and
The restrictive cool environment (12–14 C) that favors epidemics of stripe rust (17) and the ability of *P. striiformis* to survive at less than 0 C usually reduces the concurrent development of other economically important wheat leaf pathogens. Intercellular mycelia rapidly colonize leaf tissue, ramifying throughout the leaf but favoring the newly developed tissue near the axil of leaf and sheath. *M. graminicola* can infect tissues and grow in the same environment as *P. striiformis* but does not move as rapidly through the tissue. It often remains confined to the tissues first penetrated when free moisture is limited. When moisture is adequate, however, *M. graminicola* can cause lesions that expand rapidly and occupy large areas of the wheat leaf (4).

In Baart, most of the plants (93%) showed a moderately susceptible to susceptible infection type by *P. striiformis* in the absence of *M. graminicola*. When *M. graminicola* was also present in the tissue, the percentage of plants with moderately susceptible to susceptible reactions to *P. striiformis* was only 33%. Therefore, with *M. graminicola* present, plants subsequently inoculated with *P. striiformis* showed a trend away from moderately susceptible toward moderately resistant or even further to no disease symptoms. The reduced germination of *P. striiformis* urediniospores in the presence of *M. graminicola* conidia could, in part, account for this result. However, cross-protection by induction of general resistance mechanisms or nutrient depletion or imbalance are other possibilities. The interaction between *P. striiformis* and *M. graminicola* could have been an increase in resistance to *P. striiformis* caused by some unknown mechanism triggered by *M. graminicola*. The result observed was simply premature destruction of the substrate by the necrotrophic *M. graminicola*.

In the resistant cultivar Anza, without *M. graminicola* inoculation, most of the plants (78%) showed a resistant reaction to *P. striiformis*. The presence of *M. graminicola* decreased this percentage slightly (74%), but *P. striiformis* pustule production was not observed in either instance. There was no modification in the ability of *M. graminicola* to produce pycnidia in the resistant cultivar Anza when *P. striiformis* was present.

**Production of pycnidia by *M. graminicola* and uredinia and uredinio-mycelia by *P. striiformis* are mechanisms that perpetuate the pathogen from one growth cycle to another. Production of urediniospores is related to higher demands of energy that result in a rise in respiration, a drop in the C6/C2 ratio, and abortion of the Pasteur effect. In fact, in fungal diseases, sporulation places heavy demands for energy and for building blocks used as storage components of the spores (6).**

There is evidence that plants colonized by rust have an altered phloem transport with materials moving to infected areas (13,15,16). One of the earliest attempts to

---

**Table 3. Areas under disease progress curves (AUDPC) for plants inoculated with Mycosphaerella graminicola (M) alone or in combination with Puccinia striiformis (P)**

<table>
<thead>
<tr>
<th>Inoculation (time)*</th>
<th>Cultivar</th>
<th>Lakhish</th>
<th>Lemhi</th>
<th>Baart</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>O</td>
<td>7.6 (NS/NS/NS)</td>
<td>9.8 (NS/NS/NS)</td>
<td>10.3 (NS/NS/NS)</td>
</tr>
<tr>
<td>M</td>
<td>P</td>
<td>8.0 (NS/NS/NS)</td>
<td>8.1 (NS/NS/NS)</td>
<td>8.4 (NS/NS/NS)</td>
</tr>
<tr>
<td>M,P</td>
<td></td>
<td>5.5</td>
<td>5.6</td>
<td>4.5</td>
</tr>
</tbody>
</table>

*Inoculations were performed at host plant stage 12 of the decimal code (23) for time 1 and 17 days later for time 2.

**Table 4. Interactions between Puccinia striiformis (Ps) and Mycosphaerella graminicola (Mg) on spring wheat as measured by leaf dry weight increment when compared with un inoculated control**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Reaction to</th>
<th>Ps*</th>
<th>Mg*</th>
<th>Ps<em>Mg</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lakhish</td>
<td>MS*</td>
<td>28*</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Anza</td>
<td>R</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lemhi</td>
<td>S</td>
<td>1</td>
<td>3</td>
<td>25*</td>
</tr>
<tr>
<td>Baart</td>
<td>S</td>
<td>68*</td>
<td>1</td>
<td>27*</td>
</tr>
</tbody>
</table>

*Calculated by subtracting the weight of the diseased leaf from that of the control.

---

**Fig. 1. Effect of the area of leaf infected with Puccinia striiformis on leaf dry weight 26 days after inoculation. R values were: Baart, 0.81 (P = 0.05); Lemhi, 0.59 (P = 0.05); Lakhish, 0.24; and Anza, 0.18.**
quantify the flow of carbon in diseased plants was made in 1938 (6). An increase in the dry matter of rusted bean leaves was measured concomitantly with an overall drop in dry weight for the entire plant. Results of our work indicate that for the susceptible wheat cultivars Baart and Lemhi and moderately susceptible Lakhish, the presence of stripe rust increased leaf dry weight by 68, 41, and 28 mg respectively. The presence of Septoria tritici blotch and stripe rust caused an increase in dry leaf weight of only 27, 25, and 1 mg, respectively, when both pathogens were inoculated simultaneously at time 1. The leaf dry weight of the resistant cultivar Anza was not significantly modified by infection with either pathogen alone or with both at the same time. Stripe rust was reported as more harmful than mechanical defoliation in spring wheats (11). This result is consistent with the sequestering effect, which deprives other plant parts of nutrients by moving them to sporulating tissues. In our work, leaf area affected by P. striiformis was correlated with an increase in dry weight. Furthermore, it is possible that because M. graminicola apparently interrupted the sequestering effect caused by the rust, the total effect of the pathogens on the host could be less damaging than that caused by stripe rust alone.

This information suggests a danger in using multiple infection methods, as has been proposed (12,20), without further research on the physiology of the interactions. If the sequestering effect occurs by depriving other plant parts of nutrients, the behavior of these tissues toward pathogens will probably differ from that of healthy tissues.

The great variation that authors (14,17) have noted in the germination of stripe rust urediospores was also observed in this study. M. graminicola bud-spores caused a decrease in urediospore germination that could explain, in part, the reduced stripe rust observed in tissues colonized by both pathogens. An alternate explanation might be that since both organisms are stomatal penetrators, competition for stomatal openings may occur. The poor germination of P. striiformis in the presence of M. graminicola might be a survival mechanism for the urediospores, which do not germinate until suitable conditions are present. Alternatively, the urediospores could have been inhibited directly by a product of the metabolism of M. graminicola (toxin) or by other compounds produced in the host-pathogen interface.

Interaction of the two pathogens did exist because each changed its behavior when competing for the same plant tissue. When each pathogen was measured separately, each produced more disease symptoms than when the two pathogens were present together. M. graminicola seemed to act as an epistatic parasite toward P. striiformis. The peculiar pathogenic system of the biotrophic P. striiformis caused an increase in the dry leaf weight of infected seedlings that had sporulating pustules on their leaves and enabled them to sustain life and normal condition even longer than equivalent plants in control treatments with no inoculation. Necrotrophic M. graminicola caused a decrease in the leaf area affected by stripe rust, diminishing also its effect on leaf weight. It is possible that the interaction of pathogens results in a less detrimental effect to the host than that caused by the rust alone.

ACKNOWLEDGMENT

We wish to thank Colleen Mork Yahayou for valuable technical assistance.

LITERATURE CITED