Oecologia (2005) 143: 449–457
DOI 10.1007/s00442-004-1814-x

COMMUNITY ECOLOGY

F. A. Al-Naimi · K. A. Garrett · W. W. Bockus

# Competition, facilitation, and niche differentiation in two foliar pathogens

Received: 21 March 2004 / Accepted: 15 December 2004 / Published online: 12 February 2005


**Abstract** We studied competition between the obligate biotroph *Puccinia triticina* (designated here as *Puccinia*) and the facultative saprophyte *Pyrenophora tritici-repentis* (designated here as *Pyrenophora*) in older and younger leaves in a set of three host genotypes selected to be resistant to *Puccinia* only, *Pyrenophora* only, or neither. Age-related resistance is important for both of these pathogens. The facultative saprophyte *Pyrenophora* was generally a stronger competitor than the biotrophic *Puccinia*, even experiencing facilitation from the presence of *Puccinia* when *Pyrenophora* had the advantage of earlier inoculation. Both pathogen species produced the most spores when they were introduced before the competing species and more spores when introduced simultaneously compared to after the competitor. The pre-interactive niche of *Puccinia* was larger than the post-interactive niche and sporulation by *Puccinia* was substantially reduced in environments in which *Pyrenophora* had high sporulation rates. The pre-interactive niche of *Pyrenophora* was similar to the post-interactive niche and *Pyrenophora* had proportionally lower reductions in sporulation due to interspecific competition in the pre-interactive niche.

**Keywords** Facultative saprophytes · Impact niche · Obligate parasites · Requirement niche · Rust fungi

F. A. Al-Naimi · K. A. Garrett (✉) · W. W. Bockus
Department of Plant Pathology,
4024 Throckmorton Plant Sciences Center,
Kansas State University,
Manhattan, KS 66506-5502, USA
E-mail: kgarrett@ksu.edu
Tel.: +1-785-5321370
Fax: +1-785-5325392

*Present address:* F. A. Al-Naimi
Biology Department, University of Qatar,
P.O. Box 2713, Doha, Qatar

## Introduction

Competition has been studied most commonly in plants and animals, but pathogens represent a novel subject for competition studies and the definition of their ecological niches includes some unique features (da Luz and Bergstrom 1987; Adee et al. 1990; Newton et al. 1996). While a conducive abiotic environment is also necessary for successful infection by a pathogen, especially in terms of meteorological conditions such as the presence of leaf surface moisture (Huber and Gillespie 1992), a major determinant of the niche of a plant pathogen is the host genotype. Leibold (1995) has discussed the many definitions of the ecological niche and the controversy around their appropriate application. Hutchinson (1957) defined the "fundamental niche" or pre-interactive niche of a species as the set of biological and abiotic parameter values that would allow the species continued existence in the absence of competitors. For a plant pathogen, the fundamental niche is determined by the intersection of a conducive environment and susceptible host tissue. Under Leibold's (1995) definition, this could also be termed the "requirement niche". Leibold's (1995) "impact niche" describes the per capita effects of a species on its environment (Elton 1927), as opposed to the environmental requirements of a species. If this impact is measured via the effects on competing pathogen species, plant pathogens might have such effects by sequestering nutrients as well as by inducing resistance in the host (Sticher et al. 1997; Durrant and Dong 2004). Hutchinson's (1957) "realized niche", or post-interactive niche, is the set of parameters describing the environment in which an organism persists when its competitors are present, generally a smaller region in the parameter space than the fundamental or pre-interactive niche (Keddy 1989; Pulliam 2000).

The susceptibility of host tissue may vary not only with genotype but also with the age of plant tissues in the same plant. Age-related resistance has been reported for many pathogens (Heath 1996; Kolmer 1996), in

terms of both plant age and individual leaf age, and the mechanisms for this varying resistance are becoming better understood (e.g., Kus et al. 2002). Thus, for many pathogens and herbivores, an individual plant may be composed of several habitats. Microorganisms on leaf surfaces may inhabit distinct niches, as suggested, for example, by higher rates of coexistence among bacteria with more distinct profiles of carbon source utilization (Wilson and Lindow 1994). While spatial segregation may reduce competitive effects, herbivores occupying different parts of the plant may still compete by exploiting a common resource such as phloem sap. Insect herbivores also may tend to attack young and vigorous plants more frequently than old and mature plants (Price 1991). Interactions among herbivores appear frequently to be mediated through the chemistry and physiology of the host plants; the outcomes of herbivore–herbivore interactions are likely to depend on the level of host resistance to each herbivore species (Moran and Whitham 1990).

Plant host susceptibility in one plant part compared to another may differ among pathogens, in part, because of the degree to which a pathogen species is an obligate biotroph. At one extreme are the rust fungi, common pathogens in ecosystems such as tallgrass prairie (Mitchell et al. 2002; Morgan 2003; K. A. Garrett, in preparation) that tend to have quite narrow host ranges with a small number of genes determining virulence within their host species (e.g., Thrall and Burdon 2004). Rust fungi are dependent on living host tissue and generally have most success infecting plant parts which are otherwise healthy (e.g., Pretorius et al. 1988). *Puccinia triticina* Roberge ex Desmaz. f. sp. *tritici* (Eriks. and E Henn.) D. M. Henderson (= *Puccinia triticina* Eriks), causal agent of wheat leaf rust, is less common on older leaves within the canopy (Roelfs et al. 1992). By contrast, the fungus *Pyrenophora tritici-repentis* (Died) Drechs. (anamorph = *Drechslera tritici-repentis* (Died.) Shoemaker), causal agent of tan spot in wheat and several wild grass species (Krupinsky 1992; C. M. Cox, in preparation), can survive as a saprophyte and produces significantly more conidia on older leaves than younger leaves (Riaz et al. 1991; Fernandez et al. 2002) because younger leaves are more resistant to the fungus (Raymond et al. 1985; Cox and Hosford 1987).

We used these two pathogens in a study of competition and niche differentiation. Based on the characteristics of these pathogens, we constructed three main hypotheses. First, we hypothesized that *P. tritici-repentis* (designated here as *Pyrenophora*) would be a stronger competitor than *P. triticina* (designated here as *Puccinia*), in the sense that reproduction in *Pyrenophora* would be less influenced by the presence of *Puccinia* than reproduction in *Puccinia* would be by *Pyrenophora*. At least two host-specific toxins produced by *Pyrenophora* have been isolated and characterized. Ptr ToxA (formerly known as Ptr-necrosis toxin) is responsible for inducing necrosis in sensitive genotypes (Lamari and Bernier 1989). Ptr ToxB (formerly known as Ptr chlorosis toxin) induces chlorosis on sensitive genotypes (Orolaza et al. 1995). Because *Puccinia* is an obligate biotroph, tissue that has been damaged by these toxins may be less easily utilized by *Puccinia*. *Pyrenophora* has also proven a stronger competitor than the fungus *Septoria nodorum* (Adee et al. 1990), but not the fungus *Cochliobolus sativus* (da Luz and Bergstrom 1987). Second, we hypothesized that earlier infection will give a competitive advantage to either pathogen, consistent with previous studies of competition between fungal pathogens (da Luz and Bergstrom 1987; Adee et al. 1990). Third, we hypothesized that pathogens will be less affected by competition and have a greater effect on competitors in their ''fundamental niches'', or those host environments that support the highest level of reproduction in the absence of a competitor. For our system, we defined niches in terms of leaf age and host genotype. This third hypothesis is based on the idea that areas in which a pathogen reproduces more may be areas in which it is sequestering more of the resources and also in which it has had a larger impact on host tissue.

## Materials and methods

In our study, wheat seedlings were inoculated with *Pyrenophora* and *Puccinia*, singly and in combination, with infection by the two species occurring in different orders. To determine the effect of interspecific competition on uredinial formation and sporulation, spore production for both pathogens was quantified on different wheat genotypes and on leaves of different ages. Spore production in the absence of a competitor was used to define the ''fundamental niche'', that is the plant genotype and leaf ages in which the greatest number of spores was produced. These results provide information on the relative importance of different host genotypes and leaf ages for reproduction. As successful infection requires the use of a mist chamber to produce higher humidity, we also performed an experiment to partition the effects on reproduction in *Pyrenophora* of later infection by *Puccinia*, apart from the effects of the additional period of high humidity required for the second inoculation.

### Wheat genotypes and growth conditions

Three winter wheat genotypes were selected to provide a genotype supporting high reproduction in *Pyrenophora* only (2145), a genotype supporting high reproduction in *Puccinia* only (Jagger), and a genotype supporting high reproduction in both (TAM 107). In both experiments, eight seeds of a single genotype were planted in 12.2-cm-diameter plastic pots filled with a steamed mixture of silty clay loam soil and sand (1:1 by volume) and thinned to five seedlings per pot after establishment. Pots were fertilized weekly with 20–20–20 (N–P–K) water-soluble

fertilizer in the greenhouse (20 ± 5°C) for four weeks before being inoculated.

### Inoculation with *Pyrenophora*

A virulent isolate of *Pyrenophora* (race 1) was collected from an infected winter wheat field in Kansas. Conidia used for inoculation were obtained by transferring mycelial plugs from slants to V8 agar (Raymond et al. 1985), which were kept in the dark at room temperature (20 ± 2°C) for 5 days. The aerial mycelium was knocked down with a sterile, bent glass rod, and plates were placed 28 cm below four cool-white fluorescent tubes (about 35 $\mu$ mol $m^{-2}$ $s^{-1}$) for 18 h at 20 ± 2°C for conidiophore formation and then incubated in the dark for 24 h at 4°C for conidiation (Riaz et al. 1991). Conidia were harvested from petri plates by adding distilled water to the surface of the plates and dislodging conidia with a sterile, bent metal rod, and then filtered through two layers of cheesecloth. Concentrations were adjusted by adding distilled water to obtain $2\times10^3$ conidia per milliliter as a "low" concentration and $1\times10^4$ conidia per milliliter as a "high" concentration. The concentration of spores used in "high" and "low" concentrations for *Pyrenophora* and *Puccinia* was determined in preliminary tests to confirm patterns of susceptibility, growth rate, and inoculum concentrations that produced clear differences in rate of infection between high and low inoculum concentrations. Experimental plants were inoculated with conidial suspensions using an atomizer (Model 152, Devilbis Co., Somerset, PA, USA) operated at 69-kPa pressure. Plants were sprayed at a rate of 1 ml of conidial suspension per plant and dried for 30 min to allow spores to adhere to the leaves. Plants were then placed in a mist chamber for 48 h to allow infection. Two centrifugal atomizing humidifiers (Percival Manufacturing Co., Boone, IA, USA) inside the mist chamber were electrically controlled to operate for 1 min and 15 s at 10-min intervals, which produced continual leaf wetness.

### Inoculation with *Puccinia*

Urediniospores of common race MCDL of *Puccinia* were collected from wheat genotype TAM 107 in the greenhouse. A suspension of 2.0 mg of urediniospores in 1.0 ml of Soltrol 170 oil (Chevron Phillips Chemical Co., Houston, TX, USA) per plant was used to produce a "high" infection rate and 1.0 mg was used to produce a "low" infection rate. (Soltrol is commonly used in inoculations to help spores adhere to the leaf surface. Since we wanted to be able to assess the effects on *Pyrenophora* of *Puccinia* applied using Soltrol, we performed a preliminary experiment to determine whether there was a direct effect of Soltrol on *Pyrenophora* in the absence of *Puccinia*. We found no evidence ($P = 0.6$) for such an effect (S. P. Dendy, K. A. Garrett, and W. W. Bockus, unpublished)). Plants were inoculated using an air compressor operated at 100 psi. A uniform distribution of spores was obtained by spraying in an inoculation cabinet equipped with a rotating table. The inoculated plants were air dried for 1 h to ensure full evaporation of oil from plant surfaces and then incubated in the mist chamber for 48 h. For the "simultaneous inoculation" treatment, plants were first inoculated with a conidial suspension of *Pyrenophora*, and after 30 min they were inoculated with a spore suspension of *Puccinia*. For all treatments, plants were removed from the mist chamber after 48 h and placed on greenhouse benches until leaves were harvested on day 11. Pots were placed in shallow pans and watered from below to prevent free water from touching the leaves (Riaz et al. 1991).

### Assessment of sporulation on leaves

On the eleventh day after inoculation, the second (designated "older") and third (designated "younger") oldest leaves from each of the five plants in each pot were harvested. Leaves from one pot were cut into four to six pieces and placed on filter paper moistened with 2.5 ml of distilled water in a plastic petri dish, which was then sealed with parafilm with six holes to provide aeration. The petri dishes were placed approximately 30 cm below cool-white fluorescent lights. The temperature regime in the growth chamber was 25°C during a 12-h photoperiod and 16°C during a 12-h dark period for 5 days. After this 5-day period, all segments of each leaf were cut into 1-cm pieces with scissors and blended in 15 ml of distilled water in a small blender (model 91-264, Dynamic Corporation of America, New Harford, CN, USA) for 15 s. After blending, three to four subsamples were taken using a pipette and placed on a grid designed for counting conidia (six units of 0.01 ml each) (Riaz et al. 1991). The urediniospore counts were made using a hemacytometer. Conidia and urediniospores are easily distinguished by size and shape.

### Experimental design I: competition between *Pyrenophora* and *Puccinia*

In our first experiment, we applied eight treatments (Table 1) to each of the three wheat genotypes. In order to consider the effects of the order of infection, we included four types of "controls" (treatments 1, 2, 7, and 8): each pathogen infecting alone at both the earlier and later inoculation dates. We also included a low inoculation rate (treatment 3) to test whether the effects of competition were different as the inoculation rate varied. The pots were arranged in a randomized complete block design with four blocks. Blocks consisting of 24 pots were planted at approximately weekly intervals, so the complete set of treatments was evaluated at four points in time. As young and old leaves were collected from

within the same pot, leaf age was treated as applied to subplots within a split plot design. The position of the pots within a block was re-randomized once a week to minimize potential effects of position on the bench.

### Experimental design II: *Puccinia* effect versus mist chamber effect

This additional study was performed to help partition the effect of additional time in the mist chamber and the effect of the competitor *Puccinia* on *Pyrenophora*. In this experiment, we applied the additional 2 days of mist chamber time both with and without added *Puccinia* (Table 1). The same wheat genotypes were used as in our first experiment and each genotype received each treatment (Table 1). Inoculation with *Pyrenophora* (treatments A, B and C), *Puccinia* (treatment B) and handling of pots were as in the above experiment. The pots were arranged in a randomized complete block design with nine blocks each consisting of nine pots.

**Table 1** Inoculation treatments used in a study of competition between *Pyrenophora tritici-repentis* ("*Pyrenophora*") and *Puccinia triticina* ("*Puccinia*")

| Treatment | 28-day-old plants | 31-day-old plants |
|---|---|---|
| 1 | | *Pyrenophora* alone (High[a]) |
| 2 | | *Puccinia* alone (High[b]) |
| 3 | | *Pyrenophora* and *Puccinia* simultaneously (Low[c]) |
| 4 | | *Pyrenophora* and *Puccinia* simultaneously (High) |
| 5 | *Puccinia* first (High) | *Pyrenophora* after *Puccinia* (High) |
| 6 | *Pyrenophora* first (High) | *Puccinia* after *Pyrenophora* (High) |
| 7 | *Puccinia* alone (High) | |
| 8 | *Pyrenophora* alone (High) | |
| A | *Pyrenophora* (High) | No mist chamber or *Puccinia* |
| B | *Pyrenophora* (High) | *Puccinia* (High; including required[d] mist chamber time for 48 hr) |
| C | *Pyrenophora* (High) | Extra 48 h in mist chamber without *Puccinia* |

The first eight treatments were used in the main study. Treatments A, B, and C were used in a study to partition the effect on *Pyrenophora* of additional mist chamber time versus the effect of inoculation with *Puccinia*

[a] "High" concentration of *Pyrenophora* inoculum was $10^4$ propagules/ml

[b] "High" concentration of *Puccinia* inoculum was 2 mg of spores/ml of Soltrol oil

[c] "Low" concentration of *Pyrenophora* inoculum was $2\times10^3$ propagules/ml and low inoculum of *Puccinia* was 1 mg spores/ml Soltrol oil

[d] Time in an environment producing leaf wetness is required for successful infection by *Pyrenophora* and *Puccinia*

### Statistical analysis

Planned linear contrasts were performed on log spore counts in an analysis of variance in SAS (SAS Institute, Cary, NC, USA). Contrasts were used to determine the influence of leaf age, plant age, wheat genotype, and timing of inoculation on the spore production by both pathogens. Contrasts were straightforward for tests such as whether there was an overall effect of leaf age on sporulation for *Puccinia* or *Pyrenophora* or whether the presence of the competing species had an effect on sporulation for *Puccinia* or *Pyrenophora*. Other tests, such as for the influence of timing of inoculation on the effect of competition, were more complicated. As an example using the eight treatments in the order listed in Table 1 (expressed in terms of whole plot effects), the contrast to test for the influence of timing of inoculation on the competition effect was constructed for *Pyrenophora* as a 2-df contrast with the first part of the contrast being (1, 0, 0, −1, 0, 1, 0, −1) and the second part being (1, 0, 0, 0, −1, 1, 0, −1). That is, the first part compares inoculation with *Pyrenophora* first to simultaneous inoculation by comparing the difference between treatments 1 and 4 (effect of competition with simultaneous inoculation) and the difference between treatments 6 and 8 (effect of competition with *Pyrenophora* inoculated first). The second part compares inoculation with *Pyrenophora* first to inoculation with *Puccinia* first by comparing the difference between treatments 1 and 5 (effect of competition with *Puccinia* inoculated first) and the difference between treatments 6 and 8 (effect of competition with *Pyrenophora* inoculated first). These contrasts compared the absolute effects of treatments. We also performed contrasts to compare relative or proportional effects by analyzing ratios of the response with competition divided by the response without competition. For example, in the first experiment the *Pyrenophora* response in treatment 4 was divided by the response in treatment 1, the response in treatment 5 was divided by the response in treatment 1, and the response in treatment 6 was divided by the response in treatment 8.

## Results

### Spore production in the absence of competition

Spore production by both pathogens was significantly affected by leaf age and host genotype and a significant interaction was observed between wheat genotype and leaf age for both (Table 2, Fig. 1). The profile of results for both pathogens was similar for plants inoculated 13 days before spore collection or 16 days before collection (data not shown). The most important genotype-leaf age niches for reproduction of *Puccinia*, those that we defined as the fundamental, or pre-interactive, niche for *Puccinia*, were old and young

**Table 2** Factors influencing conidial production by *Pyrenophora tritici-repentis* (“*Pyrenophora*”) and urediniospore production in *Puccinia triticina* (“*Puccinia*”) when each is in the absence of competition by the other species

| Effect | df | *P*-value from AOV | |
|---|---|---|---|
| | | *Pyrenophora* | *Puccinia* |
| Main effects | | | |
| Plant age[a] | 1 | < 0.01 | < 0.01 |
| Leaf age[b] | 1 | < 0.01 | < 0.01 |
| Wheat genotype[c] | 2 | < 0.01 | < 0.01 |
| Two-way interactions | | | |
| Plant age × Leaf age | 1 | 0.79 | 0.35 |
| Wheat genotype × Plant age | 2 | 0.04 | 0.99 |
| Plant age effect, wheat genotype = TAM 107 | 1 | 0.24 | |
| Plant age effect, wheat genotype = 2145 | 1 | 0.01 | |
| Plant age effect, wheat genotype = Jagger | 1 | < 0.01 | |
| Leaf age × Wheat genotype | 2 | < 0.01 | 0. 01 |
| Wheat genotype effect, Leaf age = Young | 2 | < 0.01 | < 0.01 |
| Wheat genotype effect, Leaf age = Old | 2 | < 0.01 | < 0.01 |
| Three-way interaction | | | |
| Plant age × Leaf age × Wheat genotype | 2 | 0.77 | 0.04[d] |

[a] Plant age effect: comparison of spore production for younger plants (28-day-old plants inoculated 16 days before spore collection) and older plants (31-day-old plants inoculated 13 days before spore collection)

[b] Leaf age effect: comparison of spore production for younger (third) leaves compared to older (second) leaves

[c] Wheat genotype: comparison of spore production for three wheat genotypes: TAM 107, 2145, and Jagger

[d] The leaf age effect was also significant ($P < 0.01$) for each combination of plant age and wheat genotype

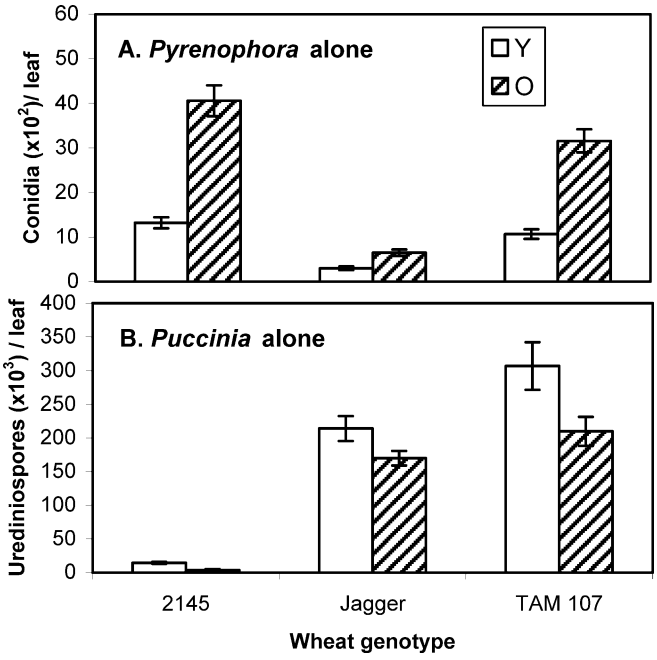


**Fig. 1** Conidia production by *Pyrenophora tritici-repentis* (“*Pyrenophora*”) when *Puccinia triticina* (“*Puccinia*”) is absent as a competitor (**a**) and urediniospore production by *Puccinia* when *Pyrenophora* is absent (**b**). Production was observed 16 days after inoculation for three wheat genotypes with varying levels of resistance for two leaf ages (*Y* young and *O* old). Bars indicate ± one standard error

leaves of TAM 107 and Jagger, with young leaves being somewhat more important (Fig. 1b). The most important genotype-leaf age niches for reproduction of *Pyrenophora*, those that we defined as the fundamental niche for *Pyrenophora*, were old leaves of 2145 and TAM 107 (Fig. 1a).

## Urediniospore production by *Puccinia* with *Pyrenophora* as a competitor

*Puccinia* was strongly affected by competition by *Pyrenophora* and the magnitude of the effect varied significantly with host genotype and timing of inoculation (Fig. 2, Table 2). Leaf age did not significantly influence the magnitude of the competition effect as a main effect, but its interactions with host genotype and timing of inoculation were significant (Fig. 2, Table 2). The highest level of urediniospore production was for *Puccinia* inoculated 3 days before *Pyrenophora* (Fig. 2b), the second highest for *Puccinia* inoculated simultaneously with *Pyrenophora* (Fig. 2a), and the least for *Puccinia* inoculated after *Pyrenophora* (Fig. 2c); that is, reproduction by *Puccinia* was greater when it was inoculated earlier relative to *Pyrenophora*. When the spore concentration for inoculation was reduced by 80% for *Pyrenophora* and by 50% for *Puccinia*, spore production in *Puccinia* was reduced by 57% to 85%, depending on the wheat genotype and leaf age (data not shown). The most important genotype-leaf age niches for reproduction, in the absence of competition by *Pyrenophora*, were young leaves of TAM 107 and Jagger (Fig. 1b), but this “fundamental niche” did not consistently exhibit the least reduction in sporulation with the addition of *Pyrenophora* (Fig. 2). While TAM 107 had the highest reproduction in the absence of competition (Fig. 1b), Jagger had the smallest reduction due to competition (Fig. 2). There was a tendency for the younger leaves, which support somewhat more reproduction in the absence of competition (Fig. 1b), to have a smaller reduction due to competition, though this trend did not hold when *Puccinia* was introduced after *Pyrenophora* (Fig. 2).

## Conidia production by *Pyrenophora* with *Puccinia* as a competitor

*Pyrenophora* was strongly affected by competition with *Puccinia* in only a few genotype-leaf age niches; the magnitude of the effect varied significantly with host genotype and timing of inoculation (Fig. 3, Table 3).

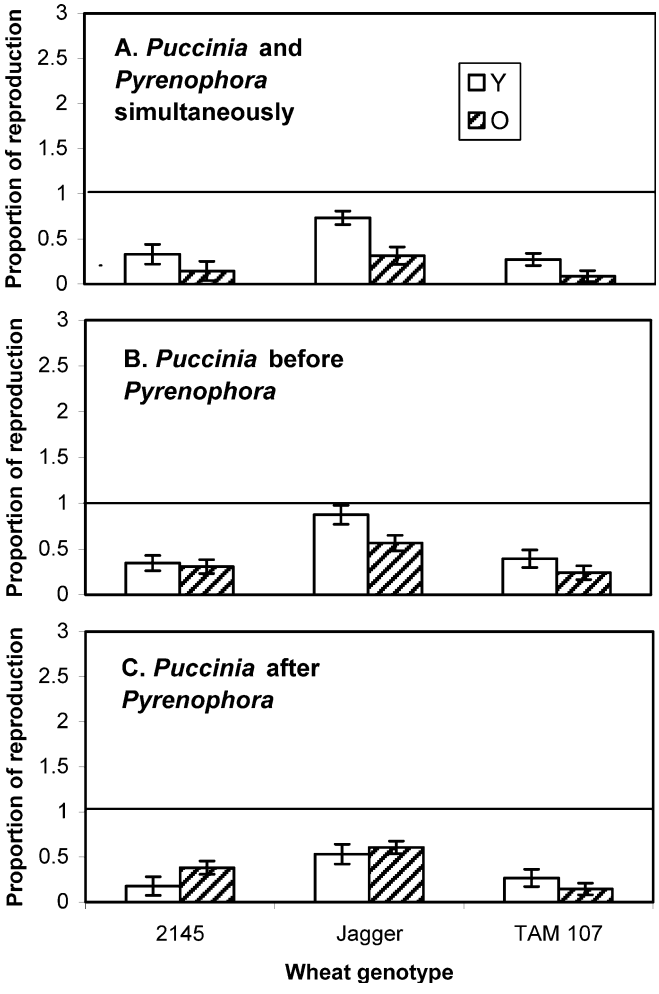


**Fig. 2** Proportion of urediniospore production by *Puccinia triticina* ("*Puccinia*") when *Pyrenophora tritici-repentis* ("*Pyrenophora*") was present as a competitor compared to when *Pyrenophora* was absent. **a** *Puccinia* was inoculated simultaneously with *Pyrenophora* and this was compared to the result when 28-day-old plants were inoculated with *Puccinia* alone. **b** *Puccinia* was inoculated before *Pyrenophora* and this was compared to the result when 28-day-old plants were inoculated by *Puccinia* alone. **c** *Puccinia* was inoculated after *Pyrenophora* for the competition treatment and this was compared to the result when 31-day-old plants were inoculated by *Puccinia* alone. *Y* younger leaves and *O* older leaves. Bars indicate ± one standard error and the horizontal line at "Proportion of reproduction" = 1 indicates the result for no effect of competition

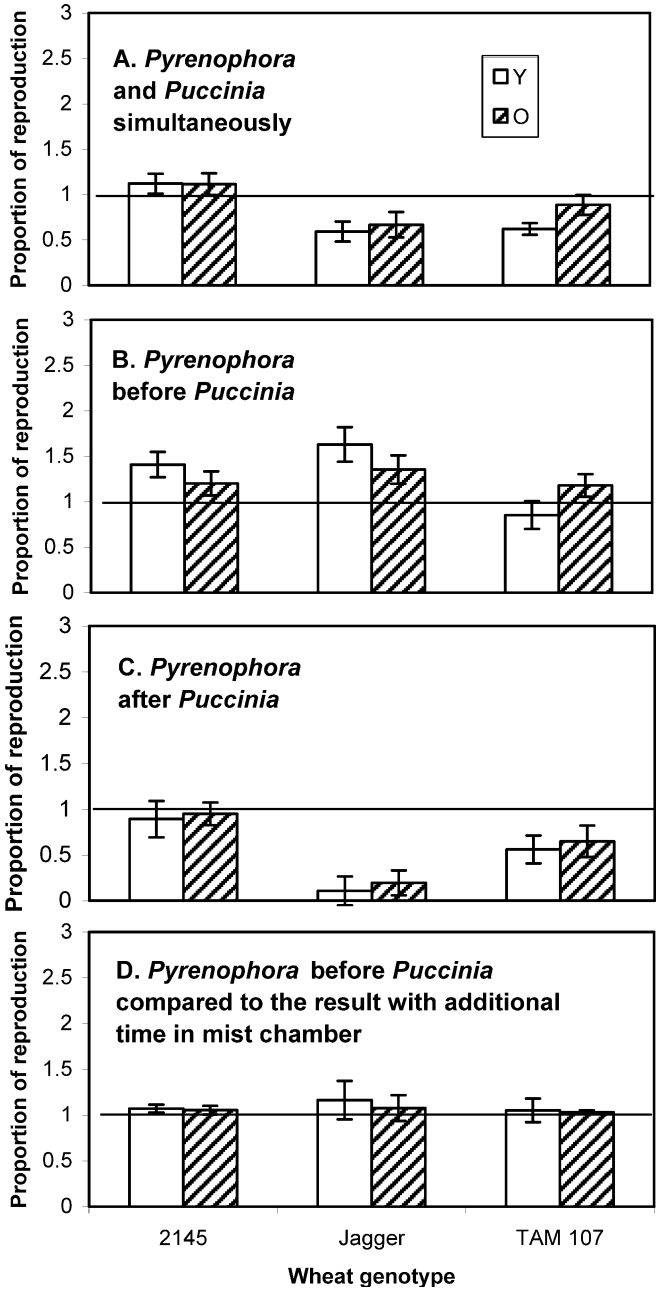


**Fig. 3** Proportion of conidial production by *Pyrenophora tritici-repentis* ("*Pyrenophora*") when *Puccinia triticina* ("*Puccinia*") was present as a competitor compared to when *Puccinia* was absent. **a** *Pyrenophora* was inoculated simultaneously with *Puccinia* and this was compared to the result when 31-day-old plants were inoculated with *Pyrenophora* alone. **b** *Pyrenophora* was inoculated before *Puccinia* and this was compared to the result when 28-day-old plants were inoculated with *Pyrenophora* alone. **c** *Pyrenophora* was inoculated after *Puccinia* and this was compared to the result when 31-day-old plants were inoculated with *Pyrenophora* alone. **d** Ratio of conidial production by *Pyrenophora* when *Puccinia* was inoculated after *Pyrenophora* compared to the results when *Puccinia* was not inoculated but the plants still received additional mist chamber time. *Y* younger leaves and *O* older leaves. Bars indicate ± one standard error and the horizontal line at "Proportion of reproduction" = 1 indicates the result for no effect of competition

When *Pyrenophora* was introduced first, later infection by *Puccinia* actually produced an increase in conidia production and this apparent facilitation was observed for all genotype-leaf age combinations except young leaves on TAM 107 (Fig. 3). The highest level of spore production was for *Pyrenophora* inoculated three days before *Puccinia* (Fig. 3b), the second highest for *Pyrenophora* inoculated simultaneously with *Puccinia* (Fig. 3a), and the least for *Pyrenophora* inoculated after *Puccinia* (Fig. 3c); that is, conidia production by *Pyrenophora* was greater when it was inoculated earlier relative to *Puccinia*. When the spore concentration for

**Table 3** Factors influencing the absolute[a] magnitude of a competition effect on spore production in *Pyrenophora tritici-repentis* (''*Pyrenophora*'') and *Puccinia triticina* (''*Puccinia*'') when the other species is present as the competitor

| Effect | df | *P*-value from AOV | |
|---|---|---|---|
| | | *Pyrenophora* | *Puccinia* |
| Overall effect of competition[b] | 1 | < 0.01 | < 0.01 |
| Leaf age | 1 | 0.11 | 0.55 |
| Wheat genotype | 2 | < 0.01 | < 0.01 |
| Timing of inoculation | 2 | < 0.01 | < 0.01 |
| Leaf age × Wheat genotype | 2 | 0.10* | < 0.01* |
| Leaf age × Timing of inoculation | 2 | 0.26 | < 0.01 |
| Wheat genotype × Timing of inoculation | 4 | < 0.01 | 0.51* |
| Wheat genotype × Timing of inoculation × Leaf age | 4 | 0.42 | 0.03 |
| Inoculum concentration[c] | | < 0.01 | < 0.01 |

[a] Relative effects were also analyzed by considering the ratio of the response without the competitor over the response with the competitor. Cases for which the *P*-values for the test for the absolute effect and the test of the relative effect were different (in the sense of being on the other size of 0.05) are indicated with an *asterisk*

[b] Overall effect of competition: the effect of competition on a given pathogen was estimated by comparison of spore production for plants inoculated only with this pathogen to spore production for plants inoculated at the same time with both pathogens. This method of estimating competition was used in all the following tests

[c] Influence of inoculum concentration on effect of competition: Estimate of how the effect of competition varies depending on whether pathogen inoculum is at a higher level ($10^4$/ml of *Pyrenophora* spores and 2 mg/ml Soltrol oil of *Puccinia*) or a lower level ($2\times10^3$/ml of *Pyrenophora* and 1 mg/ml Soltrol oil of *Puccinia*)

inoculation was reduced by 80% for *Pyrenophora* and by 50% for *Puccinia*, spore production in *Pyrenophora* was reduced by approximately 50% (data not shown). The most important genotype-leaf age niches for reproduction in the absence of competition by *Puccinia* were old and young leaves of 2145 and TAM 107 (Fig. 1a) and this ''fundamental niche'' showed the least reduction in sporulation with the addition of *Puccinia* except in the case of inoculation of *Pyrenophora* before *Puccinia* (Fig. 3). In the absence of competition, the older leaves of these two genotypes showed substantially higher reproduction than the younger leaves (Fig. 1a), but loss of reproduction due to *Puccinia* was not substantially lower for older leaves (Fig. 3).

In our second experiment, we wanted to determine what part of the apparent benefit to *Pyrenophora* from *Puccinia* was due to the additional mist chamber time versus the effect of *Puccinia* itself. In the test of mist chamber effects, the highest level of spore production was for *Pyrenophora* inoculated before *Puccinia*, the second highest was for *Pyrenophora* with additional mist chamber time but no inoculation with *Puccinia*, and the least was for *Pyrenophora* with no additional mist chamber time and no inoculation with *Puccinia* (Fig. 3d, and additional data not shown). Results for the effect of *Puccinia* compared to no additional mist were essentially the same as in the first experiment (Fig. 3b), except that the reproduction on young Jagger and young TAM 107 leaves was somewhat higher in the second experiment (data not shown). The effect of the additional mist was large, but even when accounting for it, the presence of *Puccinia* still increased reproduction in *Pyrenophora* when inoculated later (Fig. 3d, $P < 0.01$ overall and for each genotype individually).

## Discussion

Our first hypothesis was that the facultative saprophyte *Pyrenophora* would be a stronger competitor than the obligate biotroph *Puccinia*, in the sense that the presence of the competing species would have a smaller effect on production of conidia by *Pyrenophora* than on urediniospore production by *Puccinia*. This was clearly the case (Figs. 2, 3). *Pyrenophora* had proven a stronger competitor than *Septoria nodorum* in previous experiments (Adee et al. 1990), though it was strongly negatively affected by *Cochliobolus sativus* (da Luz and Bergstrom 1987). In our study, even accounting for the effect of additional time in a mist chamber in our second experiment, when *Puccinia* was introduced after *Pyrenophora* its presence actually increased production of conidia in *Pyrenophora*. This indicates that the observed facilitation was not an experimental artifact. Probably the leaf damage caused by *Puccinia* benefited *Pyrenophora*, which is adapted to utilizing senescing tissue. While facilitation of one plant pathogen by another has rarely been described, it is known to occur, for example, between nematodes and root pathogens. Many plant pathogenic bacteria enter their host plant through natural or artificial wounds caused by plant-parasitic nematodes (Bartels et al. 1998). The rupture of root cells by nematodes and the availability of necrotic tissues aid in the rapid establishment of bacteria in host tissue and favor their subsequent invasion of healthy tissues. Wilt diseases caused by *Pseudomonas* (= *Ralstonia*) *solanacearum* in various hosts have been shown to increase in incidence and severity as a result of nematode-incited wounding (Bartels et al. 1998). Likewise, herbivory by one species may make more resources available to another species. For example, grazing by mammalian herbivores may make more grass accessible to other species and stimulate grass regrowth, thereby enhancing the nutritional quality of forage for other species (Hobbs et al. 1996; Arsenault and Owen-Smith 2002).

Our second hypothesis was that earlier arrival at the host would give a competitive advantage and it was the case that earlier inoculation gave a relative advantage to both species (Figs. 2, 3). For *Puccinia*, this played out as lower reductions in sporulation due to competition when it was established prior to introduction of *Pyrenophora*. For *Pyrenophora* on 2145 and TAM 107, the two genotypes *Pyrenophora* is well adapted to use, inoculation of *Pyrenophora* prior to *Puccinia* actually

resulted in higher production of conidia than in the absence of *Puccinia*. On Jagger, which is resistant to *Pyrenophora* but susceptible to *Puccinia*, the effect of timing of inoculation on *Pyrenophora* was particularly striking. The advantage from earlier arrival at a host probably holds true for most pathogens that utilize some of the same resources. In contrast, pathogens that depend on prior damage to the physical defenses of plants, such as fungi that infect following nematode damage, may generally be more successful if introduced after other species have affected the host. It appears that *Pyrenophora* can benefit from the damage caused by *Puccinia*, especially if *Puccinia* is introduced when *Pyrenophora* is already established, but conidia production in *Pyrenophora* is decreased if *Puccinia* is established first.

Our third hypothesis was that the pathogens would be less affected by competition and would have a greater effect on the competing species in their fundamental niches. For *Pyrenophora*, it was true for most cases that the genotypes that composed the fundamental niche, in the sense of producing the most conidia when *Puccinia* was absent, also had lower percentage reductions in reproduction when *Puccinia* was also inoculated (Figs. 1, 3). This was not the case when *Pyrenophora* was inoculated before *Puccinia*, however, perhaps because later damage by *Puccinia* was particularly important for infection by *Pyrenophora* in the *Pyrenophora*-resistant Jagger genotype. There was some tendency for the older leaves, most important for *Pyrenophora* reproduction, to experience less reduction due to competition than the younger leaves, but this was not a strong effect. For *Puccinia*, reduction in spore production seemed to be equally affected by inability of *Puccinia* to use a genotype (2145) and ability of *Pyrenophora* to use a genotype (TAM 107). So, even though TAM 107 had the greatest reproduction by *Puccinia* in the absence of competition, it had a strong reduction due to competition by *Pyrenophora*. Thus, for *Puccinia* there was a shift from the ''pre-interactive'' niche composed of both Jagger and TAM 107 to a ''post-interactive'' niche of Jagger. We can also consider the ''impact'' niche defined by Leibold (1995), the per capita effects of a species on its environment. In our case, we can evaluate this in terms of the per inoculation spore effects on the plant host environment via the response of the competing species. This is an indirect measure of the effect on host nutrient availability and potential effects on host resistance responses. The impact niche of *Pyrenophora*, measured via reductions in *Puccinia*, is essentially the same, in terms of genotype, as the fundamental niche of *Pyrenophora*. The impact niche of *Puccinia*, measured via reductions in *Pyrenophora*, is reduced principally to Jagger, though there was also some reduction in TAM 107. To summarize, *Pyrenophora* was most successful in competition on the genotypes to which it was best adapted in the absence of competition. *Puccinia* was most successful in competition on the genotype to which *Pyrenophora* was least adapted.

Our experimental results, though limited to relatively young plants in a greenhouse setting, probably also bear on interactions between these two pathogens in the field. For older plants, the differences between older and younger leaves may be more pronounced, increasing the chance of coexistence of the two pathogens through niche differentiation. As discussed, abiotic environmental factors will be critical in determining whether infection occurs in any of the niches defined by plant genotype and leaf age. The abiotic environment will also determine how many spores must be produced in a plant age-genotype combination for the pathogen to have a net reproductive gain; in a harsh abiotic environment only the age-genotype combinations to which a pathogen is best adapted may be niches, in the sense of producing enough new infections to maintain the population, while in a more conducive abiotic environment even the most resistant age-genotype combinations may be niches. In the field, dispersal mechanisms will also be a factor in determining the relative success of pathogens. Rusts are adapted for long-distance dispersal of urediniospores (Eversmeyer and Kramer 2000), but some rust species require alternate hosts and the basidiospores produced on these hosts may not disperse as widely. At this stage in their life cycle, rusts may also be subject to an Allee effect (destabilizing density-dependent reproduction at low numbers) if competition drives down their population size, just as for the fungus *Tilletia indica* (Garrett and Bowden 2002).

With these caveats in mind, we can also consider how the results from this experiment might inform studies of host-frequency dependent effects on pathogen populations. Host frequency can have important effects on pathogens in natural plant communities (Mitchell et al. 2002) and can be manipulated in agricultural systems as a tool for disease management (Garrett and Mundt 1999; Mundt 2002). In a field experiment (Cox et al. 2004), *Puccinia* and *Pyrenophora* were experimentally introduced to host populations of different proportions of the genotypes 2145 (*Puccinia* resistant) and Jagger (*Pyrenophora* resistant). Disease severity decreased on the susceptible genotype as its frequency decreased and this decrease was greater for *Puccinia*, probably because of *Puccinia*'s more limited lesion size and wider dispersal of inoculum (Garrett and Mundt 1999). Based on the experiment we report here, we can speculate on how host frequency dependence would be affected if host populations included a genotype susceptible to both a rust fungus like *Puccinia* and a facultative saprophyte like *Pyrenophora*. The overall disease level would increase because of susceptibility to both pathogens in this genotype. When *Pyrenophora* is present, populations composed of the globally susceptible genotype combined with the *Puccinia*-resistant genotype would support reduced sporulation by *Puccinia* both because of the resistance and because of competition by *Pyrenophora* on the globally susceptible genotype. But *Pyrenophora* populations in such a host population could utilize both host genotypes and could even benefit from the presence of *Puccinia*.

**Acknowledgements** We thank S. P. Dendy for assistance with the experiments and R. L. Bowden, A. Jumpponen, and Oecologia reviewers for comments that improved this work. This work was supported in part by NSF grant DEB-0130692, NSF grant EPS-0236913 with matching funds from the Kansas Technology Enterprise Corporation, NSF grant EPS-9874732 with matching support from the State of Kansas, the NSF Long-Term Ecological Research Program at Konza Prairie, and USDA grant 2002-34103-11746. This is Kansas State Experiment Station Contribution No. 05-29-J. The experiments reported here were performed in the U.S.A. and comply with the current laws of the U.S.A.

## References

Adee SR, Pfender WF, Harnett DC (1990) Competition between *Pyrenophora tritici-repentis* and *Septoria nodorum* in the wheat leaf as measured with de Wit replacement series. Phytopathology 80:1177–1182

Arsenault R, Owen-Smith N (2002) Facilitation versus competition in grazing herbivore assemblages. Oikos 97:313–318

Bartels JM, Barker KR, Pederson AG, Windham LG (1998) Plant nematode interactions, Madison

Cox DJ, Hosford RM Jr (1987) Resistant winter wheats compared at differing growth stages and leaf positions for tan spot severity. Plant Dis 71:883–886

Cox CM, Garrett KA, Bowden RL, Fritz AK, Dendy SP, Heer WF (2004) Cultivar mixtures for the simultaneous management of multiple diseases: tan spot and leaf rust of wheat. Phytopathology 94:961–969

Durrant WE, Dong X (2004) Systemic acquired resistance. Annu Rev Phytopathol 42:185–209

Elton C (1927) Animal ecology. Sidgwick and Jackson, London

Eversmeyer MG, Kramer CL (2000) Epidemiology of wheat leaf and stem rust in the central Great Plains of the USA. Annu Rev Phytopathol 38:491–513

Fernandez MR, Clarke JM, Depauw RM (2002) The effect of plant height on tan spot on durum wheat in Southern Saskatchewan. Crop Sci 42:159–164

Garrett KA, Bowden RL (2002) An Allee effect reduces the invasive potential of *Tilletia indica*. Phytopathology 92:1152–1159

Garrett KA, Mundt CC (1999) Epidemiology in mixed host populations. Phytopathology 89:984–990

Heath MC (1996) Plant resistance to fungi. Can J Plant Pathol 18:469–475

Hobbs NT, Baker DL, Bear GD, Bowden DC (1996) Ungulate grazing in sagebrush grassland: mechanisms of resource competition. Ecol Appl 6:200–217

Huber L, Gillespie TJ (1992) Modeling leaf wetness in relation to plant disease epidemiology. Annu Rev Phytopathol 30:553–577

Hutchinson GE (1957) Concluding remarks. Cold Spring Harb Symp Quant Biol 22:415–427

Keddy PA (1989) Competition. Chapman and Hall, London

Kolmer JA (1996) Genetics of resistance to wheat leaf rust. Annu Rev Phytopathol 34:435–455

Krupinsky JM (1992) Aggressiveness of *Pyrenophora tritici-repentis* isolated from grass and barley hosts. Plant Dis 76:783–789

Kus JV, Zaton K, Sarker R, Cameron RK (2002) Age-related resistance in *Arabidopsis* is a developmentally regulated defense response to *Pseudomonas syringae*. Plant Cell 14:479–490

Lamari L, Bernier CC (1989) Virulence of isolates of *Pyrenophora tritici-repentis* on 11 wheat cultivars and cytology of the differential host reactions. Can J Plant Pathol 11:284–290

Leibold MA (1995) The Niche concept revisited: mechanistic models and community context. Ecology 76:1371–1382

da Luz WC, Bergstrom GC (1987) Interactions between *Cochliobolus sativus* and *Pyrenophora tritici-repentis* on wheat leaves. Phytopathology 77:355–1360

Mitchell CE, Tilman D, Groth JV (2002) Effects of grassland plant species diversity, abundance, and composition on foliar fungal disease. Ecology 83:1713–1726

Moran NA, Whitham TG (1990) Interspecific competition between root-feeding and leaf-galling aphids mediated by host-plant resistance. Ecology 71:1050–1058

Morgan GW (2003) Effects of spring burning and fungicide application on growth of warm season grasses and their pathogens in a native tallgrass prairie, greenhouse, and monoculture. M.S. Thesis, Kansas State University

Mundt CC (2002) Use of multiline cultivars and cultivar mixtures for disease management. Annu Rev Phytopathol 40:381–410

Newton MR, Kinkel LL, Leonard KJ (1996) Competition and density-dependent fitness in a plant parasitic fungus. Ecology 78:1774–1784

Orolaza NP, Lamari L, Balance GM (1995) Evidence of a host-specific chlorosis toxin from *Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat. Phytopathology 85:1282–1287

Pretorius ZA, Rijkenberg FHJ, Wilcoxson RD (1988) Effects of growth stage, leaf position, and temperature on adult-plant resistance of wheat infected by *Puccinia recondita* f.sp. *tritici*. Plant Pathol 37:36–44

Price PW (1991) The plant vigor hypothesis and herbivore attack. Oikos 62:244–251

Pulliam HR (2000) On the relationship between niche and distribution. Ecol Lett 3:349–363

Raymond PJ, Bockus WW, Norman BL (1985) Tan spot of winter wheat: procedures to determine host response. Phytopathology 75:686–690

Riaz M, Bockus WW, Davis MA (1991) Effects of wheat genotype, time after inoculation, and leaf age on conidia production by *Drechslera tritici-repentis*. Phytopathology 81:1298–1302

Roelfs AP, Singh RP, Saari EE (1992) Rust diseases of wheat: concepts and methods of disease management. CIMMYT, Mexico

Sticher L, Mauch-Mani B, Métraux JP (1997) Systemic acquired resistance. Annu Rev Phytopathol 35:235–270

Thrall PH, Burdon JJ (2004) Evolution of virulence in a plant host-pathogen metapopulation. Science 299:1735–1737

Wilson M, Lindow SE (1994) Coexistence among epiphytic bacterial populations mediated through nutritional resource partitioning. Appl Environ Microbiol 60:4468–4477