Xenoparasite-Nonhost Reactions in *Puccinia*-Gramineae Pathosystems

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ABSTRACT

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Histological studies of six species of Gramineae inoculated with five species of *Puccinia* were conducted to determine the kinds of defense systems involved in xenoparasite-nonhost interactions. Although all nonhost types had active (physiological) defense mechanisms, two exhibited quasi exclusion, but the few xenoparasites that penetrated stimulated a physiological response similar to specific resistance. Histological responses associated with cessation of fungal growth included fluorescent-collapsed mesophyll cells (specific resistance), fluorescent-noncollapsed (nonspecific resistance), and a nonfluorescent-noncollapsed type (unknown). These three defense mechanisms were observed in a single leaf of a nonhost plant inoculated with a pure race of a xenoparasite. Nonhost reaction types were clear cut, independent, and easily distinguished one from the other. The nonhost had specific and nonspecific resistance to most of the xenoparasites, indicating that these two types of resistance had a common origin. Another histological response was characterized by fluorescent-collapsed cells that did not retard radial growth of the hyphae. This reaction reduced haustorial mother cell formation and inhibited development of macroscopic symptoms in the nonhost. The nonhosts used in our study had five defense mechanisms, four physiological and one quasi exclusion type.

Additional key words: grasses, rust, small grains.

With few exceptions (2,12) obligate biotrophs such as the cereal rust pathogens (*Puccinia* spp.) are restricted to a given host species and, in some cases, are specific for a few cultivars. Resistance of the latter type is not long lasting and is easily overcome by mutations or genetic recombinations in the pathogen. In contrast, nonhost resistance is long lasting and is one of the most effective forms of disease resistance (2). In some cases, the fungus does not penetrate the plant (exclusion). Other parasites penetrate and colonize but do not complete the reproductive cycle (physiological). This latter type of interaction is common in nonhost *Puccinia*-Gramineae pathosystems.

Although Heath (2) reported the details about nonhost interactions in *Uromyces*-dicotyledon pathosystems, few studies on nonhost resistance in *Puccinia*-Gramineae pathosystems have been published (5,6,8). So, little is known about the nature of this effective defense mechanism(s) in grasses. We, therefore, initiated a study to determine the types of defense in nonhost systems and to compare colonization and reaction patterns to rust fungi from closely related, moderately related, and distantly related species of Gramineae.

MATERIALS AND METHODS

Xenoparasite. A xenoparasite is defined as a parasite capable of infecting an organism that does not normally serve as its natural host. Six species of *Puccinia* that infect grasses were used to inoculate five species of Gramineae. The species of *Puccinia* were: *P. coronata* Cda. f. sp. *lolii* (ryegrass rust, a wild collection from *Lolium multiflorum* Lam., Gainesville, FL), *P. c.* f. sp. *avenae* (oat crown rust, race 264B), *P. hordei* Otth (barley leaf rust, race 8), *P. recondita* Rob. ex. Desm. f. sp. *tritici* (wheat leaf rust, race UN14), and *P. melanocephala* H. & P. Syd. (sugarcane rust, a wild collection from *Saccharum* sp. from cultivar CL 41-223, Canal Point, FL).

Nonhost. Nonhost is defined as a plant that does not serve as the natural host for a given parasite. The nonhost plants were: *Lolium perenne* L. 'Manhattan', *Avena sativa* L. 'Fulghum', *Hordeum vulgare* L. 'Ga-Jet', *Triticum aestivum* L. 'Red Hart', *Triticosecale* Wittm. 'Beagle-82', and *Secale cereale* L. 'Gator'.

The plants were grown in a greenhouse for 4–5 wk (fourth leaf stage) and inoculated as previously described (7), except 5 mg of freshly collected spores was allowed to settle for 5 min. This procedure resulted in a deposit of about 500 spores per square centimeter on the leaves. Plants were placed in a dew chamber, maintained at 20–23 C for 16 hr, and transferred to a growth chamber maintained at 25 C, with a 14-hr light period (65,000 lx). Leaves were collected 36, 72, and 120 hr after inoculation and prepared for fluorescent microscopy as previously described (4,7). We also made longitudinal and cross sections from inoculated leaves. These sections were 10–12 μm and were prepared using a procedure described by Johansen (3), except we used a 0.3% solution of fluorescence brightener 124 (7) (Tinopal BSA; Dyestuffs and Chemical Division, Ciba-Geigy Corp., Greensboro, NC) instead of standard histological stains.

Leaf pieces (2 × 5 cm) with the midvein removed and thin sections (10–12 μm) were mounted on microscope slides in lactophenol and examined with a Zeiss standard microscope fitted with epifluorescence equipment (light source HBO 50W, exciter filter BP400-440, chromatic bean splitter FT 460, and barrier filter LP470).

Each xenoparasite-nonhost treatment consisted of one leaf from four different plants. One square centimeter of each leaf was examined giving 4 cm^2 per treatment. All of the data except haustorial mother cell/substomatal vesicle, and haustoria/substomatal vesicle are expressed as the average numbers per square centimeter. Data representing the cellular reaction and the cellular condition refer to cells beneath appressoria in which substantial vesicles developed. Fungal development and nonhost reaction were similar 36 and 72 hr after inoculation. Therefore, we only used the data obtained at the 72-hr period.

RESULTS

Colonization of nonhost by xenoparasites. Xenoparasites produced appressoria on all nonhost species, but *P. melanocephala* produced only a few appressoria on some species (Table 1). Appressoria produced by this fungus ranged from 4 per

square centimeter on *H. vulgare* to 75 per square centimeter on *A. sativa*. Sotomayor (11) also noted that *P. melanocephala* produced about the same numbers of appressoria on oats as it did on sugarcane. *P. melanocephala* germinated at a normal rate of *H. vulgare* and *L. perenne*, so the reduction in appressoria on these species resulted in quasi exclusion. *P. c.* f. sp. *lolii* and *P. r.* f. sp. *tritici* produced haustoria in all nonhost species, but *P. hordei* and *P. melanocephala* did not produce haustoria in any of them. Niks (9) also reported that haustoria did not form in wheat inoculated with the barley rust pathogen. *P. c.* f. sp. *avenae* produced

TABLE 1. Development of five species of *Puccinia* in six nonhost species of *Lolium, Avena, Hordeum, Triticum, Triticosecale,* and *Secale* 72 hr after inoculation[a]

Rust species-Nonhost species-Cultivar	Pg[b]	Appressoria	SV	Hmc/SV	Hau/SV	Hyphal growth (μm)	Cellular reaction		Cellular condition	
							Fluor.	Nonflour.	Collapsed	Noncollap.
P. coronata f. sp. *lolii*										
L. perenne	23									
Manhattan (H)[c]		76	68	11.5	0.59	227	18	50	18	50
A. sativa	33									
Fulghum		84	76	3.4	0.04	121	50	26	48	28
H. vulgare	43									
Ga-Jet		37	31	7.8	0.35	158	4	27	2	29
T. aestivum	46									
Red Hart		70	64	5.0	0.02	96	50	39	22	42
Triticosecale	46B									
Beagle-82		89	81	7.4	0.06	150	55	26	55	26
S. cereale	47									
Gator		65	58	10.9	0.40	212	39	19	39	19
P. recondita f. sp. *tritici*										
A. sativa										
Fulghum		37	32	3.0	0.06	59	28	4	18	14
H. vulgare										
Ga-Jet		50	45	2.4	1.10	57	22	23	4	41
T. aestivum										
Red Hart (H)		38	33	21.0	6.10	110	0	33	0	33
Triticosecale										
Beagle-82		20	16	6.0	0.10	65	7	9	7	9
S. cereale										
Gator		59	54	4.0	0.10	60	40	14	40	14
P. coronata f. sp. *avenae*										
A. sativa (H)										
Fulghum		60	56	20.4	0.86	390	0	56	0	56
H. vulgare										
Ga-Jet		71	65	19.0	0.70	192	5	60	5	60
T. aestivum										
Red Hart		52	46	1.6	0.00	54	43	3	21	25
Triticosecale										
Beagle-82		47	45	1.2	0.00	64	40	2	20	25
S. cereale										
Gator		63	58	6.0	0.30	160	58	0	58	0
P. hordei										
A. sativa										
Fulghum		27	22	1.6	0.00	36	17	5	12	10
H. vulgare (H)										
Ga-Jet		55	50	8.4	0.72	68	10	40	10	40
T. aestivum										
Red Hart		62	58	1.9	0.00	57	48	10	38	20
Triticosecale										
Beagle-82		43	37	1.7	0.00	34	37	0	22	15
S. cereale										
Gator		54	47	1.5	0.00	27	25	22	20	27
P. melanocephala										
L. perenne										
Manhattan		7	4	1.5	0.00	20	2	2	2	2
A. sativa										
Fulghum		75	68	1.4	0.00	20	68	0	0	68
H. vulgare										
Ga-Jet		4	2	1.5	0.00	20	2	0	2	0
T. aestivum										
Red Hart		19	16	1.2	0.00	20	16	0	7	9
Triticosecale										
Beagle-82		13	8	1.8	0.00	20	6	2	2	6
S. cereale										
Gator		10	7	1.3	0.00	20	7	0	7	0

[a] SV = Substomatal vesicle. All data except haustorial mother cells/SV (Hmc/SV) and haustoria/SV (Hau/SV) are expressed as average numbers per square centimeter. Each mean was calculated from four replications of 1 cm^2 per replicate.
[b] Pgn = phylogenetic number (1). The pgn of sugarcane is 110.
[c] (H) represents host species.

haustoria in *H. vulgare* (barley) and *S. cereale* (rye) but did not produce haustoria in *T. aestivum* (wheat) and *Triticosecale* (triticale). *P. c.* f. sp. *lolii* grew well (radial growth) and produced macroscopic symptoms on two nonhosts (barley and rye) and sporulated on one of them (rye). *P. recondita* induced macroscopic symptoms on three nonhosts (barley, rye, and triticale) and sporulated on two of them (rye and triticale). *P. c.* f. sp. *avenae* (oat crown rust) grew well in barley but did not produce macroscopic symptoms or sporulate on it. This fungus grew poorly (74 μm) in some rye leaves but grew well (316 μm) in leaves from other rye plants (data not shown). Macroscopic symptoms (chlorotic flecks, no sporulation) developed on rye leaves that had good hyphal growth, but symptoms were not observed on other nonhost species inoculated with the oat rust pathogen.

Nonhost reactions to xenoparasites. The nonhost species had four distinct reaction types. Three types stopped radial growth of hyphae at an early stage of development. These reactions included fluorescent-collapsed mesophyll cells, fluorescent-noncollapsed, and nonfluorescent-noncollapsed types. It has been reported that fluorescing collapsed cells are indicative of specific (vertical) resistance (7,8,10) and that fluorescing noncollapsed cells are indicative of nonspecific (horizontal) resistance (7,8). The fourth reaction was characterized by successive layers of fluorescent-collapsed cells that did not retard radial growth but reduced branching and haustorial mother cell formation. In addition, quasi exclusion occurred when ryegrass and barley were inoculated with the sugarcane rust fungus (Table 1). The reaction of oats to a specific xenoparasite (except *P. melanocephala*) was evenly proportioned between collapsed and noncollapsed cell types (Table 1). Oats had a fluorescent-noncollapsed reaction to the sugarcane rust fungus. Barley had a predominantly noncollapsed cellular condition to all xenoparasites except *P. melanocephala*, which gave a fluorescent-collapsed cell reaction. Wheat had a predominantly fluorescent reaction to the xenoparasites, but the cellular condition was evenly proportioned between collapsed and noncollapsed cells in any combination. The reaction of triticale was somewhat similar to that of wheat. The most common reaction in rye to xenoparasites from oats, wheat, and sugarcane was fluorescent-collapsed, but the reaction to ryegrass and barley pathogens was evenly proportioned between the collapsed and noncollapsed types. All of the nonhosts except oats gave a collapsed or predominantly collapsed reaction to the sugarcane rust fungus.

When rye was inoculated with rust from oat, contrasting effects of fluorescent-collapsed cells on hyphal growth were observed. Radial growth was retarded (range 30–70 μm) by fluorescing-collapsed cells early in the infection process, but in other infection sites, fluorescing-collapsed mesophyll cells did not retard radial growth (range 300–360 μm) as compared with the compatible check (data not shown). As the radial growth of the fungus increased, it grew through two or three zones with collapsed cells. Hyphal branching was drastically reduced as infection hyphae passed through each zone. Retardation of hyphal branching caused a reduction in the number of haustorial mother cells.

DISCUSSION

Data obtained in our study, together with the modern phylogenetic concepts of Gould (1) bring the "stabilizing selection" assumption (13) into question. Gould arranged grasses into chronological order and gave them phylogenetic numbers (pgn) according to their evolutionary kinship. Grasses used in our study were closely related (ryegrass pgn #23 and oats pgn #33); moderately related (ryegrass pgn #23 and wheat pgn #46); and distantly related (barley pgn #43 and sugarcane pgn #110). It is self-evident and commonly accepted that the rust fungi evolved concomitantly with their specific hosts. If the stabilization selection hypothesis is valid, *Puccinia* spp. from grasses that are distantly related should not have similar pathogenic properties. Nevertheless, rust fungi from moderately related and distantly related species had similar properties. For example, barley and sugarcane are distantly related, but the parasites from these two species had similar properties, such as reduced hyphal growth and the lack of haustorial development in nonhosts. Moreover these two xenoparasites incited the specific resistance reaction (fluorescent-collapsed cells) in all nonhost species except oats. Oats and wheat are only moderately related, but both species had fluorescent-collapsed cells (specific resistance) when inoculated with two xenoparasites (ryegrass and barley rust). The obligate biotrophs used in our study seem to have genes in common that condition virulence and reaction type. So genes for the vertical (specific) resistance reaction (collapsed mesophyll cells) have been conserved over a long period of time and were not lost, as predicted by the stabilizing selection hypothesis (13).

In nonspecific resistance, a golden fluorescence occurs in mesophyll cell walls, but the cells do not collapse (7,8). In specific resistance, cell walls also have golden fluorescence, but they collapse very soon after haustorial mother cell formation (7,8,10). If reports that fluorescing collapsed cells indicate specific (vertical) resistance and noncollapsed fluorescing cells indicate nonspecific (horizontal) resistance are correct, four (barley, wheat, triticale, and ryegrass) nonhost species have both specific and nonspecific resistance to all of the xenoparasites used. It therefore appears that specific and nonspecific resistance had a parallel phylogenetic development and perhaps a common origin.

Two other types of reactions were observed that stopped or retarded fungal growth. In the first case, penetration and haustorial mother cell formation did not trigger a reaction in mesophyll cell walls, but growth of the fungus was arrested early in the infection process. Histological aberrations were not observed in the nonhost or the xenoparasite. We do not have data that permit speculation on the nature of this defense mechanism. Rowell (10) noted a similar reaction in slow-rusting wheat and speculated that inhibition of the pathogen was controlled by an extracellular compound. In the second case, hyphae grew through two or three layers of fluorescent-collapsed mesophyll cells. Even though collapsed cells did not retard radial growth, they severely reduced branching of the hyphae, which in turn reduced the number of haustorial mother cells. Thus, in some xenoparasite-nonhost combinations, collapsed cells do not kill the fungus, but instead retard branching of the hyphae, which reduces the development of haustoria. Very few haustoria developed; therefore, sporulation did not occur. Retardation of branching of fungal hyphae was observed in several combinations, but was most prevalent when rye was inoculated with the oat rust pathogen.

We observed physiological and quasi exclusion mechanisms of defense to the xenoparasite. Fluorescent-collapsed and fluorescent-noncollapsed reactions stopped fungal growth early in the infection process and seem to involve recognition by the nonhost and metabolic response in mesophyll cell walls. These reactions commonly occurred in a single leaf inoculated with a pure race of a xenoparasite. The fourth reaction type was a reduction of branching of the hyphae by fluorescent-collapsed cells. Perhaps the compound that stops growth in the reduced branching system is produced too late to stop radial growth or is produced in quantities that are not sufficient to stop growth. A quasi exclusion defense system occurred in ryegrass and barley inoculated with the sugarcane rust pathogen. This system was exemplified by a sharp reduction in the number of appressoria formed, resulting in reduced penetration. But the few that formed haustorial mother cells triggered a severe fluorescent-collapsed condition. When exclusion was almost complete, some nonhosts also had a back-up defense mechanism that resembled specific resistance.

Even though some nonhost-xenoparasite combinations were incompatible, haustorial mother cells developed in all of them, indicating that defense mechanisms to *Puccinia* species from widely different grasses are active (physiological) and do not depend entirely on the exclusion process. Similar observations have been made in other nonhost studies (2,5,6). The xenoparasites used in this study have a degree of adaptation to grass species from two subfamilies in three tribes. This evidence together with the fact that the xenoparasites triggered similar defense mechanisms in nonhost leads to speculation that these fungi had a similar origin

and still have genes in common that activate specific reactions in species of Gramineae.

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