Resistance to Fungal Penetration in Gramineae

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ABSTRACT

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Twelve species in 11 tribes of the Gramineae were inoculated with incompatible leaf-infecting fungi to obtain evidence on how grasses resist epidermal penetration. We inoculated reed canarygrass, timothy, big bluestem, switchgrass, smooth bromegrass, corn, oat, wheat, and barley leaves with *Stemphylium botryosum* and examined 781 epidermal sites where the fungus had initiated direct penetration. At 779 sites, appositions developed in the epidermal cell walls and penetration was unsuccessful. At two sites the wall failed to thicken and penetration occurred. When inoculated leaves were floated on cycloheximide solutions (12 μg/ml) to inhibit leaf responses, *S. botryosum* penetrated 767 of 771 sites examined and no wall thickening was observed. Thus, cycloheximide inhibited appositions and permitted epidermal penetration in all nine species. We also inoculated the nine species with an incompatible isolate of *Curvularia lunata*. Of 1,828 penetration attempts studied, 1,800 showed appositions without penetrations, 25 showed penetrations without appositions, and three showed penetrations through thin appositions. The number of penetration attempts per 100 conidia of *C. lunata* varied among the grass species, ranging from 153 on oats to 314 on reed canarygrass. Cycloheximide treatment resulted in 1,469 penetrations without appositions and 52 appositions without penetrations. In all species, cycloheximide inhibited the formation of appositions and permitted penetration by *C. lunata*. Big bluestem, rice, bermudagrass, and Japanese lawngrass were inoculated with *Helminthosporium maydis* race T, because *S. botryosum* and *C. lunata* initiated few penetrations on those species. The sites of attempted penetration by *H. maydis* showed appositions without penetration. Cycloheximide treatment had the effect noted on other grasses and did not change the relatively low frequency of attempts. The morphology and color of appositions differed widely among grass species, but were uniform within a grass species inoculated with any of the three fungi. The results indicted that grasses have both constitutive and inducible resistance mechanisms associated with the epidermis. The constitutive mechanism restricts frequency of penetration attempts and is not highly sensitive to cycloheximide. The inducible resistance mechanism is correlated with appositional cell wall formation and is sensitive to cycloheximide. The evidence that the process of appositional wall formation acts as a general mechanism of resistance to fungal penetration in the Gramineae is discussed.

Additional key words: papillae, resistance, *Phalaris arundinacea, Avena sativa, Triticum aestivum, Oryza sativa, Zea mays.*

Appositional cell wall formation in plants is characterized by a series of molecular events set in motion by an inducing agent, mediated by an active aggregate of host cytoplasm at the induction site, and manifested by the continuing deposition of wall structural material and other unidentified compounds in a progressively thickening new wall (= apposition) at the site of induction, as well as by the modification of existing wall chemistry in a circular area (= halo) centered upon the apposition. Lignin-like compounds (10,30), callose (1,7), cellulose (30), unidentified phenolic compounds (13,23), silicon (19), and an unidentified 'basic staining material' (21) have been detected in appositions. The composition of these and of undetected components remains to be determined and apparently varies among plants (7,10,30). Within the process of appositional wall formation are contained features that may provide a mechanism of resistance to epidermal penetration by fungal pathogens. That is not to say that the process is the sole means of resistance to epidermal penetration. Nor does this contend that physical resistance of wall material to growth of the penetration peg is the necessary and sufficient condition for plant resistance. Cessation of fungal growth in the unsuccessful penetration attempts may result from growth inhibitory properties of specific metabolites at the site of appositional wall formation in conjunction with, or apart from, physical impediment. The formation of particular metabolites critical to stopping the fungus may be but part of the entire appositional wall response, and probably varies in timing and intensity, permitting some penetration attempts to succeed.

Several lines of evidence indicate that the process of appositional wall formation provides a mechanism for resistance to penetration in reed canarygrass (*Phalaris arundinacea* L.) leaves (30,32–35). The appositions contain lignified wall material, and penetration pegs usually do not pass through them. Treatment of leaves with cycloheximide inhibits activity of enzymes involved in lignification and prevents formation of appositions. Fungi readily penetrate epidermal walls of cycloheximide-treated tissues. Removal of cycloheximide from the leaves restores enzyme activity, appositional wall formation, and resistance to penetration. Prior inoculation with a nonpathogen induces a protective mechanism involving apposition formation which cannot be inhibited by cycloheximide.

Appositional cell wall thickenings at points of fungus attack have been detected in several species of Gramineae including barley (3,6,20,21), wheat (7,10,22,28,31,36), oats (26), rye (36), sorghum (31,36), and corn (18,25,27,36). Cell wall thickenings also have been associated with unsuccessful penetration attempts by incompatible and compatible fungi and with successful penetrations by compatible fungi. Therefore, we explored the possibility that the role of appositional wall formation elucidated for reed canarygrass may occur throughout the grass family. This paper reports on appositional cell wall thickening in 12 species from 11 tribes of Gramineae inoculated with leaf-infecting fungi nonpathogenic on those species and describes the effects of cycloheximide on appositional thickening and penetration.

MATERIALS AND METHODS

Plants of twelve species of Gramineae were grown in a glasshouse (Table 1). Leaves of corn, oats, wheat, and barley were obtained from 3- to 5-wk-old seedlings. Leaves from other species were obtained from regrowth 3 to 5 wk after established plants were

clipped. Pieces of leaf blade 8 mm long × 3–8 mm wide were cut from fully-expanded apical leaves and floated on water or on cycloheximide solutions (12 μg/ml) in petri dishes. About 10 leaf pieces per treatment per trial were immediately inoculated with one 7-μl drop of water containing about 15 conidia. The dishes were kept at 24 C under continuous, cool-white fluorescent light (538 lux). After 48–72 hr, the pieces were cleared and stained by boiling for 8 min in cotton blue-lactophenol (33).

Nine species were inoculated with an isolate of *Stemphylium botryosum* Wallr., which is a pathogen of legumes but not of grasses. The stained leaf pieces were examined at × 500 or × 1,250 for sites of attempted penetration (defined as epidermal wall areas, beneath appressoria or subcuticular hyphae, showing penetration pegs and/or wall thickening or discoloration). Each site was classified in one of three categories: appositional growth without successful penetration, appositional growth with successful penetration, and successful penetration without appositional growth. Penetration was considered successful if components of the fungus passed completely through the cell wall and any appositional growth and entered into the cell lumen. In each of four trials, we classified the first 25 penetration sites observed per treatment (Table 2). Fewer than 25 events could be classified in big bluestem and switchgrass. Penetration attempts that involved trichomes or stomata were not counted.

The nine species also were inoculated with an isolate of *Curvularia lunata* (Wakk.) Boed. that was pathogenic on *Agrostis palustris* Huds. but did not cause visible symptoms on the species studied here. In contrast to the *S. botryosum* inoculations in which we classified the first 100 penetration events found, in the *C. lunata* tests we classified all penetration sites associated with 25 germinated spores per treatment in each of four trials (Table 3).

Four species were inoculated with *Helminthosporium maydis*

TABLE 1. Tribes and species of Gramineae that were tested for penetration by fungi

Tribe[a]	Species	Common name	Cultivar
Agrostideae	*Phleum pratense* L.	timothy	Climax
Andropogonae	*Andropogon gerardi* Vitm.	big bluestem	NY-1145
Aveneae	*Avena sativa* L.	oats	Dal
Chlorideae	*Cynodon dactylon* (L.) Pers.	bermudagrass	Coastal
Festuceae	*Bromus inermis* Leyss	smooth bromegrass	···
Hordeae	*Hordeum vulgare* L.	barley	Trent
Hordeae	*Triticum aestivum* L.	wheat	Arthur
Oryzeae	*Oryza sativa* L.	rice	IR-8
Paniceae	*Panicum virgatum* L.	switchgrass	Blackwell
Phalarideae	*Phalaris arundinacea* L.	reed canarygrass	common
Tripsaceae	*Zea mays* L.	corn	PA-8703
Zoysieae	*Zoysia japonica* Steud.	Japanese lawngrass	···

[a]Classification and nomenclature according to Hitchcock (12).

TABLE 2. Appositional wall formation and direct penetration in leaf epidermal cells of nine species of Gramineae inoculated with *Stemphylium botryosum*

	Number of penetration sites showing the indicated result[a]					
	Leaves floated on water			Leaves floated on cycloheximide solution		
Species	Appositions without penetration	Appositions with penetration	Penetrations without apposition	Appositions without penetration	Appositions with penetration	Penetrations without appositions
Timothy	100	0	0	2	0	98
Big bluestem	31	0	0	0	0	21
Oats	100	0	0	1	0	99
Smooth bromegrass	98	0	2	0	0	100
Barley	100	0	0	0	0	100
Wheat	100	0	0	0	0	100
Switchgrass	50	0	0	0	0	50
Reed canarygrass	100	0	0	1	0	99
Corn	100	0	0	0	0	100

[a]Totals based on 25 penetration sites examined per treatment in four trials. Fewer sites were examined in big bluestem and switchgrass.

TABLE 3. Appositional wall formation and direct penetration in leaf epidermal cells of nine species of Gramineae inoculated with *Curvularia lunata*

	Number of sites showing the indicated result per 100 conidia[a]					
	Leaves floated on water			Leaves floated on cycloheximide solution		
Species	Appositions without penetration	Appositions with penetration	Penetrations without apposition	Appositions without penetration	Appositions with penetration	Penetrations without apposition
Timothy	229	0	1	13	0	134
Big bluestem	162	1[b]	4	10	0	127
Oats	149	0	4	5	0	123
Smooth bromegrass	173	0	6	2	0	150
Barley	234	0	2	1	0	168
Wheat	173	0	2	5	0	186
Switchgrass	183	0	4	7	0	169
Reed canarygrass	310	2[b]	2	9	9[b]	195
Corn	187	0	0	0	0	217

[a]Totals based on 25 germinated conidia per treatment in four trials.
[b]Appositional wall thickening was less than 1 μm and poorly developed.

Nisikado and Miyake (race T). Ten leaf pieces involving more than 100 germinated conidia were examined in each of two trials (Table 4).

RESULTS

Stemphylium botryosum. We studied 1,552 sites of attempted penetration on leaves of nine grass species inoculated with *S. botryosum.* In 779 of the 781 sites studied on leaves floated on water, appositions formed at the point of attack and the fungus did not penetrate (Table 2); *S. botryosum* penetrated two sites that had little thickening (less than 1 μm). Of the 771 penetration attempts observed on leaves floated on cycloheximide, 767 resulted in penetration through unthickened walls, and four attempts, involving three grasses, resulted in apposition formation without penetration. Big bluestem and switchgrass supported fewer than 25 penetration attempts per 10 leaves in each trial, so fewer than 100 penetration attempts were classified.

Curvularia lunata. Classification of penetration attempts by *C. lunata* appears in Table 3. Of 1,828 penetration attempts in the water treatments, 1,800 showed appositions without penetration; 25 showed penetration without appositions; and three showed penetration through thin, poorly-developed appositions. The 25 penetrations without appositions were distributed among eight grasses. Unpenetrated appositions usually were thick and well developed (Fig. 1). Each conidium usually initiated more than one penetration attempt. The frequency of penetration attempts per 100 conidia ranged from 153 in oats to 314 in reed canarygrass. Appositional thickenings most often were found in anticlinal (lateral) walls, but also were found on the face of the epidermal wall (Fig. 1).

In cycloheximide treatments, 1,530 penetration sites were found: 1,469 sites showed penetration without appositions, 52 showed appositions without penetration, and nine showed penetration through thin appositions. The frequency of penetration attempts per 100 conidia ranged from 110 in oats to 217 in corn. Frequency

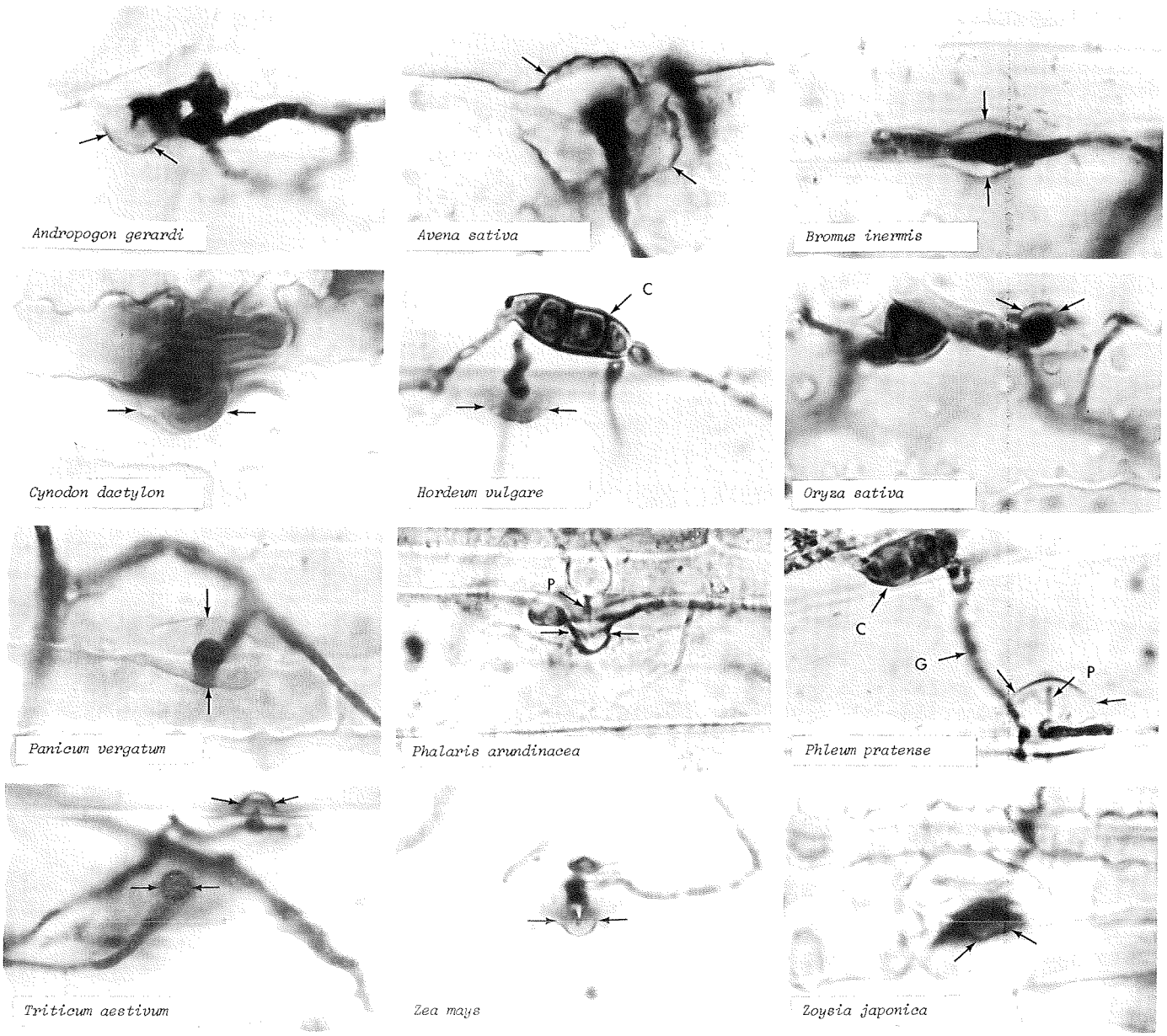

Fig. 1. Appositional thickenings in epidermal walls of leaves of 12 grass species inoculated with fungi nonpathogenic to the species. Whole mounts of leaves were stained with cotton blue-lactophenol. *Cynodon dactylon, Oryza sativa,* and *Zoysia japonica* were inoculated with *Helminthosporium maydis.* The other species were inoculated with *Curvularia lunata.* The arrows bracket the lateral limits of each appositional growth. Note the conidia (C), germ tubes (G), and penetration pegs (P) of *C. lunata* (× 700).

of successful penetration was high in all species (Table 3).

Helminthosporium maydis. In preliminary inoculations, *S. botryosum* and *C. lunata* initiated few penetration attempts on bermudagrass, rice, and Japanese lawngrass. Therefore, we used *H. maydis* because penetration attempts were more readily found. Attempted penetrations were fewer on bermudagrass, rice, and Japanese lawngrass than on big bluestem inoculated with *H. maydis* (Table 4). The leaves on water usually showed appositions without penetration. The leaves on cycloheximide usually showed penetration without appositions.

Appressoria and subcuticular hyphae. Most penetration attempts by the three fungi were initiated from appressorial cells on the epidermal surface (Fig. 2A). Occasionally, penetration attempts were initiated from subcuticular hyphae on the cell surface or wedged at the junction of adjacent cells (Fig. 2B, C). Subcuticular hyphae were more frequent in cycloheximide-treated than in untreated tissue, and some were associated with appositional growth without penetration. In cycloheximide-treated tissues, subcuticular hyphae often led to penetration without appositional thickening.

Some penetrations occurred through trichomes of corn, rice, and big bluestem treated with cycloheximide. Penetrations also occurred through stomata of corn, wheat, oats, barley, and switchgrass and were more frequent through stomata of cycloheximide-treated leaves than of untreated leaves. The penetrations through trichomes and stomata were not accompanied by appositional thickening and were not considered further in relation to epidermal resistance.

Morphology of appositions. The appearance of appositions varied among plant species but was quite uniform within species inoculated with these fungi. Well-developed appositions typical of each plant species are shown in Fig. 1. The margin was usually smoothly hemispherical or papillate in reed canarygrass, corn, switchgrass, and barley, and often irregularly sinuous in oats and smooth bromegrass. Mature appositions of oats, timothy, and big bluestem usually had a light yellow pigment. Bermudagrass appositions were light brown. Appositions of oats, timothy, smooth bromegrass, bermudagrass, rice, and Japanese lawngrass did not stain well with cotton blue.

DISCUSSION

The results indicate that grass leaves have both constitutive and inducible mechanisms of resistance to direct penetration by fungi. Consideration of the frequency of sites of attempted penetration (defined here as sites observed to have fungal penetration pegs and/or host cell wall modifications) suggests that constitutive factors may limit the number of penetrations. There were wide differences among nine grass species tested in the frequency of sites of attempted penetration by *C. lunata* or *S. botryosum*. Differences also were observed among the four species inoculated with *H. maydis*. It would be interesting to determine whether lines within species also differ. The pivotal finding was that treatment of leaves with cycloheximide to block resistance to penetration did not appreciably change the frequency of sites of attempted penetration from that observed in untreated leaves, although the percentage of successful penetrations increased dramatically. This suggests that the frequency of penetration attempts was at least partly controlled by constitutive host factors that were not altered by cycloheximide treatment. The three grass species in which penetration attempts were least frequent; ie, rice, bermudagrass, and Japanese lawngrass, were distinguished from the other species studied in having exceptionally warty and waxy outer epidermal surfaces (24). The oat leaves also were waxy. It has been suggested that epidermal wall thickness, roughness, waxiness, or other structural features may provide a background level of constitutive resistance to fungal penetration (29). Johnson's (15) report that appressoria of *Erysiphe graminis* or of *E. cichoracearum* often fail to form penetration pegs in Gramineae may reflect a kind of constitutive resistance arising from the presence of preformed inhibitory metabolites.

The finding that the infusion of presumably sublethal (33) amounts of cycloheximide into the tissues sharply increases the frequency of successful penetration attempts indicates that the untreated leaves of these grass species have an inducible resistance mechanism that can be inhibited by cycloheximide. Except for the studies on reed canarygrass, this constitutes the first evidence that epidermal resistance of Gramineae to incompatible species of pathogens is inducible.

Among the various mechanisms postulated for inducible resistance to penetration in plants, the two that have received most attention are phytoalexin formation and appositional wall thickening. No inducible resistance mechanisms have been proven to occur in the Gramineae. There are few substantiated reports of phytoalexin formation in the Gramineae (8). There is no proof that phytoalexins prevent penetration. We were unable to detect phytoalexins in reed canarygrass (33,35).

The association between cycloheximide-induced inhibition of appositional wall thickening and cycloheximide-induced promotion of wall penetration supports the hypothesis that the process of appositional wall formation functions as an inducible mechanism of epidermal resistance. The hypothesis has been studied and challenged critically without being completely resolved (1–4, 6,20). Controversy arises from repeated observation of exceptions to the association between appositional wall thickening and penetration failure; ie, instances in which previously formed appositions are penetrated, appositions form after penetration, or penetrations fail where no apposition is formed.

The correlation between apposition formation and inhibition of penetration in nonhost species of Gramineae does not prove that apposition formation is a mechanism of resistance. Cycloheximide can inhibit other metabolic processes, in addition to apposition formation, and thus might inhibit other processes that confer resistance. Additional lines of evidence are needed to determine the role of apposition formation. Examination of the chemical nature of the appositions and demonstration of an induced protection mechanism associated with apposition formation are two useful lines of investigation that were used to elucidate the relation of apposition formation to resistance in reed canarygrass (30,32–35). There are other useful lines of investigation. However, in using any approach one must be cautious in drawing conclusions. For example, Aist (2) showed that wall appositions which form in

TABLE 4. Appositional wall formation and direct penetration in leaf epidermal cells of four species of Gramineae inoculated with *Helminthosporium maydis* race T

Species	Number of events observed[a]					
	Leaves floated on water			Leaves floated on cycloheximide solution		
	Appositions without penetration	Appositions with penetration	Penetrations without apposition	Appositions without penetration	Appositions with penetration	Penetrations without apposition
Big bluestem	96	1[a]	3	0	0	100
Bermudagrass	56	0	0	9	1[b]	39
Rice	23	0	2	0	0	25
Japanese lawngrass	3	0	0	0	0	2

[a]Totals of two trials with 10 leaf pieces inoculated with about 15 conidia per leaf piece.
[b]Penetrations through small thickenings.

kohlrabi root hairs in response to mechanical wounding (bending) can exclude penetration by a parasitic fungus. However, Aist also pointed out that mechanically and fungal-induced appositions could be chemically and functionally different; thus, one should not conclude from Aist's observation on mechanically induced appositions that appositions formed in response to a parasitic fungus exclude the fungus. Defosse (7) showed histochemical differences between wound callose and the callose formed in response to a pathogen.

In our inoculations with incompatible species of pathogens, no penetrations were seen to occur through well-formed appositions, and we observed only four penetrations that passed through relatively thin (about 1 μm thick) appositions (Tables 2–4). It was not determined whether the thickening occurred prior to or after penetration. In inoculations of wheat, oats, barley, corn, rye, and sorghum with isolates of *Alternaria* (= *Macrosporium*), Young (36) observed that appositions were readily induced, but did not observe 'penetration hyphae' (= penetration pegs) that gave rise to internal hyphae. Hashioka and Kusadome (11) inoculated several nonhost grasses with the rice blast fungus and noted that penetration occurred through the outer epidermal walls and that the wall of some species was swollen at the point of the penetration. However, it was not clear whether they observed infection pegs passing through the 'papilla-like callosities' which ensheathed the infection hyphae. We are not aware of unambiguous reports showing successful penetration of nonhost grasses through well-developed appositions. Thus, we cannot rule out apposition formation as a mechanism of resistance to incompatible species of pathogens.

The present study is an investigation of the resistance of nonhost grasses to fungi which are pathogens of other species. However, the findings may be relevant to understanding epidermal resistance of plants to compatible or incompatible strains of species which are pathogenic to these species. There is clear documentation of both unsuccessful and successful penetrations associated with appositions (3,6,14). The frequency of appositions with successful penetrations varies widely. At one extreme, penetrations occurred through less than 2% of the appositions formed in reed canarygrass leaves inoculated with *Helminthosporium catenarium* (33). At the other extreme, an apposition accompanies each site of penetration of *Cochliobolus sativus* into barley and wheat, and is believed to contribute little towards host-specific resistance except when sheathed by an unidentified "dark-stained object" (14).

A recent quantitative analysis of the penetration of susceptible and resistant barley lines by the barley powdery mildew fungus revealed patterns of papilla formation and penetration failure related both to the compatibility of the host-parasite combination and to the location of the host cells (16). In an incompatible host-parasite combination, most penetration pegs terminated within appositional cells. In cells distant from stomata, very few fungi penetrated the appositions. In cells nearer stomata 11% of the germlings either entered the cell without apposition formation or penetrated through an apposition. Even in the compatible host-parasite combinations, there was a high frequency of apposition formation and low frequency of passage through appositions, and apposition formation was stronger at cells distant from stomata. Incompatibility was expressed at several points in fungal development. Thus, there was a correlation of penetration failure with apposition formation related to host specific resistance. But sometimes resistance was expressed in the absence of apposition formation, and sometimes apposition formation clearly did not result in resistance.

If, as has been suggested (3), penetration failures may be due to a deficiency in the potential of the fungus to complete penetration, rather than to appositional growth, we have still to account for reversible effects on penetration demonstrated with reed canarygrass. The facts that penetration can be turned on by application of cycloheximide to the leaf and turned off again by removal of cycloheximide (33) and that localized protection can be induced and cannot be turned off by cycloheximide (35) suggest that events in the host greatly affect whether or not the fungus completes penetration. This is not to say that variability in potential of individual fungal propagules is not also a factor. The data of Aist and Israel (3) and Johnson et al (16) support that as a factor. The outcome of each encounter between host and potential invader is dependent upon variables in both organisms and upon the environment. We believe that one defense the host may have at its command is the ability to respond by the process of apposition formation. The response may sometimes be effective; it may sometimes fail. Host cell position, and predisposition, and fungal

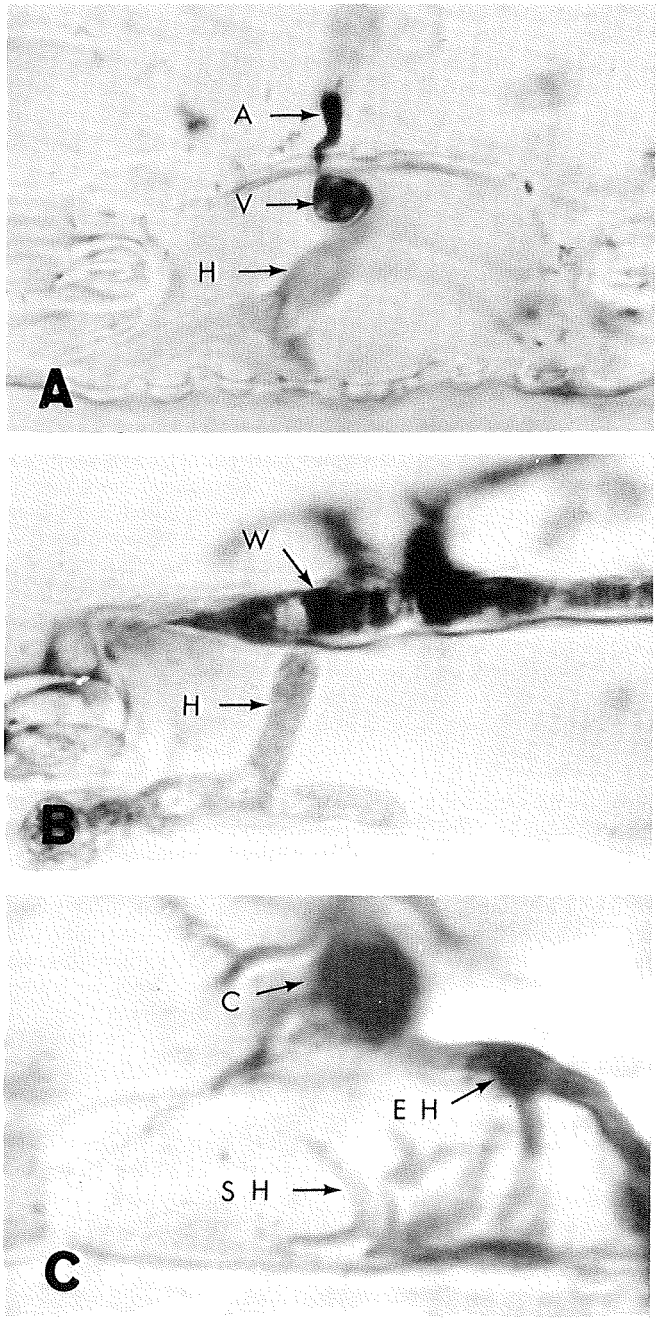

Fig. 2. Penetration of the leaf epidermis of selected species of Gramineae by leaf-infecting fungi nonpathogenic to the species being tested. **A,** Direct penetration of an epidermal cell of *Andropogon gerardi* by *Curvularia lunata*. The leaf was floated on a solution of cycloheximide (12 μg/ml). Note the noninflated appressorium (A), the primary vesicle (V), and intracellular hyphae (H). **B,** Penetration of *Triticum aestivum* leaf epidermis by *C. lunata*. The leaf was floated on cycloheximide solution. Note the subcuticular hyphal wedge (W), which initiated the penetration in the anticlinal wall and the intracellular hypha (H). **C,** Conidium (C) (out of focus), epicuticular hyphae (EH) (darkly stained), and subcuticular hyphae (SH) (lightly stained) of *C. lunata* on *Bromus inermis*. There was no penetration or wall thickening associated with this particular subcuticular hypha (× 1,472).

capabilities could account for differences in successful containment.

For the most part, the mechanism is apparently successful against incompatible species of pathogens, as tested in this study. The same general response may be variably effective against compatible species. The compatible strains of compatible fungal species may have developed ways of overcoming the mechanism. Some of the means for overcoming the mechanism are that the compatible parasite could inhibit full or timely development of the appositions; these strains could be tolerant of potentially inhibitory constituents of the apposition; or they could degrade specific structural constituents of the appositions. No host resistance mechanism has been proposed which will account more satisfactorily for the observed temporal and spatial variations in failures of individual propagules of a pathogen to penetrate the epidermis than the apposition formation hypothesis.

Holland and Fulcher (13) pointed out that the specificity of a pathogen may reflect differences in the phenolic compounds associated with the cell wall. This may apply equally to any compounds potentially deleterious to fungi. They (13) also noted that, unlike many pathogenic fungi (17), *Ophiobolus graminis* (which can readily penetrate lignified appositions of wheat [10]) also can extensively degrade lignified normal walls. Mayama and Shishiyama (23) stressed the need to identify compounds which accumulate at the infection site in order to establish their role in resistance.

Occasional failure of a mechanism does not negate its validity as a resistance response. No mechanism of induced resistance to epidermal penetration is failproof. Any postulated mechanism must account for the occasional penetrations by incompatible pathogens. The hyphae that do manage to penetrate are then confronted by other internal mechanisms that restrict spread of the fungus (8,9).

It should be informative to study the process of appositional wall formation not only as it relates to species-specific resistance, but also to genotype-specific resistance and environmentally influenced resistance. Efforts in this direction have been initiated (16).

The morphology and composition of cell walls and membranes of plants and fungi are strongly implicated as primary determinants of specificity (5). Sites of appositional growth are points of contact for those components. Possibly the appositional growth process is the mechanism that is induced by the initial encounter between recognition compounds. Specificity at the level of host species might be related to gross differences in the compounds produced at the initial encounter. The differences in natural pigmentation and stainability of appositions among the 12 species that we studied suggest that they have gross histochemical differences.

We are not aware of data indicating that any species of the Gramineae cannot form appositions. In addition to the species discussed here, appositional wall formation also has been observed in *Dactylis glomerata* L., *Festuca arundinacea* Shreb., *Sorghastrum nutans* L. (R. T. Sherwood, *unpublished*), and other species (11). We hypothesize that all species of the Gramineae characteristically respond to fungal penetration attempts by appositional wall formation, and we suggest that whatever function appositional wall formation serves in one species also may be served in all Gramineae.

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