Effects of Infection by Compatible Species or Injection of Tissue Extracts on the Susceptibility of Nonhost Plants to Rust Fungi

Michèle C. Heath

Associate professor, Botany Department, University of Toronto, Toronto, Ontario, Canada M5S 1A1.
I thank W. K. Wynn, University of Georgia, for providing the bean rust fungus isolates. French bean cultivars 780, 765, and 643 were obtained from the USDA, Beltsville, MD.
Accepted for publication 14 September 1979.

ABSTRACT

HEATH, M. C. 1980. Effects of infection by compatible species or injection of tissue extracts on the susceptibility of nonhost plants to rust fungi. Phytopathology 70:356-360.

The close proximity of established colonies of *Uromyces phaseoli* var. *typica* (the bean rust fungus), *U. phaseoli* var. *vignae* (the cowpea rust fungus), and *Puccinia helianthi* (the sunflower rust fungus) in their respective hosts increased the number of infection sites at which incompatible rust fungi produced haustoria from first-formed haustorial mother cells. Injection of extracts from susceptible rust-infected leaves of French bean had no effect on subsequent haustorium formation by the sunflower rust fungus in bean or cowpea or by the cowpea rust fungus in sunflower. However these extracts did increase haustorium production by the bean rust fungus in cowpea and by the cowpea rust fungus in bean; the effective component was of low molecular weight and partially heat labile. In both double-inoculation and injection experiments, only in the cowpea rust fungus–bean interaction did fungal growth continue beyond the formation of the first haustorium; such growth also was elicited by extracts from susceptible rust-infected cowpea leaves and by the close proximity of already growing colonies of the cowpea rust fungus induced to develop in bean leaves by a preinoculation heat shock of the nonhost tissue. It is suggested that in susceptible rusted French bean and cowpea leaves, and in interactions of French bean with the cowpea rust fungus if fungal colonies are permitted to develop by giving the tissue a heat shock, a similar or identical factor (or factors) is produced which, in French bean plants, specifically inhibits the expression of some, but not all, defense mechanisms which can prevent incompatible rust fungi from forming haustoria. However, growth of rust fungi in any plant beyond the formation of the first haustorium seems to depend on additional interactions between the two organisms. Significant differences in the response of different cultivars of French bean towards the cowpea rust fungus also were observed during this investigation.

Rust fungi attacking nonhost plants typically show poor intercellular growth and only rarely, if at all, form haustoria (4,6–8,12,19). Previously it was reported that certain preinoculation treatments of French bean, such as heat shock, increased the number of haustoria subsequently formed by the incompatible cowpea rust fungus, *Uromyces phaseoli* var. *vignae*, and also allowed this fungus to grow extensively, even after the effects of the treatments had worn off (9,11). The results of preliminary experiments suggested that, during this sustained fungal growth, the resistance of the surrounding tissue to subsequent infection by the cowpea rust fungus was reduced.

The present investigation was initiated to investigate this phenomenon further and to determine whether resistance to incompatible rust fungi also decreases during infection of a susceptible host by a compatible rust fungus.

MATERIALS AND METHODS

Double-inoculation experiments. Sunflower (*Helianthus annuus* L. 'Sunrise'), French bean (*Phaseolus vulgaris* L. 'Pinto'), and cowpea (*Vigna sinensis* (Torner) Savi 'Early Ramshorn') were grown as described previously (7). When the plants were about 11 (French bean) or 18 (cowpea and sunflower) days old the lower surfaces of their primary leaves were dusted (by means of a soft brush) with washed (5) uredospores of one of the following rust fungi: *Uromyces phaseoli* (Pers.) Wint. var. *typica* (Arth.) isolate A (the bean rust fungus); *U. phaseoli* (Pers.) Wint. var. *vignae* (Barclay) Arth. (the cowpea rust fungus): or *Puccinia helianthi* Schw. race 2 (the sunflower rust fungus). Both surfaces of the leaves were sprayed with distilled water and the plants were kept moist and dark for 24 hr after which they were returned to the normal humidity of the growth chamber and a 16-hr light, 8-hr dark cycle. Forty-eight hours later the upper leaf surfaces were similarly inoculated with uredospores of a different rust fungus. Four leaf pieces per treatment, usually from four different plants, were harvested at various intervals after this second inoculation and were stained and cleared as described previously (5). These pieces were examined under a Reichert light microscope equipped with interference-contrast optics which allowed the haustoria to be more easily detected than under bright-field optics. Although every care was taken to prevent contamination of the upper leaf surface during the first inoculation, the fact that *both* leaf surfaces were subsequently moistened ensured the germination of all viable spores present. Thus, any resulting infection sites on the upper surface subsequently could be distinguished from those of the second inoculation by the greater amount of mycelium present. In no case was such contamination greater than one infection site per square centimeter and at least 100 sites resulting from the second inoculation would normally be seen in the same area. Control plants were given the second inoculation only.

Preparation of extracts from infected and uninfected leaves. Uninfected leaves, or leaves inoculated 48 hr previously with a rust fungus pathogenic towards the particular plant species, were detached and infiltrated under reduced pressure with sterile double-distilled water at room temperature. The liquid in the intercellular spaces was then extracted by placing the leaves in centrifuge tubes half filled with clean glass marbles and centrifuging at 4 C for 20 min at 1,320 *g*. Usually the liquid was lyophilized immediately, stored at −20 C, and dissolved in sterile, double distilled water just before use. Separation of this material into fractions of high and low molecular weight was accomplished by passing the reconstituted extract through a Sephadex G-25 column. Fractions containing materials eluted in the void volume (molecular weight >5,000) were combined as were those containing materials not excluded from the gel. Each sample was then lyophilized and reconstituted in double distilled water to give a volume equal to one quarter of that of the original extract applied to the column. Isolates A and B of the bean rust fungus and French bean cultivars Pinto and 780 were used in this set of experiments. Isolate A is compatible with Pinto and 780 and isolate B is compatible with Pinto only.

0031-949X/80/04035605/$03.00/0

Bioassay of extracts. Extracts prepared as above were injected into primary leaves of 18–20 day old plants of cowpea, sunflower, and bean cultivars Pinto, 780, and 643 with a glass-needled syringe. When the injected areas no longer appeared to be watersoaked, they were inoculated, on the upper leaf surface, with uredospores of an incompatible rust fungus as described above; similarly treated, water injected, areas on separate plants served as controls. Fungal growth was examined at 24 hr and 4 days after inoculation by the same method used for the double-inoculation experiments.

RESULTS

Double-inoculation experiments. The first experiment was conducted to determine whether the sustained growth of the cowpea rust fungus, elicited in French bean by a preinoculation heat shock (9,11), is accompanied by an induced increase in susceptibility of the tissue to the same incompatible rust fungus. Heat shocked (ie, dipped in distilled water at 50 C for 25 sec) primary leaves were inoculated on their lower surfaces with the cowpea rust fungus (inoculation 1) and 2 days later, the same rust fungus was inoculated onto the upper surfaces of the same leaves (inoculation 2). When the latter infection sites were examined 24 hr later, the frequency of haustoria was much greater in sites within one-to-four palisade cells from growing colonies from inoculation 1 than at more distant sites (Table 1). By 48 hr after inoculation 2, infection sites of the latter which previously had been close to the growing colonies from inoculation 1 now were intermingled with the mycelium produced by the first inoculation and the extent of fungal growth was difficult to determine. However, as yet 'unengulfed' infection sites from inoculation 2 were seen which had many haustoria and apparently had produced as much intercellular mycelium as did infections of similar age in susceptible plants. The various stages of haustorium formation observed at the periphery of these colonies suggested that the fungus was still growing at the time that the leaves were harvested. Such growth did not seem to be due to any residual heat-shock effect since normal (6) restricted growth was observed in heat-shocked control plants which were not given inoculation 1 (Table 1). This result supports previous work which suggests that heat treatment has no effect on fungal growth if inoculation is delayed by 24 hr or more (11).

To determine whether colonies of normally compatible rust fungi similarly increase the susceptibility of the tissue towards incompatible species, an experiment similar to that described above was performed with three host species and the respective compatible rust fungus species for inoculation 1. In all cases, a significant increase in the frequency of haustoria was observed in 24-hr-old infection sites of the incompatible species close to colonies of the pathogenic fungi (Table 1). None of these haustoria developed in cells already containing haustoria of the compatible species, but the frequency of haustorium formation by the incompatible rust fungi decreased when infection sites were more than about four mesophyll cells from the periphery of the established colonies. This decrease was particularly marked for the sunflower rust fungus in French bean in which haustoria were observed *only* in cells immediately adjacent to intercellular hyphae of the bean rust fungus.

When examined 48 hr after the second inoculation, only infections of the cowpea rust fungus near colonies of the compatible rust fungus in French bean seemed to have developed beyond the growth achieved in the first 24 hr after inoculation (Table 1). Nevertheless, it is possible that continued growth of the incompatible fungi in other interactions may have occurred only where their mycelium had become intermingled with that of the compatible species. However, careful observations could provide no evidence for such growth and contrary evidence was found for the bean rust fungus in cowpea since many infection sites associated with one or two brown cells were found embedded in mycelium of the cowpea rust fungus. Such brown cells are characteristic of the bean rust fungus–cowpea interaction in which an unusually high proportion of infection sites develop haustoria: the invaded cells soon turn brown and collapse and there is no further intercellular growth (M. C. Heath, *unpublished*). Apparently the presence of colonies of the compatible fungus does not stop cell collapse and it therefore seems unlikely that it nevertheless permits further intercellular growth of the bean rust fungus.

Effect on nonhost resistance of extracts from compatible host–rust fungus interactions. The results of the above experiments suggest that prior infection by compatible rust fungi in some way counteracts the processes responsible for the absence of haustoria in nonhost–rust fungi interactions. In addition, it seems that this phenomenon is mediated by a diffusable substance(s) which can act in areas removed from the cells invaded by the compatible species. To test this hypothesis further, crude extracts from the intercellular spaces of French bean cultivar Pinto, bearing 2-day-old infections of isolate A of the bean rust fungus, were injected into uninfected Pinto leaves prior to inoculation with the cowpea rust fungus. The results given in Table 2 show that a few seemingly growing colonies

TABLE 1. Production of haustoria and growing colonies by incompatible rust fungi in nonhost plants bearing 48-hr-old, pathogenic colonies

Plant - rust fungus combinations[b]	Infection sites (%) with haustoria, 24 hr after inoculation			Growing[a] colonies seen near pathogenic rust, 48 hr after inoculation
	Within four cells of pathogenic colony	More than four cells from pathogenic colony	Control leaves (first inoculation omitted)	
French bean[b] (heat shocked) + cowpea rust fungus + cowpea rust fungus	62.5 ± 9.6[c]	10.0 ± 3.6	0[d]	Yes
French bean + bean rust fungus + cowpea rust fungus	74.5 ± 2.1	28.5 ± 14.9	0	Yes
French bean + bean rust fungus + sunflower rust fungus	28.8 ± 15.2	1.3 ± 2.6	0	No
Cowpea + cowpea rust fungus + bean rust fungus	72.6 ± 8.8[e]	58.6 ± 17.1	39.7 ± 10.8[e]	No
Sunflower + sunflower rust fungus + cowpea rust fungus	74.0 ± 5.5	31.4 ± 17.3	0	No

[a]With two or more haustoria.
[b]French bean cultivar Pinto was used throughout this experiment. The first-mentioned rust fungus is the compatible species inoculated onto the lower surface.
[c]Standard deviation of means from at least four leaf pieces.
[d]Control leaves also were heat shocked.
[e]Significantly different from each other at $P = 0.01$ (Student's t-test), but neither differ significantly from the third value.

(ie, with intercellular mycelium and haustoria in similar abundance to that developed in susceptible hosts during the same period) developed in leaves injected with the unconcentrated extract and that the number correspondingly increased if the extract was concentrated two- or fourfold. Similarly concentrated extracts from uninfected, untreated, French bean leaves, or from leaves given a heat shock 30 min before the extract was made, elicited far fewer growing colonies and essentially no more than those elicited by water alone. Growing colonies never developed in uninjected leaves, however (Table 3).

When a fourfold concentrated extract from infected leaves was fractionated on a Sephadex G-25 column, only the low-molecular-weight fraction (<5,000 daltons) had significant activity and this was reduced by about 50% by heating in a boiling water bath for 20 min (Table 2).

Injection of an unfractionated, fourfold concentrated extract from rusted French bean leaves into sunflower leaves produced no effect on the subsequent growth of, or haustorium formation by, the cowpea rust fungus (Table 4); unfortunately the effect on the bean rust fungus in this nonhost could not be evaluated because of its low frequency of penetration. Injection of a similar extract also had no effect on the growth of the sunflower rust fungus in bean or cowpea (Table 4), even when injected into bean plants 16 hr after inoculation, the time at which haustorium formation should begin. However, injection into cowpea leaves significantly increased the frequency of haustoria subsequently formed by isolate A of the bean rust fungus but no "growing" colonies developed. A similar effect on this nonhost–rust interaction was elicited by an extract from susceptible rusted cowpea leaves and the latter extract also stimulated growing colonies of the cowpea rust fungus to develop in injected French bean (Table 4). In this case, as in all cases of growing colonies of the cowpea rust fungus in French bean, the colonies resembled those produced in this plant by various preinoculation treatments (11) in that growth was accompanied by browning of haustorium-containing cells and the fungus did not sporulate.

The specificity of the extracts from successful infections of the bean rust fungus was examined further by producing unfractioned extracts from three different compatible bean rust fungus–French bean cultivar combinations. These were concentrated fivefold and injected into four French bean cultivars. The results given in Table 3 show that in three of these cultivars, all extracts increased the percentage of infection sites of the cowpea rust fungus at which haustoria formed, regardless of whether the plants were resistant or susceptible to the isolate of the bean rust fungus used to prepare the extract. Similar responses were elicited by each extract in each cultivar except that the extract from cultivar 780 infected with isolate A produced somewhat fewer haustorium-bearing sites in cultivars 780 and 765 than it did in Pinto. Results from the fourth cultivar, 643, were extremely variable and in general, fewer infection sites bore haustoria in extract-treated leaves than in those injected with water.

Although an increase in haustorium production was seen in three of the four cultivars tested, only in Pinto did the extracts elicit significant numbers of colonies which grew beyond the formation of the first haustorium (Table 3). In cultivar 765, no growing

TABLE 2. Percentage of infection sites of the cowpea rust fungus with growing[a] colonies observed 4 days after inoculation in French bean (cultivar Pinto) leaves previously injected with water or extracts from infected or uninfected leaves of this cultivar

	Source of extract			
Treatment of extract	Infected leaves[b]	Uninfected, untreated, leaves	Uninfected, heat-shocked[c] leaves	Water control
Batch 1:				
Unfractionated, unconcentrated	5.2 ± 2.7[d]	0	3.0 ± 2.6	1.4 ± 1.5
Unfractionated, concentrated × 2	14.0 ± 5.7	1.7 ± 2.1	2.3 ± 1.4	
Unfractionated, concentrated × 4	32.4 ± 6.6	0.4 ± 0.5	0.6 ± 0.8	
Batch 2:				
Unfractionated, concentrated × 4	26.7 ± 11.9			
Fraction excluded from Sephadex G-25	5.1 ± 3.7			
Fraction retarded by Sephadex G-25:				
Unheated	24.6 ± 10.6			
Heated[e]	14.2 ± 9.8			

[a]With two or more haustoria.
[b]Inoculated 48 hr previously with the bean rust fungus isolate A.
[c]Immersed in water at 50 C for 30 sec.
[d]Standard deviation of means from at least four leaf pieces.
[e]Boiling water bath for 20 min.

TABLE 3. Effect of preinoculation injection of extracts from various types of successful bean rust fungus infections on subsequent growth of the cowpea rust fungus in different French bean cultivars

	Infection sites (%) with haustoria, 24 hr after inoculation of French bean cultivar:				Infection sites (%) with growing colonies 4 days after inoculation of French bean cultivar:			
Preinoculation treatment of leaves	Pinto	780	765	643	Pinto	780	765	643
None	1.7 ± 1.7[a]	3.1 ± 3.3	6.9 ± 2.6	4.8 ± 4.2	0	0	0	0
Injection with distilled water	4.2 ± 3.0	3.7 ± 4.0	3.4 ± 5.6	18.5 ± 7.6	3.7 ± 3.0	0	0	0
Injection with extract from infections of:								
Pinto with isolate A[b]	45.5 ± 11.8	46.7 ± 10.0	41.2 ± 12.3	9.3 ± 7.8	43.7 ± 11.3	0	0	0
Pinto with isolate B[c]	47.5 ± 17.8	32.6 ± 14.7	40.4 ± 10.8	10.4 ± 3.0	45.6 ± 18.0	1.3 ± 2.9	0	0
780 with isolate A	47.2 ± 18.5	19.5 ± 10.1	16.8 ± 2.7	3.8 ± 6.6	42.1 ± 15.2	0.3 ± 0.7	0	3.2 ± 5.5
Heat shock[d]	64.2 ± 1.9	67.7 ± 7.8	52.9 ± 20.5	31.0 ± 6.6	61.2 ± 2.7	0	8.9 ± 7.3	14.4 ± 5.5

[a]Standard deviation of means from at least four leaf pieces.
[b]Pathogenic on Pinto and 780 only.
[c]Pathogenic on Pinto only.
[d]Dipped in distilled water at 50 C for 35 sec.

colonies developed even when 20-fold concentrated extract was injected. However, growing colonies were elicited by a preinoculation heat shock in all cultivars except 780. The heat shock treatment elicited fewer haustorium-bearing infection sites in cultivar 643, and more growing colonies in Pinto, than it did in the other cultivars (Table 3).

DISCUSSION

The results described in this paper show that the close proximity of established colonies of the bean rust, cowpea rust, and sunflower rust fungi in their respective hosts increases the number of infection sites at which incompatible rust fungi produce haustoria. This adds another example to the increasing list of reports which show that infection by pathogenic fungi increases the subsequent susceptibility of the tissue to either different nonpathogenic species or avirulent strains of the pathogen (14,16–18,20,22,24). In the present investigation, this effect was not restricted to cells already containing haustoria, as observed by Ouchi et al (16,17) for powdery mildew infection, but seemed to be accomplished by the diffusion of some substance from the region of the infection site. The effective agent from rusted French bean leaves was easily extracted by water infiltrated into the intercellular spaces and, like the phytoalexin "suppressor" produced by *Mycosphaerella pinodes* (14,15) and the hypersensitivity-inhibiting factor produced by *Phytophthora infestans* (2,3), it appears to be of low molecular weight (<5,000 daltons). There is as yet insufficient evidence to prove conclusively that the French bean extracts act similarly by "suppressing" responses normally prohibiting haustorium formation in nonhost plants. Conceivably the present results also could be explained in terms of the release of a "susceptibility factor" from the nonhost cells which stimulates haustorium development. However, if the latter hypothesis was true, one would expect this factor to be released in uninfected, heat-shocked French bean leaves since this treatment also increases the frequency of haustoria seen after subsequent inoculation with the cowpea rust fungus (9,11). In fact, extracts from heat-shocked leaves were no more effective than those from untreated leaves in promoting haustorium formation when used as a preinoculation treatment. Thus, it seems most likely that the extracts used in this investigation act by preventing the expression of reactions which normally inhibit haustorium formation. That haustorium formation is inhibited in nonhost tissue (rather than there being a lack of stimulation of haustorium development) is also supported by the increased number of haustoria seen in nonhosts treated with inhibitors of the synthesis of RNA or protein (10,11,20) and by the observation that the absence of haustoria in nonhost plants usually correlates either with the inhibition of fungal growth (7,8,10–12) or with some active plant response which feasibly could prevent haustorium formation (7,13).

It remains to be seen whether this example of "induced susceptibility" (1,23) determines host-parasite specificity or whether it is merely incidental to successful establishment of the compatible rust fungus in its host. In theory, it could be argued that a host plant is one whose "nonhost" defense mechanisms are ineffective towards, or not elicited by, the plant pathogen. Thus, the ability to prevent responses which normally prevent incompatible rust fungi from forming haustoria could theoretically play an important part in determining the host range of a particular rust fungus. In the present investigation this hypothesis is supported by the apparent specificity with which the crude extracts from susceptible, rusted French bean leaves had any effect of haustorium production in various nonhost–rust fungus combinations. These extracts increased haustorium production by the bean rust and cowpea rust fungi, but only in plants in which the former fungus normally produces at least some haustoria; in these situations, extracts from rusted cowpea leaves were similarly effective. In contrast, the extracts had no effect on haustorium production by the sunflower rust fungus, either in French bean or in cowpea leaves. An attractive interpretation of these results is that, due to their close relationship, the cowpea rust and bean rust fungi elicit similar "haustorium inhibiting" responses in French bean, but only the compatible rust fungus can prevent these from occurring during normal infection; however, "supression" of these responses by the extracts from rusted bean leaves allows the cowpea rust fungus to form haustoria. No similar increase in haustorium production is seen for the sunflower rust fungus because this species elicits different haustorium-inhibiting responses in this nonhost plant (11). The results of the present investigation also suggest a "suppressing factor" similar or identical to that produced by the bean rust fungus is produced by the cowpea rust fungus once it is established, either in its own host or in heat-shocked French bean (Table 1).

In accord with a role in determining host (rather than cultivar) specificity, extracts from susceptible, rusted French bean leaves allowed the cowpea rust fungus to develop haustoria in three of the four French bean cultivars tested, regardless of whether the latter were resistant or susceptible to the isolate of the bean rust fungus used to prepare the extract. However, none of the extracts had a significant effect on infection of the fourth cultivar and this cultivar also permitted fewer haustoria of the cowpea rust fungus to develop after a preinoculation heat shock. Thus, either the haustorium-inhibiting response is particularly vigorous in this cultivar or it possesses different mechanisms for resistance.

TABLE 4. Effect of preinoculation injection of extracts from susceptible, rusted French bean and cowpea plants on subsequent growth of incompatible rust fungi in various nonhost plants

Source of extract[a]: Recipient of injection: incompatible species inoculated	Infection sites (%) with haustoria 24 hr after inoculation[b]		Infection sites (%) with growing[c] colonies, 4 days after inoculation[b]	
	Extract-injected	Water-injected	Extract-injected	Water-injected
French bean infected with bean rust fungus: French bean: sunflower rust fungus	0	0	0	0
French bean infected with bean rust fungus: sunflower: cowpea rust fungus	0	0	0	0
French bean infected with bean rust fungus: cowpea: bean rust fungus	85.9 ± 8.3[d]	39.7 ± 10.8	0	0
French bean infected with bean rust fungus: cowpea: sunflower rust fungus	0	0	0	0
Cowpea infected with cowpea rust fungus: French bean: cowpea rust fungus	...[e]	...[e]	51.0 ± 16.0	0
Cowpea infected with cowpea rust fungus: cowpea: bean rust fungus	59.3 ± 9.2[d]	39.7 ± 10.8	0	0

[a] All 4 × the original concentration; isolate A of the bean rust fungus and French bean cultivar Pinto was used throughout the experiment.
[b] ± standard deviation of means from at least four leaf pieces.
[c] With two or more haustoria.
[d] Significantly different from water injected at $P = 0.05$ (Student's t-test).
[e] ... not determined.

Differences between French bean cultivars also was observed for the frequency with which the cowpea rust fungus formed growing colonies and previously was reported for the nonpathogen's behavior on the leaf surface (7). These results suggest that even in studies of nonhost resistance, it may be unwise to extend to the species any conclusion made from experiments using only one cultivar of the nonhost plant.

This investigation was initiated because of the observation that various preinoculation treatments of French bean not only increased haustorium formation by the cowpea rust fungus, but also allowed it to grow extensively, even after the effects of the treatments had worn off (9–11). In the present investigation, such continued growth was observed only in this fungus–nonhost combination and was elicited in cultivar Pinto by all the treatments which increased the number of infection sites at which first-formed haustorial mother cells formed haustoria. However this increase in haustorium production does not in itself explain why the fungus continued to grow since such was not the consequence of haustorium formation in other nonhost–rust fungus combinations examined in the present or previous (11) studies, even when established colonies of the compatible fungus were nearby and presumably producing a continuous supply of any necessary factor. Possibly sustained growth of any rust fungus requires specific substances which, for the cowpea rust fungus, but not the sunflower rust fungus, are available in both French bean and cowpea. Alternatively, only the former fungus may be able to overcome other inhibitory responses specifically elicited after the first barrier to infection (ie, inhibition of haustorium formation) has been bridged. However, it should be noted that none of the treatments described in this or previous reports (9–11) allowed French bean to become fully susceptible to the cowpea rust fungus and fungal growth always was accompanied by browning of haustorium-containing cells and no sporulation occurred.

LITERATURE CITED

1. DALY, J. M. 1972. The use of near-isogenic lines in biochemical studies of the resistance of wheat to stem rust. Phytopathology 62:392-400.
2. DOKE, N., N. A. GARAS, and J. KUĆ. 1977. Partial characterization and mode of action of the hypersensitivity-inhibiting factor isolated from *Phytophthora infestans*. (Abstr.) Proc. Am. Phytopathol. Soc. 4:165.
3. GARAS, N. A., N. DOKE, and J. KUĆ 1977. Extraction of mycelial components from *Phytophthora infestans* that inhibit the accumulation of terpenoids in potato tuber tissue. (Abstr.) Proc. Am. Phytopathol. Soc. 4:168.
4. GIBSON, C. M. 1904. Notes on infection experiments with various Uredineae. New Phytol. 3:184-191.
5. HEATH, M. C. 1971. Haustorial sheath formation in cowpea leaves immune to rust infection. Phytopathology 61:383-388.
6. HEATH, M. C. 1972. Ultrastructure of host and nonhost reactions to cowpea rust. Phytopathology 62:27-38.
7. HEATH, M. C. 1974. Light and electron microscope studies of the interactions of host and nonhost plants with cowpea rust—*Uromyces phaseoli* var. *vignae*. Physiol. Plant Pathol. 4:403-414.
8. HEATH, M. C. 1977. A comparative study of non-host interactions with rust fungi. Physiol. Plant Pathol. 10:73-88.
9. HEATH, M. C. 1977. The effect of heat treatment on nonhost interactions with cowpea rust. (Abstr.) Proc. Am. Phytopathol. Soc. 4:169.
10. HEATH, M. C. 1978. Specificity-determining interactions between cowpea rust and nonhost plants. (Abstr.) Page 203 in: Proc. Third Int. Conf. Plant Pathol., München, West Germany. Paul Parey, Berlin and Hamburg. 435 pp.
11. HEATH, M. C. 1979. Effects of heat shock, actinomycin D, cycloheximide, and blasticidin S on nonhost interactions with rust fungi. Physiol. Plant Pathol. 15: (In press).
12. LEATH, K. T., and J. B. ROWELL. 1966. Histological study of the resistance of *Zea mays* to *Puccinia graminis*. Phytopathology 56:1305-1309.
13. LEATH, K. T., and J. B. ROWELL. 1969. Thickening of corn mesophyll cell walls in response to invasion by *Puccinia graminis*. Phytopathology 59: 1654-1656.
14. OKU, H., T. SHIRAISHI, and S. OUCHI. 1977. Suppression of induction of phytoalexin, pisatin, by low-molecular-weight substances from spore germination fluid of pea pathogen, *Mycosphaerella pinodes*. Naturwissenschaften 64:643.
15. OKU, H., T. SHIRAISHI, and S. OUCHI. 1978. Contribution to the panel discussion "Elicitation and sites of formation of phytoalexins and induced resistance." (Abstr.) Page 214 in Proc. Third Int. Conf. Plant Pathol., München, West Germany. Paul Parey, Berlin and Hamburg. 435 pp.
16. OUCHI, S., C. HIBINO, and H. OKU. 1976. Effect of earlier inoculation on the establishment of a subsequent fungus as demonstrated in powdery mildew of barley by a triple inoculation procedure. Physiol. Plant Pathol. 9:25-32.
17. OUCHI, S., H. OKU, and C. HIBINO. 1976. Localization of induced resistance and susceptibility in barley leaves inoculated with the powdery mildew fungus. Phytopathology 66:901-905.
18. OUCHI, S., H. OKU, C. HIBINO, and I. AKIYAMA. 1974. Induction of accessibility and resistance in leaves of barley by some races of *Erysiphe graminis*. Phytopathol. Z. 79:24-34.
19. RAGGI, V. 1964. Affinità sistematica e affinità biologica nel caso di parassiti obbligati specializzati. Phytopathol. Mediterr. 3:135-155.
20. TANI, T., S. OUCHI, T. ONOE, and N. NAITO. 1975. Irreversible recognition demonstrated in the hypersensitive response of oat leaves against the crown rust fungus. Phytopathology 65:1190-1193.
21. TOMIYAMA, K. 1966. Double infection by an incompatible race of *Phytophthroa infestans* of a potato plant cell which has previously been infected by a compatible race. Ann. Phytopathol. Soc. Jpn. 32:181-185.
22. VARNS, J. L., and J. KUĆ. 1971. Suppression of rishitin and phytuberin accumulation and hypersensitive response in potato by compatible races of *Phytophthora infestans*. Phytopathology 61:178-181.
23. WARD, E. W. B., and A. STOESSL. 1976. On the question of 'elicitors' or 'inducers' in incompatible interactions between plants and fungal pathogens. Phytopathology 66:940-941.
24. YARWOOD, C. E. 1977. *Pseudoperonospora cubensis* in rust-infected bean. Phytopathology 67:1021-1022.