Relationship Between Heat-Induced Fungal Death and Plant Necrosis in Compatible and Incompatible Interactions Involving the Bean and Cowpea Rust Fungi

Michèle C. Heath

Professor, Botany Department, University of Toronto, Toronto, Ontario, Canada. M5S 1A1
The technical assistance of L. Babcock and K. Sault is gratefully acknowledged.
Accepted for publication 29 June 1984 (submitted for electronic processing).

ABSTRACT

Heath, M. C. 1984. Relationship between heat-induced fungal death and plant necrosis in compatible and incompatible interactions involving the bean and cowpea rust fungi. Phytopathology 74:1370-1376.

Postinoculation heat treatment of bean and cowpea leaves infected with their respective compatible rust fungi resulted in the seemingly rapid death of the fungus and the encasement of haustoria. Browning of invaded cells was rare and, although discolored flecks developed on bean leaves heated during uredium formation, this was caused by the browning of the fungus and cell walls of the plant, rather than the plant cytoplasm. These results suggest that bean and cowpea rust fungi do not release products during death that cause significant necrosis in susceptible tissue. In an incompatible combination of bean and the bean rust fungus, and in infections of the same fungus in the nonhost species, cowpea, fluorescence microscopy revealed no signs of haustorium death before that of the invaded plant cell. Postinoculation heat treatment applied to these plant-fungus combinations inhibited the normal plant cell necrotic reaction if applied early enough. Heat treatment applied later had no effect on the frequency or extent of plant browning, indicating either that the browning had been irreversibly triggered prior to heating or that the fungus had reached a stage of development at which recently-formed, constitutive, necrosis-causing, factors were released during fungal death. It is suggested that the former hypothesis is the more likely and that the initiation of plant necrosis in the incompatible interactions examined requires some activity of the living fungus.

Additional key words: *Phaseolus vulgaris, Uromyces phaseoli*, and *Vigna sinensis*.

Although plant cell necrosis (often termed the hypersensitive response) is one of the most common responses of resistant plants to fungal infection, its role in resistance and its mode of induction are still controversial issues. One hypothesis, based initially on the appearance of macroscopic brown flecks on susceptible, infected tissue after treatments that supposedly kill the fungus (1,7,18), suggests that plant cell death is a consequence of the prior death of the pathogen. This paper reexamines this concept for compatible bean and cowpea rust infections and presents evidence that the necrosis observed in specific examples of cultivar and nonhost resistance towards the former fungus is unlikely to be caused by the prior death of the haustorium or intercellular mycelium.

MATERIALS AND METHODS

French bean (*Phaseolus vulgaris* 'Pinto' and '765') and cowpea (*Vigna sinensis* (Torner) Savi 'Early Ramshorn') were grown in Metromix 220 (W. R. Grace & Co. of Canada Ltd.) in growth chambers maintained at 20–22 C and illuminated for 16 hr/day at about 15,000 lx. Plants were treated weekly with 20-20-20 fertilizer, and the growing tips were pinched out to maintain the plants at the primary leaf stage. The upper leaf surfaces of 10- to 12-day-old plants were brush-inoculated (15) with washed (11) urediospores of either the bean rust fungus (*Uromyces phaseoli* (Pers.) Wint. var. *typica* (Arth.)) or the cowpea rust fungus (*U. phaseoli* (Pers.) Wint. var. *vignae* (Barclay) Arth.). Plants were heat treated at various times after inoculation by immersing one primary leaf of each plant in deionized, distilled water at 50 C for 30, 60, or 90 sec. Leaf pieces were harvested at various times after treatment. Some were vacuum infiltrated with water or a 10^{-4} M aqueous solution of chlortetracycline hydrochloride (CTC) (Sigma Chemical Co.) and examined immediately. Others (referred to as "cleared" leaf pieces) were decolorized in boiling ethanol, cleared for several days in saturated chloral hydrate and mounted in a modification (4) of Hoyer's medium. For the detection of callose encasements, tissue was decolorized in boiling ethanol and soaked for several days in either 0.07 M K_2HPO_4, pH 8.9, or the same solution containing 0.005% aniline blue (10). Fresh or treated tissue was examined with a Reichert-Jung Polyvar microscope using epifluorescence (exciter filter BP 330-380, dichroic mirror DS 420, barrier filter LP 418 for aniline blue or CTC-induced fluorescence, exciter filter BP 450-495, dichroic mirror DS 510, barrier filter LP 520 for autofluorescence) or interference contrast optics. The total amount of intercellular hyphae at each infection site was estimated with the aid of an ocular micrometer. Unless otherwise mentioned, data were obtained from 25 or more infection sites observed by scanning two 1 cm^2 leaf pieces cut from each of three plants. Data in the text are given as the mean and standard deviation of the average value obtained from each leaf piece. Other data were analyzed by analysis of variance. Infection structures of the bean rust fungus were produced on collodion membranes as described previously (9): membrane pieces were gently mounted in double-distilled water or CTC solution.

RESULTS

Fluorescence of dying fungal infection structures and plant mesophyll cells. To find a method of detecting the onset of fungal and plant cell death in host-parasite interactions, preliminary experiments were performed to evaluate the usefulness, in this context, of autofluorescence, and the fluorescence resulting from the presence of CTC. Although the latter compound is usually used to localize membrane-located calcium (2), it was found that infection structures of the bean rust fungus, grown on collodion membranes, did not fluoresce in CTC solution as long as they exhibited the characteristic appearance and cytoplasmic movements of living hyphae (10). As infection structures senesced and died due to the absence of the host plant (24–48 hr after inoculation), the onset of death could be detected by the development of Brownian motion in cytoplasmic particles. The cytoplasm subsequently appeared to gel. Only at this stage did the cytoplasm fluoresce bright yellow in the presence of CTC. A slight yellow autofluorescence, detectable in water-mounted infection

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

structures, developed at this time, but bright yellow autofluorescence did not develop until the cytoplasm began to turn brown over 24 hr later. On fresh leaf pieces, cut from susceptible plants infected with either the bean or cowpea rust fungi and mounted in water or CTC solution, a similar sequence of events was observed in infection structures formed on the leaf surface by appressoria that were not located over stomata. When such leaf pieces were cleared, dead (but unbrowned) infection structures showed the same faint yellow autofluorescence, detectable against the general green background fluorescence of the tissue, as seen in similar infection structures formed on collodion membranes. Brown infection structures fluoresced much more brightly. Thus, these studies suggested that CTC-induced fluorescence in fresh tissue, and autofluorescence in cleared tissue, were good indicators of the death of the rust fungus; such phenomena were therefore looked for in experimental tissue.

CTC-induced fluorescence and autofluorescence of mechanically damaged plant mesophyll cells was examined at the edges of pieces of cowpea and bean leaves cut from the plants with scissors. In fresh pieces mounted in CTC solution, all cut and obviously damaged cells showed bright CTC-induced fluorescence, but no yellow autofluorescence, when examined immediately after cutting. Undamaged cells exhibited only red chlorophyll fluorescence. Examination of tissue given a single cut, and then removed from the plant at various intervals after cutting, showed that CTC-induced fluorescence of the cut edge diminished over the next 6 hr, and yellow autofluorescence in damaged, and adjacent, cells increased. However, when the same leaf pieces were cleared after a quick microscopical examination, or when additional pieces were cleared without prior examination, strong yellow autofluorescence was seen in cells adjacent to edges damaged only 15–30 min before fixing in ethanol; no such fluorescence had been visible in fresh tissue at this time. Fluorescing cells usually appeared to have coagulated contents, did not clear easily, and were not initially brown although slight browning could be detected in tissue harvested 6 hr or more after cutting. These results suggested that autofluorescence of mesophyll cells in cleared tissue was an early indicator of the physiological changes which lead to autofluorescence and browning in fresh leaves.

Compatible interaction. *Presporulation heat treatment.* Pinto bean or cowpea plants were heat treated for 30, 60, or 90 sec at 8, 24, or 48 hr after inoculation with their respective compatible rust fungi. All heat treatments appeared to stop fungal growth virtually immediately, since the total length of intercellular hyphae per infection site did not change in the 3- or 5-day period following treatment. Moreover, all values were statistically identical to those obtained from control, unheated leaf pieces harvested at the time of treatment (*unpublished*).

In general, no obvious effect of the heat treatment was observed in plant cells away from infection sites. The only exception was the development of widespread browning and collapse of mesophyll cells in a few bean leaves about 3 days after they were subjected to the 90-sec heat treatment. Leaf pieces from these plants were not examined further for host responses to infection. For the remaining plants, no differences in plant or fungal appearance were seen between leaves heated for 30, 60, or 90 sec.; therefore, data from all three heat treatments were combined.

In plants heat treated at 8 hr after inoculation, fungal growth was inhibited during the formation of the infection hypha and before the formation of the first haustorium. In tissue harvested 24, 48, or 72 hr after heat treatment, a few sites were seen where the fungal apex had apparently burst, spilling cytoplasm into the intercellular space. No cytoplasmic browning, granulation, or collapse of host cells was observed at these, or any other, infection sites (Fig. 1).

In leaves heated at 24 hr after inoculation, infection sites usually contained two (for the bean rust fungus which often forms bifurcated infection hyphae) or one (for the cowpea rust fungus) haustoria. In leaves heated at 48 hr after inoculation, infection sites of both fungi contained an average of four haustoria. In both situations, only haustorium-containing cells showed any effect of the treatment, and most commonly, the effect consisted solely of the formation of an encasement around the haustorium (Figs. 2 and 3). Encasements tended to develop faster in cowpea (Table 1) than in bean, but in both plants, the majority of haustoria was fully encased 48 hr after the heat treatment. In cowpea, a few cells containing encased haustoria became brown and slightly collapsed within 3–5 days after heating (Table 1).

Relatively few haustoria remained unencased, and in such situations, the contents of some invaded cells became slightly brown or developed granular contents. This browning or granulation developed faster in cowpea than in bean, and all unencased haustoria were in brown or granular cells by 3 or 5 days after heating (Table 1). However, even in cowpea, brown or granular cells were seen in no more than 18% of all infection sites, and in no situation did the average number of brown or granular cells significantly exceed one (Table 1) even in sites containing four to six haustoria.

This experiment was repeated twice more with the bean rust fungus and once more with the cowpea rust fungus. In all cases results were essentially identical to those described above. In no

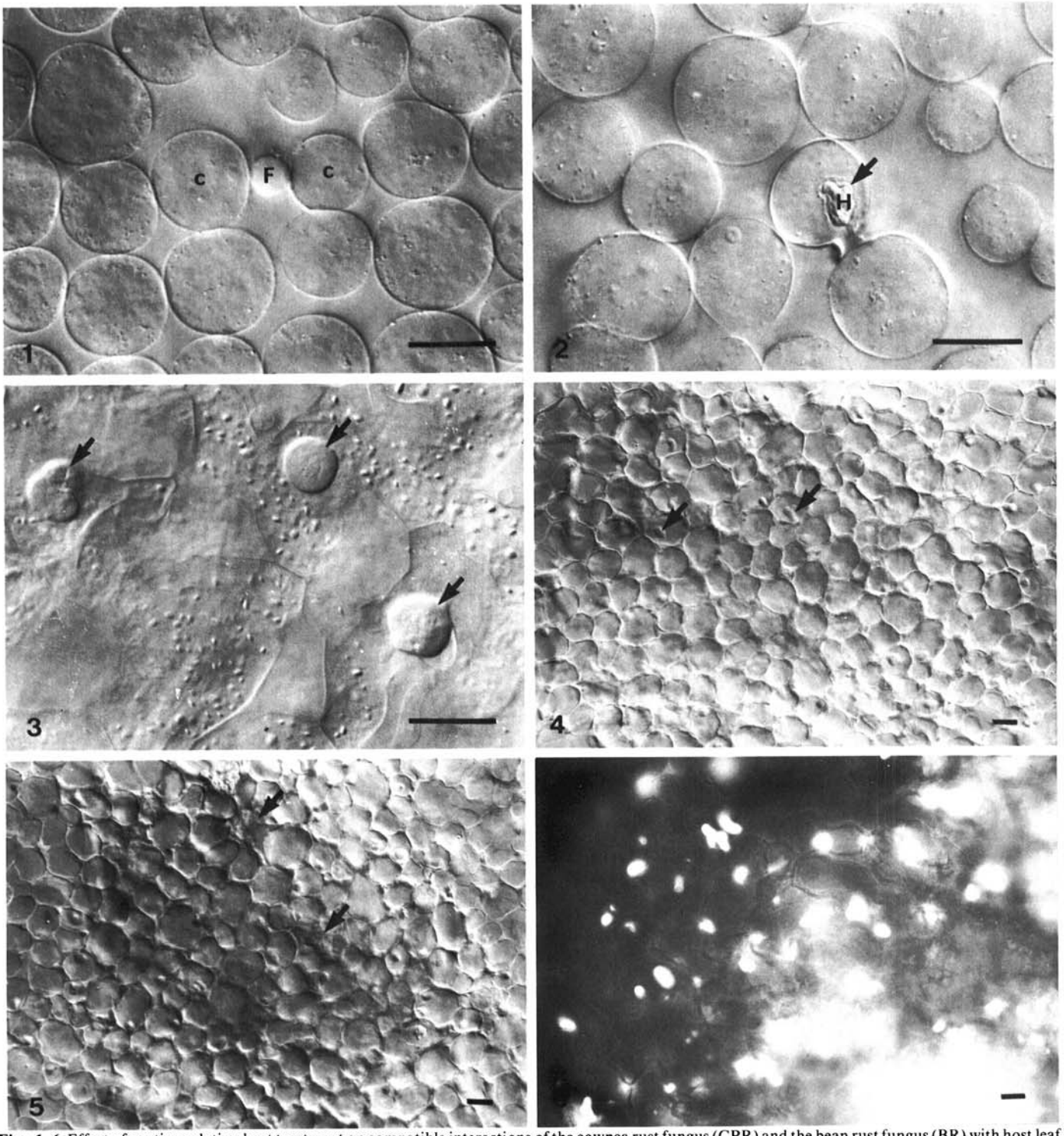
TABLE 1. Response of haustorium-containing cells after heat treatment of susceptible rust-infected French bean and cowpea leaves

Time of heat treatment after inoculation (hr)	Time of harvest after heating (hr)	Haustorium-containing cells (%)[a] with: unencased haustorium in brown or granular cell	encased haustorium, no cell browning or granulation	encased haustorium in brown or granular cell	Brown cells (mean number) per site[b]
24	24 BR[c]	0	0	0	0
	CPR	8	50	0	1.0
	48 BR	0	73	0	0
	CPR	0	100	0	0
	72 BR	0	96	0	0
	CPR	18	70	12	1.0
48	24 BR	0	4	0	0
	CPR	0	65	0	0
	48 BR	0	96	0	0
	CPR	8	87	0	1.0
	120 BR	3	97	0	1.0
	CPR	5	86	9	1.2

[a] Each value derived from observations of over 20 or 50 haustorium-containing cells for leaves heated at 24 or 48 hr, respectively.
[b] Of those sites with brown cells.
[c] BR = bean rust fungus, CPR = cowpea rust fungus.

case was any encasement of haustoria, or browning of invaded cells, seen in unheated tissue harvested at 24 or 48 hr after inoculation.

To determine how soon the fungus was killed after heat treatment, French bean leaves infected 1 or 5 days earlier with the bean rust fungus were heated for 30 sec. Leaf pieces were immediately removed and infiltrated with water or CTC and examined microscopically for fluorescence. At 10 min after heating, the intercellular fungus in CTC-treated tissue fluoresced bright yellow at about 50% of the infection sites, and this had



Figs. 1–6. Effect of postinoculation heat treatment on compatible interactions of the cowpea rust fungus (CPR) and the bean rust fungus (BR) with host leaf cells. Bars = 20 μm. Figs. 1–5 from whole mounts of cleared tissue. **1,** Infection hypha (F) of the CPR in cowpea 3 days after application of a 60-sec heat treatment given 8 hr after inoculation. No haustorium has formed and there is no browning of surrounding cells (c). **2,** A haustorium (H) of CPR in cowpea 3 days after application of a 30-sec heat treatment given 24 hr after inoculation. A thin encasement (arrow) surrounds the haustorium and the encompassing host cell contents appear normal. **3,** Three thickly-encased haustoria (arrows) of the BR 5 days after application of a 30-sec heat treatment given 48 hr after inoculation. All host cells near the infection site appear normal: the granular appearance of the host cytoplasm is due to chloroplasts remaining visible after the incomplete clearing of the tissue. **4,** BR in bean 6 days after application of a 30-sec heat treatment given 7 days after inoculation. This nonsporulating colony covers most of the area of the photograph and virtually all the haustoria (eg, arrows) are in normal-appearing cells. **5,** A sporulating colony adjacent to that shown in Fig. 4. A few light brown, collapsed host cells (arrows) can be seen at the colony center, but the majority of haustoria-containing cells appear normal. The dark shadow over the colony center is caused by the brown color of overlying immature urediospores. **6,** The appearance under UV irradiation of the edge of a colony similar to that shown in Fig. 4 but treated with aniline blue solution. All the bright fluorescent spots are haustoria.

increased to about 70% at 1 hr after heat treatment. By 5 hr after heating, the intercellular mycelium was fluorescent at virtually all sites and the cytoplasm of about 10–25% of the mesophyll cells scattered throughout the leaf was also fluorescent in younger-looking leaves. No fluorescent haustoria were observed, and it was difficult to be sure that the haustorial mother cells were fluorescing. In tissue examined 22 hr after heating, mesophyll fluorescence could no longer be detected, and all mesophyll cells looked normal under bright-field illumination. No clear yellow fluorescence of the fungus was observed in water-infiltrated, heated tissue, or in unheated tissue infiltrated with water or CTC. In tissue heated at 5 days after inoculation, haustoria did not appear to fluoresce with CTC even at 48 hr after treatment. However, some encasements, particularly those which surrounded haustoria in epidermal cells, were slightly fluorescent at this time. Such encasements showed a lesser degree of bluish fluorescence in water-infiltrated tissue.

To better determine at what stage the haustoria were killed, tissue heated at 5 days after inoculation was harvested 24 hr later, cleared, and examined for autofluorescence of the fungus. All fungal structures, including haustoria, exhibited a yellow-green fluorescence distinguishable from the green background fluorescence of the tissue. Only this latter green fluorescence was exhibited by fungus and mesophyll cells in cleared tissue harvested from infected, unheated leaves.

Heat treatment during uredium formation. Leaves of two rust-infected Pinto plants, inoculated 7 days previously and showing the flecking symptoms indicative of early uredium formation, were heated for 30 sec. By 3 days after treatment, no obvious macroscopic changes could be seen, and no brown or collapsed host cells were observed under the light microscope in or around the pustules in cleared leaf pieces.

By 6 days after heat treatment, all treated leaves bore brown flecks. Microscopical examination of cleared tissue revealed that the brown coloration primarily resided in the developing urediospores and intercellular mycelium, although plant cell walls in the center of the colony were also pale brown. Colonies in which urediospore initiation had only just begun, showed only slight browning of the intercellular mycelium at the colony center, and this was undetectable in black and white photographs (Fig. 4). In all cases, only one to seven host cells in the center of the colony had brown or collapsed contents (Fig. 5), and these appeared to be only slightly more numerous than those seen in unheated infection sites. No haustorium-containing cell, or uninfected cell, around the colony periphery showed any sign of granulation, browning, or collapse (Figs. 4,5).

Careful examination of cleared tissue harvested 3 or 6 days after heat treatment suggested that the majority of haustoria were surrounded by a thin, often barely detectable, encasement. Since haustorial encasements commonly contain callose and therefore fluoresce strongly under UV irradiation after aniline blue treatment (16,20), aniline blue-treated tissue was examined by epifluorescence to check whether, in fact, all haustoria were encased. Strong fluorescence was observed associated with all the haustoria examined (Fig. 6) while no such fluorescence was observed in heat-treated, infected tissue soaked in phosphate solution alone. No more than four fluorescent haustorial encasements per colony were observed in comparable, unheated tissue treated with aniline blue; such encasements were usually in epidermal cells only.

Incompatible interaction. *Bean cultivar 765 and the bean rust fungus.* In this combination, the bean plant responds to infection by producing brown flecks on the leaves, and no uredia are produced. Microscopically, plant cell browning is first detected in cleared tissue between 48 and 72 hr after inoculation and occurs in both haustorium-containing, and uninvaded, cells at the center of the colony. At 70% or more of the infection sites, browning of cells continues in the vicinity of the fungus, and fungal growth ceases between 72 and 96 hr after inoculation. However, by 7 days after inoculation, the remaining fungal colonies have abundant, seemingly healthy, hyphae and haustoria and exceed 100 μm in diameter. Such colonies are generally associated with a similar number of brown cells as the more restricted colonies, but these are scattered over a much larger area and most haustorium-containing cells show no indications of necrosis.

Heat treatment for 30 sec at 24 hr after inoculation caused the fungus to stop growing after the formation of the infection hypha and one or two haustoria, depending on whether the infection hypha had bifurcated. When examined in cleared tissue harvested 6 days later, 94 $\pm$4.5% of all observed haustoria were obviously encased (Fig. 7). The remaining haustoria did not appear to be encased, and were found in cells which were collapsed and had granular and/or brown contents. No browning of uninvaded cells was observed.

The same heat treatment was also applied at 48 hr after inoculation, when infection sites had one to five haustoria, just before the onset of plant browning. Two days later, every haustorium in 80 $\pm$1% (mean and standard deviation from two leaf pieces from two plants) of infection sites seen in cleared tissue was encased (Fig. 8). Up to 10 brown cells that did not contain haustoria were observed at some of these sites. The fungus at the remaining sites was embedded in a cluster of 10 or more dark-brown cells and it was impossible to observe the state of haustoria. By 5 days after heating, all sites had similar clusters of brown cells, and encased haustoria (usually only one or two per site [Fig. 9]), were detected in only 49 $\pm$14% of the infection sites observed. No encased haustoria were observed at infection sites in unheated tissue.

The average number of brown cells per infection site counted 5 days after heating in this, and two subsequent experiments, is given in Table 2. Only sites that had at least one brown cell were counted. For untreated leaves and plants, sites with large, apparently growing colonies were ignored to allow comparison of the effect of the heat-killed colonies with those that naturally stopped growing at a similar time. In all three experiments, there was no significant difference between heated and unheated leaves in the average number of brown cells per infection site.

In all heated, cleared tissue, the fungus showed a yellow-green autofluorescence that differed from the green background fluorescence of the tissue. In control, unheated tissue, both fungus

TABLE 2. Effect of postinoculation heat treatment on the browning of cells of French bean cultivar 765 resulting from infection by the incompatible bean rust fungus

Experiment	Heat duration (sec)[a]	Brown cells per infection site (avg. no.)[b]		
		Heated leaves[c]	Unheated leaves[c]	Control leaves[d]
1	30	12.4[e]	16.1	15.6
2	30	11.1	14.3	12.5
3	60	17.0	10.6	15.8

[a] 50 C applied at 48 hr after inoculation.
[b] Measured 5 days after heating: only sites showing at least one brown cell were included. For unheated leaves and plants, only nongrowing colonies were examined.
[c] Heated and unheated leaves of the same plant.
[d] Untreated plants.
[e] Means of six leaf pieces from three plants. In no experiment did the value for heated leaves differ significantly from the combined values for unheated and control leaves ($P = 0.01$).

TABLE 3. Effect of postinoculation heat treatment on the browning of nonhost cowpea leaf cells resulting from infection by the bean rust fungus

Time after heating (days)[a]	Brown cells per infection site (avg. no.)[b]		
	Heated leaves[c]	Unheated leaves[c]	Control leaves[d]
2	1.8[e]	2.1	1.9
6	1.9	2.5	1.8

[a] Heated for 30 sec at 50 C, 16 hr after inoculation.
[b] Only sites with at least one brown cell are included.
[c] Heated and unheated leaves from the same plants.
[d] Untreated plants.
[e] Means of two leaf pieces from each of three plants. F values for the comparison of all values were nonsignificant at $P = 0.05$.

and plant fluoresced a similar dull green at infection sites with no plant browning. At sites where invaded cells had begun to brown, such cells showed bright yellow autofluorescence and, in some cases, the haustoria and haustorial mother cells were similarly fluorescent. Such fluorescence rarely extended to the intercellular mycelium. Colorless, haustorium-containing cells with granular contents did not exhibit yellow fluorescence in either plant cell or haustorium. No instance of yellow autofluorescence of the haustorium was seen without similar fluorescence of the invaded cell.

Cowpea and the bean rust fungus. The cowpea is not considered to be a host species for the bean rust fungus (3). As for many examples of nonhost resistance to rust fungi (13), infection sites in this plant-fungus combination commonly lack haustoria. However, this particular combination is unusual in that the number of infection hyphae that do form a single haustorium is relatively high (15) and markedly increases in young plants (*unpublished*). In the absence of a haustorium, the infection hypha eventually shrivels and the surrounding cells appear to remain healthy, although at some sites their cell walls become brown and refractile by about 7 days after inoculation. At sites with haustoria, the invaded cell contents become brown between 24 and 48 hr after inoculation. Although a few infection hyphae produce small (30 μm) secondary hyphae, the majority do not and intercellular growth at sites with or without haustoria essentially remains at that established during the first 24 hr of infection.

In this plant-fungus combination, heat treatment was applied for 30 sec at 16 hr after inoculation. Total hyphal length at 2 days after heating was virtually identical in cleared tissue from heated leaves (62.4 $\pm$6.8 μm), unheated leaves of the same plants (59.0 $\pm$3.0 μm), and untreated plants (62.8 $\pm$2.5 μm). Thus, it appeared that intercellular growth had essentially stopped before heat treatment. At 2 days after heating, 24.6 $\pm$10.4% of the infection sites had no haustoria in heated leaves, and there was no sign of browning in surrounding plant cells. By 6 days after heating, a similar 26.2 $\pm$10.3% of the sites were also observed to have no haustoria or any browning of surrounding plant cell contents, although there was some browning of cell walls similar to that seen in untreated leaves and plants.

At sites with haustoria, only 3.7 $\pm$0.4% had haustoria that were detectably encased by 2 days after heat treatment. None of these haustoria appeared to be fully developed, and some consisted only of a peglike neck terminating in a very small body (Fig. 10). Half of these haustoria were in cells with granular or brown contents. The apparently unencased haustoria seen at other infection sites were all in cells showing granulation or browning of their contents. At a few sites, from one to 10 additional brown cells were present that did not contain haustoria. The average number of brown cells at sites with at least one brown cell did not differ between heated or unheated leaves, and values were almost identical to those obtained from the same plants 4 days later (Table 3, Fig. 11). No encased haustoria were observed in unheated tissue.

In heated, cleared tissue harvested 2 days after heat treatment, haustoria and intercellular hyphae could be distinguished from the mesophyll cells by their yellow-green autofluorescence. Examination of control, unheated plants harvested at 17 and 30 hr after inoculation revealed no instance of yellow autofluorescence of haustoria in the absence of similar fluorescence of the invaded cell. However, faint autofluorescence of colorless, haustorium-containing cells, often with granular contents, was observed with little (Figs. 12 and 13) or no detectable autofluorescence in haustorium or haustorial mother cell. Brown, haustorium-containing cells exhibited bright yellow autofluorescence, which was usually accompanied by the equally bright autofluorescence of the haustorium and haustorial mother cell (Figs. 14 and 15). Such fluorescence rarely extended into the infection hypha.

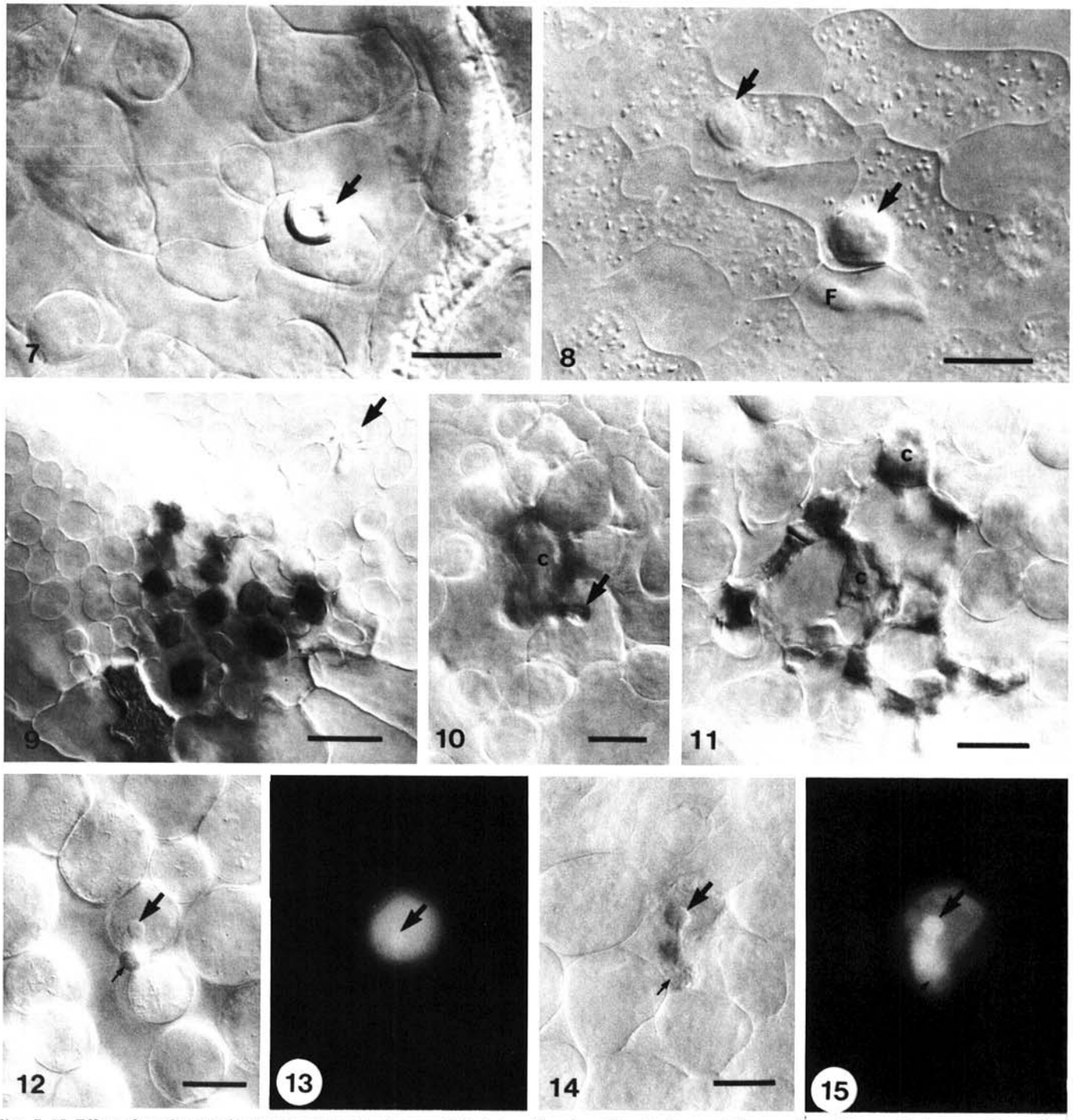
DISCUSSION

The results described here support other reports (1,5,7,8,18,26,27) that pathogenic fungi are more sensitive than their hosts to short bursts of high temperature. In all the situations tested, the bean rust and the cowpea rust fungus grew no further after the tissue was heated. That the fungus was killed rapidly is suggested by the observation that the intercellular mycelium of the bean rust fungus began to fluoresce with CTC within minutes of the heat treatment, and by the fact that autofluorescent haustoria and haustorial mother cells were detectable in cleared tissue within 24 hr of heating. Uninvaded plant cells, in contrast, showed no microscopical signs of heat damage other than a transient CTC-induced fluorescence of some cells in young leaves.

For susceptible, rust-infected cowpea and bean plants, the typical response triggered by heat-induced fungal death was the encasement of haustoria in callose-containing material. Host cell necrosis, as indicated in cleared leaves by a browning, collapse, or granulation of the cytoplasm, was not induced at all next to infection hyphae lacking haustoria and was only infrequently seen in haustorium-containing cells. While it could be argued that this may not represent the normal response of the plant to the dying fungus, but one induced by the heat treatment, several factors suggest that this is unlikely. First, other studies have shown that heat treatment temporarily abolishes, but does not change the nature of, plant defense reactions toward rust fungi (12). Second, a similar lack of plant necrosis next to fungicide-treated, haustoria-lacking, infection hyphae has been observed in compatible crown rust infections (25). Third, encasement of haustoria, rather than host necrosis, also has been reported in fungicide-treated, susceptible pea plants infected with *Peronospora pisi* (16). However, other studies with rust-infected beans suggest that heat, or chemical treatments supposed to selectively kill the fungus, result in extensive plant cell necrosis (18,23). As pointed out by Kim et al (17), results from experiments involving chemical treatments have to be interpreted with caution due to the potential phytotoxicity of some of these compounds, but Király et al (18) used heat treatments similar to those applied in the present work. Király et al (18) do not mention at what stage of infection the leaves were heated, but it seems most likely that it was just before sporulation since this was the only stage of fungal development in the present study in which heating resulted in brown flecks appearing on the leaves. Microscopical examination of these flecks revealed that it was primarily the fungus and plant cell walls that were brown, not the plant cell contents. Such discoloraton in no way resembled the intense browning of host cytoplasm typical of most studied examples of cultivar and nonhost resistance shown by beans and cowpeas to rust infection (*unpublished*). Deverall and MacLeod (5) also have observed that the macroscopic yellow-brown areas that develop in heated, infected wheat leaves do not closely resemble the symptoms induced in the same plants by incompatible races of *Puccinia graminis* and *P. recondita*. Although detailed information is not given, their description of the microscopical appearance of heat-treated infection sites in susceptible leaves does not give the impression that host necrosis was particularly widespread. No mention is made of haustorial encasements, but such structures might have been missed since, in the present investigation, they were very thin and difficult to see by conventional microscopy when the colonies were large. Kim et al (17) similarly report that the pattern of limited necrosis seen after polyoxin D treatment of susceptible (*sr*6 gene) wheat infected with *P. graminis* did not resemble the more extensive cell death induced by the same race of the fungus in a near-isogenic (*Sr*6) resistant line. Prusky et al (25) also have shown that only half of the initially compatible host cells containing fungicide-killed haustoria of *P. coronata* eventually die.

These results suggest either that the bean and cowpea rust fungi, and perhaps other rust and downy mildew fungi, do not release components during their death which cause significant necrosis in susceptible plants or that haustorial encasement prevents such components from reaching the plant cell. Therefore, if the necrosis seen in incompatible interactions is the result of the prior death of the fungus as suggested by some investigators (1,18), resistant plants either must be more sensitive than susceptible ones to such fungal components or they must lack the ability to seal off the haustorium in an encasement. However, the present results do not support either hypothesis. No plant necrosis was observed at

haustorium-lacking infection sites of the bean rust fungus in the nonhost plant, cowpea, either in heated or unheated tissue. These results are similar to those reported for other host and nonhost interactions with rust fungi (13,22,26) and indicate that resistant plants tend not to be unduly sensitive to the products of inhibited, and eventually dying, infection hyphae. Necrosis also was absent when the resistant, bean rust-infected, bean cultivar 765 was heated 24 hr or more before the expected onset of host necrosis. Instead, all



Figs. 7–15. Effect of postinoculation heat treatment on incompatible interactions involving the bean rust fungus. Bar = 20 μm. All photographs are from whole mounts of cleared tissue. **7,** Encased haustorium (arrow) in a normal-appearing cell of bean cultivar 765 6 days after application of a 60-sec heat treatment given 24 hr after inoculation. **8,** Intercellular hypha (F) and two encased haustoria (arrows) in cultivar 765 2 days after application of a 30-sec heat treatment given 48 hr after inoculation. All plant cells at the infection site appear normal: chloroplasts are still visible in these cells due to incomplete clearing of the tissue. **9,** An infection site in cultivar 765 5 days after application of a 30-sec heat treatment given 48 hr after inoculation. The fungus, except for one encased haustorium (arrow) is obscured by a cluster of host cells with dark brown contents. **10,** A small, encased, haustorium (arrow) in cowpea 6 days after application of a 30-sec heat treatment given 16 hr after inoculation. A neighboring cell (c) has browned, but not the cell containing the haustorium. **11,** An infection site in cowpea 6 days after application of a 30-sec heat treatment given 16 hr after inoculation. A small group of invaded and uninvaded cells (c) has browned and collapsed. **12,** A haustorium (large arrow) in a nonbrowned cell in untreated cowpea tissue harvested 17 hr after inoculation. The haustorial mother cell (small arrow) can be seen in optical cross-section. **13,** Autofluorescence of the haustorium (arrow) and invaded cell, but not the haustorial mother cell, shown in Fig. 12. **14,** A haustorium (large arrow) and haustorial mother cell (small arrow) in untreated cowpea tissue harvested 32 hr after inoculation. The haustorium-containing cell is slightly brown. **15,** Autofluorescence of the haustorium (large arrow), haustorial mother cell (small arrow), and invaded cell shown in Fig. 14.

haustoria became encased. Heat treatment also could prevent necrosis at those sites in the nonhost, cowpea, at which the bean rust fungus formed a haustorium, if the heat treatment was applied during the very early stages of haustorium development. Again, the young haustorium became encased.

Thus, the results suggest that in these examples of incompatible plant-fungus interactions there is a short (nonhost species) or longer (incompatible bean cultivar) stage of fungal development during which the death of the first-formed haustorium does not cause the death of the invaded cell. This may be true of other interactions, since other studies involving rust (17) and powdery mildew (8) fungi also suggest that haustorium death does not necessarily result in the death of the invaded cell in incompatible plants. The fact that such death can be inhibited when the haustorium is killed suggests that continued fungal activity is necessary for necrosis to be initiated.

If heat treatment was applied to bean rust-infected cultivar 765 or cowpea plants just before the normal onset of browning, haustoria became encased as usual. However, browning developed unhindered and to the same extent seen in unheated plants, even though fungal growth continued in unheated cultivar 765 for at least another 24 hr. Conceivably it could be argued that the fungal activity necessary to initiate necrosis represents the formation of constitutive toxic compounds at a specific stage of fungal development. If the fungus is killed at this stage by the plant or by heat treatment, cell death may occur either because of the release of these compounds from the dying haustorium or because their toxic effects are no longer negated by any counteracting process of the living fungus. Such a hypothesis requires the death of the fungus to occur before that of the plant in untreated, incompatible interactions. Fluorescence microscopy provided no evidence for such premature fungal death in cultivar 765 or in cowpea plants. Furthermore, haustoria of the bean rust fungus in the latter plant remain ultrastructurally normal for some time after the contents of the plant cells have completely disorganized (Figs. 36 and 37 in 20). Thus, the simplest hypothesis suggested by the data is that, in cowpea and cultivar 765, cell death had been irreversibly triggered by the living bean rust fungus just before heat treatment and that it continued irrespective of the subsequent death, or continued growth, of the fungus. A modification of this hypothesis is that, instead of cell death being triggered directly, the first of a series of physiological changes had been induced that would eventually result in cell death in unheated tissue; in heated plants, this change resulted in heat-induced cell necrosis. Such a change in heat sensitivity of surrounding plant cells has been reported for powdery mildew infection sites in incompatible barley (8), but it seems unlikely to explain the necrosis seen in heated cultivar 765 because treated cells were initially healthy enough to synthesize the observed haustorial encasements.

Taken altogether, the data presented here provide no evidence for the hypothesis, suggested by others (1,18), that cell death in resistant, rust-infected tissues is the result of prior death of the fungus, or that such death can cause plant cell necrosis. Instead, they, and other data (8,17,26) suggest that such necrosis requires the continued metabolic activity of the fungus and that it can be prevented by inhibiting fungal development before a "critical" stage of development is reached. This stage seems to be the early development of the first haustorium in the cowpea-bean rust fungus combination, but occurs much later when the same fungus infects bean cultivar 765. Possibly the need for a living fungus to trigger necrosis may be a general feature of most, although perhaps not all (24), interactions involving fungi that develop a sophisticated biotrophic relationship with their hosts. Interestingly, continued activity of the living pathogen also seems to be a prerequisite for the induction of necrosis in resistant, bacteria-infected plants (19,21). Such observations contrast markedly with the rather substantial body of data that suggests the constitutive components of *Phytophthora infestans* can elicit cell death in compatible and incompatible potato tissue (6,7). Whether necrotrophs, and less well-adapted biotrophs, differ from the more highly adapted biotrophs in this respect seems to be a worthwhile area of investigation.

LITERATURE CITED

1. Barna, B., Érsek, T., and Mashaâl, S. F. 1974. Hypersensitive reaction of rust-infected wheat in compatible host-parasite relationships. Acta Phytopathol. Hung. 9:293-300.
2. Caswell, A. H. 1979. Methods of measuring intracellular calcium. Int. Rev. Cytol. 56:145-181.
3. Cummins, G. B. 1978. Rust fungi on Legumes and Composites in North America. University of Arizona Press, Tucson. 424 pp.
4. Cunningham, J. L. 1972. A miracle mounting fluid for permanent whole-mounts of microfungi. Mycologia 64:906-911.
5. Deverall, B. J., and McLeod, S. 1980. Responses of wheat cells around heat-inhibited rust mycelia and associated with the expression of the *Lr*20, *Sr*6, and *Sr*15 alleles for resistance. Physiol. Plant Pathol. 17:213-219.
6. Doke, N., and Furuichi, N. 1982. Response of protoplasts to hyphal wall components in relationship to resistance of potato to *Phytophthora infestans*. Physiol. Plant Pathol. 21:23-30.
7. Érsek, T., Barna, B., and Király, Z. 1973. Hypersensitivity and the resistance of potato tuber tissues to *Phytophthora infestans*. Acta Phytopathol. Hung. 8:3-12.
8. Hazen, B. E., and Bushnell, W. R. 1983. Inhibition of the hypersensitive reaction in barley to powdery mildew by heat shock and cytochalasin B. Physiol. Plant Pathol. 23:421-438.
9. Heath, I. B., and Heath, M. C. 1976. Ultrastructure of mitosis in the cowpea rust fungus *Uromyces phaseoli* var. *vignae*. J. Cell. Biol. 70:592-607.
10. Heath, I. B., and Heath, M. C. 1978. Microtubules and organelle movements in the rust fungus *Uromyces phaseoli* var. *vignae*. Cytobiologie 16:393-411.
11. Heath, M. C. 1971. Haustorial sheath formation in cowpea leaves immune to rust infection. Phytopathology 61:383-388.
12. Heath, M. C. 1974. Light and electron microscope studies of the interactions of host and non-host plants with cowpea rust—*Uromyces phaseoli* var. *vignae*. Physiol. Plant Pathol. 4:403-414.
13. Heath, M. C. 1977. A comparative study of non-host interactions with rust fungi. Physiol. Plant Pathol. 10:73-88.
14. Heath, M. C. 1979. Effects of heat shock, actinomycin D, cycloheximide and blasticidin S on nonhost interactions with rust fungi. Physiol. Plant Pathol. 15:211-218.
15. 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.
16. Hickey, E. L., and Coffey, M. D. 1980. The effects of Ridomil on *Peronospora pisi* parasitizing *Pisum sativum*: An ultrastructural investigation. Physiol. Plant Pathol. 17:199-204.
17. Kim, W. K., Rohringer, R., Samborski, D. J., and Howes, N. K. 1977. Effect of blasticidin S, ethionine, and polyoxin D on stem rust development and host-cell necrosis in wheat near-isogenic for gene *Sr*6. Can. J. Bot. 55:568-573.
18. Király, Z., Barna, B., and Érsek, T. 1972. Hypersensitivity as a consequence, not the cause, of plant resistance to infection. Nature (Lond.) 239:456-458.
19. Klement, Z., and Goodman, R. N. 1967. The role of the living cell and induction time in the hypersensitive reaction in the tobacco plant. Phytopathology 57:322-323.
20. Littlefield, L. J., and Heath, M. C. 1979. Ultrastructure of Rust Fungi. Academic Press, New York. 277 pp.
21. Meadows, M. E., and Stall, R. E. 1981. Different induction periods for hypersensitivity in pepper to *Xanthomonas vesicatoria* determined with antimicrobial agents. Phytopathology 71:1024-1027.
22. Niks, R. E. 1983. Haustorium formation of *Puccinia hordei* in leaves of hypersensitive, partially resistant, and nonhost plant genotypes. Phytopathology 73:64-66.
23. Pring, R. J., and Richmond, D. V. 1976. An ultrastructural study of the effect of oxycarboxin on *Uromyces phaseoli* infecting leaves of *Phaseolus vulgaris*. Physiol. Plant Pathol. 8:155-162.
24. Prusky, D., Dinoor, A., and Jacoby, B. 1980. The sequence of death of haustoria and host cells during the hypersensitive reaction of oat to crown rust. Physiol. Plant Pathol. 17:33-40.
25. Prusky, D., Dinoor, A., and Jacoby, B. 1981. The fungicide or heat induced hypersensitive reaction of oats to crown rust: relations between various treatments and infection type. Physiol. Plant Pathol. 18:181-186.
26. Tani, T., Yamamoto, H., Onoe, T., and Naito, N. 1975. Initiation of resistance and host cell collapse in the hypersensitive reaction of oat leaves against *Puccinia coronata avenae*. Physiol. Plant Pathol. 7:231-242.
27. Yarwood, C.E. 1963. Heat therapy of bean rust. Phytopathology 53:1313-1316.