Capsidiol Induction in Pepper Fruit during Interactions with Phytophthora capsici and Monilinia fructicola

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ABSTRACT

The concentration of the phytoalexin, capsidiol, in pepper fruit 24 hours after inoculation with *Monilinia fructicola* was 2×10^{-4}M, sufficient in vitro to account for resistance of the fruit tissue to this fungus. The concentration tripled in the subsequent 24-hour period. However, concentrations of capsidiol were too low to explain the temporary resistance of pepper fruit tissue to *Phytophthora capsici* (isolate 18) in an initial incompatible interaction, which persisted for approximately 48 hours after zoospore inoculation.

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The accumulation of inhibitory concentrations of capsidiol during an incompatible interaction between pepper fruit and *Phytophthora infestans* was shown previously to correlate closely with the progressive restriction of hyphal growth as revealed by ultrastructural studies (2, 4). This suggested that the phytoalexin plays a significant role in disease resistance in this interaction, especially as only low, noninhibitory concentrations of capsidiol are induced in a compatible interaction with *Phytophthora capsici* (A.T.C.C. 15399) (1, 4). This paper describes parallel studies of capsidiol accumulation during interactions with *P. capsici* (isolate 18) and *Monilinia fructicola*, made in conjunction with the ultrastructural studies described in the preceding paper (3). In both cases the interactions were incompatible, but whereas with *M. fructicola* this was permanently so, with *P. capsici* resistance was temporary and invasion of tissues recommenced approximately 48 hours after inoculation (3).

MATERIALS AND METHODS.—*Phytophthora capsici* (Leonian) (isolate 18), originally isolated from a pepper fruit in North Carolina (5), was kindly supplied by R. K. Webster of the Department of Plant Pathology, University of California, Davis. It was maintained on V8 juice agar at 25 C and zoospore suspensions ($1\text{-}4 \times 10^5$ spores per ml) were prepared as previously described (3). *Monilinia fructicola* (Wint.) Honey, originally isolated from a peach fruit, was also grown routinely on V8 juice agar plates at 25 C and spore suspensions ($5\text{-}6 \times 10^5$ spores per milliliter) prepared as before (3).

Ripening fruits of sweet pepper (*Capsicum frutescens* L. var. *grossum* 'Keystone Resistant Giant') of uniform size were harvested from the field or greenhouse and immediately inoculated by injection of 10 ml of *P. capsici* or *M. fructicola* spore suspensions into the fruit cavity. Diffusates were removed from 5-10 fruits taken at random, at the time intervals indicated in the results, during 48 hours of incubation in the dark at 25 C. After recording the volume, the combined diffusates, together with water rinses from each fruit cavity, were extracted with ether as before (6). The first few cell layers lining the fruit cavity in the area in contact with the zoospore suspension were removed by first cutting the fruit into strips and then slicing off the surface layer. The slices of tissue were weighed, macerated, and extracted with ether as described previously (4, 6). Capsidiol concentrations in the ether extracts were determined by gas-liquid chromatography (4, 7). In recording the concentrations, the fresh weight of fruit tissue was regarded as water. The

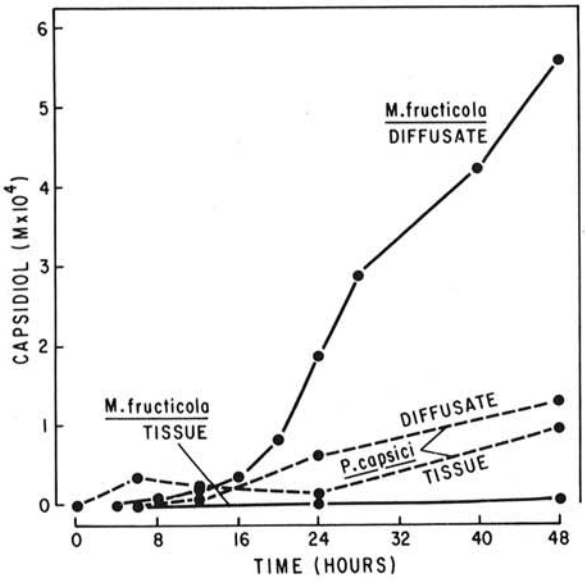

Fig. 1. Capsidiol concentrations in pepper fruit tissue (first few cell layers lining inner cavity surface) and diffusates during interactions with *Phytophthora capsici* (broken line) and *Monilinia fructicola* (solid line).

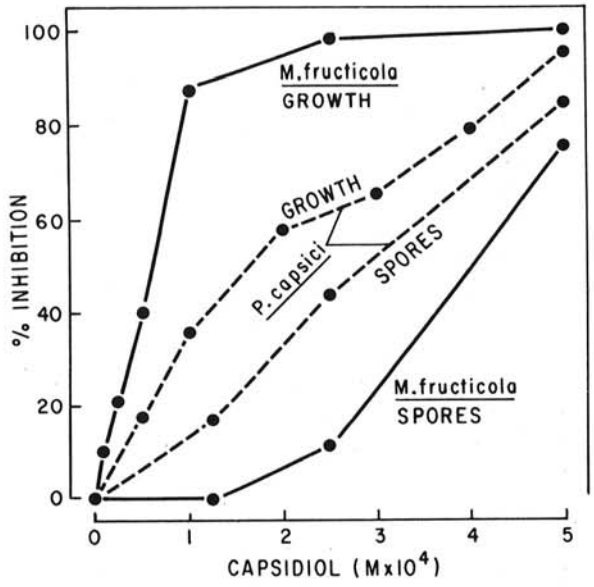

Fig. 2. Effect of capsidiol on the in vitro mycelial growth (GROWTH) and spore germination (SPORES) of *Phytophthora capsici* (broken line) and *Monilinia fructicola* (solid line).

results obtained were confirmed in duplicate experiments.

Inhibition of growth and spore germination by capsidiol in vitro was determined by standard procedures as described previously (9).

RESULTS.—In fruit inoculated with *P. capsici* (isolate 18), capsidiol was detected in small amounts in the first few cell layers after 6 hours and in the diffusate after 12 hours (Fig. 1). Capsidiol concentrations in both the diffusate and the tissue rose slowly between 12 and 48 hours, but remained low throughout. Capsidiol was detected in the diffusate from fruits inoculated with *M. fructicola* after 8 hours and subsequently increased in concentration rapidly throughout the incubation period. In contrast, only trace amounts were detected in the surface tissue (Fig. 1).

The in vitro growth of *M. fructicola* was more sensitive to capsidiol than that of *P. capsici* (isolate 18). Whereas the ED_{50} and ED_{100} values for *M. fructicola* were about 0.6 and 5.0×10^{-4}M, respectively; for *P. capsici* (isolate 18), they were about 1.7 and 10×10^{-4}M. However, the germination of *M. fructicola* spores was less affected by capsidiol than was the germination of *P. capsici* zoospores (Fig. 2).

DISCUSSION.—Previous studies of capsidiol accumulation in pepper fruit during interactions with *P. infestans*, a nonpathogen of peppers, and *P. capsici* (A.T.C.C. 15399), highly virulent on peppers, have provided correlations strongly supporting a role for this compound in disease resistance (4). The data presented here for *M. fructicola* appear to be consistent with this also. Thus ultrastructural evidence (3) indicated that invasion of pepper cells had ceased approximately 24 hours following inoculation. Capsidiol levels in diffusates had reached 2×10^{-4}M by that time (Fig. 1), sufficient to cause almost complete inhibition in vitro of mycelial growth (Fig. 2). During this period, hyphae infrequently penetrated the first few cell layers, but caused widespread cell death. There was no further spread of hyphae, but during the following 24-hour period, cells down to the seventh layer became severely damaged and capsidiol levels tripled. Evidently the agent(s) responsible for death of cells, and presumably for capsidiol production, diffused well in advance of invading and surface hyphae. Of the four interactions studied (1, 2, 3, 4), the extent of pepper cell damage was by far the greatest with *M. fructicola* and the accumulation of capsidiol the most rapid. Failure to determine significant levels of capsidiol in the tissue is surprising; possibly pepper fruit cells, which are highly vacuolate, lose their contents rapidly into the diffusate when severely damaged.

In contrast to these and the previous findings, the results obtained with *P. capsici* (isolate 18) provide an interesting exception. It is evident from the electron microscope studies (3), that invasion is halted 9-12 hours after inoculation in the third layer of cells. Capsidiol levels at that time (Fig. 1), and in fact throughout the 48-hour period during which growth is arrested, are low and insufficient to account for the inhibition on the basis of in vitro assay data (Fig. 2). However, the hypersensitive response of host cells in this interaction (3) appeared to be similar ultrastructurally to that in the interactions with *P. infestans* (2) and *M. fructicola* (3), in which higher concentrations of capsidiol were produced (4) (Fig. 1). In unpublished experiments, no evidence has been obtained for breakdown of capsidiol by *P. capsici* (isolate 18) in vitro. Thus the evidence strongly suggests that little capsidiol is induced and other factors are responsible for restriction of the growth of this isolate in hypersensitive pepper fruit cells, unless it is assumed that concentrations at the host-parasite interface are much higher than determined levels.

If capsidiol is a direct product of the hypersensitive reaction, concentration differences could reflect differences in numbers of reacting cells. Thus with *M. fructicola*, large numbers of cells in addition to those that were invaded, reacted hypersensitively and with *P. infestans*, hypersensitive cells were surrounded by slowly degenerating cells with cell border lesions (2), which may have contributed substantially to capsidiol levels in the tissue (4).

A second possible explanation of these results is that the hypersensitive and capsidiol-producing responses are separate cellular reactions, which may not always be invoked together. The recognition of a fungus as alien, as discussed, for example by Wood (10), may involve two and possibly more steps or degrees. Alternatively, *P. capsici* (isolate 18) may have the ability to specifically suppress capsidiol formation, as suggested by Varns and Kuć (8) for rishitin production in potatoes in response to virulent races of *P. infestans*. In either case it must be concluded that the hypersensitive response of pepper cells (without the production of inhibitory capsidiol concentrations) in itself provides a resistant phase, which remains unexplained. This is short lived however, for after approximately 48 hours rapid colonization of the tissue occurs.

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