The Significance of Capsidiol Induction in Pepper Fruit during an Incompatible Interaction with Phytophthora infestans

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ABSTRACT

In a time-course study of capsidiol accumulation during an incompatible interaction between pepper fruit tissue and *Phytophthora infestans*, concentrations of capsidiol in the tissue were totally inhibitory 24 hours after inoculation, as indicated by in vitro tests of mycelial growth inhibition. Capsidiol accumulated sufficiently rapidly therefore to account for the progressive restriction of hyphal growth observed in the ultrastructural study of the interaction previously reported. Capsidiol was shown to be fungistatic only, and other factors are presumably responsible for the subsequent death of hyphae in infected cells.

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Diffusates from pepper fruit inoculated with a number of nonpathogenic fungi accumulate the phytoalexin, capsidiol, in concentrations that are sufficient to inhibit these fungi in vitro (7). More specific evidence of a role for this compound in disease resistance is provided in this paper, which describes the time-course of capsidiol accumulation in diffusates and tissues in an incompatible interaction with *Phytophthora infestans*, a nonpathogen of peppers. This was carried out in conjunction with an ultrastructural study of the progress of the interaction described in detail in the preceding paper (2). For comparative purposes, details are also given of the accumulation of capsidiol in a compatible interaction with *P. capsici* for which an ultrasturctural study was reported previously (1).

MATERIALS AND METHODS.—*P. infestans* (Mont.) de Bary, isolated locally from tomatoes and *P. capsici* Leonian (A.T.C.C. 15299), were maintained on V8 juice agar at 18 and 25 C respectively, approximately the temperature optima for in vitro mycelial growth. These incubation temperatures were used also for all experimental work. Zoospore suspensions (1-4 $\times$ 10^5 zoospores per ml) were prepared as described previously (1, 2).

Ripening pepper fruit (*Capsicum frutescens* L. 'Keystone Resistant Giant') of uniform size (field or greenhouse grown) were inoculated immediately after harvest by injection of 10 ml of zoospore suspension into the fruit cavity. Diffusates were removed from five fruit taken at random at each of various intervals during three days of incubation in the dark. The diffusates were combined, volumes recorded, and together with a water rinse of each fruit cavity, extracted with ether as previously described (7). The first few cell layers lining the fruit cavity in the area of contact with the zoospore suspension were removed by cutting the fruit into strips and slicing off the surface layer. The slivers of tissue were weighed, macerated and also extracted with ether. The ether extracts were treated as previously (7) and capsidiol concentrations determined by gas-liquid-chromatography (GLC), essentially as described previously (8), except that peak areas were determined automatically using an integrator. In recording the capsidiol concentrations, the fresh weight of fruit tissue was regarded as having the same density as water. Results are based on five determinations.

Inhibition of growth and zoospore germination by capsidiol was determined by methods described previously (7, 8). The fungistatic, as opposed to fungicidal action of capsidiol, was demonstrated simply as follows. V8-juice agar plates containing inhibitory concentrations of capsidiol were inoculated with mycelial plugs (5 mm in diameter) from the periphery of actively growing colonies and incubated for periods indicated below, after which they were transferred to V8 juice agar without capsidiol, and subsequent radial growth measured.

RESULTS.—In fruit inoculated with *P. infestans*, capsidiol was detected in trace amounts after 6 hours and measurable amounts after 12 hours, accumulating rapidly, especially in the tissue, during the following incubation period. Very low amounts were detected after 2 days in fruit inoculated with *P. capsici*; fruit were too severely rotted beyond 2 days for meaningful comparisons to be made (Fig. 1).

P. infestans was much more sensitive to capsidiol than was *P. capsici* (Fig. 2), both in spore germination and growth assays. Whereas ED_{50} values for *P. infestans* were about 0.2×10^{-4} M, for *P. capsici* they were at least 10 times greater. Mycelial growth of *P. infestans* was completely inhibited by 2×10^{-4} M. Both fungi recovered rapidly from exposure to capsidiol. Thus *P. infestans*, exposed for up to 10 days to 5×10^{-4} M, which completely prevented growth, resumed growth immediately on transfer to control medium and reached the control growth rate after a further 2 days (Fig. 3). Similarly, *P. capsici* survived exposure to 1×10^{-3} M capsidiol for 4 days, after which growth commenced without a lag (Fig. 4).

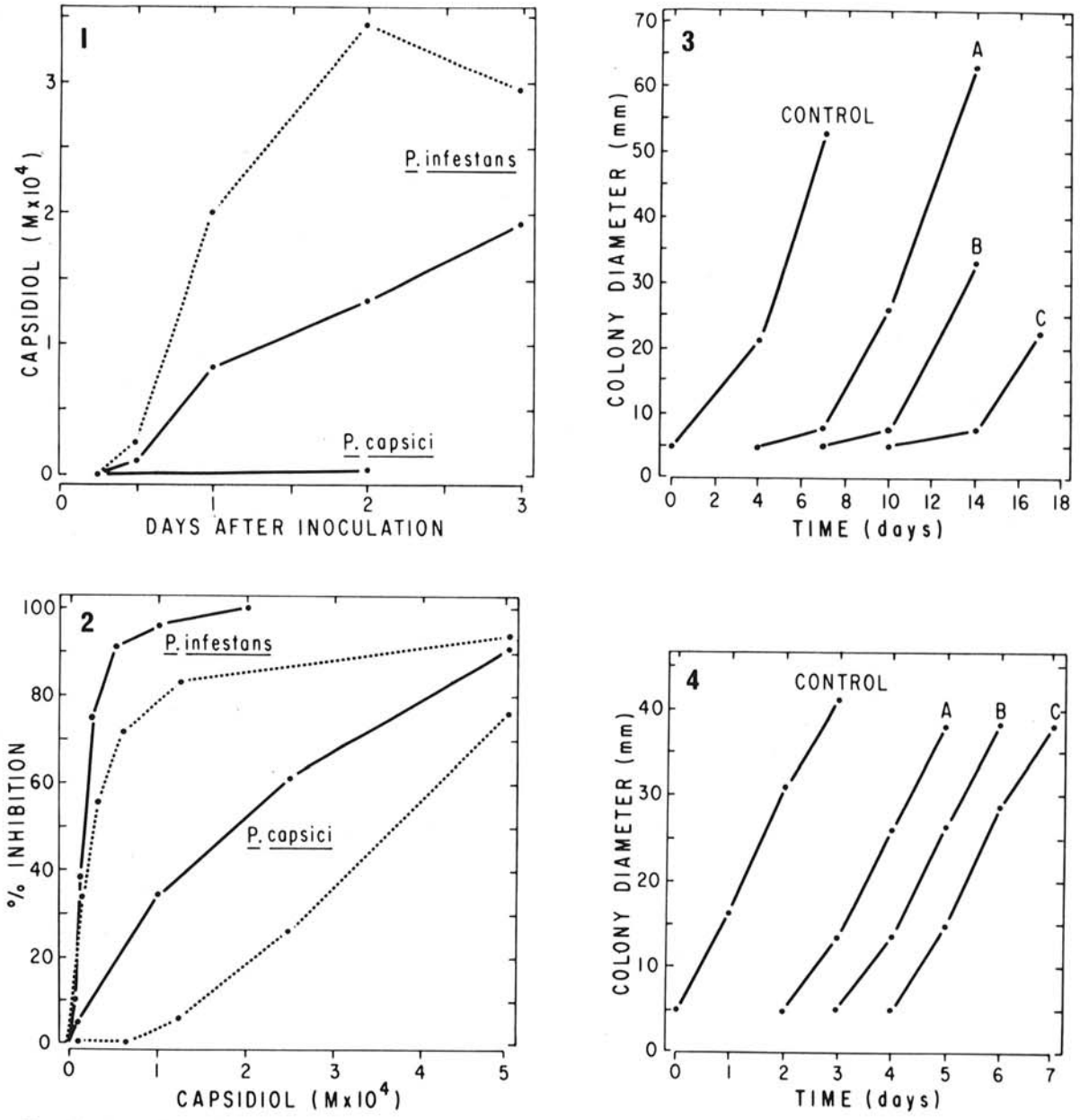

Fig. 1-4. **1)** Capsidiol induced in pepper fruit during an incompatible interaction with *Phytophthora infestans* and a compatible interaction with *Phytophthora capsici.* Concentrations in the first few cell layers lining the fruit cavity during the *P. infestans* interaction are indicated by the dotted line and in diffusates in both interactions by the solid lines. **2)** Effect of capsidiol on the in vitro mycelial growth (solid line) and zoospore germination (dotted line) of *Phytophthora infestans* and *Phytophthora capsici.* **3)** Recovery growth rates of *Phytophthora infestans* on a capsidiol-free medium after 4 (A), 7 (B), and 10 (C) days of exposure to a completely inhibitory concentration of capsidiol (5×10^{-4} M). **4)** Recovery growth rates of *Phytophthora capsici* on a capsidiol-free medium after 2 (A), 3 (B), and 4 (C) days of exposure to a completely inhibitory concentration of capsidiol (1×10^{-3} M).

DISCUSSION.—These results emphasize the considerable differences in capsidiol concentrations that accumulate in incompatible, as opposed to compatible interactions. The values in Fig. 1 probably underestimate the actual concentrations. The tendency of zoospores to collect in depressions on the inner surface of the fruit was noted previously (2) and it is unlikely that all the extracted tissue was uniformly infected. The diffusion of capsidiol from the affected tissue into the spore suspension (10 ml) must also result in a considerable dilution. Nevertheless, appreciable concentrations accumulated by 12 hours and if these are compared with the data of Fig. 2, it is evident that they approach the ED_{75} for *P. infestans*. By 1 day the concentration, at least in the tissue, is sufficient to bring about complete inhibition of growth. The situation with *P. capsici* again contrasts sharply with this, for not only

does it induce very little capsidiol, but is also very much less sensitive to it. It is unlikely that this can be explained by degradation of capsidiol by *P. capsici*, for no evidence was obtained for this in vitro (9).

Considered in relation to the progress of the resistant reaction described in the preceding paper (2), the rate of capsidiol accumulation in response to *P. infestans* appears especially significant. Thus initial infections occurred very rapidly and some cells had undergone invasion and responded hypersensitively within 4 hours following inoculation. These coincided with the period during which capsidiol concentrations were undetectable or very low. Progress of the fungus was not stopped at this stage however, but continued at a diminishing rate until 1-1.5 days following inoculation. By that time capsidiol had reached concentrations which in vitro would have completely inhibited mycelial growth. It is particularly interesting that spread of the fungus was not halted at the initial hypersensitive stage, but in the secondarily invaded, slower reacting cells. This strongly suggests that capsidiol was accumulating prior to cytoplasmic disorganization in these cells, and may well have been a product of adjacent uninfected cells which were clearly responding at the ultrastructural level. Evidence leading to conclusions similar to this has been provided for several different systems (3, 4, 5, 6). Nevertheless degenerating haustoria of *P. infestans* were only seen in cells that had died and although capsidiol, which is fungistatic only, may halt the progress of infection other factors are presumably responsible for the death of hyphae, which finally occurs in dead host cells.

LITERATURE CITED

1. JONES, D. R., W. G. GRAHAM, and E. W. B. WARD. 1974. Ultrastructural changes in pepper cells in a compatible interaction with Phytophthora capsici. Phytopathology 64:1084-1090.
2. JONES, D. R., W. G. GRAHAM, and E. W. B. WARD. 1975. Ultrastructural changes in pepper cells in an incompatible interaction with Phytophthora infestans. Phytopathology 65:
3. KITAZAWA, K., and K. TOMIYAMA. 1973. Role of underlying healthy tissue in the hypersensitive death of a potato plant cell infected by an incompatible race of Phytophthora infestans. Ann. Phytopathol. Soc. Japan 39:85-89.
4. MANSFIELD, J. W., and J. A. HARGREAVES. 1974. Phytoalexin production by live cells in broad bean leaves infected with Botrytis cinerea. Nature (Lond.) 252:316-317.
5. MÜLLER, K. O. 1958. Relationship between phytoalexin output and the number of infections involved. Nature (Lond.) 182:167-168.
6. PAXTON, J., D. J. GOODCHILD, and I. A. M. CRUICKSHANK. 1974. Phaseollin production by live bean endocarp. Physiol. Plant Pathol. 4:167-171.
7. STOESSL, A., C. H. UNWIN, and E. W. B. WARD. 1972. Postinfectional inhibitors from plants. I. Capsidiol, an antifungal compound from Capsicum frutescens. Phytopathol. Z. 74:141-152.
8. STOESSL, A., C. H. UNWIN, and E. W. B. WARD. 1973. Postinfectional fungus inhibitors from plants: fungal oxidation of capsidiol in pepper fruit. Phytopathology 63:1225-1231.
9. WARD, E. W. B., and A. STOESSL. 1972. Postinfectional inhibitors from plants. III. Detoxification of capsidiol, an antifungal compound from peppers. Phytopathology 62:1186-1187.