

Capsidiol Production in Pepper Leaves in Incompatible Interactions With Fungi

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ABSTRACT

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In incompatible interactions with *Phytophthora capsici* and *P. infestans* the concentrations of the phytoalexin, capsidiol, in hypersensitively responding pepper leaf epidermal cells was estimated on the basis of concentrations

diffusing into overlying infection droplets. Very high values were obtained (approaching molar levels) exceeding the concentration required for total inhibition of fungi in vitro by several orders of magnitude.

Although fungitoxic concentrations of the phytoalexin capsidiol are readily demonstrated in incompatible interactions in pepper fruit (5), we have failed to detect or have detected relatively low amounts in infection droplets on pepper leaves. A similar situation in potatoes has been referred to by Kuć (4) where rishitin and phytuberin were demonstrated in tubers but not in leaves. Phytoalexin production, if a fundamental mechanism in resistance, might be expected to be a general property of host cells and especially in such important sites of infection as the leaves. This report examines in more detail the situation in infection droplets on pepper leaves, particularly in relation to numbers of hypersensitively responding cells.

Droplets (approximately 0.02 ml) of spore suspensions (approximately 1×10^5 per ml) of various fungi were placed on the lower surface of detached pepper leaves (*Capsicum frutescens* L. var. *grossum* 'Keystone Resistant Giant') and incubated for 48 hours at room temperature in Pyrex trays fitted with lids lined with a pad of moistened filter paper. Droplets from a minimum of 20-30 leaves (approximately 20 ml) were removed by suction, extracted with ether, and capsidiol determined by thin-layer chromatography and gas-liquid chromatography as described in detail previously (5, 6).

Areas of leaf beneath infection droplets were excised, stained with Evans Blue (7) to aid in visualizing hypersensitive cells, and the numbers of hypersensitive cells recorded using a microscope.

An approximation of the volume of leaf epidermal cells was obtained by measuring their depth in cross section using a microscope with eye piece micrometer (mean of 50 cells from 10 leaves, 0.017 mm, range 0.013 - 0.026 mm) and, because of their highly irregular shapes, estimating their width in surface view by determining the number of cells across a measured field width. From the average value obtained (50 fields, 10 leaves, 0.0343 mm, range 0.028 - 0.043 mm), and because all the surface space is occupied, the mean surface area of an epidermal cell can be regarded as 0.0343^2 mm^2 . The average volume would then be $0.0343^2 \times 0.017 \text{ mm} = 20.17 \times 10^{-6} \text{ mm}^3$. For

calculation of capsidiol concentrations in these cells, the solid components are ignored and the total volume available for solution assumed to be the same, or $20.17 \times 10^{-9} \text{ ml}$. The mean diameter of infection droplets (0.02 ml) was found to be 4 mm; hence the surface area covered by a droplet would be $12.6 \text{ mm}^2 [\pi \times (4/2)^2]$. As the mean surface area of an epidermal cell was $0.0343^2 \text{ mm}^2 = 1.18 \times 10^{-3} \text{ mm}^2$, then the number of epidermal cells covered by a droplet is $12.6 \div 1.18 \times 10^{-3} = 10,700$. A 0.02-ml droplet of a spore suspension with 1×10^5 spores per milliliter would deposit 2,000 spores onto these cells.

Spore suspensions of many of the fungi which readily induce capsidiol in pepper fruit (5) failed to induce more than trace amounts in infection droplets on leaves and rarely caused a hypersensitive response in epidermal cells. *Monilinia fructicola* (Wint.) Honey for example [which in fruit is one of the best inducers of capsidiol and causes widespread damage to surface cells (2, 5)], only occasionally caused a hypersensitive response and capsidiol, if present, was in marginally detectable amounts. Spores germinated readily and conidiophores and chains of conidia were commonly produced at the surface emphasizing the lack of toxicity in the droplets.

Capsidiol was only produced in measurable amounts in interactions where there was a consistent hypersensitive response. Two examples of this are provided by *Phytophthora capsici* (isolate 18, an avirulent isolate obtained from R. K. Webster, Davis, California) and *Phytophthora infestans* (Table 1). On the basis of previous studies in vitro the concentrations in the droplets were insufficient to cause any inhibition of *P. capsici* [$ED_{50} 1.5 \times 10^{-4} \text{ M}$ (2)] and for *P. infestans*, which is much more sensitive [$ED_{50} 1 \times 10^{-5} \text{ M}$ (8)], only the highest value approached completely inhibitory levels. Relative to the number of epidermal cells covered by an infection droplet, the number of hypersensitively responding cells was small. However, spores that germinated and produced appressoria were invariably associated with hypersensitive host cells. There were spores with long germ tubes, but without appressoria but

TABLE 1. Capsidiol production, hypersensitive response, and estimation of capsidiol concentrations in hypersensitive cells in pepper leaves in incompatible interactions with *Phytophthora infestans* and *Phytophthora capsici*

Fungal species	Capsidiol ^a (10 ⁻⁵ M)	HR. ^b Cells	Volume ^c HR. Cells (10 ⁻⁶ ml)	Capsidiol in ^d HR. Cells (M)
<i>Phytophthora infestans</i>	1.13 ^e	260	5.25	0.04
	2.19	67	1.35	0.32
	7.34	475	9.59	0.15
<i>Phytophthora capsici</i>	1.06	50	1.01	0.21
	1.74	116	2.34	0.15
	1.09	39	0.79	0.28

^aConcentration of capsidiol in infection droplets.

^bAverage number of hypersensitively responding (HR.) cells per infection droplet.

^cVolume of hypersensitively responding cells per infection droplet, obtained by calculation from numbers in preceding column and an estimated cell volume of 20.17×10^{-9} ml.

^dCapsidiol concentration in hypersensitively responding cells calculated from the ratio of hypersensitive cell volume (Column 3) to infection droplet volume (0.02 ml), assuming that all capsidiol in the infection droplets originated in hypersensitively responding cells.

^eEach line of figures is derived from an independent experiment, capsidiol concentrations were determined in about 20 ml of infection droplets collected from 20-30 leaves, and numbers of HR. cells are based on microscopic examination of 10 or more droplets.

these did not appear to attempt penetration. Thus, although other possibilities cannot be eliminated absolutely, it seems reasonable to assume that interaction between these fungi and pepper leaf cells results only in a hypersensitive response. If it is assumed further that only the hypersensitive cells produced capsidiol the concentrations that they would have accumulated without dilution in the infection droplet, are exceedingly high, surpassing inhibitory doses by several orders of magnitude (last column, Table 1). It is probable, therefore, that cells surrounding hypersensitive ones contribute to capsidiol production either by supplying substrates or by synthesizing capsidiol in response to stimuli received from or via hypersensitive cells. In any case, the most probable pathway of diffusion to the infection droplet will be through the breaches in the epidermal layer provided by the necrotic hypersensitive cells. In fruit there was also some indication that cells other than hypersensitive ones, may contribute to capsidiol accumulation (1), and evidence strongly supporting this concept has been presented for other systems [e.g., (3)]. Nevertheless, from the calculations reported here, the capsidiol concentration could be diluted through a very large number of cells before being reduced to ineffective levels.

It is concluded, therefore, that capsidiol is produced in leaves of pepper as well as in fruit in effective concentrations. Whereas the fruit pericarp is unprotected by cuticle and epidermis, permitting fungi such as *M. fructicola* to infect, these barriers presumably preclude them from the leaf cells, and only those that can penetrate cause a hypersensitive response with the production of capsidiol. Even then the number of successful penetrations relative both to the number of spores applied and to the number of epidermal cells beneath infection droplets, is

small, hence accounting for the apparently low levels of capsidiol diffusing into infection droplets. It would be interesting to determine whether differences in the production of rishitin in potato tubers and leaves can be explained in the same way (4).

LITERATURE CITED

- JONES, D. R., C. H. UNWIN, and E. W. B. WARD. 1975. The significance of capsidiol induction in pepper fruit during an incompatible interaction with *Phytophthora infestans*. *Phytopathology* 65:1286-1288.
- JONES, D. R., C. H. UNWIN, and E. W. B. WARD. 1975. Capsidiol induction in pepper fruit during interactions with *Phytophthora capsici* (isolate 14) and *Monilinia fructicola*. *Phytopathology* 65:1417-1419.
- 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. Jap.* 39:85-89.
- KUC, J. 1972. Phytoalexins. *Annu. Rev. Phytopathol.* 10:207-232.
- STOESSL, A., C. H. UNWIN, and E. W. B. WARD. 1972. Postinfectious inhibitors from plants. I. Capsidiol an antifungal compound from *Capsicum frutescens*. *Phytopathol. Z.* 74:141-152.
- STOESSL, A., C. H. UNWIN, and E. W. B. WARD. 1973. Postinfectious inhibitors from plants: fungal oxidation of capsidiol in pepper fruit. *Phytopathology* 63:1225-1231.
- TURNER, J. G. and A. NOVACKY. 1974. The quantitative relation between plant and bacterial cells involved in the hypersensitive reaction. *Phytopathology* 64:885-890.
- WARD, E. W. B., C. H. UNWIN, and A. STOESSL. 1974. Postinfectious inhibitors from plants XIII. Fungitoxicity of the phytoalexin capsidiol and related sesquiterpenes. *Can. J. Bot.* 52:2481-2488.