Concentration of Safynol in Phytophthora-infected Safflower

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ABSTRACT

First internode stem sections of the resistant Biggs line, uniformly infected with *Phytophthora drechsleri,* contained sufficient safynol 96 hr after inoculation to completely inhibit the pathogen. The concn of safynol in nonpith tissues was 6 times greater than the concn in the pith. Phytopathology 60:1153.

The experiments reported here were conducted to determine the concn and location of safynol (3,11-tridecadiene-5,7,9-triyne-1,2-diol) in safflower (*Carthamus tinctorius* L.) uniformly infected with *Phytophthora drechsleri* Tucker.

We reported safynol to be a major antibiotic compound that accumulates in safflower hypocotyls infected by *P. drechsleri* (3). Although a 20-fold increase in concn of safynol occurred in hypocotyls during 96 hr after inoculation by means of a single cut, the amount of compound present was not sufficient to completely inhibit the pathogen. However, owing to nonuniformity of infection, considerable healthy tissue was present in the extracted hypocotyl sections. A toxic compound related to disease resistance should be present in an inhibitory concn at the portal of infection (1).

The procedures used in inoculation, incubation, and safynol determinations were similar to those previously described (3), except that more efficient inoculation procedures were devised. Briefly, 8-week-old plants of the resistant line Biggs were wound-inoculated with a virulent isolate (No. 201 of *P. drechsleri*). Inoculum consisted of lima-bean agar plate cultures incubated for 8 days at 27 C. Inoculated plants were held at 30 C for 96 hr. Infected tissue was extracted with methanol. The methanol was removed by distillation in vacuo, and the remaining aq solution was extracted with ethyl ether. Safynol was isolated from the ethyl ether solution by thin-layer chromatography, utilizing three solvent systems. Concn of safynol in absolute ethanol was determined by OD at 269 nm ($\varepsilon = 61{,}600$).

In preliminary tests, hypocotyls were inoculated by smearing a 5-mm^2 plug of inoculum into a 7-mm vertical incision made approximately one-fourth of the way through the hypocotyl just above the soil surface. Each of thirty hypocotyls was inoculated with a single incision, and an equal number inoculated with three incisions spaced an equal distance apart around the hypocotyl. Whole cross sections of the infected hypocotyls were cut 96 hr after inoculation at the upper and lower edges of the necrotic lesions which generally extended 5 mm from the incisions. Sections from hypocotyls inoculated with one incision contained 1,080 μg safynol/100 g fresh hypocotyl, corrected for 35% loss of compound during chromatography. Sections from hypocotyls inoculated with three incisions contained 2,300 μg safynol/100 g fresh hypocotyls.

Owing to the uneven growth of hypocotyls, we used first internode stems in subsequent tests to achieve greater uniformity of inoculation and infection. The reactions of hypocotyls and first internode stems of 8-week-old plants to wound-inoculation with *P. drechsleri* showed that there is little or no difference in resistance between these plant parts.

An area of the stem on the first internode was wounded with 16 pin pricks. Four vertical rows of pin pricks were spaced an equal distance apart around the stem. The four pin pricks in each vertical row were approximately 3 mm apart. The wounded area was covered with a rectangular strip of inoculum held in place with a plastic tube. Safynol was extracted 96 hr after inoculation from infected stem sections cut at the upper and lower edges of the necrosis. In three tests, 30 or more plants each, the infected stems contained an average of 3360 μg safynol/100 g fresh stem sections.

Klisiewicz & Johnson (2) reported that the resistance mechanism of Biggs hypocotyls appears to be activated upon penetration of the epidermis by *P. drechsleri.* They found that the necrotic lesions were limited to the epidermis and one or more cell layers of the cortex. Thomas & Zimmer (4) found little or no enlargement of lesions in 8-week-old plants later than 96 hr after inoculation. We compared the amount of safynol present 96 hr after inoculation in the tissues external to the pith with the amount present in the pith. Infected stem sections were split longitudinally and the pith was removed by scraping. In two tests of 30 plants each, the concn of safynol in the nonpith tissues, collectively, was 6 times greater than the concn in the pith. The pith was 25% of the stem on a fresh-wt basis.

The median effective dose (ED_{50}) of safynol required to inhibit mycelial growth of *P. drechsleri* is 12 μg/ml. Mycelial growth is completely inhibited at 30 μg/ml (3). Ninety-six hr after inoculation, the external cell layers of the first internode stem sections contained 42 μg safynol/g fresh nonpith tissues, which is more than enough to completely inhibit the pathogen. The relative rates of accumulation in resistant, moderately resistant, and susceptible safflower would be helpful in further assessing the role of this antibiotic compound.

LITERATURE CITED

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