Host Colonization and Polygalacturonase Production by Two Tracheomycotic Fungi

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ABSTRACT

Verticillium albo-atrum and *Fusarium oxysporum* f. sp. *lycopersici* produced polygalacturonase within stems of susceptible tomato and cotton, and polygalacturonase activity correlated well with the onset and development of disease symptoms. Although the test fungi were recovered from inoculated, resistant tomato stem sections, polygalacturonase activity and disease symptoms were either absent or much lower than in the susceptible tomato. Polygalacturonase production by the virulent T-1 cotton isolate of *V. albo-atrum* was much higher than by the less virulent SS-4 isolate both in vitro and in susceptible cotton stem sections.

Polygalacturonase activity and the occurrence of disease symptoms in all susceptible host-pathogen combinations investigated, coupled with the absence of both polygalacturonase activity and symptom development in all resistant combinations, suggests that polygalacturonases have a role in the pathogenesis by these fungi. Persistence of these fungi in resistant stem sections in the absence of both polygalacturonase production and symptom expression supports this idea, and indicates that resistant host tissues may regulate polygalacturonase production by these fungi. Phytopathology 60:192-195.

Induction and repression of cell wall-hydrolyzing enzymes play a role in resistance of onion seedlings to *Pyrenochaeta terrestris* (7, 8). English & Albersheim (5) have reported less synthesis of a wall-hydrolyzing enzyme by *Colletotrichum lindemuthianum* when grown on cell-wall fractions from resistant bean hypocotyls than when grown on susceptible hypocotyl cell walls. The inducible nature of the polygalacturonases (PG's) produced in vitro by *Verticillium albo-atrum* Reinke & Berth. and *Fusarium oxysporum* Schlecht. f. sp. *lycopersici* is well documented (10, 11), and the production of PG's by these fungi growing on or in susceptible host tissue has been demonstrated (3, 4, 11). However, the production of PG's by these fungi in vivo has not previously been demonstrated to correlate with either virulence of the isolates or colonization of host tissue.

The present study compares the colonization of resistant and susceptible host stem sections by various isolates of *V. albo-atrum* and *F. oxysporum* f. sp. *lycopersici,* and correlates disease symptoms with polygalacturonase production in host tissues.

MATERIALS AND METHODS.—One isolate of *V. albo-atrum* from tomato and two from cotton; SS-4, a representative nondefoliating isolate; and T-1, a highly virulent isolate causing severe defoliation (14) were used. All *V. albo-atrum* isolates were the microsclerotial type. An isolate of *F. oxysporum* f. sp. *lycopersici* race 1 from tomato was also used. These fungi were maintained on Czapek-Dox agar, and in vitro studies were made using a modified Czapek-Dox liquid medium containing 1% glucose (8). Conidia of *V. albo-atrum* were produced as described by Roth (12), and bud cells of *F. oxysporum* f. sp. *lycopersici* were harvested from 48-hr shake cultures grown on Tochini broth (10 g peptone, 20 g maltose, 0.5 g KH_2PO_4, 0.5 g $MgSO_4$ in 1 liter H_2O). All inocula were washed by centrifugation at 800 *g*, through four volumes of 0.25% maltose solution, and mycelial fragments were removed by filtering the suspension through two layers of lens cleaning paper. All inocula were adjusted to 7.5×10^4 spores/ml prior to inoculation.

Cotton (*Gossypium hirsutum* L. 'Z-106') and tomato (*Lycopersicon esculentum* Mill.) cultivars used were grown in a greenhouse under a 16-hr photoperiod at 20-25 C, and fertilized on a 5-day schedule with starter fertilizer (10-52-17 analysis).

Plants were prepared for inoculation when 35 to 40 cm tall by immersing the plants in water and cutting the stems off at soil level. Leaves on the basal 15 cm of the stems were removed, and the cut ends were immersed in 25 ml of inoculum in test tubes (2.5 cm × 15 cm). Four cotton stems or 3 tomato stems were placed in each tube. The excised plants were maintained in controlled climate facilities at 24-26 C under a 16-hr photoperiod (1,000 ft-c) supplied by a mixture of fluorescent and incandescent lights. Three hr after immersion in the inoculum, 0.5 cm was trimmed from the stem bases, and the volume of the spore suspension adjusted to 25 ml using a solution containing 25 ppm of both streptomycin sulfate and aureomycin. After 6 hr, the stems were again trimmed and placed in half-strength Czapek-Dox salts solution containing the above antibiotics. Twelve hr later, the stems were removed to a solution containing only the antibiotics for the duration of the experiments. The antibiotic solutions were replaced every 48 hr.

Intact plants used for comparison of symptom development were inoculated with *V. albo-atrum* by stem puncture (2), and with *F. oxysporum* f. sp. *lycopersici* by root dipping in inoculum prepared as described above.

To determine enzyme levels in inoculated cuttings,

0.5 cm of the stem was removed and the next 10 cm of stem tissue was inserted in an apparatus similar to that described by Bell (1). Air pressure was applied to a 1-ml aliquot of 0.01 M citrate-phosphate buffer (pH 5.0) contained in tubing attached to the basal end of the stem section. A vacuum was applied to the distal end of the stem, and liquids flushed from the stems were collected. A specific bioassay was used to determine polygalacturonase activity (9), using one cucumber pericarp section/tube. All PG activities were determined in relative units by establishing the dilution end points for the enzymes of the respective fungi, and relating these end points to that of a stock solution of a commercial pectinase (Sigma lot 76B 0560). Controls were noninoculated cuttings and boiled fluids from inoculated cuttings.

Stem samples used to determine colonization by these fungi were handled according to the methods of Stover & Waite (15). The basal 0.5 cm of the stem was removed. The next 10 cm of stem was surface-disinfected in 1% hypochlorite-1% ethanol for 1 min, then blended for 1.5 min in 100 ml of sterile deionized water/stem. A dilution series of the resulting slurry was prepared, and 1-ml aliquots were plated on Czapek-Dox agar and incubated in darkness at 24 C. *F. oxysporum* f. sp. *lycopersici* was incubated for 48 hr; *V. albo-atrum* for 84 hr. Colony counts were taken from plates yielding between 25 and 250 colonies/plate. Stem colonizations were recorded as a per cent of the number of colonies recorded after the initial 6-hr inoculation period.

All experiments were replicated three times and repeated; all values within each experiment represent averages of four determinations.

RESULTS.—For each susceptible host-pathogen combination tested, symptom development was similar in both intact plants and cuttings. Symptom expression in intact plants was slightly slower than in cuttings; however, symptoms elicited by each fungus were essentially identical in intact plants and cuttings.

The tomato isolate of *V. albo-atrum* colonized both susceptible (Bonny Best) and resistant (VF-145) tomato stem sections to approximately the same extent during these experiments (Fig. 1). Polygalacturonase activity was detectable 48 hr after inoculation of the susceptible cuttings, and increased over the duration of these experiments, but PG activity was not detected in resistant cuttings. Results obtained with *Verticillium*-resistant tomato cultivars VF Roma and VR Fireball were similar to those obtained with VF-145.

Fusarium oxysporum f. sp. *lycopersici* also colonized cuttings of both susceptible Bonny Best and resistant VF-145 tomato stems (Fig. 2). Polygalacturonase activity, detectable in both cultivars 48 hr after inoculation, persisted and increased only in susceptible cuttings. In resistant VF-145 stems, PG activity declined rapidly, and was not detectable past 4 days after inoculation, while the number of recoverable colonies of the fungus continued to increase until the 6th day after inoculation (Fig. 2). Although the per cent recovery of the fungus declined after 6 days, the fungus persisted in these cuttings for the duration of these experiments.

Polygalacturonase activity was detected in intact tomato plants 8 to 16 days after inoculation with both tomato pathogens; however, the levels of PG activity were too low for quantitative determinations.

The more virulent T-1 cotton isolate of *V. albo-atrum* produced more PG activity than the less virulent SS-4 both in culture (Fig. 3) and in inoculated susceptible cotton stems (Fig. 4). The woody nature of the cotton stems made determinations of the extent of colonization by these two isolates impossible. Polygalacturonase activity was not detected in cotton stems after inoculation with the tomato isolate of *V. albo-atrum*, and the only symptom induced in cotton by this isolate was a slight vascular discoloration.

DISCUSSION.—The appearance and increase in PG activity observed in all susceptible host-pathogen combinations were consistent with symptoms observed in the cuttings and in intact plants. In contrast, persistence of the test fungi in resistant hosts was not accompanied by either symptom expression or the production of PG's in these hosts. This suggests that multiplication of the pathogens in host tissue does not necessarily indicate successful pathogenesis, but that production of PG's (and probably other wall hydrolyzing enzymes) is required for successful pathogenesis. Additional evidence for a role for pectolytic enzymes in pathogenesis by these fungi was the higher levels of PG activity associated with the more virulent T-1 cotton isolate of *V. albo-atrum* in culture and in susceptible cotton stems.

The data presented substantiate the reports of Deese & Stahmann (3, 4) that PG activity was greater when these two fungi were grown on susceptible than on resistant stem sections. However, the methods used here showed that PG activity was localized in the host vascular system, and that differences in levels of PG activity were not solely a result of different growth rates of the respective fungi in susceptible and resistant host tissue.

Increases in the number of colonies recovered from resistant stem sections without apparent damage to host tissues may be explained by results reported by Scheffer & Walker (13). They observed that *F. oxysporum* f. sp. *lycopersici* was incapable of spreading out of vascular tissue of resistant tomato stems after inoculation. They also reported that resistant hosts differentiated new vascular tissue which was not invaded by the pathogen. It is tempting to speculate that the inability of the pathogen to invade this new vascular tissue was due to repression of the synthesis of wall-hydrolyzing enzymes by the resistant host tissue. The disappearance of pectolytic activity early after inoculation of resistant plants with *F. oxysporum* f. sp. *lycopersici* would fit this hypothesis, and is substantiated by the observations of Gothoskar et al. (6). They reported that resistance in tomato to *F. oxysporum* f. sp. *lycopersici* depends upon production of a labile substance by the host, and that it is produced only in inoculated, resistant tomato tissue. Patil & Dimond (11) have also demonstrated that repression of production of pectolytic enzymes by exogenously sup-

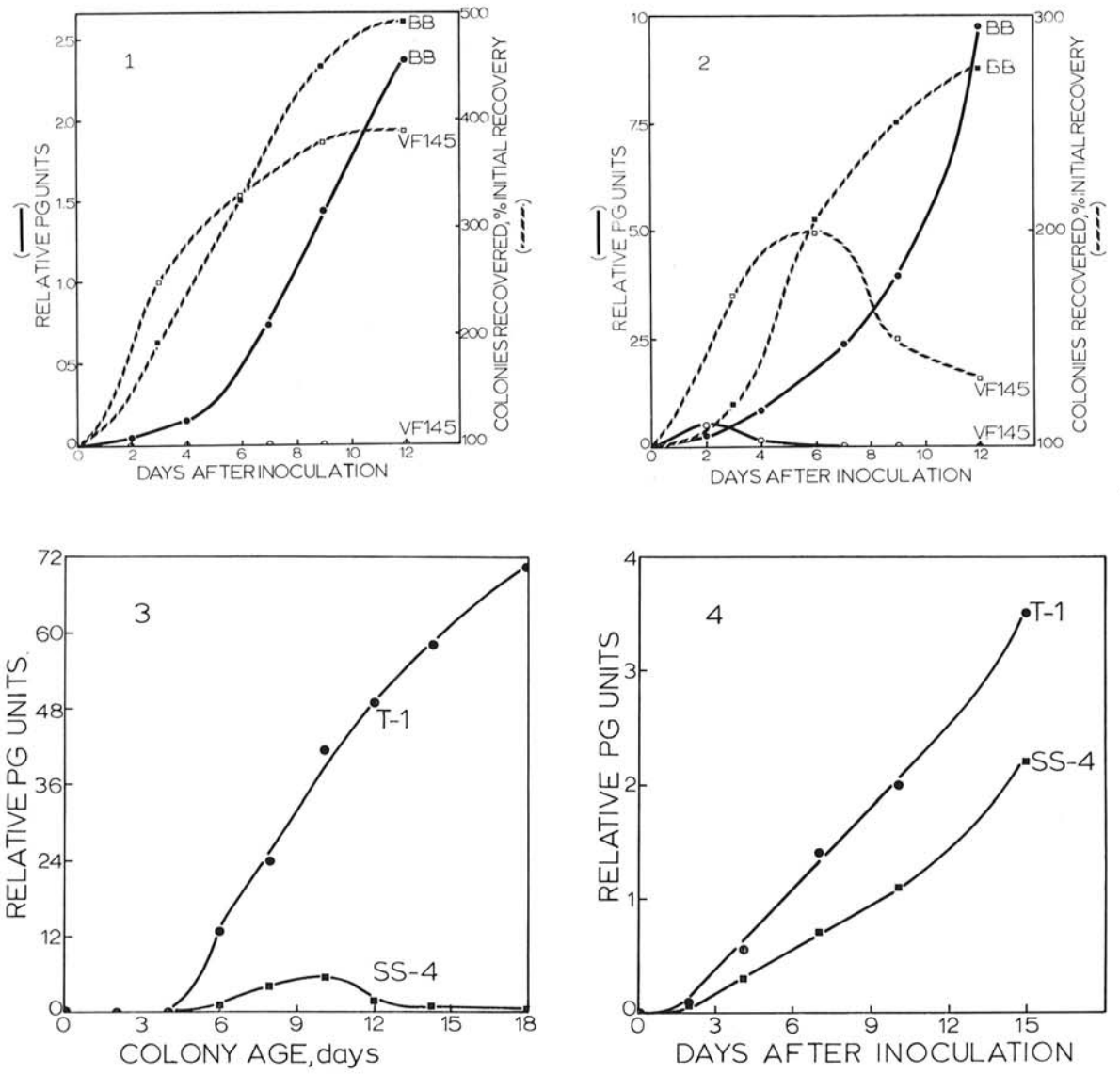

Fig. 1-4. **1)** Stem colonization (– – –) and polygalacturonase production (——) by *Verticillium albo-atrum* in susceptible Bonny Best (BB) and resistant VF-145 tomato stem sections. **2)** Stem colonization (– – –) and polygalacturonase production (——) by *Fusarium oxysporum* f. sp. *lycopersici* in susceptible Bonny Best (BB) and resistant VF-145 tomato stem sections. **3)** Polygalacturonase production by a defoliating (T-1) and a nondefoliating (SS-4) cotton isolate of *Verticillium albo-atrum* in culture. **4)** Polygalacturonase production by a defoliating (T-1) and a nondefoliating (SS-4) cotton isolate of *Verticillium albo-atrum* in susceptible (Z-106) cotton stems.

plied sugars lessened the severity of symptom expression in susceptible tomato stems inoculated with *F. oxysporum* f. sp. *lycopersici*.

The absence of both symptoms and PG activity in resistant tomato stems inoculated with *V. albo-atrum*, combined with the apparent persistence of the pathogen in these tissues, indicates that pectolytic enzymes are essential for successful pathogenesis by this fungus, probably to facilitate ramification of the pathogen through host tissues. Since PG activity was not detected in these sections, the mechanism of PG repression operative here must be present prior to infection. This may be similar to results reported by English & Albersheim (5), in which wall fractions from resistant hosts appear to regulate synthesis of a wall hydrolyzing enzyme secreted by *Colletotrichum lindemuthianum*.

Successful colonization of host tissues and concomitant pathogenesis by many plant pathogenic fungi probably depend upon synthesis of wall hydrolyzing enzymes by these pathogens. Our observations and those of Patil & Dimond (11), Horton & Keen (7), and English & Albersheim (5) substantiate this hypothesis, and suggest that one mechanism of resistance to these plant pathogens may be the suppression or regulation of production of cell-wall hydrolases by these pathogens in resistant plant tissues.

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