PROGRESS REPORT

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TRICHODERMA AS A BIOLOGICAL CONTROL FOR CYTOSPORA CANKER

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SUMMARY

Field and laboratory experiments were conducted to determine whether the fungus *Trichoderma* could be used as a protective control against *Cytospora leucostoma* infection on peach trees in Colorado. Wounds treated with *Trichoderma* had less infection than the controls. *T. aureoviride* was the only significantly effective one of the four *Trichoderma* species tested. The action of *Trichoderma* against *Cytospora* may be attributed in part to direct hyphal antagonism and to a possible production of a diffusible antibiotic.

INTRODUCTION

Cytospora canker (caused by the fungus *Cytospora leucostoma)* is a disease of peach trees in Colorado. The disease is characterized by gumming of the infected area, gradual death of the bark and eventual girdling. *Cytospora* spores germinate and their germ tubes invade the bark of the tree through wounds. These wounds may be the result of winter injury, pruning, insect feeding, hail, or mechanical injury. The spores are spread by splashing or wind-driven rain.

Cytospora has infected more than half of all Colorado peach trees, due in part to the lack of adequate prophylactants or their use by growers. Preventive measures include delayed pruning, use of wound protectants and the avoidance of mechanical injury. Eradication usually means removing the canker surgically and applying a chemical wound dressing. There is an increasing demand for effective yet ecologically safe protectants. *Trichoderma*, a common soil inhabiting fungus, has shown promise as a wound protectant against the silver leaf disease of peaches in Europe and against a number of wood rotting fungi on forest and shade trees. The experiments reported here were conducted in 1978 to explore the possibility of using *Trichoderma* as a biological control agent against *Cytospora*.

MATERIALS AND METHODS

1. Production of a diffusible antibiotic by *Trichoderma*.
Twenty-five ml of sterile malt agar were dispensed into standard plastic petri dishes. Sterile dialysis membranes were placed on the solidified medium. Plugs of sporulating *Trichoderma* mycelium (6 isolates, 4 species) were placed in the middle of the plates. After two days, an outline of the mycelial growth was sketched on the bottom of each plate. The *Trichoderma* was removed by lifting the membranes off the agar. Mycelial plugs of *Cytospora* were placed (mycelium down) in the center of the plates directly on the medium. The diameter of *Cytospora* growth was measured in three fixed directions daily. Control plates, which had not supported previous *Trichoderma* growth, were also measured. The time when *Cytospora* growth exceeded the area of previous *Trichoderma* mycelial growth was also noted.

2. Hyphal interaction between *Trichoderma* and *Cytospora*.
Malt agar plates were prepared and inoculated with one isolate of *Cytospora* and one of *Trichoderma* so their interaction could be observed. The dual culture plates were inoculated so that each of five *Cytospora leucostoma* isolates opposed the various *Trichoderma* species and isolates (see Table 1 for *Trichoderma* designations). *Cytospora* plugs were placed on the plates one day before inoculation with *Trichoderma* plugs. Plates were examined for reduced *Cytospora* growth before hyphal contact and microscopic observations made of the zone of interaction

Table 1. Ability to re-isolate *Trichoderma* from two previously inoculated peach tree wounds.

Trichoderma cultures	Re-isolation success				
	1 day	4 wks	6wks	9 wks	11 wks
T. aureoviride (A1)	++[a]	++	++	+0	+0
T. aureoviride (A2)	++	++	+0	00	00
T. harzianium (H1)	++	++	++	+0	00
T. harzianium (H2)	++	++	++	+0	00
T. harzianium (H2G)[b]	++	++	++	++	++
T. koningii (K1)	++	++	+0	++	00
T. viride (V2)	++	++	++	00	+0
Non-inoculated check	00	00	00	00	00

[a] + = growth, 0 = no growth, for each of the two replicates.
[b] Spore suspension of H2 in 20% glycerol solution.
[c] Open check wound, not inoculated with *Trichoderma*.

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after contact occurred. Also, agar plugs were removed from this zone to determine which fungus survived.

3. Field longevity of *Trichoderma*.
Wounds were made with a patch budder on July 31 on 10-year-old Elberta trees and inoculated with spore suspensions of the *Trichoderma* isolates. Samples of bark and wood were taken periodically from two trees over 11 weeks and cultured on agar to determine how long the isolates remained viable in the wounds.

4. Field protectant ability of *Trichoderma*.
Four experiments were conducted to determine how effective *Trichoderma* species are in reducing *Cytospora* infections on peach tree wounds. These experiments were completed during the summer and early fall. All wounds were made with a Jones patch budder and spore inoculations made with an atomizer. *Trichoderma* spores were applied first and *Cytospora* spores several hours later.

Mertect 340-F (TBZ) (Merck Chem. Div.), mixed 1:8 with water, and orange shellac were used for comparison as protectant paints. Other variables included 20 percent glycerol added to the spore suspension to reduce spore drying and heat-killed *Trichoderma* (A2) spores, which were included to study the effect of metabolites from inactivated spores. Wounds were examined 60 days after inoculation for gumming, callus growth and the extent of discolored sapwood under the cankered area. The latter was measured by tracing the outline of the discoloration of the diseased area onto paper and determining the area with a planimeter.

RESULTS AND DISCUSSION

1. Diffusible antibiotic effect.
Results for the diffusible antibiotic experiment are shown in Figure 1. All *Trichoderma* isolates tested apparently inhibited growth of *Cytospora leucostoma*. Isolate V2 reduced

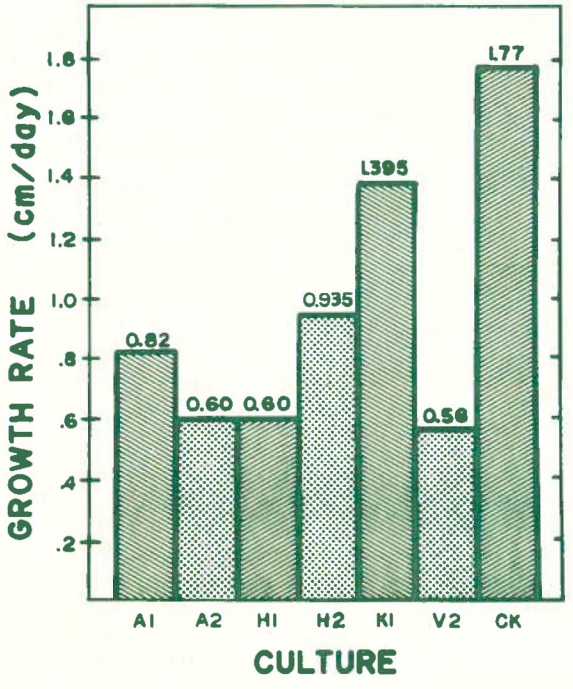

Figure 1. The *in vitro* effect of a diffusible antibiotic produced by *Trichoderma* cultures on *Cytospora* growth rate.

Cytospora growth 68 percent when compared with the control. This was followed closely by isolates A2 and H1, both of which reduced growth by 66 percent. Some competitive effect on nutrient source may also be involved with this technique.

2. Hyphal interactions.
Under microscopic examination, the isolates of *T. harzianum* (H1 and H2) showed the greatest amount of coiling around *Cytospora* hyphae. *T. aureoviride* (A1 and A2) also showed antagonistic ability by causing cellular granulation of *Cytospora* hyphae. These isolates slowed the growth of all *Cytospora* isolates, and, when in direct contact, were capable of inactivating *Cytospora* hyphae since *Cytospora* could not be reisolated from the interaction zones. Direct penetration of fungal cells did not occur.

3. Longevity of *Trichoderma* in the field.
Table 1 shows that *Trichoderma harzianum* (with added glycerol — H2G) survived throughout the 11-week test period. *T. aureoviride* (A1) and *T. viride* (V2) survived for the same period on one tree. The other isolates survived only 6-9 weeks. This indicates the possibility that *Trichoderma* can survive on wounded peach tree surfaces during the critical period after wounding when *Cytospora* infection could occur.

4. Field protectants.
Results of two tests are shown in Table 2. The presence of *Trichoderma* resulted in a slight to moderate reduction of canker development but not complete elimination of *Cytospora* from the wounds. Significant canker reduction occurred with only one isolate, *T. aureoviride* (A1), in test A. However, further research is warranted to determine if this approach to control can be improved and made practical.

Table 2. The results of two orchard tests using various *Trichoderma* spore suspensions and other protectants against *Cytospora* in Elberta peach tree wounds.

Treatment	Canker area-mm²	% Canker reduction
A. June 20 - Aug. 20		
T. aureoviride (A1)	510[a] a	83
T. aureoviride (A2)	692 abc	77
T. harzianium (H1)	866 abc	71
T. harzianium (H2)	1200 abc	60
T. koningii (K1)	1280 bc	57
Inoculated check	2978 c	—
B. Sept. 12 - Nov. 12		
T. aureoviride (A2)	682 a	54
T. aureoviride/ heat-killed (A2)	1016 a	35
T. aureoviride + gly. (A2)	656 a	58
T. harzianium (H2)	548 a	65
T. koningii (K1)	680 a	57
Gly. Soln. (20%)	1182 a	25
Orange shellac	1476 a	6
TBZ	544 a	65
Inoculated check	1570 a	—
Non-inoculated check	708 a	—

[a]Values followed by the same small letter, within each experiment, do not differ significantly (5% level DMR test).