Biological Control of Cucumber Black Root Rot by Gliocladium roseum

Arnold R. Moody and Daniel Gindrat

Plant Pathology Section, Changins Federal Agricultural Research Station, CH-1260 Nyon, Switzerland. Present address of senior author: Virginia State College, Petersburg, VA 23803.

Research supported by Grant No. 3.644.71 from the Swiss National Fund for Scientific Research.

The authors gratefully acknowledge Bio-Innovation AB BINAB, Box 54. S-190 30 Sigtuna, Sweden, for manufacturing and donating bark pellets containing *G. roseum*, and A. R. Weinhold, J. Louvet, and A. E. Rich for help in preparing the manuscript. Appreciation is also given to P. Vullioud for assistance in statistical analysis.

Accepted for publication 10 March 1977.

ABSTRACT

MOODY, A. R., and D. GINDRAT. 1977. Biological control of cucumber black root rot by Gliocladium roseum. Phytopathology 67:1159-1162.

When a black, highly organic soil was artificially infested with *Phomopsis sclerotioides,* the disease potential was consistently lower than that of a check soil infested with the same amount of *P. sclerotioides.* This antagonism to *P. sclerotioides* was destroyed by steam-air pasteurization at 60 C for 30 min. When mycelial mats of *P. sclerotioides* were buried in the antagonistic soil, *Gliocladium roseum* grew profusely on the surface of the mats. *Gliocladium roseum* was isolated from the mycelial mats and inoculum was prepared which consisted of 5-day-old actively growing cultures in a mixture of peat, soil, and nutrients. When *G. roseum* was added to an infested mineral soil, the disease potential was significantly lowered. An inoculum of conifer bark pellets containing mycelium and conidia of *G. roseum* and a nutrient source also was used. Its efficacy in soil did not last as long as that of the other inoculum.

Additional key words: soil fungi.

After it was described by van Kesteren in The Netherlands in 1965 (8), the cucumber black root rot fungus, *Phomopsis sclerotioides* Kest., has spread throughout Western Europe, and has appeared in North America (1, 5, 6, 7, 9). Where it occurs, Phomopsis black root rot is the most important problem on greenhouse cucumbers, killing the plants at about the time harvesting begins. Steam sterilization or fumigation of soil, followed immediately by planting, controls the disease. Because *P. sclerotioides* appears to be important only in greenhouses, and in fact has not been reported in more natural environments, it is possible that effective controls exist in natural environments. This work was undertaken to examine possible biological control mechanisms.

MATERIALS AND METHODS

Artificial soil infestation with Phomopsis sclerotioides.—Seven soils were examined to find one in which less Phomopsis black root rot developed; i.e., a soil in which disease potential (3) decreased over a period of time. Soils 1, 2, and 3 were mineral soils, with standard fertilization, from different greenhouses in Geneva, Switzerland, where cucumbers had been grown and *P. sclerotioides* was present. A similar soil, soil number 4, was sampled in a commercial greenhouse in Morges, Switzerland. Soil 5 was a clay-loam soil from a field near Morges. Soil 6 was a clay-loam field soil from the Changins Experiment Station and was used as a check soil in all experiments. Soil 7 was a highly-organic, black soil from Bavois, Canton Vaud, Switzerland. Cucumbers had never been grown in soils 5, 6, or 7. Each soil was infested with mycelium of *P. sclerotioides* grown in 500-ml Erlenmeyer flasks that contained 200 ml of Czapek Dox broth plus 0.2% yeast extract. The cultures were incubated for 10 days at 26 C on a rotary shaker at 150 rpm. The isolate of *P. sclerotioides* used in these experiments was obtained in 1971 from diseased cucumber roots from a greenhouse in Geneva, Switzerland, and it was among the most pathogenic in our collection. The mycelium was strained from the growth medium and blended for 30 sec with distilled water at medium speed. The inoculum was brought back to its original volume of 200 ml with additional water. This suspension was mixed directly with the soil at 10 ml/1,500 cc of soil (60 mg dry wt/1,000 cc soil), unless stated otherwise. As shown in preliminary soil infestation studies, this inoculum density yielded adequate disease development and consistent results (Fig. 1).

Measurement of disease potential.—Soils infested with *P. sclerotioides* were stored in plastic bags and the disease potential was determined immediately after soil infestation and at weekly intervals. To evaluate disease potential the soil was placed in 1,500-cc flats and 25 cucumber seeds were planted, with two to four replicates. Each experiment was repeated at least three times. Disease severity was evaluated on roots after 50 days growth in a greenhouse in which the temperature averaged as low as 18 C in winter and as high as 30 C in the summer. Disease ratings used were: 1 = healthy; 2 = slightly rotted; 3 = moderately rotted; 4 = severely rotted; and 5 = dead. A disease index then was computed by

multiplying the total number of roots in each rating class by its rating and dividing the sum of the products by the total number of roots sampled. Analysis of variance was made between replicate experiments, including F and *t* tests.

Isolation of the antagonist.—Mycelial mats of *P. sclerotioides* were obtained from liquid cultures similar to those described above. Washed mycelial mats were buried in soil samples dispensed in petri dishes. After at least 1 wk of incubation under laboratory conditions, mats were retrieved, excess soil was removed, and the mats were placed in sterile petri dishes containing a little sterile water. After about 1 wk of incubation in the laboratory, mats were examined for colonization by microorganisms.

Inoculation of soil with Gliocladium roseum.—*Gliocladium roseum* Bainier was isolated from the surface of mycelial mats of *P. sclerotioides* after the

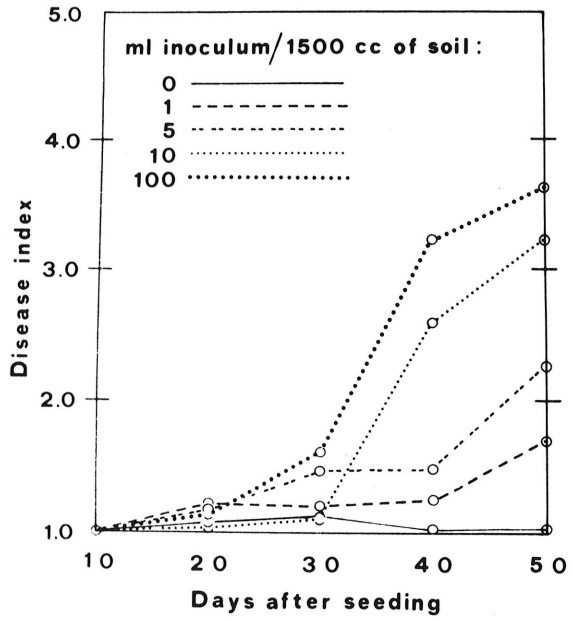

Fig. 1. Increase of cucumber black root rot caused by *Phomopsis sclerotioides* during 50 days in soil number 6 (a clay-loam field soil) infested with different quantities of 10-day-old mycelium. One ml inoculum = 6 mg dry wt. Disease index: 1 = no disease; 2 = slightly rotted; 3 = moderately rotted; 4 = severely rotted; 5 =dead. Each point is an average of three experiments with three replications of 25 seeds each or a total of more than 200 plants. Means are significantly different ($P = 0.05$) at 40 and 50 days after seeding.

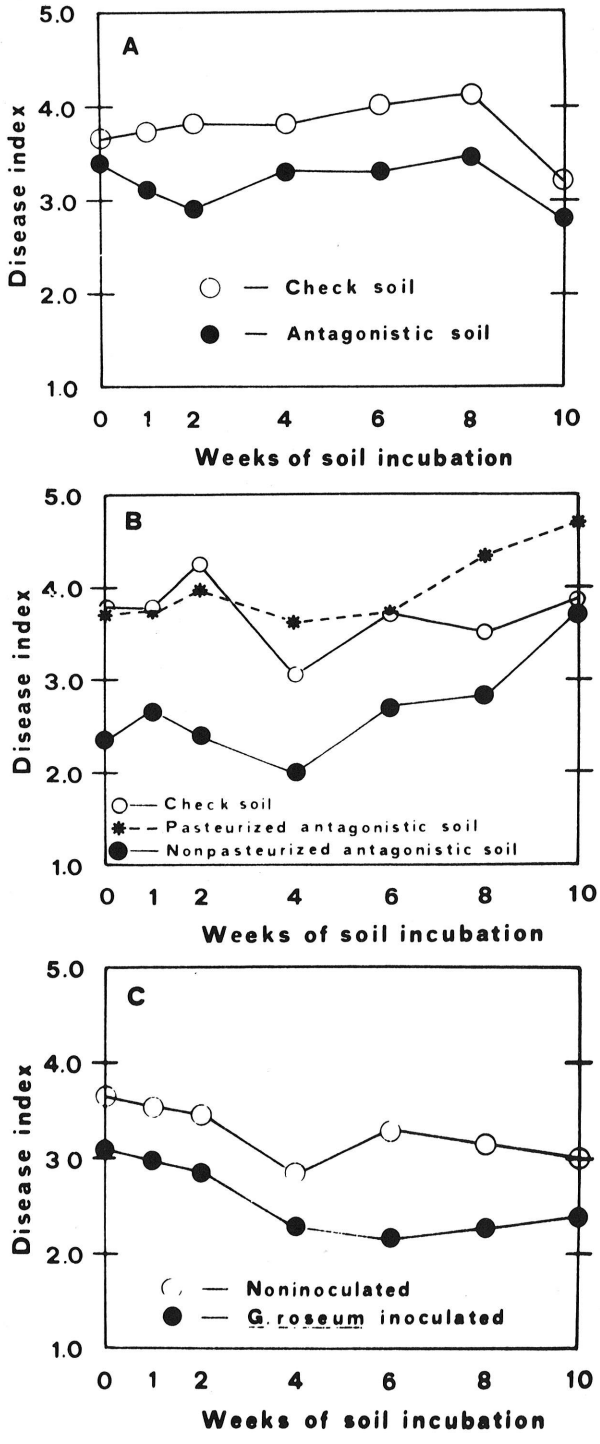

Fig. 2-(A to C). Disease indices in soils infested with *P. sclerotioides*. Soils were incubated for 0, 1, 2, 4, 6, 8, and 10 wk before planting cucumbers. Roots were rated for amount of rot after 50 days. Each disease index represents an average of three to five experiments with two or four replications of 25 seeds each. Disease index: 1 = no disease; 2 = slightly rotted; 3 = moderately rotted; 4 = severely rotted; 5 = dead. **A)** Comparison between check and antagonistic soils. Means were significantly different ($P = 0.05$) at all periods of incubation except 0, 4, and 10 wk. **B)** Comparisons among check soil (= CS), pasteurized (60 C/30 min) antagonistic soil (= PAS), and nonpasteurized antagonistic soil (= AS). For $P = 0.05$, means were significantly different: between CS and PAS only at 8 and 10 wk; between CS and AS at all times except 10 wk; between AS and PAS at all periods of incubation. **C)** Check soil with and without the addition of *G. roseum* inoculum. Means were significantly different ($P = 0.05$) at all periods of incubation.

mats had been buried in soil 7. Inoculum of *G. roseum* was grown in 500-ml bacteriological flasks filled with a horticultural potting mixture of peat (75%), soil (10%), leaf compost (10%), and sand (5%), plus 200 ml of Czapek Dox broth. In some experiments wheat grain was added at 20 g/flask. One hundred ml of Czapek Dox broth was added to the mixture before autoclaving, and an additional initially sterile 100 ml suspension of viable *G. roseum* conidia was added after the flasks had cooled. After 4 to 7 days of incubation at 25 C, the inoculum became white with mycelium and spores and was added to soil infested or not infested with *P. sclerotioides* at the rates of 160, 250, or 500 ml of inoculum/1,500 cc of soil. A horticultural potting mixture like that used in inoculum preparation was added in equal volume to the check soil. Soil infested or not infested with *P. sclerotioides* also was amended with conifer bark pellets that contained *G. roseum;* the pellets were added at 50 g/1,500 cc of soil. The pellets, approximately 0.5 to 1.0 cm long and 0.6 cm in diameter, were manufactured using cultures of *G. roseum* growing on barley kernels that were ground and mixed with conifer bark plus fresh barley flour. Control pellets contained bark plus barley flour and yielded *Penicillium* sp. when plated on petri dishes or incubated in the soil.

RESULTS

Identification of antagonistic soil.—Infested soils were incubated in plastic bags, then disease potential was measured weekly by planting seeds and rating roots after 50 days. In five separate experiments, the disease potential of soil 7 was significantly lower than that of soil 6, except after 0, 4, and 10 wk of incubation before measuring (Fig. 2-A). Disease development in the remaining soils did not appear different from soil 6. Similar results were obtained with 1 ml of inoculum/1,500 cc of soil and with 50 ml of inoculum/1,500 cc of soil.

Nature of antagonism in soil number 7.—When the antagonistic soil 7 was pasteurized with a steam-air mixture at 60 C for 30 min, the antagonism was lost (Fig. 2-B). This finding suggested that antagonism of soil 7 to *P. sclerotioides* was of biological nature.

Isolation of the antagonist.—Mats of *P. sclerotioides* buried and then incubated in soil 7 were usually completely covered with a fungus identified as *Gliocladium roseum* Bainier. Mats buried in the check soil, soil number 6, as well as those buried in the other soils rarely yielded *G. roseum.* When pure, washed mycelial mats of *P. sclerotioides* were inoculated with conidia of *G. roseum* then incubated for about 1 wk in small moist chambers under aseptic conditions, they were almost completely destroyed and disintegrated by the antagonist.

Biocontrol studies.—Although many other microorganisms such as nematodes, bacteria, fungi *(Trichoderma, Arthrobotrys, Volutella,* etc.) and protozoa could be isolated from mycelial mats of *P. sclerotioides* incubated in the antagonistic soil, *G. roseum* was chosen because it was a destructive mycoparasite of *P. sclerotioides in vitro* and it has been known as a serious candidate for biocontrol studies by other workers (4, 10).

Local mineral soil number 6, infested with *P. sclerotioides* and amended with *G. roseum* (grown in the horticultural potting mixture plus wheat grain and/or Czapek Dox broth) yielded a lower disease index than mineral soil amended with the horticultural potting mixture alone. Best results were obtained with 500 cc *G. roseum* inoculum/1,500 cc infested soil, the maximum amount of inoculum used (Fig. 2-C). The difference between the disease indices of the untreated and *G. roseum*-treated soils was 0.5 at the beginning of the experiment, 1.1 at 6 wk, and 0.6 after 10 wk.

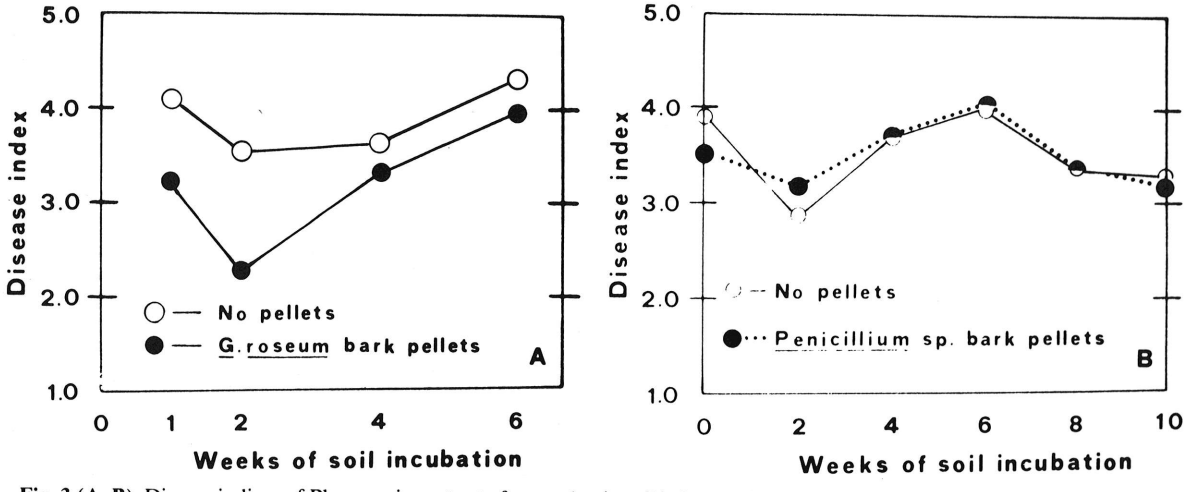

Fig. 3-(A, B). Disease indices of Phomopsis root rot of cucumber in soil infested with *Phomopsis sclerotioides* with and without the addition of bark pellets at the rate of 50 g/1,500 cc. Soils were incubated in plastic bags, then disease potential was measured by planting seeds and rating roots after 50 days. Each point represents three replications of 25 seeds each. Disease index: 1 = no disease; 2 = slightly rotted; 3 = moderately rotted; 4 = severely rotted; 5 = dead. **A)** Comparison of soil treated with *G. roseum* bark pellets and nontreated soil. No significant difference ($P = 0.05$) between means was found at 4 and 6 wk of incubation. **B)** Comparison of soil treated with bark pellets containing *Penicillium* sp. and nontreated soil. No significant difference ($P = 0.05$) was found at any period of incubation.

When infested mineral soil number 6 was treated with bark pellets containing *G. roseum* and a nutrient source, the disease index after 1 and 2 wk of incubation in soil was significantly less than that of soil not treated with *G. roseum* bark pellets (Fig. 3-A). No significant differences were found after longer soil incubations. Infested soil treated with bark pellets containing *Penicillium* sp. did not differ significantly from nontreated infested soil (Fig. 3-B). Damping off by Phycomycetes occurred when cucumber seeds were planted immediately after the soil was treated with bark pellets containing a nutrient source. This ceased to be a problem 1 wk after treatment.

DISCUSSION

An important factor in the success of *G. roseum* as a biological control agent for *P. sclerotioides* may be the use of actively growing cultures together with a nutrient source to support additional growth of the fungus in the soil. A similar observation was made by Wells et al. (11) concerning biological control of *Sclerotium rolfsii* using *Trichoderma harzianum*. However, a fresh nutrient source brought to the soil may stimulate growth of plant pathogenic phycomycetes, which are among the first colonizers of simple carbon and nitrogen compounds in soil. Delayed planting in biologically treated soil can suppress that hazard, as shown in this study.

From a practical point of view, several problems must be overcome before growers can be offered *G. roseum* as a biological control against *P. sclerotioides* or any other soilborne pathogen. Further ecological data must be obtained for determining the limits and optima of the control mechanism described here. Present studies in this laboratory concentrate on soil temperature and moisture relations. Also, the inoculum must be easy to use. Diatomaceaous earth granules have been shown to be a suitable growth substrate and delivery vehicle for *Trichoderma harzianum* (2). Other problems may be the instability of the biological preparation and the possible colonization by foreign microorganisms during manufacturing and storage. Technological aspects of biocontrol deserve as much attention as ecological ones.

LITERATURE CITED

1. ALABOUVETTE, C., and M. JAVOY. 1972. Comment lutter contre le deperissement des cucurbitacees provoque par le Phomopsis sclerotioides. Pepinieristes-Horticulteurs-Maraichers 126:1-7.
2. BACKMAN, P. A., and R. RODRIGUEZ-KABANA. 1975. A system for the growth and delivery of biological control agents to the soil. Phytopathology 65:819-821.
3. BAKER, K. F., and R. J. COOK. 1974. Biological control of plant pathogens. W. H. Freeman, San Francisco. 433 p.
4. BARNETT, H. L., and V. G. LILLY. 1962. A destructive mycoparasite, Gliocladium roseum. Mycologia 54:72-79.
5. EBBEN, M. H., and F. T. LAST. 1967. A sclerotial fungus pathogenic to cucumber roots. Plant Pathol. 16:96.
6. GINDRAT, D., and J. DAMBRON. 1971. Fletrissement et pourriture noire des racines du concombre en serre dus a Phomopsis sclerotioides. Rev. Hort. Suisse 44:334-336.
7. ORMROD, D. J., and W. D. CHRISTIE. 1972. Phomopsis root rot of greenhouse cucumbers in British Columbia. Plant Dis. Rep. 56:53-55.
8. VAN KESTEREN, H. A. 1965. A wilt disease of cucumbers and gherkins caused by a sclerotia producing fungus. Neth. J. Plant Pathol. 71:122-123.
9. VAN KESTEREN, H. A. 1967. "Black root rot" in Cucurbitaceae caused by Phomopsis sclerotioides nov. spec. Neth. J. Plant Pathol. 73:112-116.
10. WALKER, J. A., and R. B. MAUDE. 1975. Natural occurrence and growth of Gliocladium roseum on the mycelium and sclerotia of Botrytis allii. Trans. Br. Mycol. Soc. 65:335-338.
11. WELLS, H. D., D. K. BELL, and C. A. JAWORSKI. 1972. Efficacy of Trichoderma harzianum as a biocontrol for Sclerotium rolfsii. Phytopathology 62:442-447.