

GLIOCLADIUM ROSEUM

A VERSATILE ADVERSARY OF BOTRYTIS CINEREA IN CROPS

Antagonistic microorganisms are an important focus of current attempts to improve control of gray mold and other diseases caused by the pernicious pathogen *Botrytis cinerea* Pers.:Fr. in fruits, vegetables, field crops, tree seedlings, ornamental plants, and other crops (56). Microbial antagonists of *B. cinerea* have potential to help counter inadequacies, periodic failures, and concerns associated with present control practices, which generally involve cultural and sanitation measures, regulation of the microclimate, and heavy dependency on fungicides. Frequent resistance of *B. cinerea* to fungicides and inadequate host resistance (14) contribute to precarious vulnerability of many crops to the pathogen. Biological control by microorganisms also is seen as a means to avoid any risks associated with occupational exposure of workers to fungicides and with fungicide residues in harvested crops and in the environment. The filamentous fungus *Gliocladium roseum* Bainier has emerged as an effective and versatile antagonist of *B. cinerea* that, with other outstanding agents, promises to improve disease management in crops. This article attempts to provide a broad overview of *G. roseum* and insights into the roles of this remarkable fungus in natural systems, in crops, and as a biological control agent.

Morphology and Taxonomy

G. roseum is an unusual hyphomycete that produces one-celled conidia on two distinct types of conidiophores, one penicillately branched and the other verticil-

lately branched (Fig. 1A) (13,44). Colonies of the fungus on agar media are generally whitish, orange, or salmon (Fig. 1B). *G. roseum* is the anamorph of *Nectria ochroleuca* (Schwein.) Berk, an ascomycete of the Hypocreales (45). The fungus is considered generically distinct from the biological control agent *Gliocladium virens* J.H. Miller, J.E. Giddens, & A.A. Foster, now referred to as *Trichoderma virens*, and from the type species *Gliocladium penicillioides*. Domsch et al. (13) proposed that *G. roseum* belongs in the genus *Clonostachys* and suggested that it should be regarded as a collective species.

Ecology

G. roseum is common in an extraordinary range of habitats in tropical, temperate, subarctic, and desert regions of the world. It was reported in cultivated, grassland, woodland, forest, heathland, freshwater, and coastal soils, particularly those of neutral to alkaline pH (4,13,15,43). *G. roseum* is frequently associated with cysts of *Heterodera* spp., *Globodera* spp., and other nematodes in soil (6,18,24,26,36) and with sclerotia of *Sclerotinia sclerotiorum*, *Phymatotrichum omnivorum*, *Rhizoctonia solani*, *Botrytis* spp., *Verticillium* spp., and other fungi in soil and plant materials (25,51,61,62). Over the past 35 years, *G. roseum* has gained a distinctive reputation as a mycoparasite of a broad spectrum of fungi (3,30,35,37,59).

Equally remarkable are diverse associations of *G. roseum* with roots, stems, leaves, fruits, and seeds of plants. The fungus occurs on plant surfaces, such as the phylloplane of strawberry (31) and the mycorrhizoplane of European silver fir (*Abies alba*) (33). Reports point to an abundance of *G. roseum* within senescent and dead roots and foliage of a wide variety of plants and in plants weakened by

stress factors such as herbicides and disease (5,9,11,12,13,16). Intriguingly, *G. roseum* is known to colonize, without symptom production, apparently healthy roots, stems, pods, and seeds of soybean (34,46), roots of red clover (50), and leaves of strawberry (55) and raspberry (63). In these instances, the fungus apparently colonized the host as a nonpathogenic parasite, and in soybean at least, the association is systemic. Reported evidence that *G. roseum* is pathogenic to apple fruits, potato tubers, conifer seedlings, *Exacum affine*, and faba beans is considered inconclusive by the present authors (1,2,17,20,21,58). The teleomorph of *G. roseum*, *N. ochroleuca*, is found most often on branches of recently dead trees, but also on herbaceous and fleshy tissues of plants and fungi (19,44).

Collectively, the aforementioned reports underscored the cosmopolitan nature and extraordinary ecological versatility of *G. roseum*. Given this perspective, it is not surprising that the fungus has appeared repeatedly in assemblages of microbes recovered from living and dead plant tissues for evaluation in biocontrol tests against *B. cinerea* and other pathogens (28,29,40,63).

Socioeconomic Expectations of Biological Control

Development of antagonistic organisms as biological control agents is a demanding task given socioeconomic expectations that biocontrol should be efficient, dependable, cost-effective, and safe for humans, the crop, and the environment (53,54). Like other methods of disease management, biological control ideally aims to suppress disease enough that yield losses are minimized and crop quality is maintained at an acceptable level. To ascertain whether biological control agents meet the expecta-

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tions, we generally must evaluate them under epidemiological conditions similar to those of well-managed crops. Antagonists introduced to suppress *B. cinerea* might have to interact appropriately with the crop plants, pathogen, and other microorganisms under the prevailing microclimatic conditions, and in face of fertilizer and pesticide applications, tillage operations, irrigation, pruning, and other "human interferences" (27,56). Biocontrol systems of *B. cinerea* are highly dynamic, embracing growth and development of the host, infection cycles and serial dispersals of the pathogen, quantitative shifts in the biocontrol agent and indigenous organisms, and microclimatic fluctuations over time and in space.

Selection of *G. roseum*

The task of selecting an organism as a biological control agent is formidable given the staggering numbers of microbial isolates that can justify evaluation, the need to optimize effectiveness of biologi-

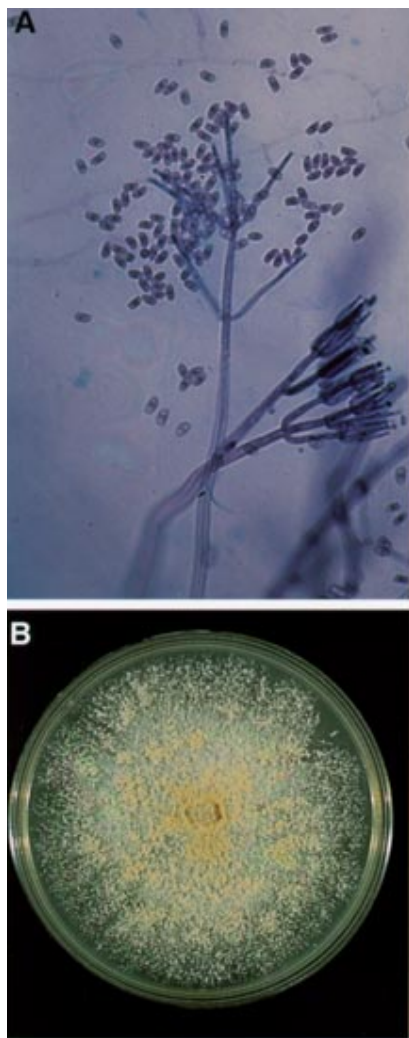


Fig. 1. *Gliocladium roseum*. (A) Verticillate and penicillate conidiophores and conidia. (B) Colony on potato-dextrose agar medium.

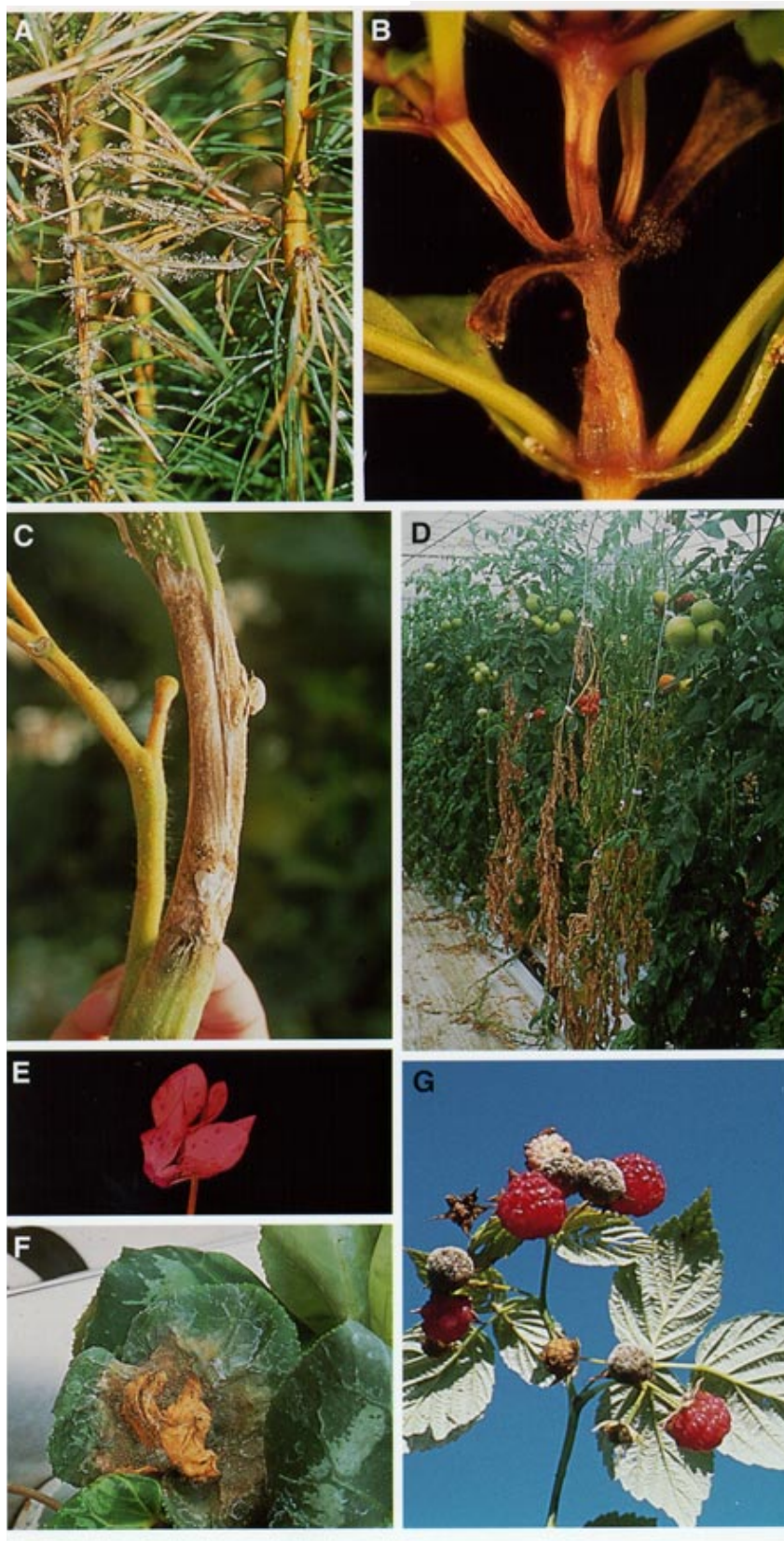


Fig. 2. Signs and symptoms of gray mold caused by *Botrytis cinerea*. (A) Lesions bearing conidiophores and conidia of *B. cinerea* on stems and needles of black spruce seedlings. (B) Lesions on stem and leaves of *Exacum affine* bearing conidiophores and conidia of *B. cinerea*. (C) Lesion arising from infection of a defoliation wound in a tomato stem. (D) Collapse of tomato plants affected by stem lesions. (E) Petal flecking in cyclamen. (F) Lesion produced in a cyclamen leaf invaded by *B. cinerea* from an adhering petal. (G) Gray mold of raspberry fruits.

cal control, and investments normally required to develop a microbe for commercial use. Whether a truly outstanding organism has been found and whether a superior microbe will turn up are lingering concerns of biocontrol investigators.

In selecting organisms against *B. cinerea*, we subscribed to the rationale that ecological adaptation of the microbe to the host plant is advantageous for effective and sustained biological control in the cropping system, and accordingly used host plants as chief sources of test organisms. Mycelial fungi, yeasts, and bacteria were isolated from living and dead leaves, stems, flowers, and fruits of pesticide-free host plants. The organisms included saprotrophs, weak pathogens, and nonpathogenic epiphytes and endophytes. Greenhouse plants, which lacked desirable diversity of microflora, were sprayed with aqueous suspensions of soils and incubated in high humidity prior to recovery of test isolates. Host plants, in effect, functioned as selective substrates and substrata of indigenous organisms, thus narrowing the field of organisms for evaluation in screening tests. For some hosts, this field was narrowed further by applying conidia of *B. cinerea* to plants and later recovering organisms only from portions of tissue on which the pathogen failed to sporulate given favorable conditions. *G. roseum* was isolated repeatedly from living and dead tissues of almost all hosts investigated.

Isolates of *G. roseum* were among hundreds of organisms tested against *B. cinerea* in each host. Sequences of tests designed to increasingly mimic the epidemiological conditions of commercial crops were conducted using detached plant parts in the laboratory, pot-grown plants in the growth room and greenhouse, and when appropriate, plants in field plots. Standard fungicide treatments served as yardsticks

of biocontrol effectiveness. As it turned out, isolates of *G. roseum* consistently ranked among organisms of outstanding effectiveness against *B. cinerea* in strawberry, raspberry, black spruce, and a range of flowers and vegetables produced in greenhouses, including begonia, geranium, cyclamen, poinsettia, *Exacum affine*, tomato, cucumber, and pepper. In almost all instances, *G. roseum* was as or more effective than fungicide treatments in leaves, bracts, stems, flowers, and fruits. A perspective of biocontrol testing against *B. cinerea* is now given as background to further consideration of biocontrol performance of *G. roseum*.

Customizing Biocontrol Tests

Because relationships of *B. cinerea* and various hosts differ widely in respect to pathways and conditions of infection, kinds of tissues affected, host age, inoculum concentration, and other factors, testing methods normally are best customized for each host. Leaves and stems require protection in some hosts to suppress gray mold on the foliage, as in conifer seedlings (Fig. 2A) and the flowering pot plant *Exacum affine* (Fig. 2B). In hosts such as strawberry, leaves are protected to control symptomless infection and spore production of *B. cinerea* when the tissues die (55,57,66). In greenhouse-grown tomato, it is important to protect wounds made in stems when lower leaves are removed to facilitate ventilation and to remove inoculum sources of *B. cinerea* (23). The defoliation wounds are prime sites of entry of the pathogen, from which lesions can develop and lead to collapse of the plants (Fig. 2C and D). Similarly, wounds made in mother and daughter plants of geranium when cuttings are prepared require protection against gray mold. Flowers require protection for a variety of reasons. Protection against petal flecking is of prime importance in flowering ornamentals like cyclamen (Fig. 2E), gerbera, and rose. Flowers of tomato, cyclamen, and geranium should be protected in part because colonized petals, when fallen, are utilized as a food base by the pathogen to aggressively attack leaves (Fig. 2F), stems, calyces, and fruits (23). Protection of strawberry and raspberry flowers blocks invasion of fruits (Fig. 2G) by mycelium of *B. cinerea* in flower tissues (52,56). Principal effects of temperature, humid periods, inoculum concentration of the pathogen, and other key variables on the host-pathogen interaction frequently can be accounted for in the design of biocontrol tests.

In a majority of tests developed in our laboratory, the pathogen and test organisms were each applied once to host tissues, usually at different times, and control was estimated in terms of signs or symptoms of disease. Conidiophore production of *B.*

cinerea provided an indirect but convenient means for quantifying infection and colonization of host tissues by the fungus (40). Tissues of leaves, petals, and stem segments used as test materials or removed from treated plants usually were incubated in high humidity or on an agar medium containing paraquat and chloramphenicol (PCA; Fig. 3A and B). By killing the tissues, this procedure circumvented problems associated with long and variable periods of quiescence or latency of *B. cinerea* and enabled the pathogen to sporulate rapidly so that tests were completed expeditiously and with improved reproducibility. Monocyclic tests were employed when the objective was to control portions of epidemics involving a single infection cycle of *B. cinerea*, but polycyclic tests involving serial infection cycles of the pathogen often were needed for evaluations under conditions similar to those of commercial crops. Because of complexity, logistics, and space requirements, polycyclic tests usually were reserved for leading biocontrol candidates like *G. roseum*.

Biological Control of *B. cinerea*

Strawberry. *G. roseum* first emerged as a powerful antagonist of *B. cinerea* in studies in strawberry in the late 1980s (39,40). Isolates of the antagonist consistently suppressed *B. cinerea* by more than 98% in assays on attached and detached leaves, petals, and stamens, and invariably performed as well as or better than did other leading organisms, such as *Trichoderma viride*, *Alternaria alternata*, *Myrothecium verrucaria*, and *Penicillium* spp., and a standard fungicide (captan). Tissues used in assays usually were inoculated with test organisms (10^7 conidia per ml of water plus surfactant for fungi), challenged after 24 h with *B. cinerea* (10^6 conidia per ml), and later incubated on PCA and assessed for sporulation of the pathogen.

The most realistic evaluations, however, were conducted in field plots established in matted-row strawberry plantings (40,53,54). Test fungi (10^6 conidia per ml) or captan was applied weekly to protect the flowers, the chief pathway to fruit invasion by *B. cinerea* in strawberry (52). Treatments were applied shortly before nightfall and dew onset, based on conjecture that dew and darkness could facilitate survival and activity of the biocontrol organisms. In four tests in cv. Redcoat, *G. roseum* suppressed incidence of *B. cinerea* in stamens and fruits by 79 to 93% and 48 to 76%, respectively, a performance equal to or better than that of other leading antagonists and the fungicide. *G. roseum* also was as effective as captan against *B. cinerea* in fruits of eight strawberry cultivars that did not differentially affect biocontrol by the antagonist. As in commercial plantings, *B. cinerea* generally was recalcitrant and

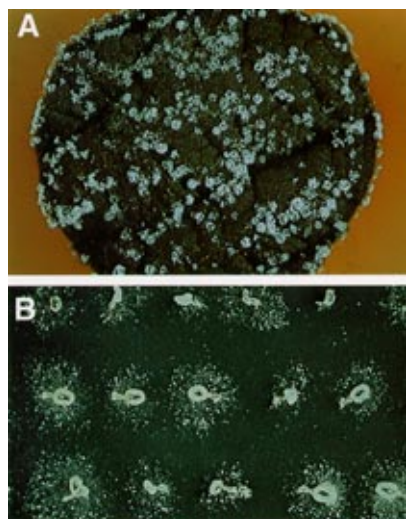


Fig. 3. Sporulation of *Gliocladium roseum* on tissues incubated on an agar medium containing paraquat. (A) Leaf disk. (B) Stamens.

sporulated on 15 to 30% or more of fruits whether the plants were treated with fungicide or *G. roseum*. A plausible explanation was that flowers of the tested cultivars remained open for only a few days, so that many would have escaped weekly treatments or were treated too late for effective control. In addition, the tests were stringent, in that plots were artificially inoculated with *B. cinerea*, and sporulation incidence of the pathogen in harvested fruits was estimated only after several days in room temperature and high humidity. Improved methods for timing and applying treatments may well improve biocontrol in the field.

Weekly applications of *G. roseum* (10^6 conidia per ml) gave good control of *B. cinerea* in Chandler strawberries grown in a plastic-covered production greenhouse in the extremely humid climate of the Serra Gaucha mountains of southern Brazil (60). Fruit losses at harvest and 4 to 5 days after harvest, averaged over 8 weeks, were reduced by 73 and 48%, respectively, in strawberries treated with *G. roseum* compared with 64 and 36% by a weekly fungicide program.

Besides flowers and fruits, *G. roseum* proved remarkably effective against *B. cinerea* in strawberry foliage, the chief inoculum source of the pathogen in strawberry plantings (52,55,57). In six field tests in Ontario, variously conducted in spring, late summer, and early fall, foliage was inoculated with *B. cinerea* (10^5 to 10^6 conidia per ml) and 2 to 5 weeks later was sprayed with inoculum of *G. roseum* or other biocontrol fungi (10^7 conidia per ml) or with chlorothalonil, a fungicide of exceptional effectiveness against *B. cinerea* in strawberry leaves (52). In most instances, *G. roseum* suppressed spore production of *B. cinerea* on the leaves by 90 to 100% and was always as effective as chlorothalonil, whereas *Penicillium* sp. and *T. viride* matched the performance of chlorothalonil in only three of the six tests. Both *G. roseum* and the fungicide suppressed the pathogen by only about 60% in semisenescence overwintered leaves.

Raspberry. Isolates of *G. roseum* generally equaled or outperformed other microbes and captan when tested against *B. cinerea* in raspberry field plots in Ontario. Strong suppression normally required treatment of flowers and of fruits, presumably because *B. cinerea* is able to infect drupelets directly as well as by invasion from flower tissues (8). In tests of leading biocontrol candidates, flower clusters of seven summer-bearing raspberry cultivars were treated with *B. cinerea* (5×10^3 conidia per ml) only, or with the pathogen in combination with *G. roseum*, *T. viride*, or *Trichothecium roseum* (all at 10^6 conidia per ml). Disease severity was estimated after each harvested flower or fruit was incubated in high humidity at 20 to 22°C for 10 days. Observations for the

cultivars, combined in the absence of interactive effects, indicated that the antagonists and captan suppressed *B. cinerea* in flowers and ripe fruits by 57 to 73% and 37 to 61%, respectively. In subsequent tests, conducted in both summer- and fall-bearing cultivars, *G. roseum* suppressed fruit

rot by 27 to 54% when applied once to flower clusters, and by 48 to 61% when clusters were treated at flowering and again when fruits were developing. Respective values for captan-treated raspberries were 18 to 25% and 23 to 40%. Under conditions of protracted flowering and



Fig. 4. Biological control of *Botrytis cinerea* in various hosts by *Gliocladium roseum* and *Trichoderma koningii*. (A) Profuse sporulation of *B. cinerea* in raspberry shoots inoculated with the pathogen only (left) compared with sporulation chiefly of *G. roseum* in shoots inoculated with the pathogen and the antagonist. (B) Suppression of gray mold symptoms in black spruce seedlings by a fungicide (chlorothalonil) and various inoculum concentrations of *G. roseum*: (left to right) seedlings treated with fungicide, 10^6 conidia per ml, 10^8 conidia per ml, 10^4 conidia per ml, and water. (C) (left to right) Begonia plants inoculated with *B. cinerea* (10^6 conidia per ml) and treated with water, *T. koningii*, *G. roseum*, and chlorothalonil. (D) (left to right) Cyclamen plants inoculated with *B. cinerea* (10^6 conidia per ml) and treated with water, *G. roseum*, *T. koningii*, and chlorothalonil.

fruiting, more than two treatments may be needed to optimize fruit rot control in raspberry crops. *G. roseum* was found also to be a powerful antagonist of *B. cinerea* in leaves and canes of raspberry (Fig. 4A; 63).

Conifer seedlings. *G. roseum*, although not among several hundred microbes recovered from conifer trees in northern Ontario, was evaluated against *B. cinerea* in black spruce seedlings because of previous strong performance in strawberry (65). In growth room tests, isolates of *G. roseum* suppressed *B. cinerea* almost completely and outperformed 132 microbial isolates from conifers; only an isolate of *T. viride* and one of *Trichothecium* sp. matched the effectiveness of *G. roseum* (65). In greenhouse studies, treatment programs were initiated to coincide with closure of seedling canopies, when gray mold epidemics usually begin. Four applications of *G. roseum* (10^6 or 10^8 conidia per ml) at 2- to 4-week intervals suppressed spore production of *B. cinerea* and gray mold symptoms on seedlings as or more effectively than did chlorothalonil treatments applied at the same times (Fig. 4B). In subsequent greenhouse tests, programs of *G. roseum* reduced incidence of killed shoots by 63 to 81% compared with reductions of 44 to 55% for chlorothalonil programs. A single application of *G. roseum* (10^6 conidia per ml) at canopy closure was found to suppress the pathogen as effectively as did programs of two to six applications of the

antagonist, or six of chlorothalonil, all at 1- to 2-week intervals starting when the canopies closed (67). Biological control activity of *G. roseum* was extraordinarily persistent, lasting 8 to 12 weeks or more.

G. roseum also gave promising results in red pine seedlings (66). Inoculum applications of the antagonist (10^8 conidia per ml) suppressed *B. cinerea* by 88% in first-year seedlings inoculated with the pathogen (10^6 conidia per ml). The antagonist also was strongly therapeutic against gray mold in second- and third-year seedlings grown in the presence of natural inoculum of *B. cinerea* in greenhouses and outdoor nursery compounds.

Greenhouse flowers and vegetables. The strong performance of *G. roseum* against *B. cinerea* in hosts as diverse as strawberry and conifers fueled speculation that the antagonist also might be effective against the pathogen in the multitude of susceptibles grown in greenhouses. For comparative studies, microbial isolates associated with greenhouse crops were obtained from foliage and flowers of plants with microflora enriched by previous treatment with suspensions of natural soils. Isolates of *G. roseum* were obtained frequently from each of 11 kinds of greenhouse plants employed in biocontrol investigations and evaluated on the same hosts from which they were recovered.

G. roseum suppressed *B. cinerea* by 94 to 100% in leaf disk assays of begonia, cyclamen, and geranium, and in stem-piece

assays of cucumber, pepper, and tomato. No tested microbe was superior to *G. roseum*, and few microbial isolates matched the performance of *G. roseum* isolates, except in cyclamen (Table 1). Isolates of *Trichoderma* spp. were among those as effective as *G. roseum* in some hosts, including one identified as *T. koningii* in begonia and another in cyclamen, and three of *T. harzianum* in geranium. Among isolates of *Stilbella*, a genus rarely if ever mentioned in biocontrol literature, one of *S. aciculosa* performed well in cyclamen, cucumber, and pepper. A diversity of microbes was highly effective in cyclamen, an unusual circumstance in our experience. Although isolates of *G. roseum* invariably gave good protection of leaves and stems against conidia of *B. cinerea*, they were less effective against petal inoculum. Petals colonized by *B. cinerea* and adhering to plant foliage are an important and formidable form of inoculum of the pathogen in cyclamen, geranium, petunia, tomato, and other hosts (23,49). In our experience, biological control is achieved more easily by treating flowers against conidia of *B. cinerea* than by treating foliage against mycelium of the pathogen in diseased petals. In cyclamen, for example, treatment of flowers with *G. roseum* prior to inoculation with *B. cinerea* and subsequent use of the petals as inoculum on foliage suppressed the pathogen in the leaves by more than 90%; in contrast, only 40 to 50% control was attained in leaves

Table 1. Proportion of tested isolates of various microbes that suppressed *Botrytis cinerea* as effectively as did isolates of *Gliocladium roseum* in leaf disk assays of begonia, cyclamen, and geranium, and in stem-piece assays in cucumber, pepper, and tomato^a

Microbes	Proportion of effective isolates					
	Begonia	Cyclamen	Geranium	Cucumber	Pepper	Tomato
<i>Acremonium</i> sp.	0/3	...	0/1	0/2	0/2	0/2
<i>Alternaria</i> sp.	1/10	3/4	0/5	0/3	0/2	0/2
<i>Aspergillus</i> sp.	0/1	...	0/3	0/2	0/2	0/2
Bacteria (unidentified)	0/10	0/2	0/2	0/3	0/4	0/3
<i>Botryosporium longibractiatum</i>	0/1
<i>Cladosporium</i> sp.	0/6	...	0/4	0/1	0/1	...
<i>Colletotrichum gloeosporioides</i>	1/1	0/1
<i>Epicoccum purpurascens</i>	1/4	2/3	0/3	0/2	0/2	0/2
<i>Fusarium</i> sp.	0/4	1/3	0/1	0/1	0/1	0/2
<i>Glioccephalis</i> sp.	0/1
<i>Myrothecium</i> sp.	0/3
<i>Paecilomyces</i> sp.	...	1/1	0/1	0/2	0/2	0/2
<i>Penicillium</i> sp.	0/15	1/1	0/5	0/4	0/6	0/5
<i>Phialophora</i> sp.	...	0/1
<i>Phoma</i> sp.	0/1	3/6	...	1/2	0/2	0/1
<i>Physalospora</i> sp.	0/1	1/1	0/1	0/1	0/1	0/2
<i>Pithomyces</i> sp.	0/2	0/1	0/1	...
<i>Stilbella</i> sp.	...	1.1	...	1/1	1/1	0/1
<i>Trichoderma</i> sp.	6/15	1/3	3/8	0/5	0/4	0/5
<i>Verticillium</i> sp.	0/1
Yeast (unidentified)	1/6	0/2	0/4	0/3	0/2	0/3
Mycelial fungi (unidentified)	0/2	...	0/2	0/3	0/2	0/2
Totals	10/86	14/29	3/41	2/36	0/35	0/33

^a Inoculum concentrations were: 10^6 conidia per ml for *B. cinerea*, *Alternaria* spp., and *Epicoccum purpurascens*; 10^7 cells per ml for yeasts; and 10^8 CFU/ml for bacteria. Number of *G. roseum* isolates tested in begonia, cyclamen, geranium, cucumber, pepper, and tomato were four, four, four, three, two, and three, respectively. These isolates suppressed *B. cinerea* in the respective hosts by 100, 98, 100, 100, 100, and 100%. None of the other microbial isolates was more effective than *G. roseum*.

treated with *G. roseum* and inoculated with colonized petals.

Performance of *G. roseum* against *B. cinerea* varied considerably in flowers of ornamental plants in the greenhouse, and in several instances was inferior to that of the best isolates of *Trichoderma* spp. and to standard fungicides (Fig. 4C and D). For example, *G. roseum* suppressed *B. cinerea* by 49 and 68%, respectively, in fully opened and senescent flowers of begonia (*Begonia × hiemalis*) compared with 95 and 93%, respectively, for an isolate of *T. koningii*, and 85 and 95% for chlorothalonil. Substantial differences were found in biocontrol effectiveness among isolates of *G. roseum* from begonia and seven other hosts when tested against *B. cinerea* in petals but not in leaves of begonia. In cyclamen petals, *G. roseum* and an isolate of *T. koningii* suppressed the pathogen by 75 and 90%, respectively. In geranium petals, *G. roseum* and *T. harzianum* were 32 to 44% and 44 to 86% effective, respectively. Petal flecking caused by *B. cinerea* in gerbera was reduced by >90% by *G. roseum* and by *T. harzianum*, but neither antagonist effectively controlled petal blight. Under some conditions, however, *G. roseum* suppressed the pathogen in petals of various hosts by as much as 85 to 95%. Flower age, pollen, temperature, humid periods, inoculum concentration, and method of inoculum application are among the many variables that substantially influence biocontrol by *G. roseum* and other biocontrol agents in flowers and should be considered in a biocontrol program.

B. cinerea often utilizes fallen tomato petals as food bases from which it invades

green or ripening tomato fruits, either directly or by first attacking calyces attached to the fruit (23). When petals were inoculated with the pathogen (5×10^5 conidia per ml) and positioned near calyces on attached green fruits, almost all calyces became blighted and 95% of the fruits developed rot (Fig. 5A and B). Calyx blight and fruit rot were controlled almost completely, however, when the petals were treated with *G. roseum* a few hours prior to inoculation with the pathogen (Fig. 5C and D).

Plant wounds are another theater of aggressive biological control by *G. roseum*. The antagonist strongly suppressed *B. cinerea* in cuttings and mother plants of geranium, in wounds on tomato stems when lower leaves are removed, and in sun scorch wounds on leaves of *Exacum affine*. In geranium, dipping fresh cuttings bearing snapping and deleafing wounds into inoculum of *G. roseum* (10^7 conidia per ml) controlled gray mold almost completely and as or more effectively than did iprodione (Fig. 6A and B). Cuttings treated with *G. roseum* developed a profusion of healthy roots and were more vigorous than were the fungicide-treated cuttings. In contrast, cuttings inoculated with *B. cinerea* developed large lesions, and about one-quarter of them quickly died. In tomato, application of *G. roseum* (10^7 conidia per ml) to fresh deleafing wounds protected the stems for several months after application. *B. cinerea* was recovered from 70% of wound sites 3 months after deleafing when fresh wounds were inoculated with the pathogen (5×10^5 conidia per ml) only, but from less than 2% of wounds treated

with *G. roseum* 24 h before inoculation with the pathogen. *G. roseum* was extraordinarily persistent in the living stems and was recovered from more than 98% of wound sites 3 months after inoculation. Sun scorch wounds in *Exacum* leaves are major sites of invasion by *B. cinerea* (Fig. 7A). Treatment of artificial scorch wounds made by momentary use of a flame and of natural sun scorch wounds using *G. roseum* (5×10^7 conidia per ml) reduced gray mold severity induced by *B. cinerea* (10^5 conidia per ml) by 97 to 100% (Fig. 7B).

Variables Influencing Effectiveness of *G. roseum*

An understanding of interactions among *G. roseum*, *B. cinerea*, host plants, microclimatic factors, and other components of the biological control systems is fundamental for optimizing suppression of the pathogen in crops. Highlights of what is known of these interactions are now considered.

Development stage of host organs.

Development of leaves from the newly expanded stage to early senescence generally had little effect on performance of *G. roseum* against *B. cinerea* in foliage of strawberry, begonia, and cyclamen. Expanding leaves of begonia and cyclamen were of such low receptivity to *B. cinerea* that biocontrol was difficult to measure. In all three hosts, biocontrol effectiveness was 30 to 70% lower in senescent leaves compared with presenescent leaves, and almost zero in dead leaves.

Biological control of *B. cinerea* in flowers presents special challenges because of the complexity of flower growth, development, and structure. In general, various organs of flowers develop and senesce over different times, are differentially suscepti-

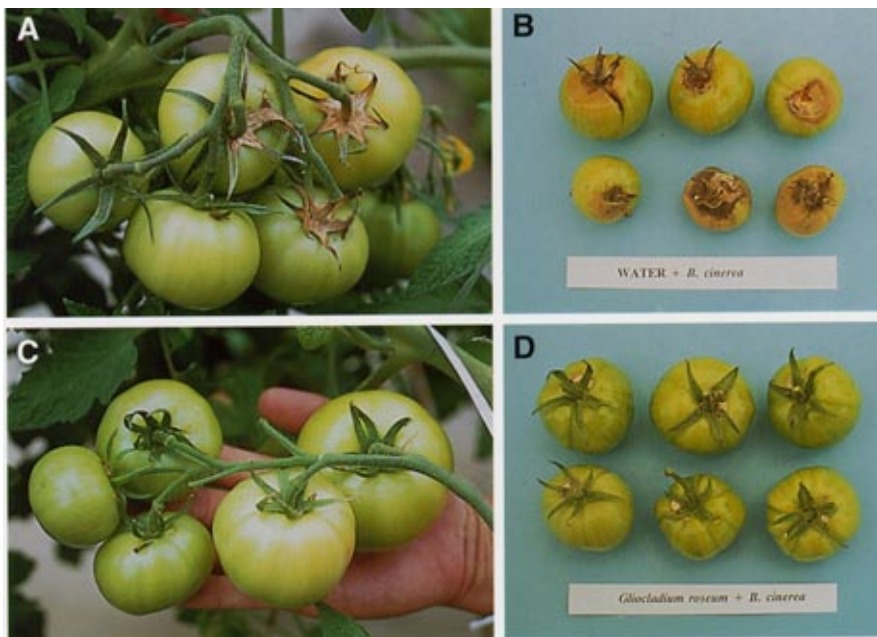


Fig. 5. Protection of calyces and fruits of tomato against *Botrytis cinerea* in colonized tomato petals by treatment of petals with *Gloecladium roseum*. (A) Calyx blight initiated from diseased petals. (B) Fruit rot developing from diseased calyces. (C) Calyces protected by *G. roseum* applied to petals. (D) Fruits protected by *G. roseum* in adhering petals.

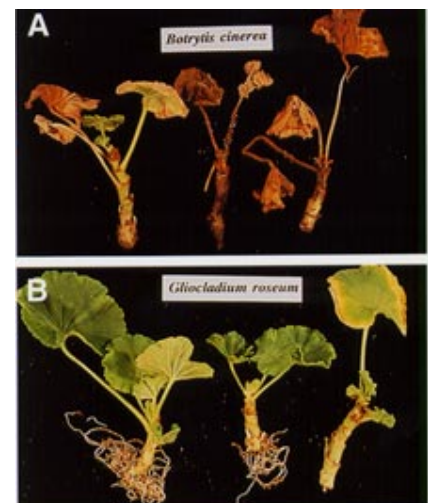


Fig. 6. Biological control of gray mold in geranium cuttings by *Gloecladium roseum*. (A) Cuttings inoculated with *Botrytis cinerea* only. (B) Cuttings inoculated with *B. cinerea* and with *G. roseum*.

ble to *B. cinerea*, and interact differently with microbial antagonists (49,63). Insights into effects of flower age on biocontrol of *B. cinerea* were provided in studies of partially opened, fully opened, and senescent flowers of begonia (*Begonia* × *hiemalis*) that were inoculated with the pathogen (10^6 conidia per ml) and challenge-inoculated after 24 h with *G. roseum* or *T. koningii* (each at 10^7 conidia per ml) (Fig. 8). Susceptibility to *B. cinerea* increased in the successive stages of flower development. *G. roseum* increasingly suppressed the pathogen by 0, 49, and 68%; whereas *T. koningii* was consistently suppressive (93 to 95%) at the successive stages.

Inoculum concentration. An understanding of inoculum concentration of *G. roseum* in relation to control of *B. cinerea* is fundamental to establishing appropriate biocontrol recommendations in crops. As might be anticipated, the relationships vary with crop, type and age of plant organ, pathogen concentration, microclimatic conditions, and other factors. By inference, optimal concentration of *G. roseum* fluctuates in the crop over time and spatially on foliage, flowers, and fruits. As is commonplace in disease management, settling on an inoculum concentration of *G. roseum* that provides satisfactory control of *B. cinerea* in a crop will inevitably demand compromise and approximation.

Studies under controlled conditions provided perspectives of inoculum requirements of *G. roseum* for controlling *B. cinerea* in several crops. When foliage and flowers of begonia and cyclamen were inoculated with various concentrations of *B. cinerea* (0 to 10^6 conidia per ml) and of *G. roseum* (0 to 10^8 conidia per ml) in all combinations, good biocontrol was achieved mainly when concentration of the antagonist was the same as or greater than that of the pathogen. In similar studies in raspberry, high levels of control (90 to 100%) were obtained in leaves for all

combinations of 10^3 to 10^6 conidia of *B. cinerea* per ml and 10^4 to 10^8 conidia of *G. roseum* per ml, but in stems, stamens, and stigmas, only when concentration of the antagonist was 10 or 100 times greater than that of the pathogen (64). Unexpectedly, *G. roseum* at 10^8 conidia often was less effective than at 10^7 or 10^6 conidia in stamens and stigmas, a circumstance also observed in foliage of black spruce in the growth room. Apparently, it can be important not to exceed optimal concentrations of *G. roseum* for biocontrol in crops, especially when conditions favor survival of the antagonist. In the greenhouse and growth room, application of 10^7 conidia of *G. roseum* per ml strongly suppressed *B. cinerea* applied at 10^6 conidia per ml in various organs of begonia, cyclamen, *Exacum*, geranium, gerbera, cucumber, pepper, raspberry, strawberry, tomato, and several other hosts. In each instance, concentration of the pathogen was sufficient to produce consistently severe disease in the absence of the antagonist.

Inoculum concentration of 10^6 to 10^8 conidia of *G. roseum* per ml normally provided good control of *B. cinerea* in crops in the field and in greenhouses. Inoculum containing 10^6 conidia per ml usually was sufficient in strawberry, begonia, and cyclamen; 10^7 conidia per ml gave best results in raspberry; and 10^7 to 10^8 was near optimal in black spruce (28,29,40,55,67). Applications of *G. roseum* at 5×10^6 conidia per ml, timed using a disease forecasting system, suppressed leaf blight caused by *B. squamosa* in field plots of cooking onions by 50 to 58% (22).

Temperature. Observations of temperature in relation to biocontrol activity of *G. roseum* against *B. cinerea* were compiled from separate studies in five hosts to facilitate comparison of data (Fig. 9). In general, the level of biocontrol was high at 20 and 25°C, and also at 30°C in hosts in which measurable infection was obtained, but was progressively less at 15 and 10°C.

Cool temperatures reduced biocontrol only marginally in leaves of strawberry and begonia and in raspberry stamens, moderately in leaves of raspberry, cyclamen, and geranium and in begonia petals, but markedly in petals of cyclamen and geranium (55,63; unpublished). Differential effects of temperature on biocontrol in different host organs were evident in each of four hosts in which two organs were investigated. From additional tests of several *G. roseum* isolates on flowers of cyclamen and geranium, different patterns of reduced biocontrol at cool temperatures were attributable chiefly to the host but not to isolates of the antagonist.

Environmental water. Relationships of atmospheric humidity, dew, rain, irrigation, or other forms of environmental water to biological control of *B. cinerea* in crops by *G. roseum* are poorly understood. Available evidence indicates that environmental water is of paramount importance in the survival, germination, and growth of *G. roseum* on plant surfaces and in penetration of plants by the fungus. Conidia of *G. roseum* failed to germinate on dry foliage of black spruce seedlings in growth chambers at 12, 20, and 28°C, and their ability to germinate, estimated by recovery on potato-dextrose agar medium, decreased sharply with time after application to seedlings (Fig. 10). In black spruce and probably other hosts, a humid period is needed within a few hours of application of *G. roseum* in order to optimize growth of the antagonist and biological control. Several hours of surface wetness suffices in leaves and flowers of hosts that we have investigated, but relationships of form (droplets, films) and duration of wetness to biocontrol have yet to be explored. Similarly, it is not known whether humidity near saturation in the absence of surface moisture is conducive to biological control. In field studies, natural and simulated rain shortly after application of *G. roseum* to straw-



Fig. 7. *Exacum affine*. (A) Sun scorch wounds on leaves. (B) Biological control of gray mold in plants with scorch wounds. At 3 weeks after treatment, disease was severe in water checks (left column), moderately severe in plants treated with iprodione (Rovral) or *Trichoderma harzianum* (second and third columns, respectively), and controlled almost completely by *G. roseum* (right column).

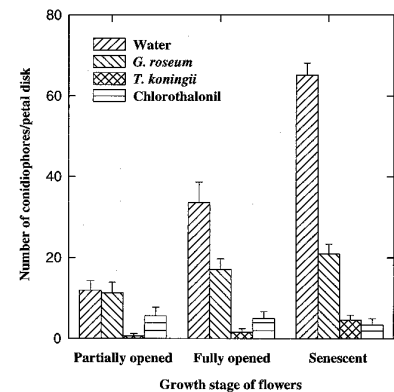


Fig. 8. Conidiophore production of *Botrytis cinerea* in petals of begonia flowers of different growth stages that were inoculated with the pathogen and treated with water plus surfactant, *Gliocladium roseum*, *Trichoderma koningii*, or chlorothalonil.

berry plants depleted inoculum density of the antagonist on the leaves and reduced the effectiveness of biological control (7). Besides effects on *G. roseum*, effects of environmental water on *B. cinerea* and on host plants also could influence biological control (48).

Relationships of *G. roseum* with Host Tissues

Conidia of *G. roseum* are able to germinate in moisture on the host surface, pro-

duce simple germ tubes and superficial hyphae, and penetrate into leaves, stems, petals, and stamens of various kinds of plants. Superficial development of the antagonist is best known from studies conducted under continuous high humidity at 21 to 23°C on leaves, stems, and stamens of raspberry (63). A large proportion (70 to 90%) of conidia germinated at 4 to 12 h after inoculation and produced narrow (1 to 1.5 µm diameter) germ tubes that elongated rapidly after 12 and 16 h on stamens and stems, respectively, and slowly at 12 to 24 h on leaves. Short branches (1 to 5 µm long) developed on germ tubes and hyphae after 16 h and penetrated host tissues directly. Remarkably, verticillate and penicillate conidiophores of *G. roseum* developed from thickened superficial hyphae on stems, stamens, and leaves at 32 to 72 h (Fig. 11A) and produced abundant conidia at 40 to 72 h. The antagonist thus has potential to reproduce in the epiphytic phase, a situation that, to our knowledge, is novel for biocontrol agents applied to foliage and flowers and that could contribute to sustained control of *B. cinerea* in the crop. Evidence of penetration by *G. roseum* in several hosts is presumptive and based on recovery of the antagonist from tissues that were inoculated and later surface-sterilized. Such evidence was obtained in black spruce, red pine, *Exacum affine*, geranium, gerbera, petunia, cucumber, and tomato, as well as in raspberry.

Postpenetration development of *G. roseum* in plant foliage and flowers generally is unclear. Whether the antagonist remains strictly localized or colonizes tissues more extensively may be a function of the type, age, and physiological status of the host tissues. Circumstantial evidence

based on recovery of *G. roseum* from tissues at various distances from inoculation sites indicated that the antagonist remained localized for at least 3 months in stems of vigorously growing tomato plants (*G. Peng, unpublished*). The antagonist progressively colonized senescent leaves and flowers of strawberry, begonia, cyclamen, and geranium, and frequently sporulated within a couple days after the tissues died (38,56; D-W Li and *G. Peng, unpublished*). While senescence and stress apparently favor progressive colonization of host tissues by *G. roseum*, the antagonist also is known to colonize vascular elements of vigorous soybean and red clover plants (34,46,50). In our experience and in almost all other reports, host tissues infected by *G. roseum* remained symptomless. In the few examples of lesion production by *G. roseum*, such as in bean hypocotyls (20), host plants were under stress-inducing conditions and were treated with heavy doses of inoculum, often as mycelium. Long-term persistence in symptomless tissues is a key feature of the parasitic activity of *G. roseum* in stems, leaves, petals, and some other tissues of numerous host plants.

Modes of Action of Biological Control

Competition for nutrients or substrate and mycoparasitism are presumed modes of antagonism of *G. roseum* toward *B. cinerea* in host plants. Nutrient competition was thought to be important on the phylloplane of strawberry, raspberry, begonia, and cyclamen (55,57,63; D.-W. Li, *unpublished*). Substrate competition was considered a key mode of biocontrol of *B. cinerea*

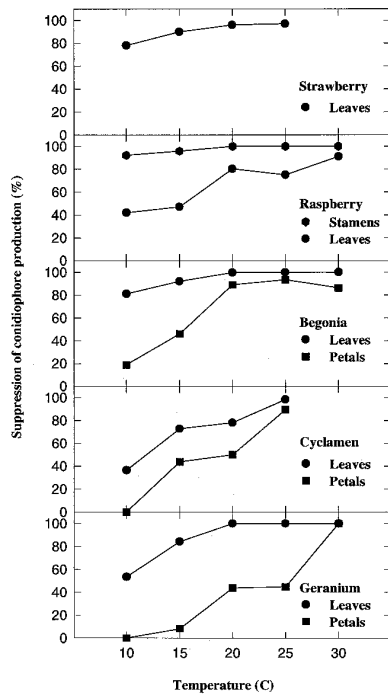


Fig. 9. Observations from separate studies in five hosts for effects of temperature on conidiophore production of *Botrytis cinerea* in leaves and flower parts that were inoculated with the pathogen and treated 24 h later with *Gliocladium roseum*. Isolates of the pathogen and antagonist used in each study were from the respective hosts.

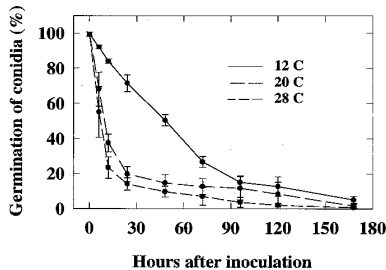


Fig. 10. Germination of *Gliocladium roseum* conidia after periods of incubation at various temperatures on dry foliage of black spruce seedlings. Percent germination was estimated after conidia were recovered from the foliage and placed on potato-dextrose agar medium.

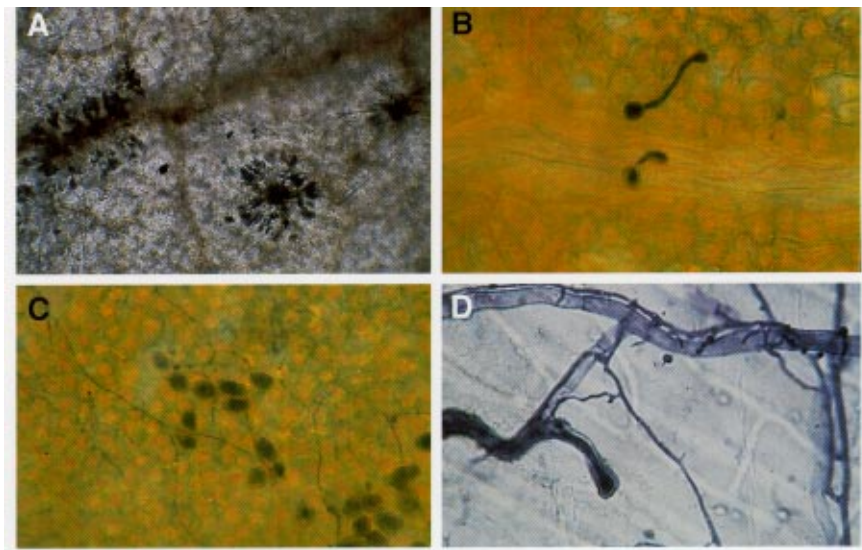


Fig. 11. Development and interactions of *Gliocladium roseum* and *Botrytis cinerea* in raspberry. (A) Verticillate and penicillate conidiophores of *G. roseum* on living leaf tissue. (B) Conidial germination and appressorium formation of *B. cinerea* on a leaf in the absence of *G. roseum*. (C) Lack of conidial germination of *B. cinerea* on a leaf in presence of *G. roseum* hyphae. (D) Narrow hyphae of *G. roseum* entwining and invading larger hyphae of *B. cinerea*.

by *G. roseum* in senescing leaves of strawberry and black spruce (55,66). Evidently the antagonist colonized and exploited the senescent tissues much more rapidly than did *B. cinerea* and largely precluded colonization by the pathogen. This mode of action is important chiefly because it contributes to inoculum suppression of *B. cinerea*. *G. roseum* is a well-known mycoparasite of hyphae, spores, sclerotia, and fruiting bodies of numerous fungi, including *B. cinerea* (3,10,61). While a majority of studies were conducted on agar media, recent observations on raspberry indicated that mycoparasitism can be an important mode of biocontrol of *B. cinerea* on host plants (63).

Notably different interactions of *G. roseum* and *B. cinerea* were found on leaves, stems, and stamens of raspberry maintained under continuous high humidity (63). On leaves, *G. roseum* strongly suppressed germination and germ tube growth of *B. cinerea* but rarely parasitized the pathogen (Fig. 11B and C). On stems, however, conidia of *B. cinerea* frequently germinated and produced well-developed germ tubes and superficial hyphae regardless of nearby conidia, germ tubes, or hyphae of *G. roseum*. Antagonism on stems was manifested as modest suppression of germ tube growth and intense parasitism of *B. cinerea* by *G. roseum*. During persistent high humidity (32 to 72 h), short hyphal branches of *G. roseum* contacted, grew on, or coiled around, and in many instances, invaded conidia and hyphae of *B. cinerea* (Fig. 11D). Affected cells generally lacked contents, and some hyphae collapsed. Whether or not cells of *B. cinerea* were alive when invaded was not determined. Continuity of superficial and intracellular hyphae indicated that many hyphae within those of *B. cinerea* were those of *G. roseum*, as opposed to fine regrowth hyphae of the pathogen developing within damaged cells from septal pores of adjacent undamaged cells (10). On surfaces of stamens, *G. roseum* generally did not suppress germination, growth, or production of appressoria and infection cushions of *B. cinerea*, and was not observed to parasitize the pathogen. However, the antagonist strongly reduced colonization incidence of stamens by the pathogen. Differential availability and composition of nutrients and of antifungal substances in exudates on the host surface were advanced as hypotheses to explain the extraordinary variation in interactions of *G. roseum* and *B. cinerea* on the different raspberry tissues.

Besides competition and mycoparasitism, *G. roseum* possibly antagonizes *B. cinerea* through antibiosis and induced resistance. The antagonist produces an array of fungal inhibitors and wall-degrading enzymes (10,37,38), some of which could function in overlapping scenarios of antibiosis and mycoparasitism. Loss of turgor and lysis of *B. cinerea* hy-

phae, each commonly induced by *G. roseum*, can be considered in the context of both of these modes of antagonism. In general, antibiosis appears to function only over short distances, implying that the antagonist must have access to nutrients close to the pathogen and that nutrient competition could underpin antibiosis (10). While *G. roseum* is able to produce at least one antifungal metabolite, no evidence was found to indicate a role of the metabolite in biological control. On the contrary, mutants of *G. roseum* that produced high or intermediate levels of the metabolite, or none at all, did not differ in biocontrol effectiveness against *B. cinerea* in strawberry leaves (38). Induced resistance in host plants by *G. roseum* is an intriguing possibility, especially in view of prolonged associations of the antagonist with living tissues of many hosts. Collectively, available information underscores a high flexibility of *G. roseum* in modes of biocontrol.

Application Methods and Strategies

A critical challenge in practical biological control is development of reliable methods and strategies for applying the biological control agent in the cropping system. Extraordinary attention to detail is needed to optimize biological control under the full range of conditions in the crop and to minimize risks that errors will be made when the system is in the hands of growers. Methods and strategies generally have to be worked out on a crop-by-crop basis to account for crop-specific conditions such as production methods and epidemiologic factors. Integration of biological control with other crop protection practices is often called for and may differ from farm to farm or region to region. For optimizing effectiveness of *G. roseum* against *B. cinerea*, considerable progress has been made in terms of formulation of inoculum and methods to time, target, and deliver the inoculum in several crops. There should be no illusions, however, regarding the enormity of tasks that lie ahead if we are to take full advantage of the antagonist in disease management.

Inoculum of *G. roseum* can be produced easily and cheaply on sterilized grains of wheat and other cereals. In our laboratory, high concentrations of the antagonist (1 to 5×10^9 conidia per g) are routinely produced within 35 days on wheat grains maintained at 20 to 23°C. After an initial period of mycelial growth (14 to 18 days), cultures are allowed to slowly dry, a process that promotes production of penicillately branched conidiophores, which yield more conidia than do verticillate conidiophores. The verticillate form usually predominates under persistent high humidity. Besides conidia, mycelial fragments of *G. roseum* recovered from wheat grains after 15 days were found to be effective against

B. cinerea in strawberry. Conidia from wheat grains have been stored for more than a year at 3 to 5°C and for 3 months at 20 to 23°C without significant loss in viability or biocontrol effectiveness, findings of considerable importance for commercialization of the antagonist. Prior to application to crops, conidia of *G. roseum* can be suspended in water plus a surfactant such as Triton X-100 or formulated as a powder with talc or another carrier (41,54).

The best method of application of *G. roseum* ordinarily depends on the kinds of host tissues that require protection. Spray application to deposit fine droplets of inoculum uniformly on target surfaces is appropriate for general treatment of foliage and flowers in many crops, but more specialized techniques often are needed for targeting specific tissues. Wounds on geranium cuttings are easily treated by momentary immersion of cuttings in inoculum, while deleafing wounds of greenhouse tomato and cucumber can be treated with a hand sprayer, brush, or cloth. Devices that simultaneously deleaf and spray inoculum onto the deleafing wound can contribute to efficiency of these operations. Flowers of strawberry and raspberry have been successfully treated with *G. roseum* by means of bees employed as vectors of inoculum (41,63).

Few concepts in plant disease management can be considered more “environmentally friendly” than the use of bees to deliver a biological control agent to flowers for controlling a flower-infecting pathogen. In recent studies (41,63), honeybees (*Apis mellifera* L.) effectively vectored inoculum of *G. roseum* to flowers of strawberry and raspberry, and bumblebees (*Bombus impatiens* Cresson) efficiently vectored the antagonist to raspberry flowers (Fig. 12A and B). For vectoring, a powder formulation of *G. roseum* was placed in an inoculum dispenser mounted on the hive. Dispensers for use with honeybees and bumblebees were of different design to accommodate structural differences in hives (Fig. 12C to E); in both instances, however, bees were contaminated with inoculum only when leaving the hive. In field plots, honeybees and bumblebees trapped when emerging from dispensers containing inoculum with 5×10^8 to 1×10^9 CFU of *G. roseum* per g each carried several hundred thousand colony-forming units of the antagonist. Honeybees delivered 300 to 27,000 CFU per flower in strawberry and 600 to 2,100 CFU per flower in raspberry; whereas bumblebees vectored 450 to 2,400 CFU per flower in raspberry. Bee-vectored inoculum effectively controlled *B. cinerea* in petals, stamens, and flowers of strawberry and in flowers of raspberry. However, raspberry fruits were not adequately protected, presumably because *B. cinerea* is able to infect drupelets directly as well as by invasion from flowers.

Efficiency of bee vectoring can be affected by competing nectar sources outside the crop, periods when individual flowers are open, climatic factors, spatial relationships of hives and the crop, and other variables (53,54). Honeybees are easily attracted away from strawberries, but raspberries are a favored nectar source. Bumblebees readily patronize both crops and, unlike honeybees, forage in cool weather (e.g., 6 to 16°C). When conditions are favorable, bee vectoring can have the potential advantage of daily delivery of inoculum to freshly opened flowers. Combination of inoculum delivery and host pollination by bees is feasible in many crops.

Optimal timing of *G. roseum* applications against *B. cinerea* remains to be worked out for almost all crops. Good timing of treatments sometimes is easy to establish, as in protection of deleafing and other artificial wounds, but is more difficult for fast-growing foliage and flowers. Residual biocontrol activity of a *G. roseum* treatment may depend heavily on epiphytic and endophytic relationships of the antagonist with specific host tissues and the ability of the antagonist to remain active and spread in host populations. Endophytic establishment can obviate the need for further application of *G. roseum* to treated tissues (55,66), but does not necessarily

protect new growth. In many, perhaps a majority, of instances, empirical studies under conditions representative of commercial crops are suitable for developing treatment programs. In some crops, it may be feasible to adapt disease prediction systems to facilitate timing of *G. roseum* applications, as was done in onion (22).

Outlook and Future Research

A strong case can be made for continued exploration and development of *G. roseum* as a biological control agent. The antagonist has a remarkable record of satisfying socioeconomic expectations of efficiency, dependability, cost-effectiveness, and safety in biological control. Strong performance of *G. roseum* against *B. cinerea* in hosts as taxonomically diverse as strawberry, geranium, tomato, and black spruce suggests that the antagonist may effectively suppress the pathogen also in many crops in which biocontrol has yet to be investigated. *G. roseum* also justifies further evaluation against other pathogens (32,42,47), particularly in view of its broad ecological adaptation in plants and its wide-ranging mycoparasitic competence. Indeed, knowledge of the activity spectrum of the antagonist, at present fragmentary, is central to integration of the antagonist into disease management programs. *G. roseum* has advantages of abundant production and

long-term viability of inoculum, attributes of key importance for commercial use. Moreover, stickiness of the spores facilitates handling and formulation of inoculum while minimizing any risks such as allergies associated with occupational exposure of workers. Fortunately, *G. roseum* does not grow at human body temperature, and standard ocular, dermal, and feeding tests of *G. roseum* inoculum failed to produce toxicological effects in rabbits and rats. In vectoring studies, no evidence was found that *G. roseum* was harmful to bees.

Introduction of *G. roseum* into crop systems should not present ecological risks, especially in view of wide distribution of the fungus in plants and soils in many regions of the world and abundant evidence that *G. roseum* is not normally pathogenic to plants. In many instances, treatment of crops would simply augment natural populations of the antagonist. Effectiveness of *G. roseum* against *B. cinerea* in a broad range of crops can be expected to increase market potential of the antagonist, counter difficulties of commercial interests with minor or specialty crops commonly attacked by *B. cinerea*, and generally improve prospects for successful implementation of biological control.

G. roseum beckons numerous lines of intriguing and useful research, both fundamental and applied. A much clearer per-



Fig. 12. Bee vectoring of *Gliocladium roseum* inoculum from inoculum dispensers to flowers. (A) Honeybee on raspberry flower. (B) Bumblebee on raspberry flower. (C) Inoculum dispenser (painted black) mounted on a honeybee hive. (D) Inoculum dispenser mounted on the front of a bumblebee hive. (E) Inoculum dispenser for bumblebees with top removed and inoculum-filled tray partly opened. When leaving the hive, bees enter the dispenser through the lower of two rear holes, crawl through the inoculum and into the upper chamber, and emerge from the front hole.



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spective is needed of microclimatic variables, host factors, fungicides, insecticides, and other components of cropping systems in relation to population density and activity of *G. roseum* in crops, especially from the perspective of integrating biological disease control with other production and protection practices. Genetic variability, stability, and relationships of the pool of fungi referred to as *G. roseum* are largely unexplored but could harbor solutions to problems such as reduced effectiveness of biocontrol of *B. cinerea* at low temperature. Highly tantalizing is the endophytic phase of *G. roseum* that is known to exist in some plants (46,50,55,63). How widespread is this phenomenon among plant species? What relationships exist between the endophyte and the host tissues? Can the endophyte induce resistance in the host to pathogenic organisms such as *B. cinerea*? Almost nothing is known of *G. roseum* in plants except that it did not alter electrolyte leakage, chlorophyll level, and photosynthetic rate in black spruce (68). From the viewpoint of human needs, priority should be given to exploiting *G. roseum* as a biologically and ecologically flexible antagonist for suppression of *B. cinerea* and other destructive pathogens in cropping systems.

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