Interaction Specificity of the Biocontrol Agent *Sporothrix flocculosa*: A Video Microscopy Study

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ABSTRACT

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Sporothrix flocculosa, a biocontrol agent reported to act by antibiosis, had a differential effect against four target fungi, based on video microscopy records of the interactions. Two of the fungi, *Botrytis cinerea* and *Cladosporium cucumerinum*, were very susceptible and reacted ahead of the advancing colony of *S. flocculosa*. Typical reactions included retraction of the plasmalemma, aggregation of the cytoplasm, and cell death. When *S. flocculosa* was inoculated directly onto a colony of *B. cinerea*, conidia of the biocontrol agent developed toward the pathogen, and the hyphae of *B. cinerea* ceased cytoplasmic activity and became highly vacuolated within 8 h. Individual conidia of *S. flocculosa* produced several germ tubes, but did not attack live, necrotic, or dead hyphae of the pathogen. The other two tested fungi, *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Idriella bolleyi*, were unaffected by the presence of *S. flocculosa*. These results correlated with previous observations that *C. cucumerinum* and *F. oxysporum* f. sp. *radicis-lycopersici* were, respectively, sensitive and tolerant against purified toxins of *S. flocculosa*. This indicates that the host range of *S. flocculosa* is dictated by host sensitivity to its toxins.

Additional keywords: biological control, host specificity.

Biological control agents of fungal diseases are known to exert their activity by substrate competition, antibiosis, or direct parasitism (23). While some agents have been associated strictly with one of these modes of action, others have been reported to rely on two or all of them (9). In the latter cases, there have been examples of either a concomitant or a sequential manifestation of these mechanisms (2). Ascertaining with precision the exact, or main, mode of action of a putative biocontrol agent is of critical importance, because it underlies future developments in terms of selection of superior candidates and formulation of the agent (21).

Sporothrix flocculosa Traquair, Shaw & Jarvis is a promising candidate for the biological control of powdery mildews (3,4,17). It is believed to act exclusively by antibiosis (14–16) and, up to now, four antifungal molecules, three of them being fatty acids, have been isolated and characterized from culture filtrates of *S. flocculosa* (5,8). Electron microscopic observations have suggested that these antifungal molecules interfere with membrane permeability (15,16).

Because of the difficulties inherent to the manipulation of powdery mildew fungi in vitro, it has been difficult to generate reproducible information pertaining to the activity, specificity, and other characteristics of *S. flocculosa*. Recently, Benyagoub et al. (6) demonstrated that the level of sensitivity varied among fungi exposed to purified toxins produced by *S. flocculosa*. For instance, *Cladosporium cucumerinum* Ellis & Arth. appeared to be very sensitive and *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *radicis-lycopersici* W. R. Jarvis & Shoemaker fairly resistant, while *S. flocculosa* itself was virtually unaffected by its own metabolites. This suggested that susceptibility of fungi to *S. flocculosa* could be dictated by their sensitivity to its toxic metabolites. This sensitivity appeared to be linked with the lipid composition of tested fungi. Whether this specificity of the toxins affects or correlates with the relationship of *S. flocculosa* in interaction with fungi is unknown. This information would be useful in establishing the potential of *S. flocculosa* as a biocontrol agent, its host range and, eventually, the possibility of resistance development by susceptible fungi.

In recent years, the use of video microscopy has proven extremely useful for studying the host range and aggressiveness of mycoparasites, the differential susceptibility of plant pathogens to mycoparasites, and the chronological events of the interactions between plant pathogens and fungal antagonists or even between two putative biocontrol agents (12,18,20). Most of these studies have involved observations of mycoparasitism characterized by hyphal interactions. However, video microscopy comparisons of interactions between biocontrol agents reported or known to act by antibiosis and host fungi have never been carried out. Considering the previous contributions of this technique in furthering our understanding of biocontrol agents, the objectives of this study were to apply the video microscopy approach to the analysis of the chronological stages and the extent and specificity of the antagonistic activity of *S. flocculosa* against target fungi. Based on their reported relative sensitivity to *S. flocculosa* metabolites, *C. cucumerinum* and *F. oxysporum* f. sp. *radicis-lycopersici* were

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selected as target fungi. *Botrytis cinerea* Pres.:Fr. and *Idriella bolleyi* (R. Sprague) Arx (=*Microdochium bolleyi* (R. Sprague) de Hoog & Hermanides-Nijhof), two other representatives of the phyllosphere and rhizosphere, respectively, were also tested.

MATERIALS AND METHODS

Fungi. *S. flocculosa* was grown in indented flasks containing yeast malt peptone dextrose broth (YMPD) (3, 3, 5, and 10 g/liter) under constant agitation (approximately 100 rpm) or in yeast malt peptone dextrose agar (YMPDA) in petri dishes, and it was used in all experiments. *B. cinerea* (isolated from strawberries by P. O. Thibodeau, Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec [MAPAQ], Québec) and *C. cucumerinum* (Centre for the Land and Biological Resources Research, Ottawa, Ontario) were used as model fungi, because of their ease of manipulation in vitro and their reported susceptibility to both *S. flocculosa* and its toxic products (6). In addition, *F. oxysporum* f. sp. *radicis-lycopersici* (obtained from P. O. Thibodeau, MAPAQ), reported for its relative resistance to *S. flocculosa* (6,16), and *I. bolleyi* strain T560, reported for its biocontrol abilities (11), were studied in interaction with *S. flocculosa*. All four test fungi were grown on potato dextrose agar (PDA) at 25°C.

Interactions. Fungal interactions were studied, based on methodology previously described (18). Large coverslips (44 × 64 mm) were dipped into molten PDA or nutrient agar (NA) and deposited in the center of petri dishes half-filled with technical agar (20 g/liter). The slides were inoculated with two PDA disks (5 mm) of a test fungus placed side by side roughly 2 cm apart and confronted with *S. flocculosa* inoculated either as agar disks or as a 10-µl drop of a spore suspension (approximately 5×10^6 CFU/ml). Each disk or drop was placed approximately 2 cm directly across each of the two agar disks of the test fungus. The test fungus and *S. flocculosa* were inoculated simultaneously on the dishes. In addition, *B. cinerea* or *S. flocculosa* were interchangeably inoculated 48 h before being confronted against one another. The dishes containing the coverslips were then incubated at 25°C and observed at different time intervals postincubation ranging from 12 to 72 h.

To evaluate if nutrients affected the outcome of the interactions, water agar (WA) (20 g of Bacto agar [Difco Laboratories, Detroit]/liter) and a one-fourth dilution of PDA (PDA/4) were also used as agar film on the coverslips.

In the case of the *Botrytis-Sporothrix* combination, in which mycelial contact never took place when both fungi were set apart, the interaction was also studied by placing a spore suspension of *S. flocculosa* at the margin of a 2-day-old mycelial colony of *B. cinerea* growing on PDA. Observations were made from 3 to 12 h later.

Experimental design. Before being observed under the microscope, all interactions were observed macroscopically for colony

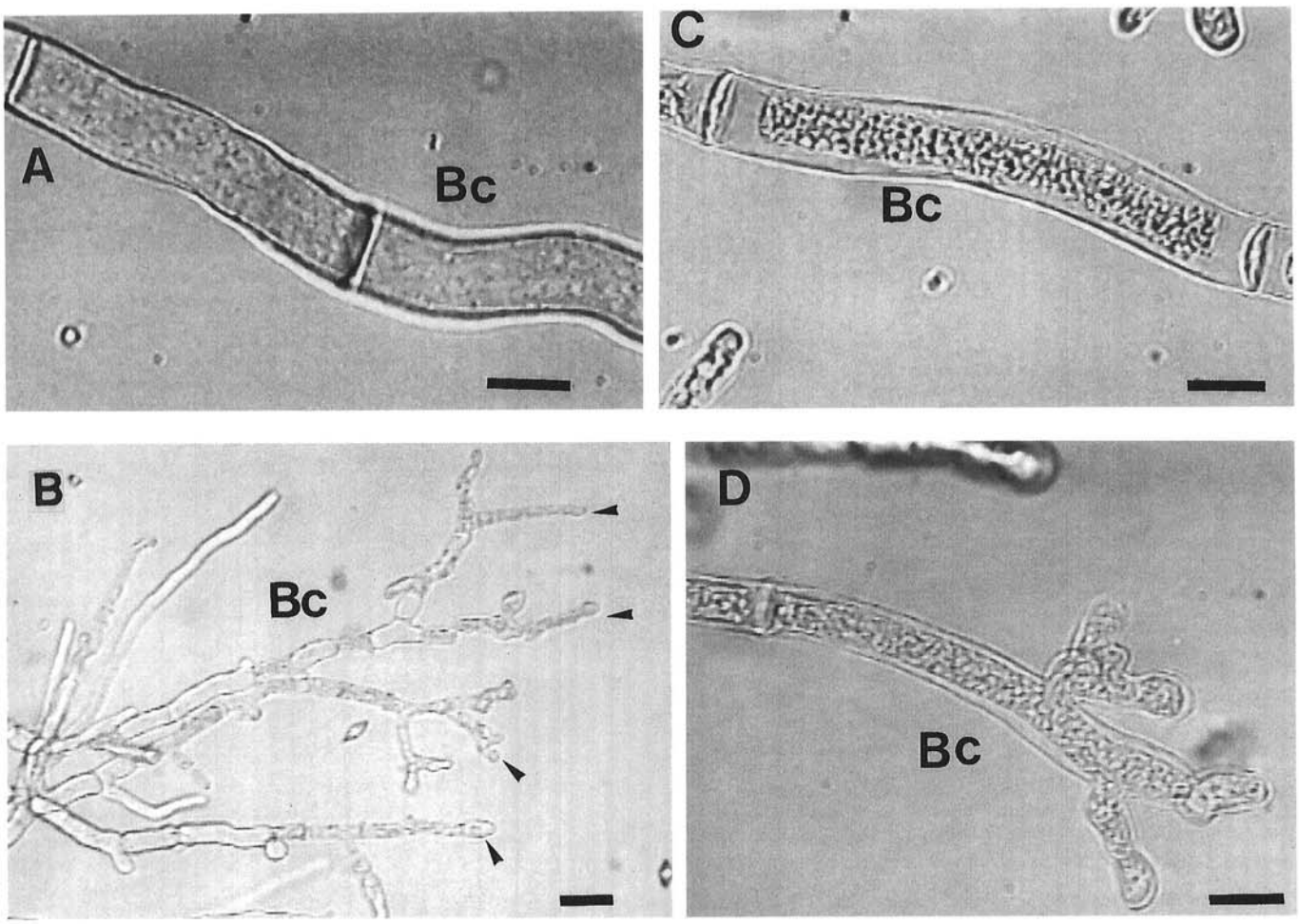

Fig. 1. Video microscopic observations of *Botrytis cinerea* (Bc) hyphae. **A,** Typical *B. cinerea* hypha growing on a synthetic medium, characterized by regular septations and intense cytoplasmic streaming. 700×. Bar = 10 µm. **B,** *B. cinerea* hyphal tips of a colony 48 h after development toward an incoming colony of *Sporothrix flocculosa*. Note the short and stubby septate segments and the irregular branching. All hyphal tips are necrotic and contain an aggregated cytoplasm (arrowheads). 250×. Bar = 20 µm. **C,** Hyphal segments of *B. cinerea* at the front end of a colony exposed to *S. flocculosa*. The plasmalemma has retracted from the cell wall and the cytoplasm is granular and inactive. 700×. Bar = 10 µm. **D,** Time-lapse microscopic study of the interaction between a conidial suspension of *S. flocculosa* starting at 2 h after being inoculated at the margin of a 48-h-old colony of *B. cinerea* showing a dead hyphal tip of *B. cinerea* with several budding segments. 700×. Bar = 10 µm.

diameter, zones of antibiosis, and other signs of interest. Colony diameters of each fungus were measured after 48 h on all three nutrient media. For each fungus on each medium, 16 colony diameters were recorded and averaged. Statistical analyses were carried out with the software SuperANOVA (Abacus Concepts, Inc., Berkeley, CA) and means were separated by Duncan's multiple range test ($P \leq 0.05$).

Fungal interactions were then studied microscopically through the agar film under a 100, 250, or 700× magnification. The interactions were recorded using an S-VHS F15 color video camera (Panasonic Co., Secaucus, NJ) attached to a Orthoplan microscope (E. Leitz, Inc., Rockleigh, NJ). The camera was attached to a S-VHS BT AG-6720 video recorder (Panasonic Co.), which in turn was connected to a S-VHS BT-M1420PY color video monitor (Panasonic Co.). Interactions were recorded on master broadcast S-VHS video cassettes. Video cassettes were transcoded into compatible format by Panavideo Inc. (Québec). Videotape photographs of the events were made into Polaroid photographs by Panavideo Inc.

For each experiment, all possible combinations were replicated, and each combination was studied in a minimum of four separate experiments. Unless specified otherwise, all reported results were consistently observed in all samples studied. To ascertain that recorded observations were not artifacts induced by in vitro conditions, all possible combinations of interaction were used as controls, including self-interactions.

Time-lapse recordings of *S. flocculosa-B. cinerea* and of *S. flocculosa-I. bolleyi* were carried out to study the chronology of some of the phenomena characterizing the interaction.

RESULTS

Macroscopic observations. Based on visual observations, the interactions could be readily segregated into two groups. Both *B. cinerea* and *C. cucumerinum* were hindered in their development in the presence of *S. flocculosa*. This was characterized by a complete and permanent arrest of radial development occurring roughly 2.0 mm from the edge of the *S. flocculosa* colony after 48 h where a clear zone of lysis could be observed. The opposite end of the colony of both test fungi was apparently unaffected, and their mycelium reached the limit of the glass coverslip within 3 to 4 days. When *B. cinerea* was inoculated in close proximity to a 48-h-old colony of *S. flocculosa*, no radial growth occurred in the direction of the antagonist.

By contrast, *F. oxysporum* f. sp. *radicis-lycopersici* and *I. bolleyi* did not show obvious signs of growth inhibition. The latter was particularly insensitive to the presence of *S. flocculosa*, as it grew right through its colony (or vice versa) with neither fungus inhibited. In the case of *F. oxysporum* f. sp. *radicis-lycopersici*, its radial development facing *S. flocculosa* appeared to be inhibited initially (between 24 and 48 h), as evidenced by a more extensive

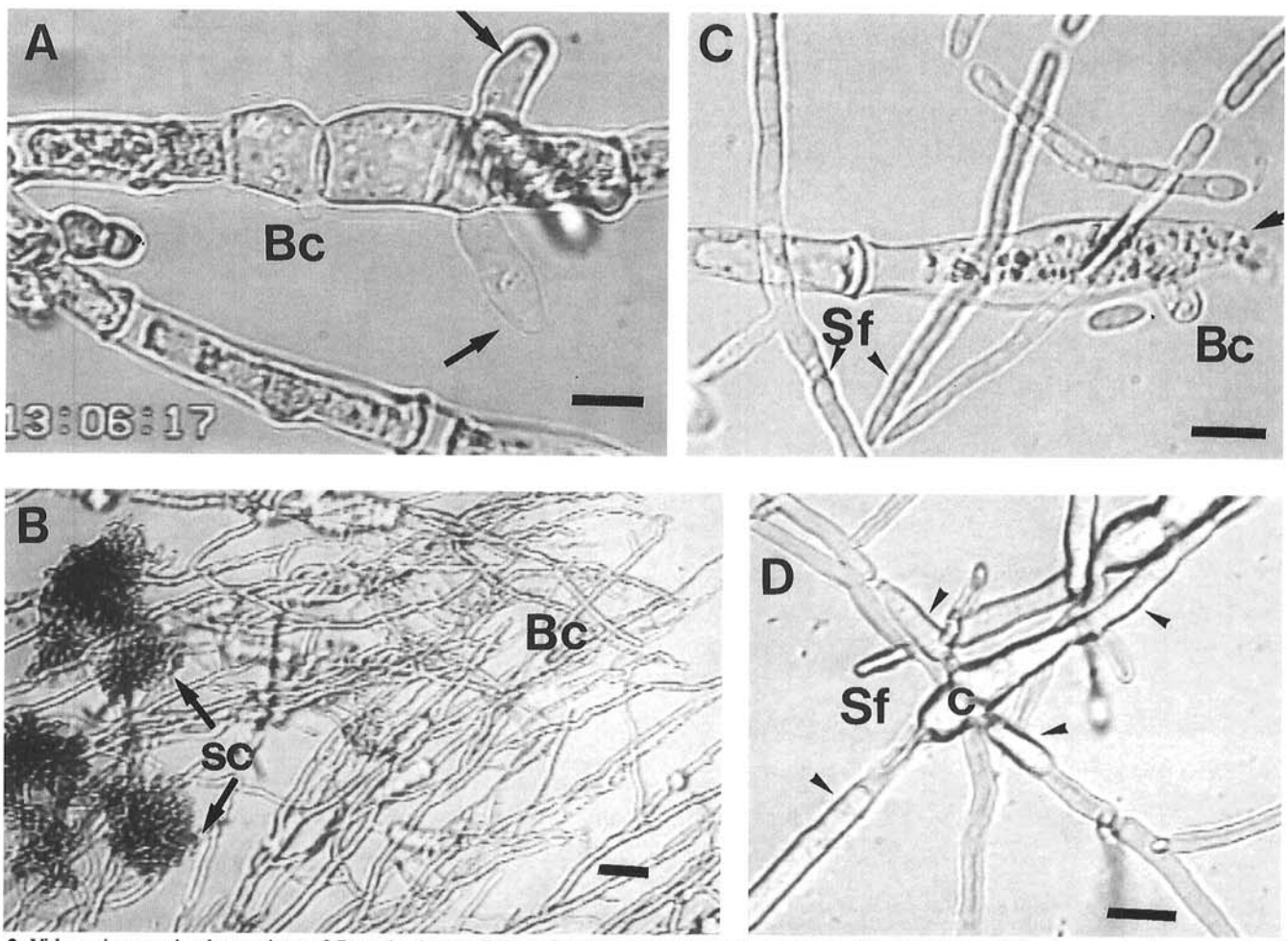

Fig. 2. Video microscopic observations of *Botrytis cinerea* (Bc) confronted with *Sporothrix flocculosa* (Sf). **A,** Hyphae of *B. cinerea* showing a small viable budding segment (arrows). 700×. Bar = 10 µm. **B,** Low magnification of a *B. cinerea* colony growing toward *S. flocculosa*. Note the formation of sclerotia (sc) by the pathogen. All hyphae ahead of the sclerotial front are collapsed. 100×. Bar = 50 µm. **C,** Typical hyphal tip of *B. cinerea* 12 h after inoculation of a spore suspension of *S. flocculosa* at the margin of a *B. cinerea* colony. The hyphal tip (arrow) is characterized by a retracted plasmalemma and granular cytoplasm as *S. flocculosa* (arrowheads) develops in proximity. Note that hyphal segments before the tip are highly vacuolated and show no physiological activity. 700×. Bar = 10 µm. **D,** A conidium (c) of *S. flocculosa* developing in a colony of *B. cinerea*. The conidium has developed as many as four germ tubes (arrowheads) developing in all directions. 700×. Bar = 10 µm.

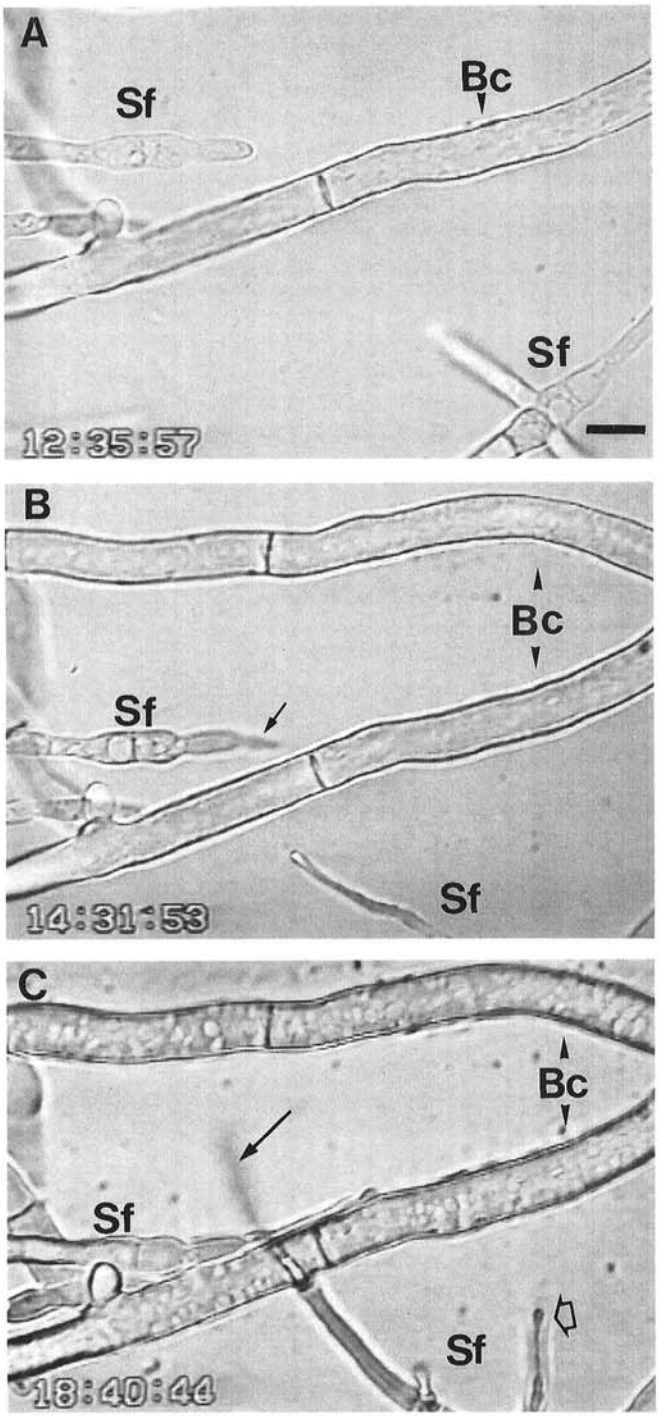

Fig. 3. Time-lapse microscopic study of the interaction between a conidial suspension of *Sporothrix flocculosa* (Sf) starting at 2 h after being inoculated at the margin of a 48-h-old colony of *Botrytis cinerea* (Bc). Bar = 10 µm. **A,** At 2 h, there is no obvious damage to *B. cinerea* hyphae as cytoplasmic streaming is regular. Two *S. flocculosa* conidia can be seen germinating toward *B. cinerea*. **B,** At 4 h, a conidium of *S. flocculosa* coming into close proximity of *B. cinerea* has altered its plane of development to move around the underside of the encountered hypha (arrow). Another *S. flocculosa* hypha can be observed moving toward *B. cinerea*. At this stage, the intensity of cytoplasmic streaming has been considerably reduced in *B. cinerea* hypha. **C,** Four hours later, two *S. flocculosa* hyphae have come in contact with *B. cinerea*, although they did not penetrate the pathogen, but altered their plane of development (arrow). Another *S. flocculosa* hypha can be seen moving toward *B. cinerea* (open arrow). At this time, *B. cinerea* hyphae are highly vacuolated and cytoplasmic streaming has stopped completely.

radial growth at the opposite end. However, its mycelium was nonetheless capable of sustained growth, and it intermingled with the opposing colony of *S. flocculosa* after 48 h.

In all four interactions, the growth of *S. flocculosa* was never reduced.

When the interactions were studied on low-nutrient media (WA and PDA/4), there was a significant reduction ($P \leq 0.05$) in radial development of all fungi as measured after 48 h compared with richer media. For instance, *S. flocculosa* averaged 1.05 and 1.02 cm of growth on PDA and NA, respectively, compared with 0.84 and 0.65 cm of growth on WA and PDA/4, respectively. *B. cinerea*, for example, grew 1.7 and 1.5 cm on PDA and NA in contrast to 0.55 and 0.60 cm on WA and PDA/4. However, after 72 h, the outcome of the interaction was not visually altered, as similar zones of lysis were observed with *B. cinerea* and *C. cucumerinum*, while hyphal contact could be observed with *F. oxysporum* f. sp. *radicis-lycopersici* and *I. bolleyi*.

All self-interactions indicated compatibility between the advancing colonies. In addition, there was no macroscopic evidence of interplay when target fungi were coupled in different combinations against one another.

Microscopic observations. S. flocculosa-B. cinerea. *B. cinerea* was markedly affected by the presence of *S. flocculosa*, and it never came closer than 2 mm in front of the opposite mycelial colony. When observed unchallenged or from the control end of the interaction, its mycelium was characterized by a dense and streaming cytoplasm with regular septa roughly 50 to 100 µm apart (Fig. 1A). By contrast, mycelial development toward *S. flocculosa* was rapidly modified. Within 24 h, first reactions were typified by intense and irregular branching accompanied by short and stubby septate segments (Fig. 1B). After 48 h, altered hyphae were found a distance from the front of the interaction and showed a detached plasmalemma surrounding an aggregated cytoplasm (Fig. 1C). Hyphal tips were invariably shrunken, being either empty or containing granular and retracted remnants of the cytoplasm (Fig. 1D). In several instances, viable segments budded in the stress zone (Fig. 2A), but, at this stage, these efforts never led to sustained development by *B. cinerea*. There was a remarkable consistency with the extent of damage as the delimitation between necrotic and viable hyphae followed a near-straight line across the colony. Under low magnification, this zone coincided with the formation of sclerotia by *B. cinerea* (Fig. 2B).

When a conidial suspension of *S. flocculosa* was deposited directly at the edge of a colony of *B. cinerea*, the same reactions described above were observed, albeit more rapidly. Within 12 h, all hyphal tips of *B. cinerea* in the vicinity of the antagonist were typified by a granular and condensed cytoplasm (Fig. 2C). Observations of individual conidia of *S. flocculosa* revealed an interesting phenomenon. It appeared that conidia that were deposited directly into a fungal colony (*B. cinerea*, in this case) were able to produce several germ tubes developing in different directions (Fig. 2D).

To study the evolution of the degradation events, a zone of interaction was placed under time-lapse observation 2 h after *S. flocculosa* had been deposited at the margin of a *B. cinerea* colony. At the selected site, *B. cinerea* hyphae were still active, as evident by intense cytoplasmic streaming, and *S. flocculosa* conidia were in an early stage of germination (Fig. 3A). As *S. flocculosa* developed toward *B. cinerea*, it appeared to move around or away from the opposing hypha of *B. cinerea*, its hyphal tip going out of focus (Fig. 3B). At this stage, alterations of *B. cinerea* hyphae were evident by a reduction in cytoplasmic streaming. Four hours later, another hypha of *S. flocculosa* had come in contact with *B. cinerea*; as in the previous case, it changed its plane of development and did not penetrate the pathogen hypha, but grew over it (Fig. 3C, closed arrow). At this moment, the pathogen cells were highly vacuolated and cytoplasmic streaming had completely stopped (Fig. 3C).

S. flocculosa-C. cucumerinum. *C. cucumerinum* reacted to the presence of *S. flocculosa* much the same as *B. cinerea* did. When confronted by the antagonist, advancing hyphae of *C. cucumerinum* were rapidly vacuolated or emptied of their cytoplasmic content. The control end of the colony was dense and composed of healthy and rapidly developing hyphae (Fig. 4A and B). By contrast, the hyphae at the interaction end were sparsely dispersed and highly vacuolated, when not dead. In a response similar to the formation of sclerotia by *B. cinerea*, *C. cucumerinum* reacted to the presence of *S. flocculosa* by sporulating abundantly, starting at a point expanding nearly straight across the colony (Fig. 4C). All hyphae ahead of the front of sporulation were necrotic and seemingly dead, as suggested by the absence of cytoplasmic streaming.

S. flocculosa-I. bolleyi. *I. bolleyi* is a rhizosphere inhabitant that has been reported for its biocontrol potential against soilborne fungi (11). During in vitro confrontations, neither *I. bolleyi* nor *S. flocculosa* was affected by the presence of the other. When studied under time-lapse, it was interesting to observe that neither fungus attempted to alter its course in an effort to avoid an incoming hypha. The fungi rather grew directly over or under any hyphae they encountered, but no clear signs of antagonism by either organism were detected (Fig. 5A). However, at the site of contact, cytoplasmic streaming of *I. bolleyi* was somewhat perturbed, resulting in the formation of a clear vacuolated zone appearing directly across the tip of the incoming hypha (Fig. 5A, arrows). This reaction appeared to subside within a few minutes as *S. flocculosa* moved forward (Fig. 5B).

S. flocculosa-F. oxysporum *f. sp.* radicis-lycopersici. As in the previous combination, neither fungus appeared to be altered in its development as a result of the interaction. However, the fungi were apparently growing on a different plane, instead of directly confronting each other as observed with the interaction *S. flocculosa-I. bolleyi*. For this reason, it was impossible to generate a clear picture of the two fungi in the same plane of view. In any event, both fungi displayed a normal morphology and development when observed individually at sites of overlapping.

DISCUSSION

The need to obtain a strong basic understanding of the properties of a biocontrol agent cannot be overstated considering that technological transfer of microorganisms into biopesticides is lagging behind anticipated expectations (1). In the case of *S. flocculosa*, which has shown good potential as a biofungicide against powdery mildew (3,4), previous studies have relied mostly on chemical studies (6) and electron microscope observations (14–16) to decipher its behavior and mode of action. In this work, video microscopy was used to study the chronological progress of the interaction, the developmental processes of *S. flocculosa*, and the differences between compatible and incompatible interactions. As such, this information can prove helpful in increasing our understanding of the antagonist and its commercial potential.

Based on our results, *S. flocculosa* displayed the typical features of a biocontrol agent acting by antibiosis, activity which was specific against two of the four target fungi, namely *B. cinerea* and *C. cucumerinum*. To date, four fatty acids with antibiotic activity have been isolated and characterized from liquid cultures of *S. flocculosa* (5,8). Whether all four are involved simultaneously in the antagonistic process observed here is uncertain, but cell reactions of affected fungi are in line with the reported mode of action of some toxic fatty acids (19). Indeed, rapid cell lysis preceded by cytoplasmic vacuolation and then plasmalemma retraction, without any apparent cell wall alteration, are reactions consistent with the diffusion of a toxic component through the cell wall and interfering with membrane permeability (7).

Antibiosis may offer an advantage over parasitism in the sense that it kills host cells very rapidly (13). In our study, hyphae of *B. cinerea* were inactivated by *S. flocculosa* in less than 8 h when directly challenged with the antagonist. Reports of parasitism by biocontrol agents usually cover a period of 5 to 7 days under the best conditions for complete control of a pathogen (22). From a practical point of view, this is of critical importance, especially with powdery mildews, which take less than 72 h to complete a life cycle under greenhouse conditions. This might explain recent reports of the better ability of *S. flocculosa* at controlling powdery mildews in commercial conditions compared with potential biocontrol agents reported as being strict parasites of powdery mildews (10).

Another interesting point was the observation that nutrients did not affect the outcome of the interaction. As expected, *S. floccu-*

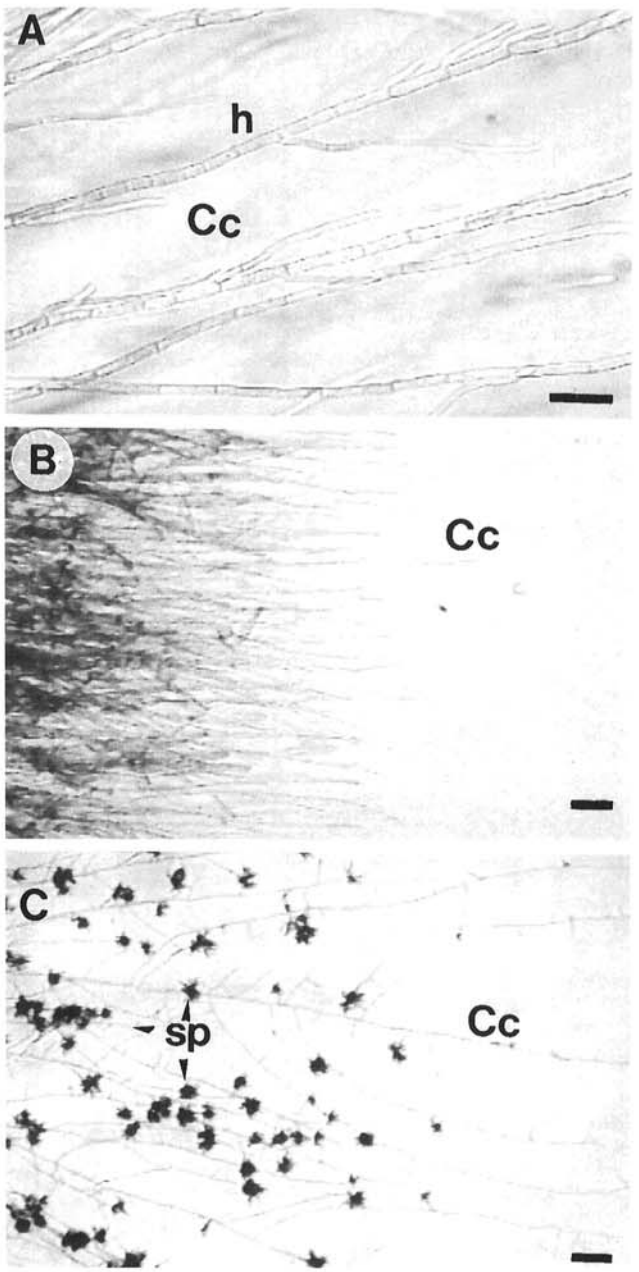

Fig. 4. Video microscopic observations of *Cladosporium cucumerinum* (Cc) in confrontation with *Sporothrix flocculosa* (Sf). **A,** Typical hyphae (h) of *C. cucumerinum* at the control end of the interaction showing regular septations and branching. Bar = 10 µm. **B,** Low magnification of the end of a *C. cucumerinum* colony away from the antagonist. It is typified by a dense and regular mat of hyphae. Bar = 50 µm. **C,** Interaction end of the same *C. cucumerinum* colony confronted by an advancing colony of *S. flocculosa*. Mycelium is sparse and mostly degraded. Note the formation of sporulating colonies (sp). Bar = 50 µm.

losa radial growth was retarded on a nutrient-poor medium compared with PDA. However, sensitive fungi (namely *B. cinerea*) did not seem to benefit from this, as they were affected in the same manner regardless of the medium on which the interaction took place, although it might be said that their growth was equally reduced. These results are of interest for the commercial formulation in which *S. flocculosa* would be released. Current trends, especially in the case of strong saprophytes and competitors such as *Trichoderma* spp., emphasize amending the formulation with an exogenous source of carbon and nitrogen to provide the biocontrol agent with an early advantage (21). Since *S. flocculosa* is obviously not a good competitor or saprophyte, but maintains its antagonistic activity in nutrient-poor media, a nutrient-enrichment of its formulation might be a disadvantage.

Another phenomenon worthy of consideration was the behavior of *S. flocculosa* when a spore suspension was directly confronted with mycelium of *B. cinerea*. Single spores were seen to produce germ tubes in several directions (Fig. 2D), multiplying the efficacy of a single unit. This pattern differed from when a conidium on a medium without another fungus produces a single germ tube. It would, thus, be important to assess whether this peculiar proliferation is attributable to the presence of a fungus perceived as an intruder, which would confer a tremendous advantage to the biocontrol agent.

Of the four interactions studied, two distinct sets of events emerged: *B. cinerea* and *C. cucumerinum* being sensitive to the presence of *S. flocculosa*, and *F. oxysporum* and *I. bolleyi* being apparently unaffected by the antagonist. These results provide strong evidence that *S. flocculosa* antibiotics are not generic antifungal compounds, but that they affect selectively a certain number or group of fungi. Interestingly, Benyagoub et al. (6) reported a similar specificity when using purified toxins against *C. cucumerinum* and *F. oxysporum* f. sp. *radicis-lycopersici*. According to their work, the sensitivity to the fatty acid antibiotics produced by *S. flocculosa* was related to membrane fluidity; that of *C. cucumerinum*, a phyllosphere fungus, being higher than what is commonly found in rhizosphere fungi such as *F. oxysporum* f. sp. *radicis-lycopersici*. This suggests not only that antibiosis is the main mode of action of *S. flocculosa*, but also that sensitivity to antibiotics will dictate the host range of the antagonist.

From an ecological point of view, inferences from our in vitro studies are consistent with the description of *S. flocculosa* as an epiphyte. It would appear that *S. flocculosa* has a restricted ecological niche in the phylloplane and that it protects it by means of releasing antibiotics. These antibiotics can diffuse around the colony and, thus, repel eventual invaders. This mode of action does not seem to depend on substrate or food availability, but would, rather, be part of the intrinsic properties of the organism. In fact, *S. flocculosa* has a very limited development on synthetic media and on leaf surfaces as well, which would put it at a great disadvantage if it had to rely on competition and fast colonization rate to protect its niche. Based on results from this study, it is also apparent that *S. flocculosa* is not a hyperparasite. On the other hand, its development appeared to be stimulated by the presence of an opposing fungus, as observed in the case against *B. cinerea*. Interestingly, a similar phenomenon has been reported in greenhouse studies against powdery mildew, in which epidemiological data have shown significantly higher numbers of *S. flocculosa* on leaves infested with powdery mildew than on control leaves (C. Labbé and R. R. Bélanger, *unpublished data*). This might indicate that the fungus has a certain ability to regulate its own population in response to external stimuli. As such, inquiries into the precise nature of these stimuli should prove useful in optimizing a commercial formulation.

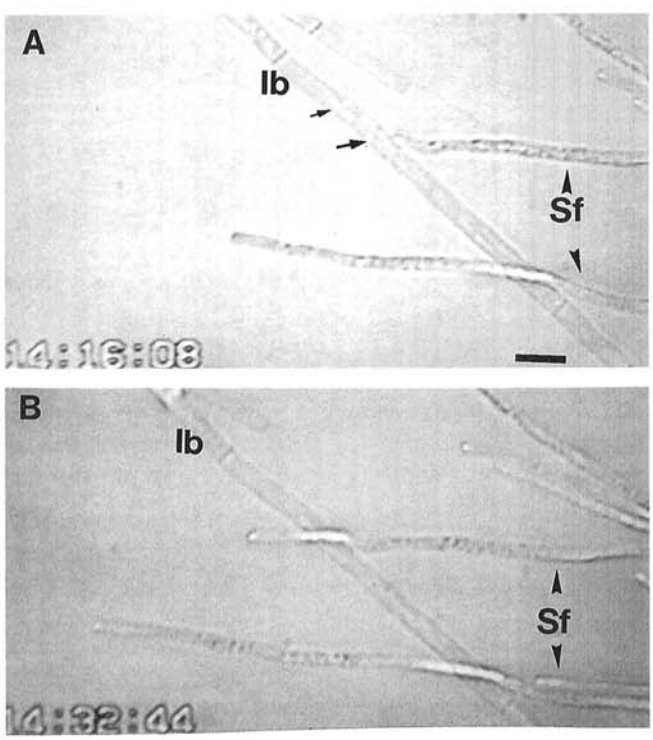

Fig. 5. Time-lapse microscopic study of the interaction between hyphae of *Idriella bolleyi* (Ib) and *Sporothrix flocculosa* (Sf). Bar = 10 µm. **A,** Hyphae of both fungi appear to intermingle without obvious damage on either part. Note that the growth of *S. flocculosa* is not altered by *I. bolleyi* hyphae. As a *S. flocculosa* hypha comes into contact with *I. bolleyi*, cytoplasmic streaming of *I. bolleyi* hypha is temporarily perturbed as evident by a localized vacuolation (arrows). **B,** A few minutes later (about 15 min), as *S. flocculosa* hyphae moves onward, the vacuolation within *I. bolleyi* hypha subsides, and regular cytoplasmic streaming resumes.

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