Factors Influencing Antagonism of *Chaetomium globosum* to *Venturia inaequalis*: A Case Study in Failed Biocontrol

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ABSTRACT

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The apple scab antagonist *Chaetomium globosum*, applied as ascospores to apple leaves in the growth chamber or field, did not colonize the leaf surface even where scab control was observed. In further growth chamber trials, heat-killed ascospores controlled scab as effectively as viable spores. Antagonism of *C. globosum* ascospores or culture extract to *Venturia inaequalis* was not observed when either was applied several days in advance of the pathogen to seedlings or agarose-coated slides. Inhibitory activity of culture extract in vitro declined somewhat over time or when incubated at pH 5.5, 7.0, or 8.8; activity was substantially reduced at pH 11.1 or when extract was dried or aerated for 6 days. The results suggest that antibiotics from *C. globosum* ascospores can diffuse passively onto the leaf surface and inhibit infection by *V. inaequalis*. On the phylloplane they can be degraded abiotically, thus accounting for loss of biocontrol. This does not preclude additional mechanisms for the loss of control.

Up to 90% reduction in apple scab severity has been observed consistently in growth chamber studies when ascospores of some strains of *Chaetomium globosum* Kunze ex. Fries are applied to apple seedlings concurrently with conidia of *Venturia inaequalis* (Cke.) Wint. (anamorph, *Spilocaea pomi* Fr.) (1,3,12). Frequent field applications over three seasons have resulted, however, in only 0–25% disease reduction (3,13). Antibiosis has been implicated as the mechanism of antagonism to *V. inaequalis* (12) as well as to other pests (21,33). Though *C. globosum* has been an effective biocontrol agent against seed rots, seedling blight, and bean seed fly in small-scale field trials (10,19,23,24), the failure to demonstrate sustained and effective apple scab suppression outside of controlled environments is more representative of past biological control work in general (2,28).

Schroth and Hancock (28) point out that many factors in field situations may lead to inconsistency between indoor and field trials and that an understanding of antagonist physiology and ecology is essential to developing successful biocontrol. Several variables potentially influential in the *C. globosum–V. inaequalis* system have been studied. *C. globosum* ascospores germinate poorly in the absence of complex media (9,11) and on apple leaves, even where apple scab biocontrol has occurred (13). Subsequent growth in culture and antibiotic production are markedly influenced by strain and culture conditions (4,6,8,12,17,30). Antibiotics are often unstable when applied in the field (14,26), and at least one class of compounds associated with scab control by *C. globosum* (13) can be inactivated by bacteria, oxidation, mild reduction, or elevated pH (7,15,20,34). Tveit and Moore (32) found that protection from *Helminthosporium victoriae* Meehan & Murphy by an unidentified *Chaetomium* sp. on oat seedlings decreased at high temperatures.

If advances in biological control are to be made, the basis for both successes and failures needs to be known. Failures are rarely explored. The objective of this study was to investigate some of the factors most likely to influence biocontrol of apple scab by *C. globosum*, including relative extent of leaf colonization in the field and growth chamber, timing of application with respect to the pathogen in the growth chamber, and antibiotic stability under various physical conditions.

MATERIALS AND METHODS

Microbial culture. The most effective *C. globosum* strains tested for apple scab biocontrol, QM 103a and NRRL 6296 (12), and a local isolate from apple leaves, ATCC 58917 (= F-6), were maintained on potato-dextrose agar (PDA) under mineral oil at 4 C (31). Six-wk-old PDA cultures were chopped, then agitated in approximately 20 ml per plate 0.01 M phosphate buffer (pH, 7.0) on a platform shaker for 1 hr at 4 C and 220 excursions per minute. The resulting liquid was filtered through two layers of cheesecloth and centrifuged at 10,000 *g* for 10 min. Culture extract was obtained by passing the supernatant through a 0.8-μm membrane filter (Millipore Corp., Bedford, MA) to remove spores and mycelial fragments. The remaining pellet was resuspended and centrifuged once more to obtain washed ascospores, which were then stored at 4 C in phosphate buffer for approximately 18 hr before use. Spores were checked periodically after this storage period to ensure viability. For *C. globosum* germination experiments, where culture extract was not required, ascospores were obtained in an identical manner except that agitation time was reduced to 20 min and a 250-μm-mesh brass sieve (W. S. Tyler, Inc., Mentor, OH) was substituted for the cheesecloth for filtration. Conidia of *V. inaequalis* strain 365 (olivaceous brown, wild-type; supplied by D. Boone, Department of Plant Pathology, University of Wisconsin-Madison), were produced on cheesecloth wicks at 16 C (22). Passage through host plants was performed periodically to maintain conidial rather than mycelial production.

Seedling trials. All seedling assays were done with 3-wk-old apple plants grown from open-pollinated McIntosh seeds as described previously (1). A 20-ml glass atomizer (DeVilbiss Co., Somerset, PA) was used to spray plants to runoff. *C. globosum* ascospores and *V. inaequalis* conidia were applied in phosphate buffer at final concentrations of 2×10^6 spores per milliliter and 5×10^5 conidia per milliliter, respectively, and, when applied together, were mixed immediately before application. Plants were placed in a randomized complete block design with four blocks in a Plexiglas infection box. Conditions favorable for infection (19 C, 100% RH for 16–24 hr; followed by 19 C, 90% RH) (1) were provided for 2 to 3 wk after inoculation with *V. inaequalis*. Seedlings were then rated for disease severity on a scale from 0 to 4, 0 representing no observable symptoms and 4 designating collapse of shoots with widespread coalesced lesions. Disease was also evaluated by

measuring leaf areas electronically with a leaf area meter (LI-3100, Li-Cor, Lincoln, NE) (12), on the premise that seedlings of the same age will grow at a rate inversely proportional to the level of disease.

Role of leaf colonization by *C. globosum*. The importance of leaf colonization by *C. globosum* was assessed by measuring germination of viable spores and hyphal elongation on apple leaves sampled from the field and growth chamber, and by determining the efficacy of dead ascospores in reducing scab severity.

Three-year-old McIntosh saplings were sprayed to runoff using a 950-ml trigger sprayer (Scientific Products, Chicago, IL) containing 2×10^6 spores per milliliter of *C. globosum* in phosphate buffer with 0.01% spreader-sticker (Plyac, Hopkins Agricultural Chemical Co., Madison, WI), as part of 1984 field tests for scab biocontrol (3). Single trees, sprayed weekly beginning May 18 with either strain QM 103a, NRRL 6296, or ATCC 58917, were chosen for sampling. Three leaves were removed randomly from each of the three trees immediately before, 2 hr after, and 6 days after the fifth *C. globosum* application (June 14). Leaves were fixed in Formalin-acetic acid-ethanol (FAA) and stored at 4 C. Three leaf disks (7 mm^2) were removed randomly from the central portion of each leaf, stained in aqueous cotton blue, and both surfaces scanned by light microscopy at 160×. The number of *C. globosum* spores, number germinated (including spores showing only the vesicle from which the germ tube originates), and hyphal elongation as a proportion of spore length were recorded for each entire disk. Constant disk area allowed calculation of spore density as number of spores per square millimeter of leaf surface. Disks on which germination was difficult to determine because of the presence of other fungi were reevaluated by scanning electron microscopy to improve depth perception. Observations of control leaves from trees sprayed with buffer alone confirmed that background *C. globosum* contributed by air spora was negligible. Control of apple scab by the applied populations was not observed at any time during the season (3).

In growth chamber trials, seedlings were treated with strain QM 103a or NRRL 6296, alone or with *V. inaequalis*. The second oldest leaf from eight plants of each treatment was sampled after the spray liquid had dried. The remaining plants were placed in the infection box, and the second oldest leaf from eight additional plants (two from each of four blocks) was sampled at 7 and 14 days after inoculation. Leaves were fixed and evaluated as for the field studies, except that only one disk was removed from each leaf. Observation of control plants sprayed with buffer indicated that background air spora was negligible. Biocontrol on *C. globosum*-treated plants was confirmed in the growth chamber by comparing disease severity and leaf area with seedlings sprayed with *V. inaequalis* alone as described previously.

Several techniques to kill *C. globosum* ascospores were attempted, to eliminate the possibility of apple leaf colonization. Heat treatment in a water bath at 60 C for 15 min with agitation gave at least 98% mortality, in agreement with Chapman and Fergus (11). Seedlings were treated with either heat-killed or viable ascospores with *Venturia* conidia, heat-treated or untreated culture extract with *Venturia*, or *Venturia* alone. Twelve plants received each treatment before placement in the infection box, and disease was rated as described above after 16 days. The experiment was done once with strains QM 103a and NRRL 6296, and again with strains QM 103a and ATCC 58917.

***C. globosum* application in advance of the pathogen.** In the field, several days may elapse after each antagonist application before the arrival of *Venturia* inoculum. The longevity of applied *C. globosum* as a biocontrol agent was evaluated under controlled conditions by applying *V. inaequalis* to seedlings and agarose-coated microscope slides previously treated with either ascospores or culture extract of strain QM 103a.

Seedlings were marked with a pinhole to identify the youngest leaf before they were treated with either *C. globosum* spores with *V. inaequalis*, *V. inaequalis* alone (12 plants each), or *C. globosum* alone (48 plants). All seedlings were placed in the infection box and given the usual environmental regimen. Subsequently, sets of 12 plants that had received *Chaetomium* alone were removed randomly after 1, 3, 5, or 7 days, inoculated with *V. inaequalis* conidia, exposed to 19 C, 100% RH for 24 hr, and returned to the infection box. Each plant was evaluated for disease 14 days after it was inoculated with *V. inaequalis*, as described previously. Assessments were made only for leaves present at the time of treatment with *C. globosum*.

Inoculum and space limitations precluded the use of plants sprayed with *V. inaequalis* only (no prior *Chaetomium* treatment) at the four delayed treatment dates. This assumes that delayed *Venturia* inoculation has no effect on disease incidence in the absence of *Chaetomium*. These assumptions were supported by repeat experiments using only one 7-day delayed *Venturia* application, rather than the four delay periods described above, which did include treatments without *Chaetomium*. Additionally, a 7-day delay experiment was conducted wherein culture extract, applied in 0.01% Plyac with a chromatography sprayer, was substituted for ascospores. This minimized the possibility of antibiotic dilution over time through lateral diffusion across the phylloplane, a potential source of short-lived biocontrol when ascospores are applied.

Germination of *V. inaequalis* conidia was assessed 48 hr after application to sterile agarose-coated microscope slides (1) previously spread with 10 μl of strain QM 103a spore suspension or culture extract using a sterile glass rod. Slides were incubated under the conditions of the infection box in petri-dish moist chambers (1). Five random transects of at least 20 conidia each were observed on each of three replicate slides per treatment. Germination (i.e., any germ tube development) and germ tube growth as a proportion of conidium length were recorded. In one experiment, treatments of 0, 2×10^6, 4×10^6, 1×10^7, and 2×10^7 *Chaetomium* spores per milliliter were spread over the slide surface either simultaneously with 10 μl of *V. inaequalis* (5×10^5 conidia per milliliter), or 1, 3, 5, or 7 days later, for a total of 25 treatments (five concentrations × five times). A second experiment involved culture extract or buffer control and delays of 0, 1, 3, 5, 7, 10, and 16 days before *Venturia* application (14 treatments: two formulations × seven times). Culture extract was sterilized by passage through a 0.45-μm membrane filter (Millipore Corp., Bedford, MA), and aseptic conditions were maintained throughout both experiments. Inoculum for these experiments was from a single harvest of *V. inaequalis* stored at −20 C, and aliquots were thawed as needed immediately before use.

Antibiotic stability in vitro. A simple bioassay (12) was used to determine the stability of antibiotic substances in culture extract of *C. globosum* under various conditions. Seventy microliters of sterile culture extract treated variously as described below was added to a 5-mm-diameter well cut in the center of a petri plate containing weak PDA (WPDA; 1). *Venturia* conidia from frozen suspensions were then cross-streaked over the well. Inhibition zones (distance from edge of well to coalesced *Venturia* colonies) were measured 10 days after incubation at 24 C. Control plates with 70 μl of sterile phosphate buffer were also prepared and inoculated. Each experiment consisted of five replicate plates except for the drying assay, in which three were used.

Aging. In two separate experiments, 1-ml aliquots of extract from strain QM 103a or NRRL 6296 were maintained in 3-ml vials in darkness in the infection box and bioassayed for activity at 0, 2, 6, and 18 days after preparation. In the case of strain NRRL 6296, additional samples were taken at 11 and 14 days.

pH. Strain QM 103a culture extract was maintained in 5-ml aliquots in darkness at 24 C, 100% RH, and pH values of 5.0, 7.0, 8.8, and 11.1 with potassium hydrogen phthalate, KH_2PO_4, tris(hydroxymethyl)-aminomethane, and Na_2HPO_4 buffer solutions, respectively (25). These were sampled at 1, 5, and 13 days after preparation, neutralized with HCl or NaOH, and bioassayed. A bioassay was also performed on WPDA adjusted to the above pH values, with freshly prepared culture extract at pH 7.0.

Drying. Three milliliters of strain QM 103a culture extract was allowed to evaporate completely at 24 C in darkness in a sterile petri dish, which required 6 days. The residue was reconstituted immediately in 3 ml of buffer and compared by bioassay with

another 3-ml sample, which had been maintained at constant volume under similar conditions.

Aeration. One hundred milliliters of sterile QM 103a culture extract was aerated aseptically with filtered, humidified air for 6 days in a 500-ml Erlenmeyer flask, in darkness, at 24 C. It was then bioassayed and compared with a nonaerated control.

Statistical analysis. All experiments were evaluated by analysis of variance with the appropriate number of factors. Multiple comparisons were performed by the Newman-Keuls method. Occasional missing points were estimated after Zar (35). Log_{10} transformations of spore density data from leaf colonization experiments were used to stabilize variance for both field and growth chamber trials. In addition, paired Student's t tests analyzed differences between adaxial and abaxial spore densities. Significance levels are at $\alpha = 0.05$ unless stated otherwise.

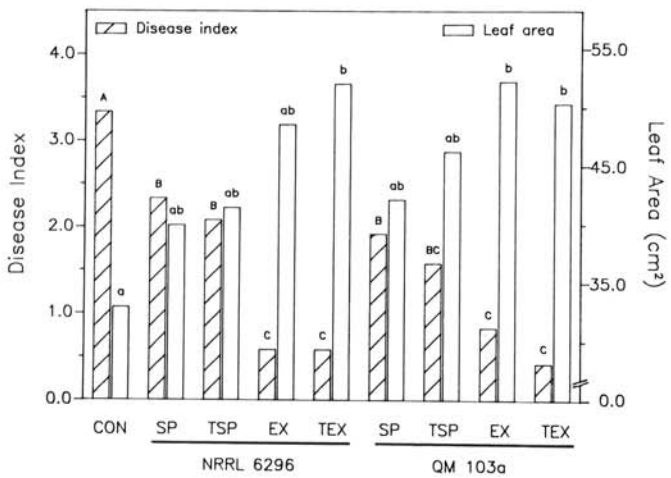

Fig. 1. Antagonism to *Venturia inaequalis* by viable and heat-killed *Chaetomium globosum* ascospores (two strains) and culture extract treated identically on apple seedlings under controlled conditions, as quantified by disease index and leaf area measured 17 days after inoculation. Disease index ranged from 0 (no observable symptoms) to 4 (shoot collapse with widespread coalesced lesions). Bars labelled with same letter are not significantly different by the Newman-Keuls method at $\alpha = 0.05$. CON = control treatment without *C. globosum*; SP = viable spores; TSP = heat-treated spores; EX = extract; TEX = heat-treated extract.

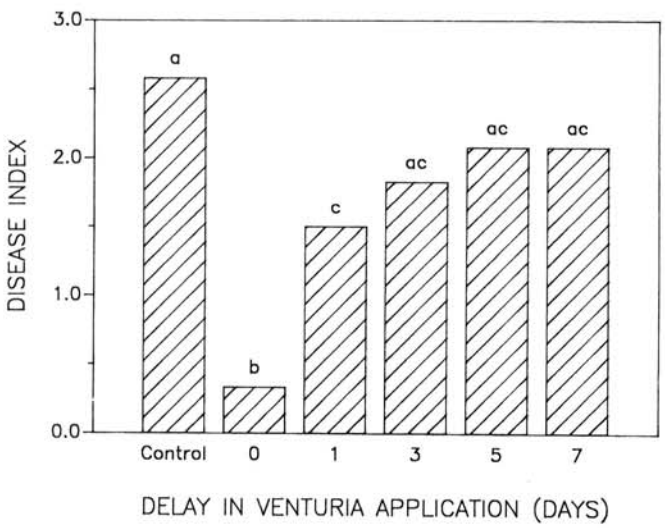

Fig. 2. Antagonism to *Venturia inaequalis* under controlled conditions, where application of the pathogen to apple seedlings pretreated with *Chaetomium globosum* QM 103a ascospores was delayed. Antagonism was quantified by disease index and leaf area measured 17 days after inoculation with *V. inaequalis*. "Control" refers to *V. inaequalis* treatment without *C. globosum*. Disease index ranged from 0 (no observable symptoms) to 4 (shoot collapse with widespread coalesced lesions). Bars labelled with same letter are not significantly different by the Newman-Keuls method at $\alpha = 0.05$.

RESULTS

Leaf colonization. In both field and growth chamber studies, mean *C. globosum* ascospore germination was less than 2% at all sample times and for all strains. Standard deviations varied between 0.06 and 2.35. Fewer than 1% of the ascospores produced hyphae longer than one spore length, and no extensive epiphytic colonization was observed.

Heat-killed ascospores of strains QM 103a and NRRL 6296 reduced scab severity significantly and as effectively as viable spores (Fig. 1). This confirmed that germination was unnecessary for biocontrol in the growth chamber. Neither viable nor heat-killed ATCC 58917 spores reduced scab severity, as expected from previous work (12). Culture extracts were not influenced by heat treatment and were more effective than spores. Results based on disease index were paralleled by assessments based on leaf area, but differences among treatments were not always significant for the latter (Fig. 1).

The number of *C. globosum* ascospores per unit leaf area did not change significantly over time in either the growth chamber or field but, due to great variability in spore density, power to detect such differences was low. Mean densities for each treatment combination ranged from 31.6 (SD = 16.7) to 99.3 (SD = 68.5) spores per square millimeter in the growth chamber and were substantially lower in the field, from 6.7 (SD = 5.6) to 33.8 (SD = 21.4) spores per square millimeter. Spore density on the abaxial surface was lower than that on the adaxial surface ($P < 0.01$) in both situations.

***C. globosum* applications in advance of the pathogen.** Neither *C. globosum* ascospores nor culture extract reduced scab severity significantly, as assessed by disease index, when *Venturia* inoculation was delayed by 7 days, even though simultaneous application resulted in 70–100% control. Evaluations based on leaf area also demonstrated loss of control over time, though the increases in leaf area observed on seedlings treated concurrently with the pathogen and *Chaetomium* were significant for culture extract, but not ascospores. Results of the time series of delayed *V. inaequalis* applications to seedlings treated with *Chaetomium* ascospores indicate that control may be lost in 1–3 days (Fig. 2).

Evaluation of germination inhibition on agarose slides demonstrated a loss of antagonism similar to that found on seedlings. Where extract was used, a strong ($P < 0.025$) time × extract interaction occurred; reduction in germination of over 20%, observed when *Venturia* was applied concurrently with extract, was lost by the 3rd day (Fig. 3). The effect was more pronounced when the percentage of conidia producing a germ tube longer than one conidium length was the criterion.

Although all *V. inaequalis* inoculum originated from the same frozen suspension, and each aliquot was thawed immediately before use, a decline in germination of controls over time occurred. A similar though more erratic pattern was observed in the equivalent experiment with ascospores (Fig. 4). This may be due to a dehydration of the thin agarose layer with time, though high humidity was constantly maintained. Germination of conidia was generally lower in the experiment using ascospores, possibly because the frozen suspensions originated from a different *Venturia* culture. This assay was further complicated by germination of the *Chaetomium* inoculum by day 3.

Inhibition of germination corresponding to concentrations of *C. globosum* ascospores was observed when applications of fungi were simultaneous (Fig. 4). Importantly, this antagonism declined markedly when *Venturia* application was delayed by only 1 day. Thereafter, some inhibition continued, particularly at high initial *C. globosum* concentration, though the unexpected *Chaetomium* germination and growth must be considered in that additional antibiotic was presumably elaborated. A similar pattern of rapid decline in germination inhibition was evident when conidia producing germ tubes longer than one spore length were

considered. In addition, a significant time × *Chaetomium* concentration interaction occurred.

Antibiotic stability in vitro. Aging. Sterile culture extracts stored in the infection box did not lose their inhibitory activity over time. Strain NRRL 6296 showed a significant increase in inhibition zone radius of 6.8 mm (SD = 2.0) at day 0 to 9.7 mm (SD = 0.5) at day 2, which then declined to 8.3 mm (SD = 0.5) at day 6 and did not change significantly through day 18. Activity of strain QM 103a did not change significantly over time.

pH. A small but significant decline in activity was observed in pH trials where QM 103a extract was maintained at pH 7.0 for 13 days. However, temperatures were approximately 5 C higher for this experiment than for that conducted in the infection box. Extract stored at pH 5.0 and 8.8 showed a greater loss in activity, and storage at pH 11.1 destroyed all antagonism within 1 day (Table 1). When freshly prepared extract at pH 7.0 was bioassayed on media of pH 5.0, inhibition zones were significantly (23%) smaller than those on neutral media. *V. inaequalis* did not grow on media of pH 8.8 or 11.1.

Drying/aeration. The most pronounced reduction in culture extract inhibition occurred when extract was dried or aerated. In the former case, a mean inhibition zone radius of only 1.8 mm (SD = 1.3) was observed, 74% below that produced by the undried control ($P < 0.01$). Aeration caused a 62% decrease in inhibition zone radius ($P < 0.01$), from 14.7 mm (SD = 1.8) to 5.6 mm (SD = 1.3).

DISCUSSION

Three central phenomena were demonstrated by these experiments: *Chaetomium* germination, or even viability, was not necessary for biocontrol; this control was short-lived in the growth chamber and in vitro; and the antibiotics produced by *C. globosum* lost their activity when exposed to certain simple physical regimens. Because loss in biocontrol occurred under controlled conditions, it was possible to evaluate systematically some factors potentially responsible.

The first opportunity for a breakdown in control, namely physical removal of inoculum by such agents as wind or rain, is excluded in the growth chamber. The ability of ascospores to adhere to the phylloplane and remain there was demonstrated by spore density evaluations, though leaf-to-leaf variability was high. After application, poor epiphytic colonization by *C. globosum* might then result in decreased antagonism. However, germination by spores known to be viable apparently had little or no role in successful biocontrol, corroborating earlier observations of Cullen et al (13). The efficacy of ungerminated spores, heat-killed spores, and culture extract supports antibiosis as the mechanism of control (12). Further, it suggests passage of antibiotic substances, already present in ascospores, into the suspension medium and/or onto the leaf surface. Thus, biocontrol by *C. globosum* ascospores may simply result from application of what are in effect "fungicide packets."

The diffusible antibiotic hypothesis is further supported by the documented occurrence of antibiotic within *C. globosum* ascospores, identical to that found in culture extract (12). We have also prepared strain QM 103a ascospore suspensions and after 1 to 2 days detected antagonistic activity to *V. inaequalis* in the concentrated cell-free liquid. Passive seepage of antibiotic is more likely than active synthesis and/or secretion by the ungerminated spores, since the latter would require living spores for effective biocontrol, which was not the case. Conceivably, membrane damage and subsequent seepage occurred, resulting in biocontrol as an artifact of heat treatment. An active mechanism, however, seems inconsistent with ecological reasoning, which argues that it would be disadvantageous for the organism to expend energy and resources on a substrate that is only minimally used, if at all.

It therefore appears that biocontrol may be effective only for a brief period when leaked antibiotic is present in sufficiently high concentration on the leaf surface to inhibit infection by *Venturia*. Toxicity may then be lost, perhaps through dilution of antibiotic by diffusion across or into the leaf, through degradation of the antibiotic, or both.

The possibility of lateral diffusion was eliminated where culture extract was uniformly applied to seedlings or agarose slides, yet biological activity against *V. inaequalis* was rapidly lost. Similarly, high concentrations of *Chaetomium* ascospores applied to agarose slides would be expected to provide toxic concentrations of antibiotic over the entire surface even after complete lateral diffusion, but antagonism was observed to decline substantially after only one day. Though this decline did not continue, *Chaetomium* growth occurred and antibiotic production was therefore likely. If absorption into the leaf were instrumental in control loss, then the thin agarose layer would not be expected to produce the same effect. Vertical and lateral diffusion, and the resulting dilution, are thus ruled out as likely mechanisms for loss of antibiotic toxicity.

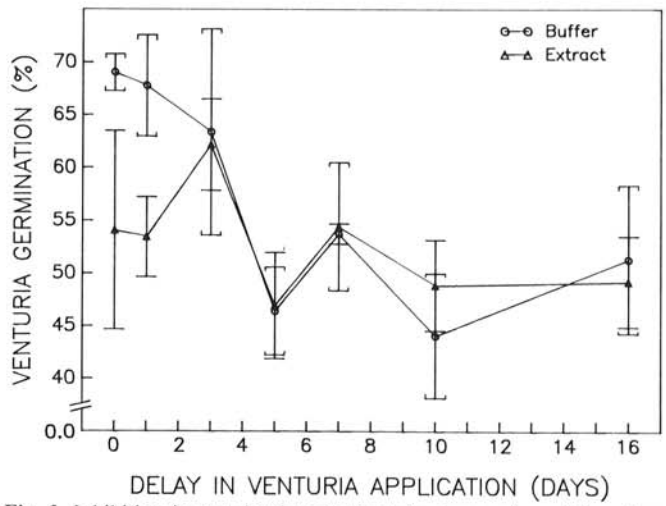

Fig. 3. Inhibition in germination of *Venturia inaequalis* conidia, where application to sterile agarose-coated slides prespread with *Chaetomium globosum* QM 103a culture extract was delayed. Vertical lines represent one standard deviation.

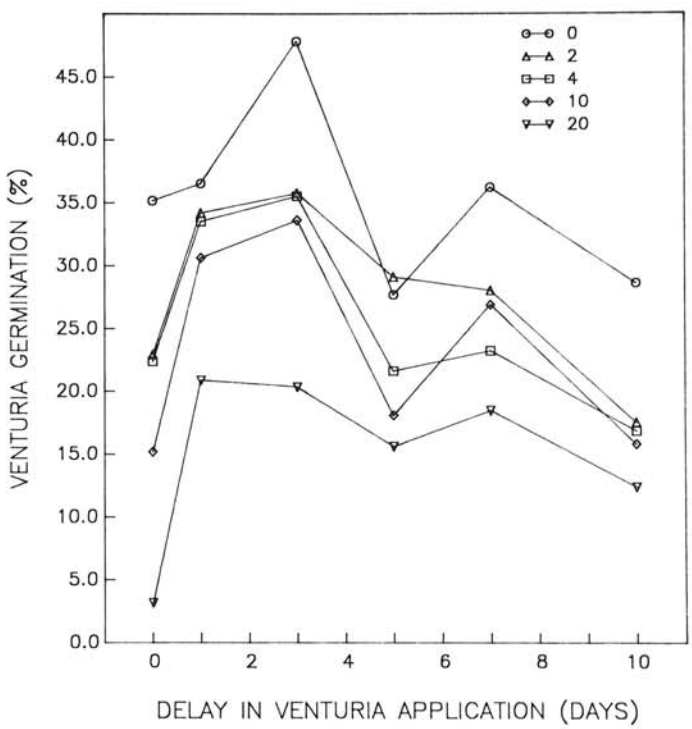

Fig. 4. Inhibition in germination of *Venturia inaequalis* conidia, where application to sterile agarose-coated slides pretreated with *Chaetomium globosum* QM 103a was delayed. Symbols represent antagonist concentrations in ascospores per milliliter × 10^6.

TABLE 1. Inhibition of *Venturia inaequalis* growth by neutralized culture extract of *Chaetomium globosum* stored in buffer of varying pH

pH	Storage time (days)			
	0	1	5	13
5.5	13.6 ± 1.1[a]	10.5 ± 0.4	8.0 ± 0.4	8.1 ± 1.0
7.0	13.6 ± 1.1	11.8 ± 1.4	10.0 ± 0.4	10.8 ± 0.8
8.8	13.6 ± 1.1	7.4 ± 0.7	6.1 ± 0.2	7.4 ± 0.5
11.1	13.6 ± 1.1	0 ± 0.0	0 ± 0.0	0 ± 0.0

[a] Mean inhibition zone radius of five replicate WPDA plates ± 1 standard deviation 10 days after inoculation.

The remaining explanation for loss of control consistent with our results is antibiotic degradation. This could occur by various mechanisms acting simultaneously on one or several compounds known to be produced by *C. globosum* and possibly present on the leaf surface. At least 19 antibiotic substances have been isolated from *C. globosum* (6,18,27,29).

Sensitivity of epidithiadiketopiperazines (e.g., chetomin) to bacterial and alkaline (pH = 8.3) degradation was reported previously (7,16,20,34), but our work demonstrated loss of antagonism under aseptic conditions (agarose slides) and indicated that substantial antagonistic activity of culture extract was retained after storage at pH 8.8 for 13 days.

Repeated condensation and evaporation of water on seedling leaves occurred in the growth chamber and may deactivate the antibiotic as was the case for a single drying of culture extract. Though complete drying is routine for isolation of the antibiotics noted above and does not eliminate activity, the concentrations are undoubtedly much higher than those on apple leaves. Common to the seedling, agarose slide, drying, and aeration experiments reported here was the exposure of a large liquid surface area to air, followed by a pronounced loss of antagonism. Both cochliodinol (often associated with *C. globosum*) and epidithiadiketopiperazines are degraded easily by mild oxidation (5,15,34). Thus, oxidation may be a major factor in loss of biocontrol.

Work by Cullen and Andrews (12) has implicated chetomin as the compound responsible for apple scab biocontrol by some strains of *C. globosum*. Isolation, identification, and quantification of antibiotics actually present on the phylloplane after treatment with *Chaetomium* are necessary to verify that degradation occurs in situ and to follow degradation dynamics. The large number of potentially active compounds makes this a formidable task. Our attempts to quantify their combined activity with the bioassay described (12), using concentrated washings of *C. globosum*-treated leaves, have not proved sensitive to the levels of antibiotic present. Mechanisms suggested here may interact, perhaps synergistically, with several others leading to a loss of antagonism over time, and then only in the controlled environment situation.

It is inappropriate to extrapolate these results to the complex field situation. Spore densities on field leaves were substantially lower than those in the growth chamber, perhaps due to weathering or poor adhesion to mature leaves. Obviously, antibiotics can be affected in the field by a much wider range of physical and biotic factors.

Nevertheless, this work indicates that control is ephemeral even without these additional influences, that physical mechanisms alone can account for loss of activity, and that effective biocontrol requires the presence of active antibiotic coincident with *Venturia* inoculum.

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