Development of *Colletotrichum gloeosporioides* f. sp. *clidemiae* and *Septoria passiflorae* into Two Mycoherbicides with Extended Viability

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ABSTRACT

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Two potential mycoherbicides were formulated for extended viability: one, containing *Colletotrichum gloeosporioides* f. sp. *clidemiae* as the active ingredient, was effective against Koster's curse (*Clidemia hirta*); and the other, containing *Septoria passiflorae*, was effective against banana poka (*Passiflora tripartita* var. *tripartita*). Microcycle conidiation of both fungi occurred on the surface of solid media inoculated with spore suspensions ≥1 × 10^6 conidia per ml, ca. 1.67 × 10^4 conidia per cm^2. In 4 days, *C. g.* f. sp. *clidemiae* produced 5 × 10^6 conidia per cm^2 after incubation at 25°C under continuous illumination on the surface of potato-dextrose agar adjusted to 3% agar. In 3 weeks, *S. passiflorae* produced 8.6 × 10^7 conidia per cm^2 after incubation on 10% Gerber Mixed Cereal for Baby agar, while on agitated potato-dextrose broth the production was 3.9 × 10^7 conidia per ml at 21°C under continuous illumination after 4 days incubation. Spores of *C. g.* f. sp. *clidemiae* and *S. passiflorae,* harvested by scraping the surfaces of solid cultures, were mixed in kaolin, dried, and stored at −18 and 1°C. They maintained greater than 84% viability for over 4 months and greater than 95% viability for 6 months, respectively. Spores of *S. passiflorae* harvested from liquid culture and stored at ≤1°C, mixed in kaolin and/or by lyophilization, maintained 97% viability for ≥1 year. *C. g.* f. sp. *clidemiae* spores produced in liquid culture had low viability and were killed by lyophilization. Viability was optimally maintained in both fungi when they were stored at −18°C. Viability of spores of both fungi stored as a kaolin formulation at 22°C was short-lived. No significant differences in pathogenicity were found in spores as a kaolin formulation after 4 months of storage. The shelf life of stored *C. g.* f. sp. *clidemiae*–kaolin was not affected by rehydrating in a 30% sucrose solution; whereas significant loss of viability occurred when the spore–kaolin mixtures were rehydrated in sterile distilled water (SDW). Rehydration of the mycoherbicide containing *S. passiflorae* in SDW did not decrease its activity. Both fungi produced a significantly higher number of lesions when applied to host plants suspended in 2% sucrose–0.5% gelatin solution than in SDW. Also, the number of lesions produced increased linearly with increases in inoculum. There was no significant difference in pathogenicity between freshly harvested spores and kaolin–spore mixtures stored for 4 months.

Additional keywords: bioherbicide, carrier, clay

The noxious weed *Clidemia hirta* (L.) D. Don., also referred to as Koster's curse, is a member of the *Melastomataceae* family. Since its introduction into Hawaii in 1941, it has spread to all the major islands, crowding out many endemic plant species (12). *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. f. sp. *clidemiae,* originally isolated on *C. hirta* from Panama, was proposed as a possible biocontrol agent for this weed in Hawaiian forests because of its specificity to *C. hirta* (12). The pathogen sprayed on microplots in 2% sucrose–0.5% gelatin solution at 1 × 10^6 conidia per ml has been highly effective in field control of *C. hirta* since its release from quarantine in late 1986. However, as with typical bioherbicide fungi, it requires more than one application to kill plants effectively (11).

The perennial woody vine *Passiflora tripartita* (Juss.) Poir var. *tripartita* Holm-Nie. Jörg. & Law. (= *Passiflora mollissima* Neal), commonly referred to as banana poka, was introduced into Hawaii in 1921 as an ornamental (3). Since its introduction, banana poka has become established at mid to high elevations (800 to 2,200 m) on all major islands in Hawaii. By 1981, this weed covered over 500 km^2 of land on the islands of Kauai and Hawaii (15). *Septoria passiflorae* Syd. was proposed as a possible biocontrol agent based on host range studies conducted on six *Passiflora* spp. introduced to Hawaii (14). This pathogen, incorporated in 2% sucrose–0.5% gelatin solution at 1 × 10^6 conidia per ml and sprayed, was aggressively pathogenic on banana poka and mildly pathogenic to *Passiflora foetida* L., a weed in lowland forests of Hawaii.

Mycoherbicides, to be commercially viable, must be produced easily and survive storage for long periods without appreciable loss of viability and/or pathogenicity. The ability of *Colletotrichum* spp. conidia to infect and remain viable for long periods of time is directly related to the formation of a conidial matrix (4,6–8). This water-soluble matrix is produced by *Colletotrichum* spp. growing on solid surfaces and is composed of sugars and proteins (4,6–8). Both protease and cellulase are present in this matrix and are believed to be involved in fungal penetration (4,7). The removal of this matrix can also significantly reduce viability of spores within 24 h (6).

S. passiflorae sporulates in pycnidia and also on single conidiophores, but no gelatinous matrix is produced. The research reported here describes procedures for the production, formulation, and efficacy testing of two foliar mycoherbicides, one containing *C. g.* f. sp. *clidemiae* as the active ingredient effective against *Clidemia* and the other having *S. passiflorae* as the active ingredient effective against banana poka. Results of efficacy testing of formulated inoculum are also reported.

MATERIALS AND METHODS

Production of the active ingredients. Spores of *C. g.* f. sp. *clidemiae* with the water-soluble matrix intact were produced by growing the fungus on the surface of Difco potato-dextrose agar (PDA). This was adjusted to 3% agar to increase the water absorption capacity of the medium, which eliminated water remaining on the plate surface when the spore suspension was added. Conidia of *S. passiflorae* were produced on: (i) PDA in 9-cm petri plates; (ii) potato-dextrose broth (PDB) in 250 ml Erlenmeyer flasks, made with 200 g of washed white potatoes (unpeeled and cut into 1-cm cubes, steeped for 1 h in water at 60°C, and strained through four layers of cheesecloth), 20 g of dextrose, and water to a volume of 1 liter, adjusted to pH 6.5 (E. E. Trujillo, *unpublished*); and (iii) 10% Gerber cereal agar (GCA) in 9-cm petri plates (medium made with Gerber Mixed Cereal for Baby at 100 g/liter [Gerber Product Co., Fremont, MI 49412] and Difco agar at 18 g/liter). Microcycle conidiation (1,9) in both fungi was induced by evenly distributing a 0.5-ml spore suspension containing ≥1 × 10^6 conidia per ml (11) on the surface of the solid medium or by adjusting the concentration of spores in the liquid medium to ≥1 × 10^6 conidia per

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ml of broth. Uniform distribution of inoculum on the surface of solid media was accomplished by placing the spore suspension at one edge of the plate and shaking the plate back and forth by hand with a slight downward slope toward the opposite edge. Media inoculated with *C. g.* f. sp. *clidemiae* and *S. passiflorae* were incubated under cool-white fluorescent light (2,160 lux) at 28°C for 4 days and 21°C for 3 weeks, respectively. Conidia were harvested from the solid medium with cell scrapers (Costar, 205 Broadway, Cambridge, MA 02139). The number of conidia produced by both fungal species was determined on three plates of each medium tested by serial spore dilution in water and counted with a hemacytometer.

Erlenmeyer flasks containing 50 ml of PDB and capped with cotton plugs were used for *S. passiflorae* microcycle conidiation in liquid culture. Three flasks were inoculated with spore suspensions of *S. passiflora* containing 1×10^6 conidia per ml and placed on a rotary shaker at 108 rpm (15-cm [6-in] throw). Flasks were incubated for 4 days at 21°C under continuous cool-white fluorescent light (2,160 lux). A 100-µl sample was removed daily from each flask, and the spore concentrations were determined with a hemacytometer. Tests were repeated.

Storage and shelf life of mycoherbicides. Kaolin (J. T. Baker Chemical Co., Phillipsburg, NJ 08865, N. F. Powder), Fuller's Earth (J. T. Baker), and talc (J. T. Baker) were evaluated as carriers of *C. g.* f. sp. *clidemiae* conidia with the water-soluble matrix intact. Approximately 104 mg of wet conidia were mixed with 1 g of carrier for a concentration of approximately 1×10^9 conidia per g of clay. Conidia–clay mixtures were spread onto plastic sheets to a depth of 0.5 cm, air-dried under forced air (24 to 37 m/min [80 to 120 ft/min]), 65% relative humidity, and 26°C for 24 h in a chemical hood. The percentage of water retained in the two mycoherbicides after air-drying was determined on three 1-g samples of spore-carrier mixture by following Gardner's procedures (2). The percentages of water content in spore–kaolin mixtures of mycoherbicides containing *C. g.* f. sp. *clidemiae* and *S. passiflorae* after air-drying were 3 and 2%, respectively. Conidia–carrier mixtures were pulverized, placed in glass jars, sealed with parafilm, and stored at –18, 1, and 22°C. Viability of conidia was determined after 7 days of storage for each treatment. Samples of 0.1 g from each treatment were dissolved in 0.9 ml of 30% sucrose, and subsequently a serial 10-fold dilution was made in 30% sucrose. Five 20-µl samples were removed from the 10^{-5} dilution (containing approximately 150 to 200 conidia per 20 µl) and placed onto PDA plates. Plates were then incubated under cool-white fluorescent light (2,160 lux) at 28°C for 24 h to allow for germination of the spores. Germinated and nongerminated spores were counted under magnification of ×40. Treatment means of viable spores were compared by an LSD test (Tukey's).

Conidia of *S. passiflorae* harvested from PDA and GCA media were stored as a kaolin formulation following the same procedures as those used for *C. g.* f. sp. *clidemiae*. Conidia of *S. passiflorae* produced in PDB were centrifuged at 6,000 × *g* for 10 min, the supernatant was decanted, and the spore pellet was mixed with 2 g of kaolin to a concentration of 1×10^9 conidia per g.

To determine viability of *C. g.* f. sp. *clidemiae* and *S. passiflorae*, spore–carrier mixtures stored at –18, 1, and 22°C, were sampled and rehydrated at monthly intervals, and spore counts were made as previously described.

Lyophilization of both *C. g.* f. sp. *clidemiae* and *S. passiflorae* conidia was tested as a procedure for storage of both active ingredients. Spores of both fungi were harvested from PDA plates incubated as previously stated, placed in 10% skim milk, freeze-dried, sealed under vacuum, and stored at 1°C. Viability of lyophilized conidia was determined for three 1-ml ampules of both *C. g.* f. sp. *clidemiae* and *S. passiflorae* after 24-h and 1-year storage. Viability of the active ingredients after storage was determined as previously described.

Viability of *C. g. clidemiae* harvested in sterile distilled water (SDW) and in other solutions. The addition of polysaccharides and proteins to *C. g.* f. sp. *clidemiae* was evaluated to determine whether they enhanced viability in storage. Conidia were harvested from three PDA plates by washing spores from the surface of the medium with 3 ml of SDW and mixing 1, 10, and 100 mg/ml solutions of xanthan gum (Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178, Cat No. G-1253), guar gum (Sigma, Cat No. G-4129), or porcine mucin (Sigma, Cat No. G-2378) with 1 g of kaolin. Mixtures were then air-dried as previously described. Spore viability was evaluated as previously described after 7 days of storage at 1°C. Also, six solutions of guar gum at 1 and 10 mg/ml with mucin at 1, 10, and 100 mg/ml were tested.

Effects of rehydration on clay-stored mycoherbicides. The sensitivity of conidia in stored mycoherbicides to osmotic stress upon rehydration was determined by mixing 100-mg samples of kaolin–conidia mixtures in SDW and/or solutions of 10, 20, 30, 40, or 50% sucrose for 5 min. Spores were diluted in their respective sucrose solutions, and viability of spores was evaluated as previously described. Shelf-life determinations were done at 24 h and at 1, 3, and 5 weeks. This experiment was repeated once. Tukey's LSD test was used to compare means of germination percentages of different treatments.

Pathogenicity of stored mycoherbicides. The concentration of conidia needed for optimum lesion development for both mycoherbicides was determined by adjusting a suspension of freshly harvested conidia to 1×10^6 conidia per ml in 2% sucrose–0.5% gelatin (11). Subsequently, serial 10-fold dilutions of the initial suspensions were made to comprise theoretically zero spores per ml. Serial spore dilutions were sprayed onto plants of both species and incubated at optimal humidity and temperature until symptoms developed (12,14). Lesions were counted, and linear regression lines were generated by the least-square method at the 0.05 level of significance.

The pathogenic activity of both mycoherbicides was tested after 4 months of storage on 20-cm-tall *C. hirta* and *P. t.* var. *tripartita* plants growing on Supersoil (Rod McLellan Co., South San Francisco, California 94080) in 15.5-cm pots. Test plants were sprayed with *C. g.* f. sp. *clidemiae*–kaolin and *S. passiflorae*–kaolin mixtures in SDW and/or 2% sucrose–1% gelatin solution at 1×10^6 conidia per ml till runoff. Clidemia plants were incubated at 25°C for 24 h in plastic bags, then placed in a greenhouse at 28°C until symptoms developed. Banana poka plants were incubated at 100% relative humidity in 50-cm-diameter spherical clear plastic terraria (12) placed in a quarantine room at 20°C under cool-white fluorescent lights (2,160 lux). After 24 h, terraria were vented and kept under fluorescent lights on a 12-h day–night cycle. Lesion counts were made using a dissecting microscope on three fully expanded leaves randomly selected from each plant. The pathogens were reisolated from lesions on representative leaves. Treatment means were compared by Tukey's LSD test.

RESULTS

Production of the active ingredients. Inoculations of *C. g.* f. sp. *clidemiae* on PDA surfaces at 8.8×10^3 conidia per cm^2 (inoculum, 1×10^6 conidia per ml) produced 5×10^6 conidia per cm^2 after 4 days of incubation at 28°C under cool-white fluorescent lights.

Average values for *S. passiflorae* on PDA and GCA after 3 weeks were 1.2×10^7 and 8.6×10^7 conidia per cm^2, respectively. Microcycle conidiation of *S. passiflorae* without hyphal development occurred in PDB inoculated with $\geq 1 \times 10^6$ conidia per ml. At lower inoculum concentrations, sporulation and abundant mycelial growth occurred, resulting in massive hyphal flocculation and low spore yields. Spore production in the agitated PDB was 3.9×10^7 conidia per ml after 4 days of incubation at 21°C under cool-white fluorescent lights.

Storage and shelf life of mycoherbicides. The differences in viable CFU of *C. g.* f. sp. *clidemiae* in the three clays tested

after 7 days of storage at 1°C were significant, Tukey's LSD $P \leq 0.05$. Only 32% CFU survived in Fuller's Earth as compared to 99% for both kaolin and talc. Subsequently, kaolin was used in all further mycoherbicide formulations for spore storage in this study.

Viability of *C. g.* f. sp. *clidemiae* conidia scraped from PDA plates and stored as a kaolin formulation was greater than 84% CFU after 4 months of storage (Fig. 1), and viability dropped slightly thereafter. Conidia of *S. passiflorae,* produced on solid and in liquid culture and stored as a kaolin formulation, retained more than 95% CFU viability after 6 months. The highest viability and extended shelf life of the active ingredients of both mycoherbicides was achieved at −18°C storage. Neither mycoherbicide maintained extended viability when stored at 22°C (Fig 1).

Spores of *S. passiflorae* when lyophilized maintained a viability of 99 and 97% CFU after 24 h and 1 year of storage, respectively. However, shelf life of lyophilized *C. g.* f. sp. *clidemiae* was <1% CFU after 24 h storage.

Viability of *C. g.* f. sp. *clidemiae* harvested in SDW and in other solutions. Attempts to enhance viability of *C. g.* f. sp. *clidemiae* conidia with the addition of different solutions of xanthan gum, guar gum, or mucin were not successful.

Effects of rehydration on clay-stored conidia. No significant difference in shelf life was observed when *S. passiflorae* conidia were rehydrated in SDW or sucrose solutions. However, after 5 weeks of storage, spores of *C. g.* f. sp. *clidemiae* rehydrated in SDW declined in viability (≥ 25% CFU) compared to samples rehydrated in 30% sucrose (Fig 2). The highest viability of *C. g.* f. sp. *clidemiae* conidia (≥90% CFU) was obtained when conidia–clay mixtures were rehydrated in 30% sucrose. Significant differences in mean comparisons, Tukey's LSD, are shown by different letters (Fig. 2). Similar results were observed when the experiment was repeated.

Pathogenicity of stored mycoherbicides. Freshly harvested *C. g.* f. sp. *clidemiae* conidia, when suspended in 2% sucrose–0.5% gelatin solution and sprayed onto *Clidemia* plants, produced fourfold more lesions per cm^2 compared to fresh spores suspended in SDW (Fig. 3). Furthermore, there was no significant difference in the number of lesions between inoculated plants sprayed with stored *C. g.* f. sp. *clidemiae*–kaolin and/or freshly harvested spores of *C. g.* f. sp. *clidemiae* in 2% sucrose–0.5% gelatin solution.

No differences were observed between numbers of lesions produced by freshly harvested *S. passiflorae* conidia and/or those stored as a kaolin formulation applied in 2% sucrose–0.5% gelatin solution to banana poka plants. However, the lesion counts of the previous treatments were significantly different from lesion counts of freshly harvested conidia of *S. passiflorae* when applied as SDW suspensions (Fig. 3).

Plants inoculated with suspensions of freshly harvested spores of *C. g.* f. sp. *clidemiae* and *S. passiflorae* in 2% sucrose–0.5% gelatin at 10^6 to 10^4 conidia per ml developed lesions 0.5 to 1 mm in diameter 7 or 14 days after spraying, respectively. The mean number of lesions produced by spraying with conidial suspensions of from 10^6 to 10^3 conidia per ml is shown (Fig. 4). Host plants sprayed with 10^3 conidia per ml or less did not produce lesions.

No significant differences were observed between the numbers of lesions produced on leaves of target plants when either fungus was applied at the same rate as freshly harvested conidia or as kaolin formulations stored for 4 months at −18°C.

DISCUSSION

Previously, Cascino et al. (1) reported that microcycle conidiation by *C. gloeosporioides* in liquid media yielded 1 × 10^7 to 2 × 10^7 conidia per ml. The spore yield of *C. g.* f. sp. *clidemiae* we obtained on PDA (1.3 × 10^7 conidia per ml of solid medium) was comparable to production reported in liquid culture. Conidia of *C. g.* f. sp. *clidemiae* were also produced at 2.42 × 10^7 conidia per ml of PDB in a highly aerated bioreactor (E. E. Trujillo, *unpub-*

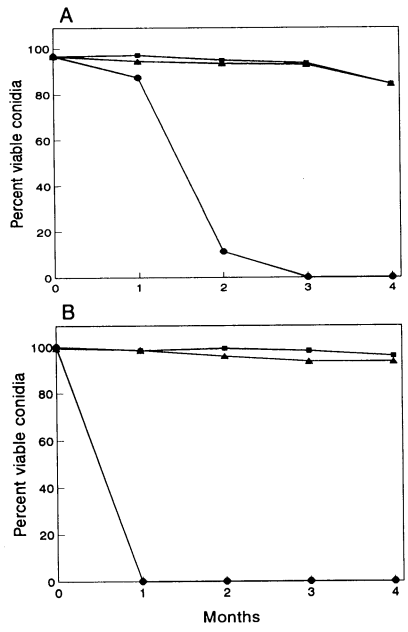

Fig. 1. Monthly viability of **(A)** *Colletotrichum gloeosporioides* f. sp. *clidemiae* and **(B)** *Septoria passiflorae* stored in kaolin at different temperatures. Storage temperatures: −18°C (■), 1°C (▲), 22°C (●). Range of standard deviation for (A) was 0–4.01 and for (B) was 0–2.26.

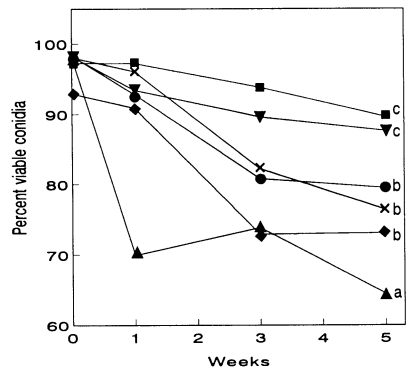

Fig. 2. Effect of rehydration in different sucrose solutions on the germinability of *Colletotrichum gloeosporioides* f. sp. *clidemiae* stored in kaolin at 1°C. Viability of *C. g.* f. sp. *clidemiae*–kaolin mixture determined 24 h after drying and 14 days thereafter. Sucrose concentrations: 0 (▲), 10 (●), 20 (▼) 30 (■), 40 (×), and 50% (◆). Means having different letters at 5 weeks are significantly different, $P \leq 0.05$ (Tukey's LSD).

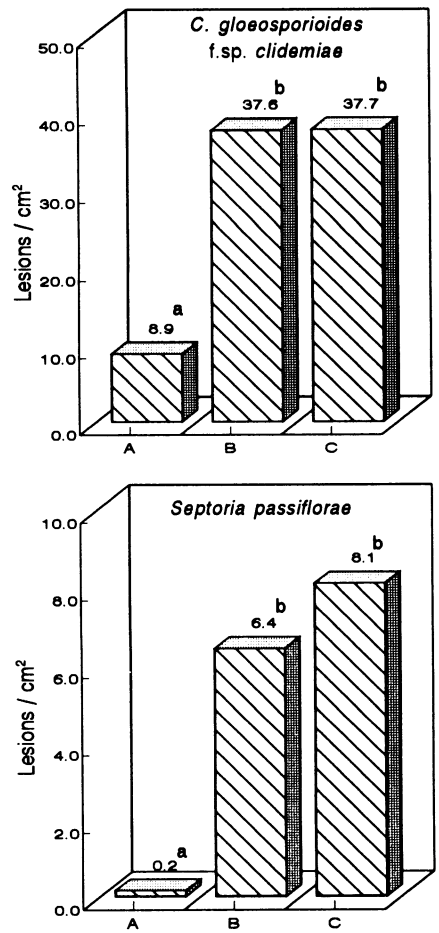

Fig. 3. Number of lesions per cm^2 produced by *Colletotrichum gloeosporioides* f. sp. *clidemiae* on *Clidemia hirta,* and by *Septoria passiflorae* on *Passiflora tripartita* var. *tripartita* when applied in water or sugar gelatin. A, Freshly harvested conidia in water. B, Freshly harvested conidia in a 2% sucrose–0.5% gelatin solution. C, Kaolin-stored *C. g.* f. sp. *clidemiae* rehydrated in 30% sucrose and applied in a 2% sucrose–0.5% gelatin solution, and kaolin-stored *S. passiflorae* applied in a 2% sucrose–0.5% gelatin solution. Means having different letters are significantly different, $P \leq 0.05$ (Tukey's LSD).

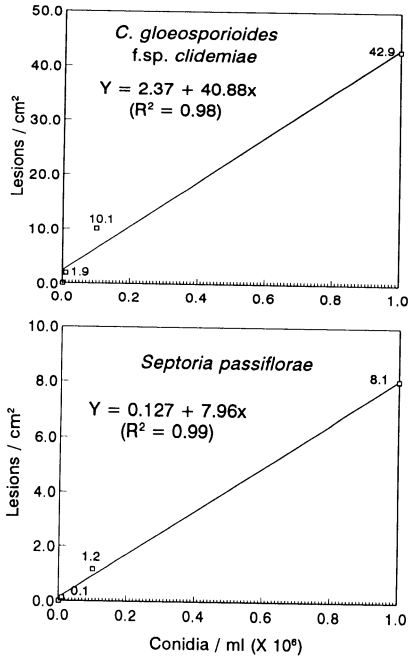

Fig. 4. Number of lesions produced on *Clidemia hirta*, top, and *Passiflora tripartita* var. *tripartita* leaves, bottom, using different application rates of conidial suspension of *C. g.* f. sp. *clidemiae* and *Septoria passiflorae,* respectively. Rates of mycoherbicides applied were from 1×10^6 to 1×10^3 conidia per ml in a 2% sucrose–0.5% gelatin solution.

lished). However, the shelf life of spores of the *Clidemia* pathogen produced in liquid culture is short compared to conidia produced on dry surfaces. Bioreactor-produced *C. g.* f. sp. *clidemiae* spores sitting in liquid had a shelf life of ≤24 h; and upon air-drying, viability was lost within 24 to 48 h.

Microcycle conidiation occurred readily in *S. passiflorae* in PDB when the inoculum level in the broth was $\geq 1 \times 10^6$ conidia per ml. The spore-carrying capacity of 3.9×10^7 conidia per ml under the reported conditions was similar to that observed by other investigators for *Colletotrichum* spp. (1,9). The active ingredient of *S. passiflorae* produced in the fermentation tank readily survived air-drying, lyophilization, and storage in kaolin. The *Septoria* spp. appear to have the best potential to become economically viable bioherbicides produced with fermentation technology, provided their performance in field tests is as effective as it was in laboratory tests (13,14).

Viability of *C. g.* f. sp. *clidemiae* can be maintained for ≥4 months when stored in kaolin. However, conidial viability is only enhanced when spores are stored at low temperatures (1 to −18°C), with the highest percentage of viability at the lowest temperature. The water-soluble matrix that envelops the conidia of *C. g.* f. sp. *clidemiae* is produced on solid media but is either not produced or is thinner in the liquid culture. The matrix inhibits desiccation and enhances spore viability. Conidia that were washed off PDA surfaces or harvested from shake culture were denatured upon drying, resulting in low viability. Poor yields and/or shelf life of *Colletotrichum* spp. spores produced with fermentation technology impact directly on the manufacturing cost of these bioherbicides. This and limited marked demand appear to be responsible for the cancellation of the production of Collego and BioMal (5,10). The production of *Colletotrichum* spp. on PDA agar surfaces coupled to air-drying of scraped spores as a kaolin formulation significantly improves shelf life. However, the technology for low-cost, continuous production of mycoherbicides on dry surfaces must be developed to replace fermentation technology, which is not reliable for fungi, e.g., *C. g.* f. sp. *clidemiae*.

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LITERATURE CITED

1. Cascino, J. J., Harris, R. F., Smith, C. S., and Andrews, J. H. 1990. Spore yield and microcycle conidiation of *Colletotrichum gloeosporioides* in liquid culture. Appl. Environ. Microbiol. 56:2303-2310.
2. Gardner, W. H. 1965. Water content. Pages 82-99 in: Methods of Soil Analysis, Part 1. C. A. Black, ed. American Society of Agronomy, Madison, WI.
3. LaRosa, A. M. 1984. The biology and ecology of *Passiflora mollissima* in Hawaii. Tech. Rep. 50, CPSU/UH. Dep. Bot., Univ. Hawaii, Honolulu.
4. McRae, C. F., and Stevens, G. R. 1990. Role of conidial matrix of *Colletotrichum orbiculare* in pathogenesis of *Xanthium spinosum.* Mycol. Res. 94:890-896.
5. Mortensen, K., and Boyetchko, S. 1994. Update of registered bioherbicides, BioMal. IBG News 3:5.
6. Nicholson, R. L., and Moraes, W. B. C. 1980. Survival of *Colletotrichum graminicola:* Importance of the spore matrix. Phytopathology 70:255-261.
7. Porter, F. M. 1969. Protease, cellulase, and differential localization of endo- and exopolygalacturonase in conidia and conidial matrix of *Colletotrichum orbiculare.* Phytopathology 59:1209-1213.
8. Ramadoss, C. S., Uhlig, J., Carlson, D. M., Butler, L. G., and Nicholson, R. L. 1985. Composition of the mucilaginous spore matrix of *Colletotrichum graminicola,* a pathogen of corn, sorghum, and other grasses. J. Agric. Food Chem. 33:728-732.
9. Slade, S. J., Harris, R. F., Smith, C. S., and Andrew, J. H. 1987. Microcycle conidiation and spore-carrying capacity of *Colletotrichum gloeosporioides* on solid media. Appl. Environ. Microbiol. 53:2106-2110.
10. Templeton, G. 1994. Update of registered bioherbicides, Collego. IBG News 3:5.
11. Trujillo, E. E. 1992. Bioherbicides. Pages 196-209 in: Frontiers in Industrial Mycology. G. F. Leatham, ed. Chapman & Hall, New York.
12. Trujillo, E. E., Latterell, F. M., and Rossi, A. E. 1986. *Colletotrichum gloeosporioides,* a possible biological control agent for *Clidemia hirta* in Hawaiian forests. Plant Dis. 70:974-976.
13. Trujillo, E. E., and Norman, D. J. 1995. Septoria leaf spot of lantana from Ecuador: A potential biological control for bush lantana in forests of Hawaii. Plant Dis. In press.
14. Trujillo, E. E., Norman, D. J., and Killgore, E. M. 1994. *Septoria* leaf spot, a potential biological control for banana poka vine in forests of Hawaii. Plant Dis. 78:883-885.
15. Warshauer, F. R., Jacobi, J. D., LaRosa, A. M., Scott, J. M., and Smith, C. W. 1983. The distribution, impact, and potential management of the introduced vine *Passiflora mollissima* (Passifloraceae) in Hawaii. Tech. Rep. 48, Univ. Hawaii Coop. Natl. Park Resour. Stud. Unit Univ. Hawaii, Honolulu.