Proliferation of *Talaromyces flavus* in Soil and Survival in Alginate Pellets

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We thank David Fox for competent assistance and Larry Douglas, consulting statistician, Statistical Consulting and Analysis Team, USDA, Beltsville, for consultations and statistical analyses.
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Accepted for publication 1 July 1986 (submitted for electronic processing).

ABSTRACT

Papavizas, G. C., Fravel, D. R., and Lewis, J. A. 1987. Proliferation of *Talaromyces flavus* in soil and survival in alginate pellets. Phytopathology 77:131-136.

Conidia of *Talaromyces flavus* in alginate-bran pellets stored for 15 wk survived better at 5 and 15 than at 25 C. Survival of ascospores was affected less by temperature than was survival of conidia. Ascospore viability declined moderately during the first 3 wk, with no further decline from the 3rd to the 15th wk. The propagule type and initial inoculum level in pellets, the type of bulking agent used to make the pellets, and the initial concentration of pellets added to soil each significantly affected fungal survival in and proliferation into soil from the pellets. Population densities in soil originating from conidia in alginate-bran pellets were higher than those originating from ascospores, especially when the former propagule was added initially at 5×10^7 or 1×10^8 conidia per gram of dry pellets or when pellets were added to soil at 0.25 and 0.5% (w/w) rather than at 0.1%. For instance, at 16 wk, recovery of colony-forming units from soil was about 800% of the original numbers of conidia added with bran in the pellets. Although ascospores in alginate-bran pellets also resulted in greater numbers in soil, the total number of colony-forming units recovered was always smaller than that obtained with conidia. In no case did conidia or ascospores added with Pyrax as the bulking agent result in great numbers in soil. In contrast, dried fungal biomass (largely mycelium) of *T. flavus* proliferated in soil when added as alginate-Pyrax pellets but not as alginate-bran pellets. Yellow-orange, fertile cleistothecia of *T. flavus* developed within 3–5 days on alginate-bran pellets containing conidia or ascospores placed on or in soil, but not on alginate-Pyrax pellets containing fungal biomass. The metabolic activity of soil was high when amended with pellets containing bran (with or without propagules) and low when amended with pellets containing Pyrax as the bulking agent. Bran was the best bulking agent in all tests performed with conidia or ascospores.

Talaromyces flavus (Klöcker) Stolk & Samson (anamorph: *Penicillium dangeardii* Pitt, usually reported as *P. vermiculatum* Dangeard) is an ascomycete widely distributed in soils of temperate and subtropical areas (7) that is antagonistic against several soilborne plant pathogens (2,6,18,21,23). The agent suppressed Verticillium wilt and increased yield of eggplant in the greenhouse and in agricultural production systems (17). When applied either as a dust to potato seed pieces or as alginate-Pyrax pellets in furrow, *T. flavus* suppressed potato wilt (*V. dahliae* Kleb.) in field tests (5,8). A single application of *T. flavus* in pellets suppressed the wilt for two growing seasons (8). Also, *T. flavus* suppressed damping-off of cotton induced by *Pythium ultimum* Trow in natural soil in the greenhouse (G. C. Papavizas, *unpublished*).

If *T. flavus* is to be used for biocontrol, effective inoculum preparations and delivery systems must be developed and tested for survival and establishment in soil and for efficacy against selected soilborne pathogens. Some progress has already been made in developing technology for growing large amounts of *T. flavus* and other biocontrol fungi. Papavizas et al (19) used

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inexpensive liquid media such as that containing molasses and brewer's yeast to produce viable inocula of *T. flavus* with a deep-tank fermentation system that simulates large-scale industrial production. Also, encapsulation technology in the form of biopreparations containing spores, mycelia, or mixtures of the two were described recently. The encapsulation of viable inoculum is based on the reaction between aqueous solutions of sodium alginate and certain cations (e.g., Ca^{++}) to form gels (4,14,25). The ease with which *T. flavus* can be incorporated into sodium alginate gels with a clay carrier in an aqueous system was exploited by Fravel et al (10) to prepare pelletized formulations of *T. flavus* and other microorganisms. The technique was further refined by incorporation of nutrient carriers (e.g., bran) into the alginate pellets to provide a food base necessary for proliferation of some antagonists (15).

The objective of this study was to investigate the effect of formulation procedures on survival and proliferation of alginate-encapsulated propagules of *T. flavus* in soil and in storage.

MATERIALS AND METHODS

Isolate and soils. The wild-type isolate of *T. flavus* (TF1), previously shown to have biocontrol ability against Verticillium wilt of eggplant in the field (5,8,17), was used in all experiments. Elsinboro sandy loam (pH 6.5) and Rumford loamy sand (pH 6.8) from Beltsville, MD, were used. The soils were brought to the greenhouse 2 mo before the research was initiated from fields kept in fallow for 2 yr. The untreated soils were kept moist in a bench until used.

Production of inoculum. For production of conidia, isolate TF1 was grown in petri plates on a molasses-corn steep liquor medium (25 g of glucose, 25 g of NaCl, 5 g of corn steep liquor, 50 g of molasses, 15 g of agar, 1 L of water) (24) under continuous fluorescent light (700 μEin/m^2/sec) at room temperature for 2 wk. Conidial suspensions were prepared by gently scraping the agar surface submerged in sterile distilled water with a cotton-tipped applicator. Cleistothecia were produced by *T. flavus* after 3 wk on Difco potato-dextrose agar (PDA, Difco Laboratories, Detroit, MI) in petri plates at 30 C in the dark. Cleistothecia were scraped from the plates, suspended in water, and crushed in a Ten Broeck tissue grinder. The ascospores were filtered through sterile lens paper. The number of conidia or ascospores in the aqueous suspensions was determined with a hemacytometer. To determine the number of viable conidia or ascospores, dilutions of the spore suspensions were made directly onto PDA and a selective medium (TF medium) previously used for isolation and enumeration of *T. flavus* from soil (16). Ascospores were also produced by growing TF1 for 3 wk on a corncob-cornmeal medium (150 g of Grit-O' Cobs granules grade 2040 [The Andersons, Maumee, OH], 60 g of cornmeal, 50 ml of water). The preparations were air-dried for 3 days and ground in a Wiley mill to pass through a 425-μm (40-mesh) screen. Colony-forming units in these preparations were determined by the dilution-plate method on the TF medium. Only cleistothecia developed on the corncob-cornmeal medium.

In addition to conidia and ascospores, fungal biomass containing mycelial fragments only was produced by growing TF1 in 16-L fermentors as described previously (19). After 7 days of growth, the fermentor biomass was separated from the aqueous broth by filtration through a cotton muslin filter on an 18-cm-diameter Büchner funnel. Unless otherwise indicated, the mats were air-dried for 3 days and ground in a Wiley mill to pass through a 425-μm-mesh screen. The resulting powder was encapsulated with sodium alginate and a bulking agent (bran or Pyrax).

Conidia, fermentor biomass, and ascospores (from PDA or from the corncob-cornmeal medium) were used to prepare alginate-propagule-Pyrax or alginate-propagule-bran pellets as described previously, with calcium chloride as the gellant (10,15,25). Pyrax, a pyrophyllite (hydrous aluminum silicate, pH 7.0, R. T. Vanderbilt Co., Norwalk, CT), does not provide any known nutrients. The viable population size of *T. flavus* in the pellets was determined before exposure to soil, and in some experiments after recovery from soil, by disintegrating the pellets in sterile water in a Tissumizer (model SDT 1810, Tekmar Co., Cincinnati, OH) and assaying for colony-forming units by dilution plating.

Survival in storage. Bran pellet lots from some propagule formulations were subdivided into 10-g samples, placed in 50-ml vials (four replications), covered loosely with screw caps, and stored at 5, 15, and 25 C in the dark. After 1, 3, 7, 10, and 15 wk, 200-mg samples were removed from the vials, suspended in sterile distilled water, crushed in a Tissumizer, and adjusted with additional water to make dilutions of 1 in 10^5, 1 in 10^6, and 1 in 10^7. While the liquid was agitated by a magnetic stirrer, 1-ml aliquots were removed from the containers and spread on the TF medium (five plates per replication, four replications). The plates were incubated for 7 days in the dark at 30 C, and colonies were counted to determine the number of viable colony-forming units per gram of preparation.

Survival and multiplication in soil. Alginate-propagule-bran and alginate-propagule-Pyrax pellets were added to 400-g (dry weight equivalent) portions of natural soils in 600-ml beakers at 0.5% (w/w). Because all pellets were made with the same number of viable propagules per gram of pellets, the presumed number of colony-forming units at 0 time was 1×10^4 per gram of soil. The soil matric potential was adjusted to approximately −0.3 bar by adding an appropriate amount of water. The relationship between moisture content and matric potential was previously determined for each soil with a pressure plate apparatus (Soil Moisture, Inc., Santa Barbara, CA). Pellets were added to soil in containers, and the containers were then covered with cellophane to prevent water evaporation. The soils were assayed periodically for viable colony-forming units by the dilution-plate method (dilutions 1:1,000 or higher) on the TF medium.

In other tests, portions of Elsinboro sandy loam and Rumford loamy sand were placed in 90 × 15 mm petri plates (60 g of soil per plate, wet weight) and the soil surfaces were smoothed with a spatula. Pellets were placed on the soil surface (10 per plate) and pushed into the soil with a glass rod so that only one-half of each pellet was exposed. In other plates, pellets were pushed into the soil and covered with approximately 5–7 mm of soil. The plates were placed in large polypropylene trays, covered with aluminum foil, and incubated at room temperature in the dark. At several time intervals, the pellets were recovered, air-dried for 10–15 min, crushed in a Tissumizer, and assayed for total colony-forming units. Pellets were also placed on glass slides and examined with a stereoscopic microscope to observe sporulation patterns.

All experiments had four replications and were repeated once. Analysis of variance was conducted using either a 2 × 3 or 3 × 3 factorial analyses (at each assay time) to discern the effects of spore type, bulking agents, and initial inoculum level or temperature.

Biomass assay and metabolic activity. To determine the growth of *T. flavus* from pellet preparations into soil, alginate-bran or alginate-Pyrax pellets of conidia or ascospores were each added to moist (−0.3 bar) Elsinboro sandy loam (100 g oven dry weight equivalent) at 1% (w/w) in 7 × 8.5 cm glass jars covered with Saran wrap punctured to permit air exchange. After 1 wk of incubation at 19–21 C, soil within each container was mixed, air-dried, ground in a Wiley mill, and sieved to pass through a 425-μm-mesh screen. Growth into soil was determined by a modification of a method used to estimate the development of filamentous fungi containing chitin in plant tissue (22). Briefly, 10-g air-dried soil portions were placed in 2 × 19 cm test tubes and the soils hydrolyzed with 30 ml of KOH (120 g/100 ml). The amounts of additional extractants and reagents used were increased 10-fold over those in the original description by Ride and Drysdale (22). The method is based on the alkaline deacetylation of chitin to chitosan, the glucosamine residues of which are susceptible to deamination with nitrous acid. The aldehyde formed is determined colorimetrically and expressed as microgram of glucosamine per gram of soil to approximate fungal weight. The experiment was performed twice with three replicates.

Metabolic activity in soil resulting from amendment with alginate-propagule pellets was estimated by measuring CO_2 evolution with a modification of the alkaline trap method (20).

Soils were amended with pellets and placed in 7×8.5 cm glass jars as before, and 5 ml of standard 0.2 N NaOH in a beaker was placed in each jar. Residual NaOH was titrated with standard 0.2 N HCl after precipitation of Na_2CO_3 with $BaCl_2$. After each assay, fresh NaOH was added to each beaker. Evolution of CO_2 is reported as milligrams of CO_2 released from four replicates of each treatment at 5, 22, 30, 48, 75, and 146 hr.

RESULTS

Survival in storage. The data from the test on survival of propagules in alginate-bran pellets stored at 5, 15, and 25 C were analyzed as a 2 × 3 factorial experiment (two propagule types, three temperatures), with each week of assay analyzed separately by using colony-forming units as the dependent variable. Except for the week 1 sampling, there were significant effects by spore type ($P \leq 0.01$) and temperature ($P \leq 0.01$) as well as a significant spore type and temperature interaction ($P \leq 0.01$). Survival of ascospores from PDA was affected less by temperature than was survival of conidia (Fig. 1). Ascospore viability declined moderately during the first 3 wk at all three temperatures, with no appreciable decline from the 3rd to the 15th wk. Results with ascospores plus spent, ground corncob-cornmeal medium were similar to those obtained with ascospores from PDA. In contrast, colony-forming units in pellets containing conidia were reduced more at 25 than at 5 and 15 C. After 15 wk at 25 C, approximately 94% of the conidia in the pellets lost viability. At 5 and 15 C, approximately 48 and 62% of the conidia lost viability at 15 wk, respectively.

Survival and multiplication in soil. Alginate-bran and alginate-Pyrax pellets containing conidia, ascospores, or fermentor biomass were added to Elsinboro sandy loam and the soil was assayed for colony-forming units at 0, 1, 2, 5, 7, 12, 16, and 24 wk. The data were analyzed as a 2 × 3 factorial experiment (two bulking agents, three kinds of propagules), with each assay period analyzed separately.

A significant effect of propagule type on survival and proliferation was observed only at the 7- and 16-wk samplings ($P \leq 0.05$), although there was a trend toward propagule type affecting survival at other sampling times ($P \leq 0.10$) (Fig. 2). Survival and proliferation of *T. flavus* were greater when bran was used as a bulking agent than when Pyrax was used ($P \leq 0.05$). For instance, population densities of *T. flavus* increased greatly in soil when added as conidia or ascospores in pellets with bran as the bulking agent, but not with Pyrax. Even after 24 wk of incubation, there were approximately 6.3×10^6 cfu per gram of soil amended with alginate-conidia-bran pellets, compared with only 1.7×10^3 cfu per gram of soil amended with alginate-conidia-Pyrax pellets. At 16 wk, detection of colony-forming units was about 800% of the original numbers of conidia added with bran. Similar results were obtained with ascospores except that the total number of colony-forming units recovered was always smaller than that obtained with conidia. In contrast, proliferation of fermentor biomass was greater in soil when added as alginate-Pyrax pellets than as alginate-bran pellets.

A second test was performed with bran only as the bulking agent combined with conidia, ascospores, or fermentor biomass. In this test, fermentor biomass was used in four different forms: dry mat ground as before; wet mat ground immediately after separation from the medium; mat ground in and used with the spent fermentation liquid; and wet mat allowed to conidiate under continuous fluorescent light, then ground and used.

Analysis of variance followed by Duncan's multiple range test indicated that conidia in alginate-bran pellets proliferated more than ascospores or fermentor biomass in similar preparations (Table 1). Thus, at 5 wk, detection of colony-forming units was about 120-fold and 13-fold greater than the original numbers of conidia and ascospores added to soil, respectively. *T. flavus* added as fermentor biomass preparations in alginate-bran pellets declined. This experiment was also done with Rumford loamy sand, with similar results.

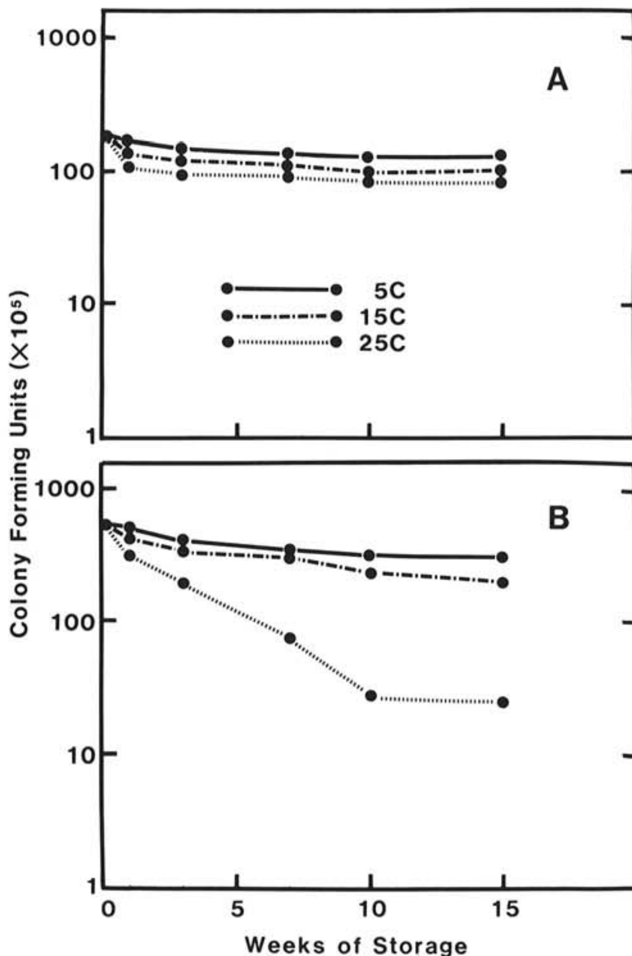

Fig. 1. Shelf life of *Talaromyces flavus* **A,** ascospores in alginate-bran pellets and **B,** conidia in alginate-bran pellets as affected by length of storage at various temperatures. Each point is the mean of four replications (semilog scale).

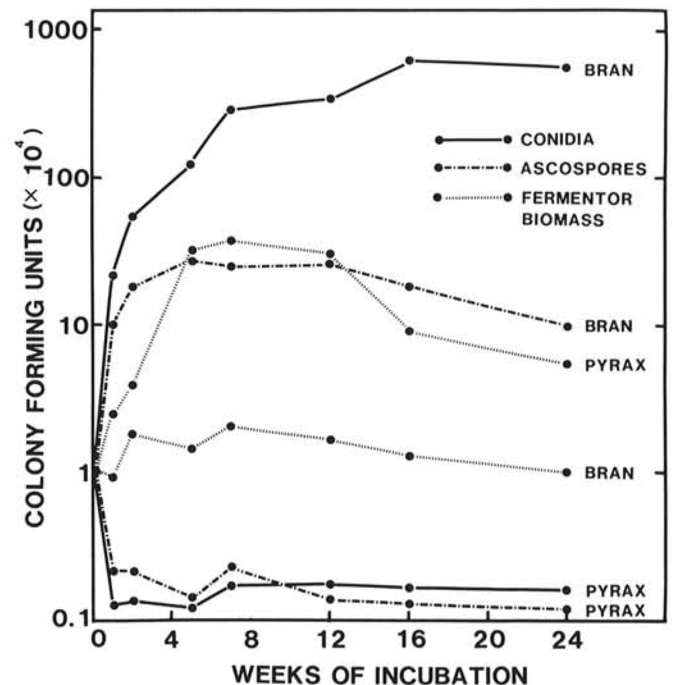

Fig. 2. Proliferation of propagules from alginate pellets of *Talaromyces flavus* in soil as affected by length of incubation and interaction of kind of spore × bulking agent (Pyrax or bran). Pellets, added at 0.5% (w/w, air-dried basis), provided 1×10^4 presumed colony-forming units per gram of soil at 0 time. Each point is the mean of four replications (semilog scale).

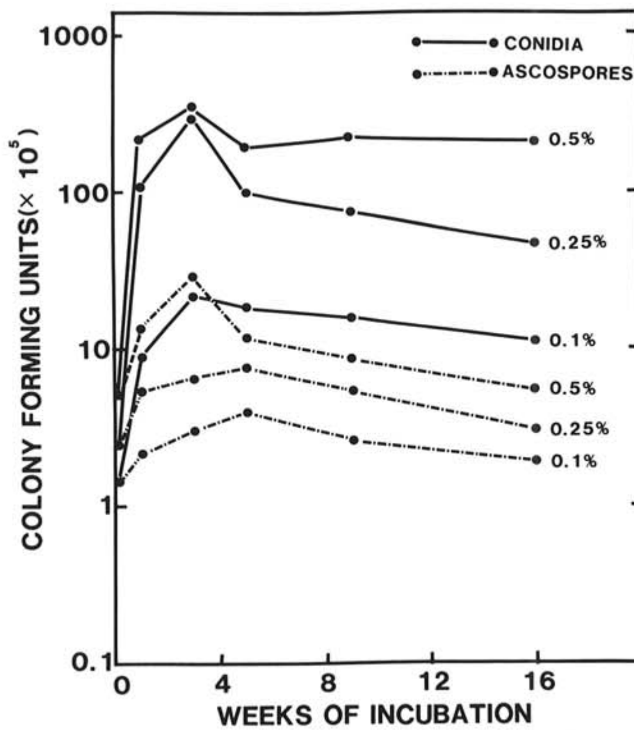

Fig. 3. Proliferation of propagules from alginate pellets of *Talaromyces flavus* in soil as affected by length of incubation, spore type, and initial inoculum concentration. Pellets, added at 0.1, 0.25, and 0.5% (w/w, air-dried basis), provided 1.4×10^5, 3.5×10^5, and 7.0×10^5 presumed colony-forming units per gram of soil at 0 time. Each point is the mean of four replications (semilog scale).

The alginate-bran pellets containing conidia or ascospores (from PDA or from the corncob-cornmeal medium) were added to both soils at 0.1, 0.25, and 0.5% (w/w, dry weight equivalent), and the soils were assayed for colony-forming units on the TF medium at 0, 1, 3, 5, 9, and 16 wk. The data were analyzed as a 3×3 factorial experiment (three pellet-bran preparations, three concentrations) for each assay period separately, using both colony-forming units or percentage of change from the 0 time figures as dependent variables. Analysis of variance indicated that spore type and initial inoculum level significantly affected survival and proliferation at all dates ($P \leq 0.05$) (Fig. 3). Again, conidia in alginate-bran pellets proliferated more in soil, especially when pellets were added at 0.25 and 0.5%, than ascospores in similar preparations. The highest numbers with conidia and ascospores were obtained after 5 and 7 wk, respectively. After 7 wk, populations in all treatments declined slowly until the 16th wk. Although the highest concentration (0.5%) resulted in the highest numbers in soil at all assay periods, the lowest concentration (0.1%) also allowed considerable proliferation. No differences were observed between the two soils.

Bran and Pyrax pellets containing conidia or ascospores at 1×10^7, 5×10^7, and 1×10^8 propagules per gram of pellets were added to Elsinboro sandy loam at 0.5% (w/w), and the soil was assayed for colony-forming units at 0, 1, 3, and 5 wk. Colony-forming units at 0 time assay of soil were less than the presumed numbers of propagules per gram of pellets in all cases (Table 2). No proliferation occurred in soil from alginate-Pyrax pellets containing either conidia or ascospores, even at 1×10^8 per gram, and from alginate-bran pellets containing ascospores. Only bran pellets with conidia at all three concentrations proliferated in soil.

Observations and assays of individual pellets. Typical yellow-orange cleistothecia of *T. flavus* developed in 3–5 days on all conidia-bran pellets placed on the surface of the soil. Cleistothecia were also observed on 33% of bran pellets prepared with

TABLE 1. Proliferation of propagules from alginate-bran pellets[x] of *Talaromyces flavus* in soil as affected by kind of propagule

Propagule	Colony-forming units[y] per gram of soil ($\times 10^3$) 1 wk	3 wk	5 wk	7 wk	12 wk	16 wk
Conidia	138 a[z]	692 a	1,192 a	1,029 a	673 a	593 a
Ascospores	3 b	9 b	29.7 b	140 b	6.9 b	11 b
Fermentor biomass						
Dry	2 b	3 b	3 b	1.9 c	0.3 b	1 b
Wet	1 b	3.3 b	3 b	1.2 c	1.0 b	0 b
In medium	0 b	3.3 b	2 b	1.0 c	1.0 b	1 b
Wet, sporulating	1 b	1.7 b	2.3 b	2.0 c	0 b	0 b
Bran only (control)	0.3 b	0 b	0 b	0.3 c	2.0 b	7 b

[x] Added to soil at 0.5% (w/w).
[y] Presumed at 0 time to be 1×10^4 per gram of soil.
[z] Means in a column for each assay period followed by same letter are not significantly different according to Duncan's multiple range test, $P \leq 0.05$.

TABLE 2. Colony-forming units from alginate-pellets[z] of *Talaromyces flavus* buried in soil as affected by spore kind and initial concentration and by kind of bulking agent in pellets

Kind of spore, bulking agent	Theoretical spore concentration per gram of pellets	Colony-forming units per gram of pellets at 0 time	Colony-forming units per gram of soil ($\times 10^3$) 1 wk	3 wk	5 wk
Conidia, bran	1×10^7	2.5×10^5	33.3	338.7	184.0
	5×10^7	8.9×10^5	108.0	330.0	267.0
	1×10^8	1.6×10^6	221.0	489.3	373.0
Conidia, Pyrax	1×10^7	2.9×10^5	2.3	1.0	1.7
	5×10^7	4.0×10^5	2.0	0.7	2.3
	1×10^8	1.3×10^6	1.0	0.7	2.0
Ascospores, bran	1×10^7	2.5×10^5	2.7	0.7	1.3
	5×10^7	8.3×10^5	1.3	1.3	5.0
	1×10^8	1.3×10^6	0.7	2.7	5.0
Ascospores, Pyrax	1×10^7	2.3×10^5	1.7	1.0	0.7
	5×10^7	7.2×10^5	1.3	1.7	1.7
	1×10^8	1.3×10^6	1.3	1.7	2.0

[z] Added to soil at 0.5% (w/w).

ascospores. Bran pellets prepared with fermentor biomass, and Pyrax pellets prepared with all three kinds of propagules, did not develop cleistothecia. Several cleistothecia crushed on a microscope slide contained mature, germinable ascospores.

Pellets placed on or in soil were recovered individually after 7 and 21 days and air-dried, excess soil was removed, and colony-forming units of *T. flavus* were determined. The numbers of colony-forming units per gram of pellets were greater with bran pellets containing conidia ($P \leqslant 0.05$) than with bran pellets containing ascospores or Pyrax pellets containing ascospores or conidia (Table 3). As many as 8.4×10^7 and 1.8×10^8 cfu per gram of pellets were recovered from bran-conidia pellets after 21 days burial in soil and on the soil surface, respectively. Only 1.4×10^3 cfu were recovered from alginate-conidia-Pyrax pellets.

Biomass assay and metabolic activity. The metabolic activity measured as glucosamine (microgram per gram of soil) of the fungi of soil amended with alginate pellets was higher ($P \leqslant 0.05$) when bran was the bulking agent used in the pellets than when Pyrax was used. Glucosamine contents ranged from 34.4 to 38.0 μg per gram of soil in soils containing bran pellets and from 21.2 to 25.2 μg in soils containing Pyrax pellets. The bran pellet treatment without propagules of *T. flavus* was just as effective in increasing metabolic activity in soil as the bran pellets containing conidia or ascospores. Also, propagule type had no effect on the activity of Pyrax pellets. Evolution of CO_2 also indicated that pellets containing bran increased metabolic activity in soil irrespective of their propagule content. Decomposing bran pellets evolved 168–198 mg of CO_2 during 146 hr, whereas less than 20 mg of CO_2 was evolved by Pyrax pellets during the same period. There was no difference in CO_2 evolution among raw soil and pellets containing Pyrax with or without *T. flavus*.

DISCUSSION

Shelf life of conidia of *T. flavus* in alginate-bran pellets was shorter at 25 C than at lower temperatures. In contrast, shelf life of ascospores was not affected by temperatures ranging from 5 to 25 C. This was not unexpected, since ascospores are known to be more resistant than conidia to high temperatures. Exposure of aqueous suspensions of presumably dormant ascospores to 53 C for 15 min, or even exposure to 70 C for 1 min, increased germination (12). In contrast, conidia exposed to 53 C for 30 min were killed. The present study corroborates the findings of Fravel et al (10) that ascospores survived longer than conidia in alginate pellets with Pyrax as the bulking agent or in Pyrax alone (11). Ascospores were also found to survive better in the field and to be more compatible with fungicides than conidia (9).

Addition of alginate pellets containing conidia of *T. flavus* and bran as the bulking agent to soil resulted in a rapid increase of activity. The bran, in close association with the rapidly germinating conidia in the pellets, supplied an adequate and easily accessible food base for fungal growth and sporulation in soil. No appreciable activity or proliferation was observed when the nutrient-free Pyrax was used as the bulking agent with conidia and ascospores or when fermentor biomass was used to make pellets with bran. Similar results were observed recently in a study on soil proliferation of several isolates of *Trichoderma* spp. and *Gliocladium virens* from alginate pellets (15). The burst of activity with conidia in alginate-bran pellets may be explained by assuming that these fast-germinating propagules (conidial germination reaches almost 100% within 2–3 hr [G. C. Papavizas, *unpublished*]) take complete possession of the substrate before indigenous microorganisms can colonize it. Rapid possession of a substrate in soil is very important for survival and saprophytic growth of a fungus (3). In contrast, the slow-growing wet or dry mycelial fragments, the only propagules present in the fermentor biomass of *T. flavus* (19), are unable to possess the substrate (bran) rapidly and may become colonized themselves by indigenous soil microorganisms. In fact, microscopic observations of alginate-fermentor biomass-bran pellets partially or entirely buried in soil revealed extensive growth of fungi of the Mucorales group on the pellets. These hypotheses may also explain why fermentor biomass proliferated with Pyrax but not with bran as the bulking agent in

TABLE 3. Proliferation of propagules on alginate pellets of *Talaromyces flavus* placed on soil surface or buried in soil as affected by kind of propagule and bulking agent

Placement of 20 pellets	Propagule, bulking agent	Colony-forming units[y] per gram of soil ($\times 10^3$)	
		1 wk	3 wk
On soil surface	Conidia, bran	75,500 a[z]	181,500 a
	Conidia, Pyrax	320 b	1,200 b
	Ascospores, bran	480 b	4,900 b
	Ascospores, Pyrax	40 b	600 b
Buried in soil	Conidia, bran	61,300 a	84,000 a
	Conidia, Pyrax	280 b	1,400 b
	Ascospores, bran	100 b	19,800 b
	Ascospores, Pyrax	80 b	300 b

[y] Presumed at 0 time to be 5×10^7 per gram of pellets.
[z] Means in a column for each assay period followed by same letter are not significantly different according to Duncan's multiple range test, $P \leqslant 0.05$.

the pellets. The behavior of fermentor biomass of *T. flavus* in alginate pellets is in marked contrast to that observed with fermentor biomass of *Trichoderma* or *Gliocladium* (1,19). Fermentor biomass of the latter two fungi containing mycelial fragments and an abundance of chlamydospores increased in soil 100-fold (from approximately 10^4 to 10^6) when added with traces of food base.

Activity and proliferation in soil from bran pellets containing ascospores were intermediate between those containing conidia, which burst into great activity, and those with fermentor biomass, which did not. This difference was not unexpected, since ascospores of *T. flavus* do not germinate rapidly and in great numbers (12,13). If ascospores are not heated to a temperature higher than 50 C for a few minutes, only about 5–10% germinate (12). Their low germination rate, even in the presence of a food base, may place these propagules at a disadvantage compared with conidia of *T. flavus* or with propagules of fast-growing soil saprophytes.

Proliferation in soil from bran-conidia pellets, but not from Pyrax-conidia pellets, is preceded by enhanced metabolic activity of *T. flavus*, as shown by the high CO_2 evolution when such pellets are added to soil and by the development of fertile cleistothecia on pellets partially or completely buried in soil. Production of cleistothecia and the great increase (800%) in colony-forming units per gram of pellets recovered from soil over initial levels indicates that growth must have preceded the formation of cleistothecia, with subsequent production of ascospores and conidia on the substrate. It remains to be seen whether alginate-conidia-bran pellets will be more effective than alginate-conidia-Pyrax pellets or pellets containing ascospores or fermentor biomass in suppressing Verticillium wilt and other diseases.

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