Liquid culture production of desiccation tolerant blastospores of the bioinsecticidal fungus *Paecilomyces fumosoroseus*

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**MARK A. JACKSON**, **MICHAEL R. MCGUIRE**, **LAWRENCE A. LACEY**

**AND STEPHEN P. WRAIGHT**

1 USDA*, Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL 61604

2 USDA, Agricultural Research Service, European Biological Control Laboratory, Montpellier, France

3 MycoTech Corporation, Butte, MT, U.S.A.

Liquid media with differing carbon concentrations and carbon-to-nitrogen ratios were tested for production of desiccation tolerant blastospores of *Paecilomyces fumosoroseus*. While all media tested supported sporulation in submerged culture, high blastospore concentrations (5.8 x 10^8 spores ml^-1) were produced in media containing 80 g glucose l^-1 and 13-2 g Casamino acids l^-1 (MS medium) and a significantly higher percentage (79%) of these blastospores survived air drying. Media containing glucose concentrations greater than 20 g l^-1 and Casamino acid concentrations between 13-2 and 40 g l^-1 supported maximal production of desiccation tolerant blastospores. All 23 isolates of *P. fumosoroseus* grown in MS medium produced high concentrations of desiccation tolerant blastospores. When stored at 4 °C, more than 60% of the lyophilized blastospores produced in MS medium were still viable after 7 months storage while less than 25% of the air-dried blastospores survived after 90 d storage. Standard whitefly bioassays were performed to compare air-dried blastospores of *P. fumosoroseus* ARSEF 4491 with solid substrate-produced conidia of *Bacillus bassiana* ARSEF 252. Air-dried blastospores of *P. fumosoroseus* gave LD₅₀s of 60 and 113 blastospores mm⁻³ for the silverleaf whitefly (*Bemisia argentifolii*) in two separate bioassays with potency ratios (LD₅₀ B. bassiana/LD₅₀ P. fumosoroseus) of 3-9 and 3-8, respectively. These results have demonstrated that high concentrations of blastospores of *P. fumosoroseus* can be rapidly produced in liquid culture, remain viable following drying, and infect and kill silverleaf whitefly.

*Bemisia tabaci* (Gennadius) (sweetpotato whitefly, cotton whitefly) has been reported to attack over 600 plants in warm climates and greenhouses worldwide (Cook, 1993; Smith, 1993; Lacey, Fransen & Carruthers, 1995). The ability of this polyphagous phloem-feeding insect to reproduce rapidly can lead to heavy infestations which are capable of killing the host plant. The silverleaf whitefly, *Bemisia argentifolii* (Bellows & Perring), also known as the B-biotype of *B. tabaci*, has been especially damaging to global agriculture in general and to U.S. agriculture in particular (Gill, 1992; Cook 1993). The development of resistance to a wide variety of insecticides and the devastating effects pesticides have on natural enemy complexes further exacerbates the problem. Parasitic and predacious insects and entomopathogenic fungi are being evaluated as potential biological control agents for this pest. In general, the overuse of insecticides in cropping systems lead to heavy infestations which are capable of killing the host plant.

Many strains of *P. fumosoroseus* and *B. bassiana* have been isolated that are aggressive pathogens of numerous species of insects including *B. tabaci* and *B. argentifolii* (Lacey, Kirk & Hennessey, 1993; Puterka, Humber & Poprawski, 1994; Lacey *et al.*, 1995). The feasibility of using these fungi as biocontrol agents against sweetpotato or silverleaf whitefly is dependent on numerous biological constraints, including the ability to produce high concentrations of stable propagules at a reasonable cost (Jaronski, 1986; Latgé *et al.*, 1986). On solid substrates, *B. bassiana* isolates produce abundant aerial conidia which are amenable to storage as dry preparations. In submerged liquid culture, *P. fumosoroseus* and *P. farinosus* isolates are reported to produce high concentrations of blastospores (Inch *et al.*, 1986; Inch & Trinci, 1987). Blastospores produced by various entomopathogenic fungi are typically larger than aerial conidia, are not amenable to simple drying techniques and tend to perish more rapidly during storage (Inch *et al.*, 1986; Bidochka, Pfeifer & Khachatourians, 1987; Lane, Trinci & Gillespie, 1991; Hegedus *et al.*, 1992). The lack of effective methods for producing desiccation-tolerant blastospores of *P. fumosoroseus* has led to...
Production of desiccation tolerant blastospores

...of commercial production processes which rely on the liquid culture production of vegetative biomass of *P. fumosoroseus* (Eyal et al., 1994).

Our research has focused on developing liquid culture techniques for producing desiccation tolerant blastospores of *P. fumosoroseus*. In this study, we evaluated various liquid culture media for producing blastospores based on spore yield and stability as a dry preparation. A liquid culture medium is reported which supported the rapid production of high concentrations of desiccation-tolerant blastospores capable of infecting and killing silverleaf whitefly.

**MATERIALS AND METHODS**

**Strains and culture conditions**

Isolates of *P. fumosoroseus* used in this study were obtained during foreign exploration by staff (L. Lacey and A. Kirk) of the USDA/ARS, European Biological Control Laboratory, Montpellier, France (Lacey et al., 1993), and domestic surveys by S. Wraight (Texas isolates) and T. Poprawski (California isolates) [Table 1]. Most isolates are maintained in ARS' entomopathogenic fungal culture collection (ARSEF), Ithaca, NY (Humber, 1992). Stock cultures were grown as single spore isolates on potato dextrose agar (PDA) for 21 d at room temperature, cut into 1 mm × 2 agar plugs and stored in 10% (v/v) glycerol at −80 °C. All isolates were routinely cultured at room temperature (ca 25°C) on PDA. Conidial suspensions, for inoculating submerged cultures, were prepared by flooding 14 to 21-d-old PDA Petri dish cultures with sterile, deionized water. *P. fumosoroseus* conidial inoculum for all submerged cultures provided a final concentration of 5 × 10⁶ conidia ml⁻¹. Liquid cultures (100 ml in 250 ml Erlenmeyer flasks) were grown at 28°C and 300 rpm in a rotary shaker incubator (INNOVA 4000, New Brunswick Scientific, Edison, NJ, U.S.A.). A minimum of duplicate flasks was used for all treatments and all experiments were repeated at least twice.

**Media composition**

Media with various carbon concentrations, carbon-to-nitrogen (CN) ratios, and nitrogen sources were tested for their impact on *P. fumosoroseus* ARSEF 4491 blastospore yield and blastospore stability. The basal component of all media tested contained per litre: KH₂PO₄, 2.0 g; CaCl₂, 2H₂O, 0.4 g; MgSO₄, 7H₂O, 0.3 g; CoCl₂, 6H₂O, 37 mg; FeSO₄, 7H₂O, 50 mg; MnSO₄, H₂O, 16 mg; ZnSO₄, 7H₂O, 14 mg; thiamin, riboflavin, pantothenate, niacin, pyridoxamine, thiotic acid, 500 μg each; folic acid, biotin, vitamin B₁₂, 50 μg each. All media had an initial pH of 5.5 and pH was uncontrolled during culture growth. Glucose was the only carbohydrate tested and was autoclaved separately.

<table>
<thead>
<tr>
<th>P. fumosoroseus isolates</th>
<th>Yield* (10⁸ blastospores ml⁻¹)</th>
<th>Blastospore germination+ (%)</th>
<th>Viable freeze-dried spores (10⁸ blastospores ml⁻¹ medium)</th>
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<td>88</td>
<td>6 - 0</td>
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<td>88</td>
<td>3 - 9</td>
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<td>89</td>
<td>4 - 8</td>
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<td>6.5</td>
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<td>5 - 8</td>
</tr>
</tbody>
</table>
| Table 1. Yield and desiccation tolerance of liquid-culture-produced blastospores of various isolates of Paecilomyces fumosoroseus isolated from Bemisia tabaci

* Blastospores collected from 4-d-old cultures.
+ Spore germination determined by 6 h germination assay in potato dextrose broth after freeze drying.
* Fisher's protected least significant difference.
§ Not significantly different.
In preliminary studies, various production media were tested (Table 2). The medium which contained the basal components with 80 g glucose l⁻¹ and 13.2 g Casamino acids l⁻¹ (Difco, vitamin-free) (MS medium) produced high concentrations of desiccation tolerant blastospores of *P. fumosoroseus* and was used subsequently to produce blastospores for drying, storage, and bioassay experiments. The effect of various concentrations of glucose or Casamino acids on spore yield and desiccation tolerance was evaluated by growing cultures of *P. fumosoroseus* ARSEF 4491 in basal media with 80 g glucose l⁻¹ and various amounts of Casamino acids (5–50 g l⁻¹) or in basal media with 13.2 g Casamino acids l⁻¹ and various amounts of glucose (10–80 g l⁻¹).

Twenty-three isolates of *P. fumosoroseus* were screened for their ability to produce high concentrations of desiccation tolerant blastospores. All isolates were grown in MS medium. Four replicate flasks were used for each treatment and all experiments were repeated at least twice.

**Substrate utilization**

High-performance liquid chromatography (hplc) was used to measure glucose concentrations in culture supernatants, as previously described (Jackson, Slininger & Bothast, 1989). The concentration of 15 amino acids was measured in culture supernatants as o-phthalaldehyde derivatives using reverse-phase hplc, as previously described (Jackson & Schisler, 1982; Willis, 1987). These amino acids were L-aspartic acid, L-glutamic acid, L-serine, L-histidine, glycine, L-threonine, L-arginine, L-alanine, L-tyrosine, L-methionine, L-valine, L-phenylalanine, L-isoleucine, L-leucine, and L-lysine. The sum of the concentrations of the 15 amino acids was used as an approximation for the amino acid concentration in the culture supernatant.

**Drying and storage studies**

Blastospores were dried using two methods; air-drying and freeze-drying. All air-drying experiments involved mixing the whole cultures or conidial suspensions with 5 % (w/v) diatomaceous earth (HYFLO, Celite Corp., Lompoc, CA, U.S.A.), vacuum-filtering the suspension on filter paper (Whatman No. 1) to remove the excess liquid, and drying the filter cake in a biological containment hood (25–40 % r.h.) overnight to 1–5 % (w/w) moisture. The moisture content of the dried blastospore: diatomaceous earth preparations was determined with a moisture analyzer (Mark I, Denver Instruments, Tempe, AZ, U.S.A.). In experiments where various media were tested, whole cultures were harvested after 3 d growth, mixed with 5 % (w/v) diatomaceous earth, and air-dried. For air-drying experiments where stability during storage and biocontrol efficacy were being evaluated, blastospores were separated from the mycelia by passing the culture broth through a double layer of cheesecloth twice prior to drying.

For storage studies, air-dried blastospore preparations were kept in sealed plastic bags at temperatures of 4 °C or 22 °C. The viability of air-dried blastospores of *P. fumosoroseus* was assessed by adding ca 50 mg of a dried blastospore preparation to 50 ml of potato dextrose broth in a 250 ml baffled, Erlenmeyer flask. After 6 h incubation at 28 °C and 300 rpm in a rotary shaker incubator, 100 blastospores were microscopically evaluated for germ-tube formation (× 500).

For freeze-drying experiments, blastospore suspensions were mixed (1:1 v/v) with a solution containing 20 % (w/v) lactose and 2 % (w/v) bovine serum albumin (BSA) to produce a blastospore mixture in 10 % lactose and 1 % BSA. Freeze-drying was performed in a tray dryer (Durstop-MP, FTS Systems) using an automatic-eutectic drying programme. This programme determined the eutectic point of the sample, set drying conditions based on this information, monitored the primary and secondary drying process, and determined when the drying process was completed. Vials (10 ml) containing 2 ml blastospore suspensions were used in all studies. At the end of the freeze-drying cycle, vials were sealed under vacuum and stored at 4 °C or 22 °C.

The viability of freeze-dried blastospores was assessed in two ways. For long-term storage studies, blastospore survival was measured by plate counts on PDA incubated at 28 °C. Duplicate plates of the appropriate dilution (30–300 colonies) were counted as colonies became visible (2–3 d incubation). Prior to plating, freeze-dried blastospores were rehydrated for 1 h at room temperature with sterile 0.004 % (w/v) phosphate buffer (pH 7). All dilutions were made with 0.004 % (w/v) phosphate buffer. When screening various strains of *P. fumosoroseus* for blastospore production and stability after drying, the viability of freeze-dried blastospores was assessed by the 6 h germination test previously described for air-dried blastospores. It should be noted that the blastospore survival values presented in Table 1 were calculated by multiplying yield and viability of each of the four replicates and averaging these values to obtain the mean survival values.

A comparison of the 6 h germination assay and the plate count method showed no significant differences in viability when comparing recently dried blastospore preparations. Difficulties in plating blastospore–diatomaceous earth preparations made the 6 h germination assay the method of choice for air-dried blastospore preparations.

**Bioassays**

Bioassays of liquid-culture produced blastospores of *P. fumosoroseus* strain ARSEF 4491 were conducted with 15-d-old *B. argentifolii* third-instar nymphs provided by the USDA-APHIS Biocontrol Laboratory in Mission Texas from a colony maintained on hibiscus (*Hibiscus rosa-sinensis* L.). Air-dried blastospore–diatomaceous earth preparations were mixed in water containing 0.01 % (v/v) Tween 80 and then sprayed onto the whitefly-infested hibiscus leaves in a Potter Spray Tower® (Burkard, Herts, U.K.). The concentrations of spores in the sprayed suspensions were adjusted to produce low, medium and high deposits (doses) of ca 40, 200 and 1000 spores mm⁻² of leaf surface. Each of the three doses was applied to two replicate leaves: 40–50 nymphs on each leaf were monitored for response to the spray treatments. The sprayed leaves were held in sealed plastic bags at 100 % r.h. for the first 24 h after treatment and then maintained, with petioles constantly embedded in water-saturated cotton, for
Production of desiccation tolerant blastospores

an additional 7 d in ventilated (screen covered) Petri dishes. Aerial conidia of *B. bassiana* strain ARSEF 252 produced on a proprietary solid substrate by Mycotech Corporation were sprayed as a control in each experiment. The *B. bassiana* control served as an internal standard by monitoring the impact of slight differences in assay conditions, plant age, etc. on whitely mortality. Additional control groups were sprayed with 0.01% (v/v) aqueous Tween 80 solution alone and with Tween 80 solution containing diatomaceous earth mixed with spent, filtered media. During each assay, samples of all spore suspensions were sprayed onto plates of Sabouraud dextrose agar, and incubated (ca 10 h for blastospores and 16 h for conidia) at 25° to determine viability. Spore doses were ultimately adjusted for the measured rates of viability. LD50s (expressed as spores mm−3) were calculated by probit analysis (Finney, 1971). Potency ratios (LD50 of *B. bassiana*/LD50 of *P. fumosoroseus*) were calculated to determine the relative effectiveness of blastospores of *P. fumosoroseus*.

**Statistical analysis**

Statistical analyses of data on blastospore yield and desiccation tolerance were performed using a one-way analysis of variance and means were separated using Fisher's protected least significant difference (LSD). Prior to variance analysis, data normality was confirmed using Wilks Shapiro/Rankit Plot Analysis.

**RESULTS**

**Nutrition and blastospore production**

Under shake-flask culture conditions, significantly higher concentrations of blastospores of *P. fumosoroseus* (isolate ARSEF 4491) were produced in the medium (MS medium) containing a high concentration of carbon (80 g glucose 1−1) and nitrogen (13.2 g Casamino acids 1−1) (Table 2). When cultures were grown in basal media supplemented with glucose and Casamino acids in which the carbon concentration was held constant (4 g carbon 1−1) and the C:N ratio varied, lower concentrations of desiccation sensitive blastospores were produced. In media with 4 g carbon 1−1, blastospore concentrations were not significantly different regardless of the C:N ratio of the medium. Desiccation tolerance was only slightly better in blastospores produced in media with a C:N ratio of 10:1 when compared with blastospores produced in media with a C:N ratio of 80:1 (Table 2). When a medium using a synthetic amino acid mixture as a nitrogen source was tested, blastospore yield and desiccation tolerance were significantly lower than those obtained with the MS medium, but not significantly different from the blastospore yields and desiccation tolerance found in cultures grown in the media containing 4 g carbon 1−1 and C:N ratios of 10:1 or 30:1.

When cultures were grown in basal media with 13.2 g 1−1 Casamino acids and various glucose concentrations, maximal blastospore production occurred in media with more than 20 g 1−1 glucose (Table 3). Glucose concentration did not significantly influence desiccation tolerance under the conditions of this test. Analysis of variance of the number of blastospores surviving air-drying showed that media containing 40, 60 or 80 g 1−1 glucose produced significantly (P < 0.05) more desiccation tolerant blastospores compared with media with 10 or 20 g 1−1 glucose. Using basal medium with 80 g 1−1 glucose and various Casamino acid concentrations, optimal production of desiccation tolerant blastospores occurred in

| Table 2. Effect of media composition on blastospore yield and desiccation-tolerance in liquid cultures of *Paecilomyces fumosoroseus* ARSEF 4491* |
|------------------|------------------|------------------|
| Glucose (g 1−1) | Casamino acids (g 1−1) | Amount of carbon (g 1−1) | C:N Ratio | Yield† (10⁶ spores ml−1 medium) | Blastospore germination‡ (%) |
| 80.0 | 13.2 | 39 | 36:1 | 580 | 79 |
| 30.0 | 8.6 | 30:1 | 94 | 30 |
| 17.8 | 5.0 | 46 | 6 |
| 9.2 | 2.0 | 15 | 3 |
| 13.6 | 3.2 | 130 | 32 |
| FPLSD* (P < 0.05) | | | | 110 | 26 |

* All media contained the basal components listed in the materials and methods.
† 3-d-old cultures.
‡ Blastospore germination after air-drying overnight with air (r.h. 20–30%).
§ Synthetic amino acid mixture, mix-C (Jackson & Slininger, 1993).
|| Fisher's protected least significant difference.

| Table 3. Effect of glucose concentration on blastospore yield and desiccation tolerance in cultures of *P. fumosoroseus* ARSEF 4491 grown in basal media with 13.2 g 1−1 Casamino acids |
|------------------|------------------|------------------|
| Glucose (g 1−1) | Yield* (10⁶ blastospores ml−1 medium) | Blastospore germination† (%) | Viable spores after air-drying (10⁶ blastospores ml−1 medium) |
| 10 | 2.6 | 72 | 2.0 |
| 20 | 4.4 | 55 | 2.8 |
| 40 | 6.2 | 68 | 4.3 |
| 60 | 7.2 | 58 | 4.2 |
| 80 | 8.1 | 53 | 4.2 |
| FPLSD* (P < 0.05) | 2.1 | NSD§ | 1.2 |

* Blastospores collected from 4-d-old cultures.
† Germination determined by 6-h germination assay in potato dextrose broth after air-drying.
§ Fisher's protected least significant difference.

In Table 3, the effect of glucose concentration on blastospore yield and desiccation tolerance in cultures of *P. fumosoroseus* ARSEF 4491 grown in basal media with 13.2 g 1−1 Casamino acids is shown. Glucose concentrations were not significantly different regardless of the C:N ratio of the medium. Desiccation tolerance was only slightly better in blastospores produced in media with a C:N ratio of 10:1 when compared with blastospores produced in media with a C:N ratio of 80:1 (Table 2). When a medium using a synthetic amino acid mixture as a nitrogen source was tested, blastospore yield and desiccation tolerance were significantly lower than those obtained with the MS medium, but not significantly different from the blastospore yields and desiccation tolerance found in cultures grown in the media containing 4 g carbon 1−1 and C:N ratios of 10:1 or 30:1.

When cultures were grown in basal media with 13.2 g 1−1 Casamino acids and various glucose concentrations, maximal blastospore production occurred in media with more than 20 g 1−1 glucose (Table 3). Glucose concentration did not significantly influence desiccation tolerance under the conditions of this test. Analysis of variance of the number of blastospores surviving air-drying showed that media containing 40, 60 or 80 g 1−1 glucose produced significantly (P < 0.05) more desiccation tolerant blastospores compared with media with 10 or 20 g 1−1 glucose. Using basal medium with 80 g 1−1 glucose and various Casamino acid concentrations, optimal production of desiccation tolerant blastospores occurred in
Substantial utilization of glucose and the total number and percentage of blastospores surviving freeze-drying were not significantly different for any of the strains tested (Table 1).

A comparison of 23 strains of P. fumosoroseus showed that MS media supported the rapid production of high concentrations of desiccation tolerant blastospores (Table 1). Blastospore yield in all but three of the strains tested was not significantly different, and the total number and percentage of blastospores surviving freeze-drying were not significantly different for any of the strains tested (Table 1).

media with 15–40 g l⁻¹ Casamino acids. Media containing 50 g l⁻¹ Casamino acids produced so few blastospores that air-drying was not possible.

Blastospore production by cultures of P. fumosoroseus (isolate ARSEF 4491) in MS medium occurred rapidly and prior to glucose or amino acid exhaustion (Fig. 1). Blastospore yields of P. fumosoroseus (isolate ARSEF 4491) reached a maximum concentration, 3.4×10⁸ blastospores ml⁻¹, after 4 d growth. Substrate utilization studies showed that the MS medium contained excess carbohydrate (Fig. 1). While Casamino acids were essentially depleted after 4 d growth, more than 70 g glucose l⁻¹ remained.

A comparison of 23 strains of P. fumosoroseus showed that MS media supported the rapid production of high concentrations of desiccation tolerant blastospores (Table 1). Blastospore yield in all but three of the strains tested was not significantly different, and the total number and percentage of blastospores surviving freeze-drying were not significantly different for any of the strains tested (Table 1).

Desiccation-tolerance and stability of blastospores of P. fumosoroseus

A high percentage of blastospores of P. fumosoroseus (isolate ARSEF 4491) produced in MS media survived air-drying (79 ± 12.4%) and freeze-drying (86 ± 10.6%). There were no significant differences in initial survival after air-drying or freeze-drying for blastospores of P. fumosoroseus produced in MS medium.

After 3 months storage at 4°, over 68% of the freeze-dried, vacuum sealed blastospores remained viable (Fig. 2). Only 1% of the freeze-dried P. fumosoroseus blastospores stored at 22° were viable after 30 d. Air-dried blastospores of P. fumosoroseus preparations were less stable than freeze-dried preparations, with a 70% loss in blastospore viability after 30 d and over 95% loss after 4 months storage at 4° (Fig. 3). After 30 d storage at 22°, none of the air-dried P. fumosoroseus blastospores germinated.

Whitefly bioassays

Air-dried, liquid culture produced blastospores of P. fumosoroseus significantly infected whitefly nymphs (Table 5). These air-dried blastospores had potency ratios (LD₅₀ Beauveria bassiana/LD₅₀ P. fumosoroseus) which showed they were nearly four times more effective than the B. bassiana ARSEF 252 standard strain in inciting disease in B. argentifolii (Table 3). Controls using 0.01% Tween and the Tween solution with diatomaceous earth in spent culture medium did not significantly influence B. argentifolii health or development.

DISCUSSION

These studies have demonstrated that high concentrations of desiccation tolerant blastospores of numerous isolates of P. fumosoroseus can be rapidly produced in submerged culture in media containing appropriate concentrations of glucose and Casamino acids. While glucose concentrations in excess of
Production of desiccation tolerant blastospores

Table 4. Effect of Casamino acid concentration on blastospore yield and desiccation tolerance in cultures of *P. fumosoroseus* ARSEF 4491 grown in basal media with 80 g l⁻¹ glucose

<table>
<thead>
<tr>
<th>Casamino acids (g l⁻¹)</th>
<th>Yield* (10⁸ blastospores m⁻³ medium)</th>
<th>Blastospore germination† (% of 6 h)</th>
<th>Viable spores after air-drying (10⁸ blastospores m⁻³ medium)</th>
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* Blastospores collected from 4-d-old cultures.
† Germination determined by 6 h germination assay in potato dextrose broth after air-drying.
§ Not determined. Too few spores were produced to conduct air-drying.

20 g l⁻¹ were required for maximal spore yield, desiccation tolerance was dependent on the Casamino acid content of the medium (Tables 3 and 4). Casamino acid concentration between 13-2 and 40 g l⁻¹ were necessary for maximum desiccation tolerance by blastospores of *P. fumosoroseus* (Tables 2, 4).

Blastospore production by *P. fumosoroseus* strains in MS media occurred rapidly and prior to the exhaustion of Casamino acids or glucose (Fig. 1) although Casamino acids were being rapidly depleted during days 3 and 4 when maximum sporulation was occurring. Sporulation during the third and fourth days of culture growth occurred on hyphal tips and also in a microcyclical fashion from germinating blastospores. Microcyclic sporulation may have contributed to the high concentrations of blastospores obtained in MS media. These results support studies with various isolates of *P. fumosoroseus* and other entomopathogenic fungi which showed that blastospore formation was not always dependent on nutrient depletion (Inch et al., 1986; Latgé et al., 1986). Unlike previous studies (Eyal et al., 1994), a significant number (ca 80%) of the blastospores produced under these nutritional conditions survived both air- and freeze-drying.

Nitrogen depletion may have limited sporulation since spore concentrations did not significantly increase after amino acids were depleted from the MS medium (Fig. 1). It is interesting to note that significant glucose utilization did not occur until after the amino acids were exhausted. Amino acids appeared to be preferentially used by *P. fumosoroseus* (isolate ARSEF 4491) as a carbon and nitrogen source. Further work is needed to determine the significance of these nutritional conditions on blastospore formation and desiccation tolerance.

The C:N ratio of the medium did not significantly influence the spore yield or desiccation-tolerance of *P. fumosoroseus* blastospores when cultures were grown in media with lower carbon concentrations (Table 2), or in media with differing amounts of glucose and Casamino acids (Tables 3, 4). In previous studies on sporulation in submerged culture by the bioherbicidal fungus *Colletotrichum truncatum*, the C:N ratio of the media had a significant effect on spore yield (Jackson & Bothast, 1990; Jackson & Schisler, 1992). Media with a carbon concentration of 4 g carbon l⁻¹ and different C:N ratios influenced *C. truncatum* spore yield and biocontrol efficacy (Schisler, Jackson & Bothast, 1990). Conversely, the MS medium, which supported high spore yields and desiccation tolerance in the *P. fumosoroseus* isolates tested in this study, totally inhibited sporulation by *C. truncatum* in submerged culture while promoting the formation of microsclerotia (Jackson & Bothast, 1990; Jackson & Schisler, 1995). In view of these differences, it is prudent to evaluate a wide range of media in order to optimize nutritional conditions for mass production of fungal biocontrol agents.

Air- and freeze-drying methods showed that 79 and 86%, respectively, of *P. fumosoroseus* blastospores produced in MS media survived drying. These survival rates were significantly higher than those for blastospores produced in the other media tested (Table 2). Past reports on the instability of blastospores during drying suggests that those blastospores were produced under suboptimal nutritional conditions. This is the first report of nutritional conditions which support the rapid production of high concentrations of desiccation-tolerant blastospores of *P. fumosoroseus*. Studies with other fungi have shown that growth and sporulation in liquid and solid media with reduced water activity enhanced spore survival by increasing the intracellular accumulation of trehalose and polyhydroxy alcohols (Jin, Harman & Taylor, 1991; Hallsworth & Magan, 1994). Our results suggest that desiccation tolerance in *P. fumosoroseus* blastospores is not dependent on reduced water activity as glucose concentration had no effect on desiccation tolerance (Table 3). Desiccation tolerance in *P. fumosoroseus* blastospores appears to be dependent on the nutrients provided by Casamino acids (Table 4). Experiments are currently in progress to determine the effect of the nutritional elements in Casamino acids on desiccation-tolerance in forming *P. fumosoroseus* blastospores.

The long-term survival of dried blastospore preparations was significantly better at 4°C compared with 22°C (Figs 2, 3), and freeze-dried preparations were more stable than air-dried blastospores. It should be noted that freeze-dried blastospores were stored under vacuum while air-dried blastospores were stored in sealed plastic bags. Whether freeze-drying or the storage conditions were responsible for increased stability is uncertain.

The LD₅₀ of the air-dried blastospores of *P. fumosoroseus* against silverleaf whitefly (Bemisia argentifolii) was significantly better at 4°C compared with 22°C (Figs 2, 3), and freeze-dried preparations were more stable than air-dried blastospores. It should be noted that freeze-dried blastospores were stored under vacuum while air-dried blastospores were stored in sealed plastic bags. Whether freeze-drying or the storage conditions were responsible for increased stability is uncertain.

Table 5. Efficacy of air-dried blastospores of *Pseudomonas fumosoroseus* ARSEF 4491 for controlling silverleaf whitefly (*Bemisia argentifolii*)

<table>
<thead>
<tr>
<th>Blastospore preparation</th>
<th>LD₅₀ (spores mm⁻³)</th>
<th>LD₅₀ (Spores mm⁻³)</th>
<th>Potency ratio†</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fumosoroseus</em></td>
<td>60 (79-37)</td>
<td>233 (310-86)</td>
<td>3-9</td>
</tr>
<tr>
<td><em>B. bassiana</em></td>
<td>113 (146-87)</td>
<td>433 (1402-234)</td>
<td>3-8</td>
</tr>
</tbody>
</table>

* Potency ratio is a measure of the relative efficacy of blastospores of *P. fumosoroseus* in infecting and killing *B. argentifolii* under the unique conditions of each bioassay (LD₅₀ *B. bassiana*/LD₅₀ *P. fumosoroseus*).
dramatic improvement in biocontrol efficacy of *P. fumosoroseus* blastospores compared with *B. bassiana* conidia was somewhat unexpected. In general, aerial produced conidia of various *P. fumosoroseus* isolates have given potency ratios of 0.5–1.5 when compared with *B. bassiana* (S. Waight, unpublished data). In this study, air-dried, liquid culture-produced blastospores of *P. fumosoroseus* had potency ratios of 3.9 and 3.8 (Table 5). While Hegedus et al. (1992) showed that blastospore preparations of *B. bassiana* were more infective than aerial conidia on the migratory grasshopper, *Melanopus sanguinipes*, a study by Lane et al. (1991) suggested that the hydrophobic nature of *B. bassiana* conidia increased the adherence of these propagules to the cuticle of the green leafhopper, *Nephotettix virescens*, and significantly improved the biocontrol efficacy of conidia compared with *B. bassiana* blastospores. It is possible that the adhesion issue is less important when dealing with sessile *B. argentifoli* nymphs rather than mobile insects. It is also likely that the rapid germination rate of blastospores compared to aerial conidia is responsible for the improved efficacy of these propagules in infecting and killing *B. argentifoli* nymphs.

While numerous questions remain unanswered concerning the effect of nutrition on blastospore stability, the optimal drying and storage conditions, and the efficacy of *P. fumosoroseus* blastospores in field conditions; these studies have demonstrated that many strains of *P. fumosoroseus* rapidly produced high concentrations of desiccation-tolerant blastospores in liquid culture when media containing appropriate levels of carbon and nitrogen are used. Under field conditions where free-moisture requirements represent a significant constraint to biocontrol efficacy, rapid germination by liquid culture produced blastospores should further enhance the ability of these propagules to infect and kill silverleaf whitefly and other susceptible insect pests.

The authors wish to recognize the significant technical contribution of Angela R. Payne throughout these studies.

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Delortia palmicola and two new species from wood submerged in a freshwater stream in Australia

TEIK-KHIANG GOH¹ AND KEVIN D. HYDE²

¹ Department of Applied Biology and Chemical Technology, Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong
² Department of Ecology and Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong

Delortia, represented by the type, D. palmicola, is redescribed from fresh decaying palm material collected in north Queensland, Australia. Two new species, D. tumidoapicis and D. aquatica, collected from wood submerged in a freshwater stream, also in north Queensland are described. The three species are illustrated with light micrographs and line diagrams and discussed in relation to each other.

Delortia Pat. was monotypic, represented by D. palmicola Pat. (Patouillard & Gaillard, 1888). The species forms white slimy sporodochia on decaying parts of palms and the conidia are hyaline and helical (Patouillard & Gaillard, 1888; Pirozynski, 1972). There are several reports of this fungus (Pirozynski, 1972), but no further species have been described.

During a survey of the fungi occurring in north Queensland we collected Delortia palmicola (on decaying palm material), and two further species from submerged wood with characteristics resembling Delortia. One of the species had conidia which were similar, but smaller, than D. palmicola, whilst the other had conidia that were morphologically distinct, having a swelling in the terminal cell. In all three

Figs 1–6. Delortia palmicola. Fig. 1. Squash mount of a portion of the sporodochium showing conidiophores and conidia. Figs 2–4. Close-up of conidiophores and developing conidia. Note the hyaline gelatinous sheath around the conidia (arrowed). Figs 5–6. Conidia. Note the hyaline gelatinous sheath around the conidia (arrowed). Bars: 1 = 50 μm, 2–6 = 10 μm.