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Mass production of *Fusarium oxysporum* (M12-4A), a biocontrol agent for *Striga hermonthica*

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Abstract. Microconidia, macroconidia, and chlamydospores of *Fusarium oxysporum* (M12-4A) were produced on moist sorghum straw incubated at 24, 28 and 32°C. Temperature affected the number of the different types of propagules produced. Maximum production of microconidia occurred at 32°C and macroconidia at 24°C. There was no significant difference in mean colony forming units (CFU) and chlamydospore production at the three temperatures. Overall production of infective propagules was greatest at 28°C. Substrate also affected the types of spores produced. All substrates supported abundant mycelial growth at 28°C and full colonization of the substrate occurred within 10 days. Maximum production of microconidia occurred on sorghum fibres, maximum production of chlamydospores occurred on straw, whereas the highest number of CFU and macroconidia occurred on glumes. When straw or glumes were soaked overnight, *F. oxysporum* sporulated abundantly, producing microconidia and macroconidia and, later, chlamydospores. In these studies CFU values generally peaked 10-15 days after inoculation (DAI), macroconidia at 15-20 DAI, and production of chlamydospores did not start until 10 DAI but then increased linearly. Microconidia numbers tended to be higher early, then declined. Isolate M12-4A grown on sorghum straw or glumes survived and remained viable for at least 12 months when stored at room temperature. Mass production of *F. oxysporum* (M12-4A) was optimized on soaked, sorghum glumes at 28°C. These colonized sorghum glumes are effective vehicles to deliver this biocontrol agent to soils infested with *Striga hermonthica*.

Introduction

An isolate of *Fusarium oxysporum* (Schlecht) Snyder and Hansen from Mali, western Africa is a selective and effective biocontrol agent of *Striga hermonthica* (Del.) Benth. (Ciotola *et al.* 1995). The inoculum was produced on sorghum, *Sorghum bicolor* (L.) Moench., straw and the colonized straw was used as the delivery vehicle. In the sahelian climate, incorporation of the biocontrol inoculum in the soil has major advantages over foliar applied inoculum because it is protected from desiccation and temperature fluctuations are minimized. Soil inoculum also promotes infection and control of striga during its subterranean development phase (Ciotola *et al.* 1995). The preferred substrate material should be an adequate food source for growth and sporulation of the fungus, while being readily available, inexpensive and not requiring any further processing prior to delivery of the final product. The goal is to have a 'product' that can be produced at the village level as a cottage industry or by on-farm production.

The objective of this research was to determine optimal conditions for the small-scale production of *F. oxysporum* on materials that are inexpensive, abundant and locally available to subsistence farmers in western Africa. The effects of different sorghum substrates, incubation temperatures and moisture regimes on the production of various spore types were determined.

Materials and methods

The trials were conducted in the quarantine research laboratory at McGill University. *Fusarium oxysporum* (isolate M12-4A) was grown on potato dextrose agar

(PDA) plates incubated at 28°C under fluorescent and incandescent light for two to three days. Mycelium plugs were taken from the leading edges of the plate colonies and used to inoculate production flasks.

Effect of temperature

Seven grams of sorghum straw (dry stems cut into 2 cm³ pieces) were placed in 250 ml Erlenmeyer flasks with 28 ml water. The flasks were autoclaved for 20 minutes at 121°C (15 psi). After cooling, each flask was inoculated with two agar plugs from the starter cultures of *F. oxysporum*. Inoculated flasks were incubated in growth chambers with constant light (380 $\mu Em^{-2}s^{-1}$ of photosynthetic active radiation) at 24, 28 or 32°C. Flasks were shaken by hand once a day to allow full fungal colonization of the sorghum straw. The experimental design was a split-plot with six replicates and the experiment was conducted three times. The main plots were three levels of constant temperature (24, 28 and 32°C) and the subplots were four growth-durations, 5, 10, 15 and 25 days after inoculation (DAI). Forty-eight flasks were placed in each of the three growth chambers.

Surface colonization of the straw by mycelium was visually assessed 2, 3 and 4 DAI. After five days, *F. oxysporum* mycelia began to colonize the internal portion of the straw pieces, thus visual estimation of substrate colonization was discontinued. After 5, 10, 15 and 25 DAI, 12 flasks were randomly selected from each temperature treatment level. In six flasks selected from each temperature level, the number of microconidia, macroconidia and chlamydospores were determined by washing the flasks twice with 25 ml distilled water and the conidial densities estimated with the aid of a haemocytometer.

The second set of six flasks was removed from the temperature treatment and the flasks were left at room temperature (28°C). Half of the contents of each flask was removed, placed in a glass petri dish, air dried for three days, ground to a fine powder with an electric coffee grinder and sieved for uniformity of particle size (250 µm). This powder was mixed with sterilized sand (0.01 g of powder: 0.99 g of sand) and 0.5 g of the mixture was plated onto PDA plates. The number of colony-forming units (CFU) was counted after 16 h with the aid of a stereomicroscope. The other half of the colonized inoculum was retained in the flasks and was used to determine inoculum viability at one month intervals for one year. At each time period, dried sorghum pieces (0.5 cm³) were placed onto PDA. Viability was assessed as mycelium growth.

Effect of substrate

Seven grams of sorghum straw, sorghum fibres (thread-like) or sorghum glumes were placed in 250 ml Erlenmeyer flasks with 28 ml water, or 21 ml water with the glumes. Forty-eight flasks of each substrate were prepared, autoclaved, inoculated and incubated at 28°C. Inoculated flasks were arranged in a split-plot design with six replicates. Substrate type and growth duration were the treatment factors examined. The trial was conducted twice. Data were collected as described above.

Effect of moisture

Sorghum straw pieces (2 cm³) were moistened as follows: (i) soaking overnight (7 g of straw in excess water overnight and then the free water was discarded); (ii) adding 21 ml of water to 7 g of straw; and (iii) adding 28 ml water to 7 g of straw. Thirty-two replicates of each moisture treatment were prepared in 250 ml Erlenmeyer flasks, autoclaved, inoculated and incubated at 28°C as described previously. Spore production in four flasks and colony-forming units in four flasks were determined 5, 10, 15 and 25 DAI. Each treatment had four replicates and the experiment was conducted twice. Data were collected as described above.

Data analysis

Prior to analysis of variance, the data (+0.5 to accommodate zero values) were square-root transformed. For each of the three experiments, the results of the replicate trials were pooled because homogeneity of variances was confirmed with Bartlett's test (Gomez and Gomez 1984). Differences in mean values were determined at $P<0.05$ according to the Student-Newman-Keuls test.

Results and discussion

Propagules of *F. oxysporum* (M12-4A) were produced over the range of temperatures tested. Temperature and growth-duration significantly affected the type of propagules produced, but there was no interaction between temperature and growth-duration. Colonization of straw pieces was most rapid at 28°C. After five days of incubation, most of the substrates were fully colonized or the mycelium was colonizing

the interior of the straw pieces. Thus, differences amongst the treatments could not be distinguished visually.

Temperature did not significantly affect the overall number of CFU (Fig. 1). Microconidia production was significantly lower at 24°C, macroconidia production significantly lower at 32°C, and differences in chlamydospores were not significant. When microconidia and macroconidia are considered together, highest production occurred at 28°C.

For all temperature treatments, maximum CFU occurred between 5-10 DAI (Diarra 1995). The highest production of microconidia occurred at five DAI at 28°C and 32°C and then decreased. Macroconidia production peaked between 15 to 20 DAI at 24°C and 28° C. Chlamydospore production began 10 DAI at the two warmer temperatures and then increased linearly. From these results, 28°C was the temperature treatment chosen for further production studies.

Substrate type and growth-duration significantly affected spore production and colony-forming units and no interaction was observed between these two factors. Maximum CFU and macroconidia production occurred on glumes, whereas highest chlamydospore production was on sorghum straw and the highest microconidia production occurred on sorghum fibres (Fig. 2). Sorghum glumes were colonized more rapidly by *F. oxysporum* than sorghum straw and fibres, but all were fully colonized after five days.

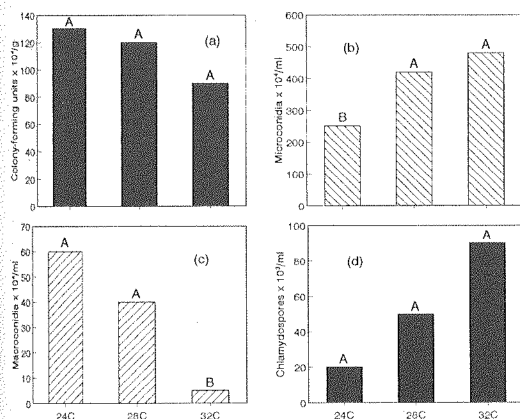

Fig. 1. Effect of temperature on spore production and colony-forming units of *Fusarium oxysporum* (M12-4A): (a) colony-forming units; (b) microconidia; (c) macroconidia; and (d) chlamydospores. Data are means of three trials. Histogram bars having the same letter show data that are not significantly different (Student-Newman-Keuls test, $P < 0.05$)

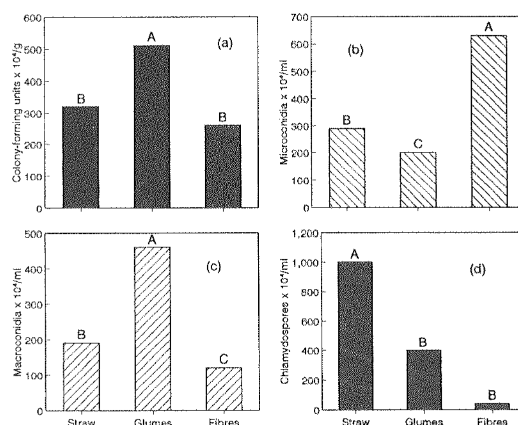

Fig. 2. Effect of sorghum straw, glume and fibre substrates on spore production and colony-forming units of *Fusarium oxysporum* (M12-4A) at 28°C: (a) colony forming units; (b) microconidia; (c) macroconidia; and (d) chlamydospores. Data are means of two trials. Histogram bars having the same letter represent data that are not significantly different (Student-Newman-Keuls test, $P < 0.05$).

Peak CFU occurred seven DAI when inoculum was grown on fibres, at 15 DAI when grown on straw, and 18 DAI when grown on glumes (Diarra 1995). Production of microconidia peaked at 15 DAI when grown on sorghum fibres or glumes, but declined linearly after five DAI when grown on straw. Macroconidia production was greatest between 15-20 DAI for all three substrates and chlamydospore production was still increasing at 25 DAI for all substrates.

Spore production was affected by moisture level and growth duration, but no interaction between these factors was detected. Colonization was more rapid on sorghum straw (2 cm^3) soaked overnight than on straw prepared in a 3:1 or 4:1 ratio with water. However all substrates were fully colonized after five days. Moisture level did not affect the number of CFU or the number of chlamydospores (Fig. 3). The highest production of microconidia and macroconidia occurred on straw soaked overnight. Maximum CFU was obtained between 8-17 DAI for all moisture regimes (Diarra 1995). Microconidia production on soaked straw and the 3:1 moisture regime peaked at 10 and five DAI, respectively, then decreased, whereas microconidia production in the 4:1 regime was still increasing linearly at 25 DAI. Macroconidial production was highest at 18-20 DAI for all three moisture regimes. Chlamydospore production began 10 DAI and was still increasing at 25 DAI for all three

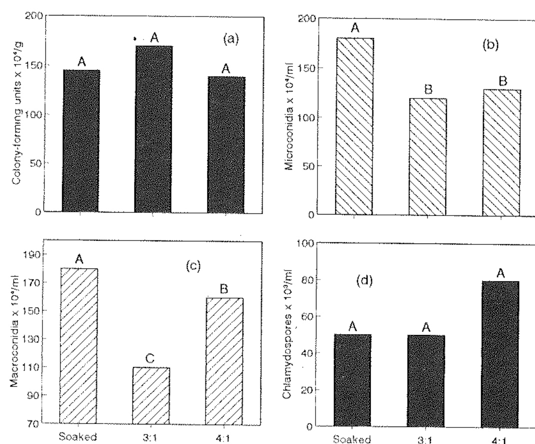

Fig. 3. Effect of moisture regime on spore production and colony-forming units *Fusarium oxysporum* (M12-4A) at 28°C: (a) colony-forming units; (b) microconidia; (c) macroconidia; and (d) chlamydospores. Data are means of two trials. Histogram bars having the same letter represent data that are not significantly different (Student-Newman-Keuls test, $P < 0.05$).

moisture regimes. Soaking overnight was also suitable for the sorghum glume substrate (data not presented). Soaking overnight, was the best moisture regime for substrate preparation, and was the simplest approach as it did not require a water measurement.

Several workers have grown other *Fusarium* spp. over a range of temperatures or on various substrates to produce biocontrol inoculum. For example, the optimum temperature for two other striga pathogens, *Fusarium nygamai* and *F. semitectum* var. *majus*, was alternating 30°C / 20°C day/night (Abbasher 1994).

When the *F. oxysporum* (M12-4A) was grown on sorghum straw or sorghum glumes, it survived and remained viable for at least 12 months when stored at room temperature in a dry state.

Hildebrand and McCain (1978) used wheat straw as a substrate to mass produce *F. oxysporum* f. sp. *cannabis* for the control of marijuana (*Cannabis sativa* L.). The highest production of infective propagules occurred on sorghum glumes and was the best substrate for *F. oxysporum* growth and sporulation. Glumes are abundant, available, inexpensive and do not require any further processing prior to delivery or application of the biocontrol inoculum to the field. Glumes are considered a waste material after threshing, as they have virtually no value for farmers, especially compared to straw that could be used for livestock feed. In addition, glumes, unlike straw, do not require cutting and therefore no additional labour is required to prepare the substrate. Hence glumes, which are soaked overnight in water and incubated at 28°C (a temperature easily attainable under shade in Mali during the growing season), are proposed as the basic system for *F. oxysporum* mass production and delivery of the 'product' to the field.

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