Technique to Induce Sporulation in *Thanatephorus cucumeris*

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ABSTRACT

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A new, reliable, and rapid technique to induce sporulation in anastomosis group (AG) 1 and AG2-2 of *Thanatephorus cucumeris* (= *Rhizoctonia solani*) is reported. This technique makes use of fungistatic stress by mancozeb to stimulate the production of basidiospores. No environmental conditions (except the incubation temperature of 25 or 30 C) need to be adjusted in this technique.

Additional key words: perfect state

A number of in vitro techniques to induce sporulation in *Thanatephorus cucumeris* (Frank) Donk. (= *Rhizoctonia solani* Kühn) have been reported (1,3–6, 8–10). The methods described, such as controlling the nutrient status of growth media by transferring cultures from rich to minimal media (nutrient stress) (1,3–5,8) and controlling environmental conditions such as temperature, light, humidity, pH, and CO_2 levels of the atmosphere (1,4–6,8), do not always prove reproducible, and some are tedious. Good control and reliability in the method of production of basidiospores of *T. cucumeris* within a relatively short time would be useful to workers who undertake studies on single-basidiospore isolates of selected anastomosis groups (AG). The principle underlying the technique described in this paper is the application of fungistatic stress, which stimulates the fungus to produce its sexual stage.

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MATERIALS AND METHODS

A 1.5-cm-diameter filter paper disk (Whatman No. 1) soaked in a 5% aqueous suspension of formulated

mancozeb (Dithane F-45, 37% flowable formulation, or Dithane M-45, 80% wettable powder formulation) was placed on the surface of 2% distilled water agar (WA) in a 100-mm-diameter plastic culture plate about 2–3 cm from the periphery. The plates were inoculated with a 5-mm-diameter agar plug cut out of a 3- to 4-day-old culture of *R. solani* (a single isolate each of AG1, AG2-1, AG2-2, AG3, AG4, or AG5) grown on either potato-dextrose agar (PDA), cornmeal agar (CMA), or WA and placed inverted on the surface of the agar about 3 cm from the filter paper disk. The plates were then incubated at 25 or 30 C in the dark. In the case of AG1 and AG4, the experiment was repeated at least five times with three replicates each time, whereas in the case of AG2-1, AG2-2, AG3, and AG5, the experiment was repeated once with three replicates each time.

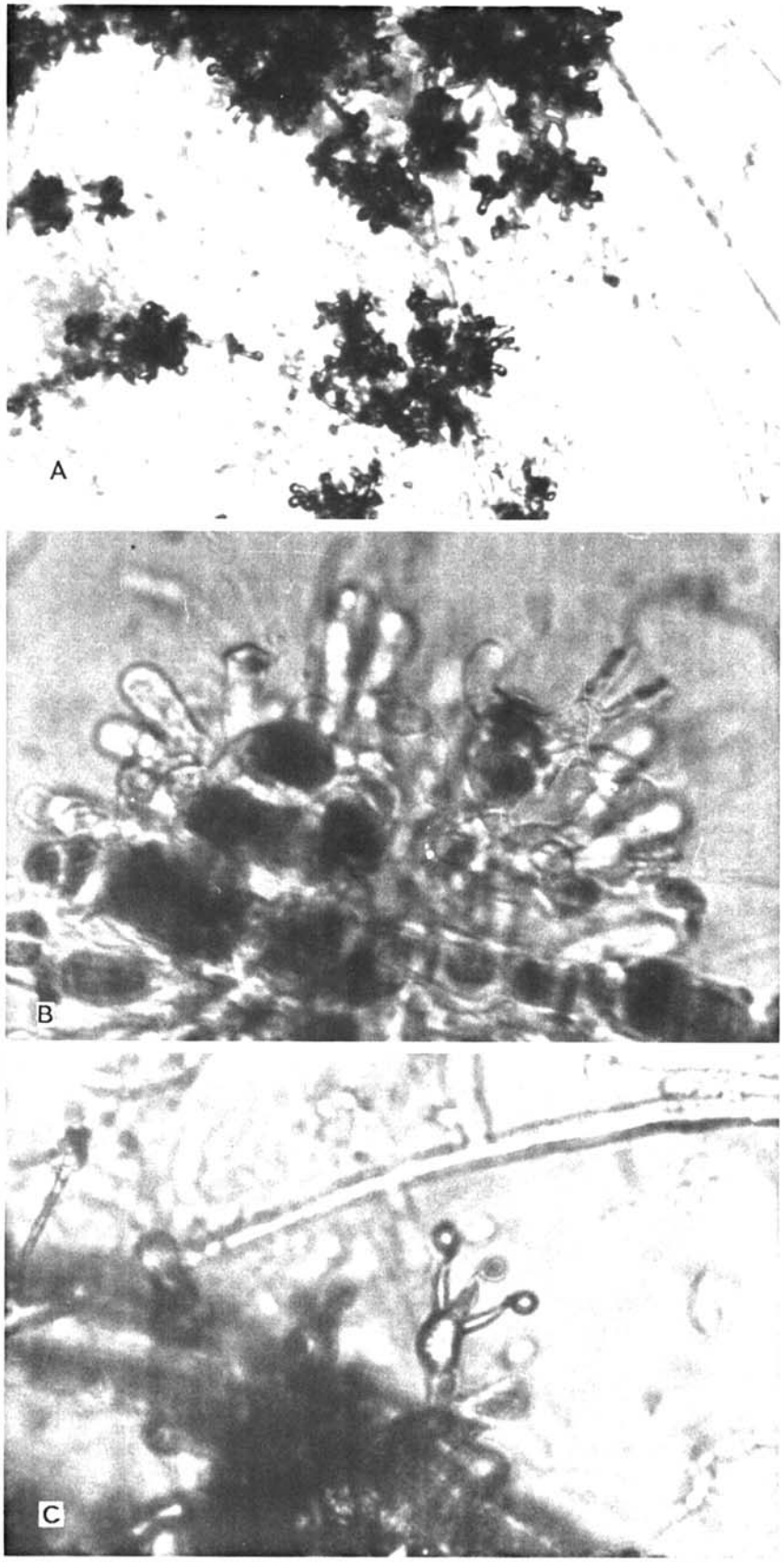

Fig. 1. Anastomosis group 1 of *Thanatephorus cucumeris* showing sporulation: **(A)** Clusters of basidia and probasidia under low magnification (174×). **(B)** Stages of development of basidium in a single cluster (1,388×). **(C)** Single basidium with basidiospores borne on sterigmata (1,348×).

RESULTS AND DISCUSSION

After 5–7 days of incubation, there was

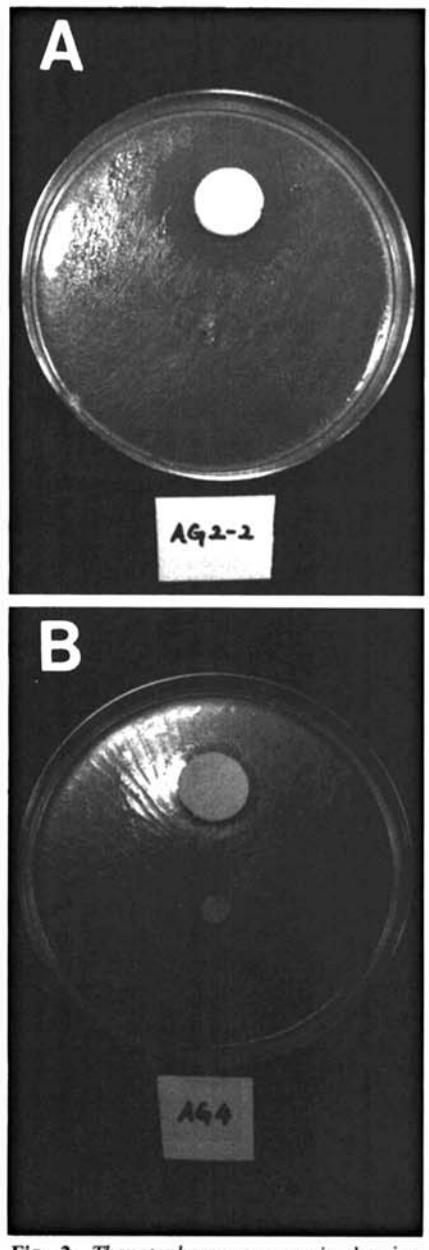

Fig. 2. *Thanatephorus cucumeris* showing zone of inhibition in response to mancozeb: **(A)** Anastomosis group (AG) 2-2 showing wide zone of inhibition and sporulation around margin. **(B)** AG4 showing narrow zone of inhibition and no sporulation.

profuse branching and cell division of the hyphae starting to produce the hymenium (7). After 10 days of incubation, several stages of basidium formation were observed. Tiny black spots developed (which proved to be clusters of basidia), and when examined under the microscope, stages of development of basidia, basidia bearing the basidiospores on sterigmata, and dispersed basidiospores were observed (Fig. 1).

After 10–15 days at 25 or 30 C, AG1 (microsclerotial form) and AG2-2 developed basidia and basidiospores in clusters around the zone of inhibition in response to mancozeb (Fig. 2A). Subsequently, basidiospores developed even farther from the margin of the zone of inhibition in AG1.

AG4 isolates failed to produce basidia even after 1 mo of incubation, though there was active branching and cell division in the hyphae around the zone of inhibition. Similarly, AG2-1, AG3, and AG5 showed profuse branching of hyphae in response to mancozeb but did not sporulate.

This is a reliable and reasonably quick technique to produce the sexual stage of *R. solani* belonging to AG1 and AG2-2. The inability to produce a sexual stage in AG2-1, AG3, AG4, and AG5 may be due in part to the fact that mancozeb is not as effective a fungistat on these isolates. This statement is based on the observation that, in these four AG, the zone of inhibition in response to mancozeb was narrower than those in AG1 and AG2-2 (Fig. 2A vs. B).

In this technique, only two incubation temperatures (25 and 30 C) were used as opposed to other techniques reported (2,4–6,8), and this may be another reason why AG2-1, AG3, AG4, and AG5 failed to sporulate in this study. The initial growth medium of the culture also can be varied as described elsewhere (1,3–5,8). This technique has advantages over others (1,3–6,8–10) although it does not apply at this stage to all AG of *R. solani*.

The principle of applying fungistatic stress to stimulate sporulation as reported with this technique may be extended to other AG using other fungistatic chemicals.

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