Protection of *Fusarium* and *Verticillium* Propagules from Selected Biocides Following Ingestion by *Pristionchus lheritieri*[1]

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Abstract: Spores of *Fusarium oxysporum* f. *lycopersici* or *Verticillium dahliae* (potato strain) ingested by *Pristionchus lheritieri* (a saprozoic nematode) survived exposure to otherwise lethal solutions of free chlorine, thiabendazole or the methyl ester of 1-butylcarbamoyl-2-bendimidazole carbamic acid. Viability of mechanically-freed or defecated spores were the same. *Key Words: Pristionchus lheritieri, Fusarium oxysporum* f. *lycopersici, Verticillium dahliae,* Spores, Biocides, Protection.

Even though many investigators probably have observed fungus spores within saprozoic nematodes, the possible rôle of this relationship in plant disease epidemiology has received little attention. Jensen (1) presented evidence that spores of two well-known genera of plant pathogens, *Fusarium* and *Verticillium* survive passage through the nematode's alimentary canal.

We have become interested in this interrelationship from the standpoint of possible protection of fungus spores after ingestion by nematodes. The constant association of saprozoic nematodes would provide an excellent opportunity for ingestion of fungus spores, and the resulting protection of the nematode body should help the fungus survive adverse environmental conditions, including biocides. This study considers one aspect of the interrelationship of saprozoic nematodes and fungus pathogens, the protection of ingested fungus spores from certain biocides.

MATERIALS AND METHODS

Pristionchus (*Diplogaster*) *lheritieri* (Maupas) Paramonov was the nematode vector; race R5–6 *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. and Hans. and *Verticillium dahliae* Kleb. (potato strain) were the fungus components of the biological system. Biocides consisted of free chlorine (obtained from sodium hypochlorite), TBZ® (thiabendazole) and Benlate®[1] (1-butylcarbamoyl-2-bendimidazole carbamic acid, methyl ester).

Following ingestion of *Fusarium* (microconidia) or *Verticillium* (conidia) spores by nematodes placed in Petri dish cultures of fungi, the nematodes were washed with tap and distilled water in a 400-mesh strainer to eliminate most external spores. Washed nematodes were added to the concentrations of the three biocides indicated in Fig. 1–4. Beakers containing these treatments were placed upon a magnetic stirrer to maintain uniform suspensions during the course of the experiment. At various time intervals (2, 15, 30, and 60 min) 1 ml samples were withdrawn from each treatment. A duplicate set of beakers containing adjusted spore suspensions (usually 2000 spores per ml) served as controls. The contents of these beakers received the same biocide treatments and sampling procedure as those containing nematodes which had ingested spores.

Treatments were terminated in various ways according to the kind of biocide used. When chlorine was used, sodium-thiosulfate was added to neutralize free chlorine. When Benlate or TBZ were used, treated samples

Received for publication 24 February 1970.

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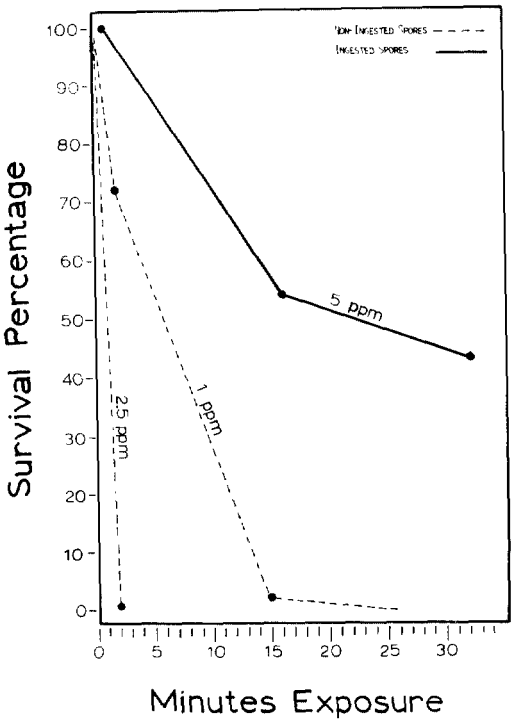

FIG. 1. Survival of ingested (by *Pristionchus lheritieri*) and non-ingested *Fusarium oxysporum* f. *lycopersici* spores in various concentration × time exposures to free chlorine.

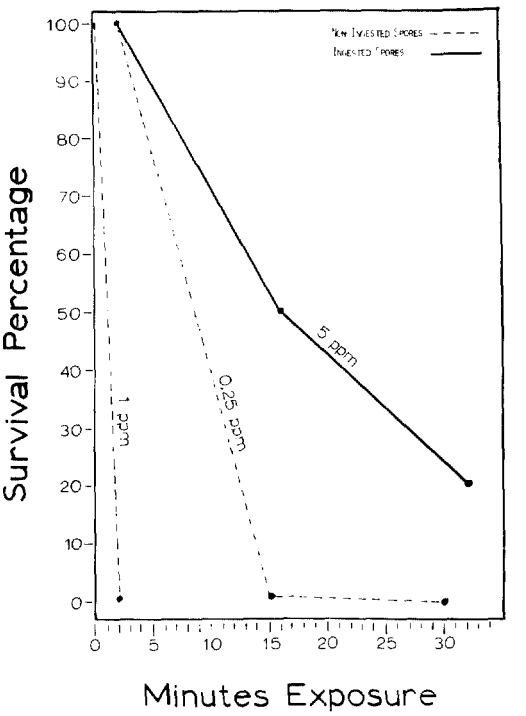

FIG. 2. Survival of ingested (by *Pristionchus lheritieri*) and non-ingested *Verticillium dahliae* (potato strain) spores in various concentration × time exposures to free chlorine.

were emptied into a 400-mesh strainer and rinsed with tap and distilled water. Later, the nematode carriers (10 pre-adult females for *Fusarium* and 5 for *Verticillium*) were removed from neutralized or washed samples, squashed beneath a dissecting needle in sterile water and then added to Petri dishes. Cooled (45–50C) potato dextrose agar containing a trace of streptomycin-sulfate (0.27 gm of 37% streptomycin-sulfate per liter) was added to these plates which were rotated vigorously to insure even distribution and mixture of contents. Another portion of nematodes from each neutralized or washed sample was placed directly upon the agar to verify spore defecation and survival. After appropriate incubation times for growth, fungus colonies were counted.

Control samples also were processed in different ways depending upon the nature of the biocide used. Samples receiving the chlorine treatment were neutralized with sodium-thiosulphate. Samples of treatments containing Benlate and TBZ received no special treatment to reduce the biocide carry-over because dilution with the culture media should have been adequate to minimize this effect. Control samples then were processed as were samples containing nematodes. Colony determinations and numbers were made by microscopic examination, special media and an electronic colony counter.

RESULTS

Although *Fusarium* and *Verticillium* spores tolerated minute concentrations of free chlo-

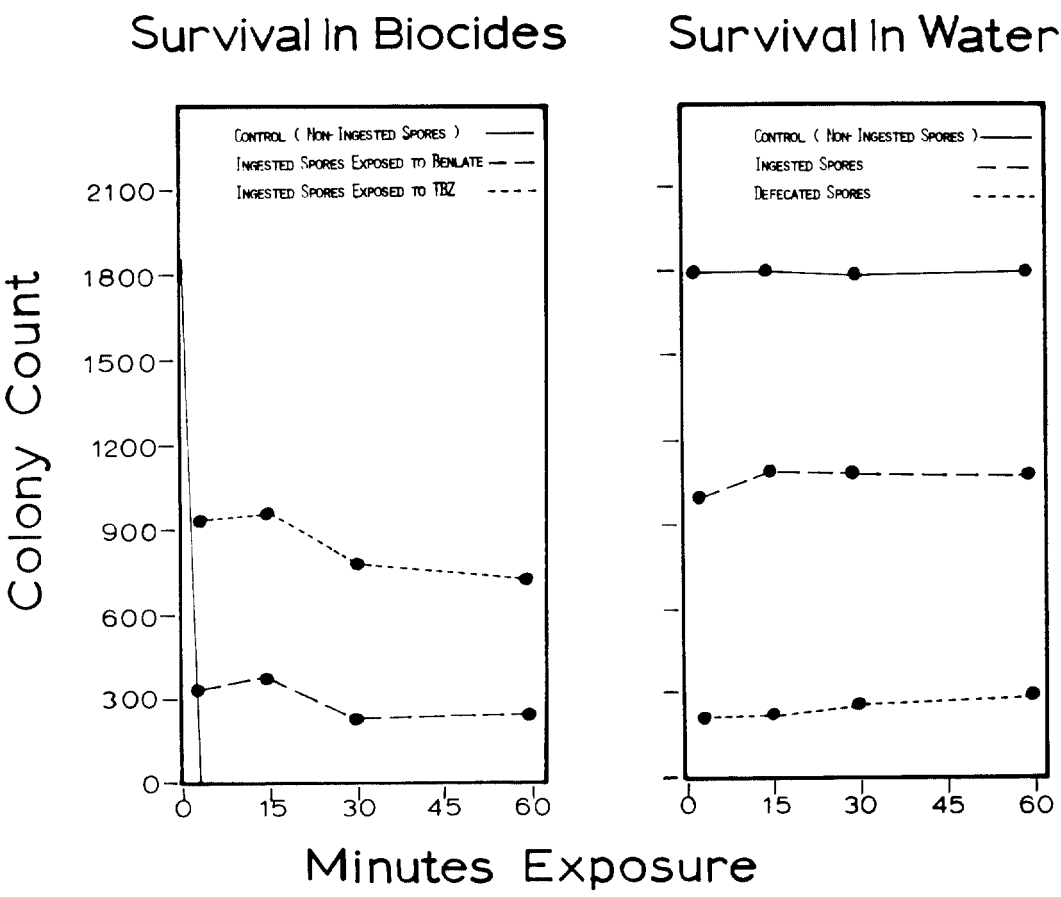

FIG. 3. Survival of ingested (by *Pristionchus lheritieri*) and non-ingested *Fusarium oxysporum* f. *lycopersici* spores in 100 ppm Benlate and TBZ and in water.

rine for brief time intervals, they failed to survive a two-minute exposure to 2.5 ppm or 1.0 ppm, respectively (Fig. 1–2). Many ingested spores, however, tolerated a 5 ppm concentration for 30 min (Fig. 1–2).

Fusarium and *Verticillium* spores also differed greatly in their tolerance to Benlate and TBZ. The lethal concentration and exposure with both materials was standardized at 100 ppm for *Fusarium* and 10 ppm for *Verticillium* in a two-minute exposure (Fig. 3–4). Death of ingested spores increased with longer exposure to the biocides, but a 60-minute exposure failed to kill all the spores of either fungus. More ingested *Fusarium* spores survived treatment with TBZ than with Benlate, whereas the reverse was shown with *Verticillium*. A 60-minute exposure at these same levels appeared to have little effect upon ingested spores (Fig. 3–4) as compared with elimination of non-ingested spores in the controls.

DISCUSSION

Ingestion, passage and defecation of fungus spores through the alimentary canal of nematodes gives these propagules added protection against certain biocides. Although free chlorine causes a reduction of ingested

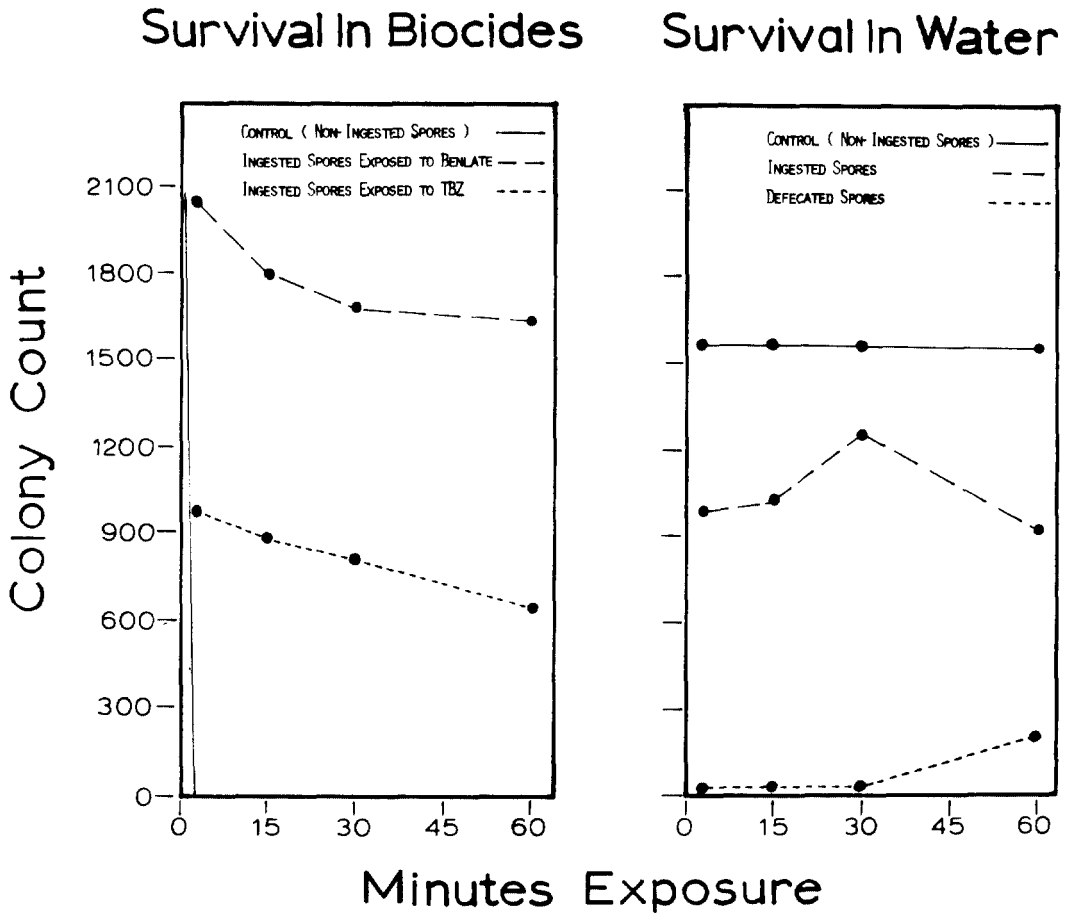

FIG. 4. Survival of ingested (by *Pristionchus lheritieri*) and non-ingested *Verticillium dahliae* (potato strain) spores in 10 ppm Benlate and TBZ and in water.

spore survival as time was extended (Fig. 1–2), survival to 16 hours was measured in preliminary experiments reported elsewhere (2). Another preliminary observation indicated that ingested spores survived a 50 ppm concentration to 60 min which was much higher than the 5 ppm recorded in Figures 1 and 2. *Fusarium* spores tolerate higher chlorine concentrations and longer exposures than do those of *Verticillium*.

Investigations with the two fungicide compounds gave similar results: potential nematode vectors tolerated biocide concentrations lethal to exposed spores. Also there was a ten-fold difference in biocide tolerance which favored *Fusarium* over *Verticillium* spores. Ingested *Fusarium* spore survival in water indicates very little change in colony count resulting from defecation (Fig. 3). Colony counts of ingested *Verticillium* spores, however, are erratic and actually show an increase then a rapid decrease as defecation increases (Fig. 4).

Thus, nematodes not only tolerate concentrations and exposures in excess of those lethal to fungus spores, but they can shield ingested spores from fungicidal treatments far in excess of recommended application

rates. The greater motility of nematodes compared to the movement of spores also favors wider dispersion of ingested spores. Critical examination of spores or colony growth to determine if passage through the vector's alimentary tract has had any effect upon the spores has not been made.

LITERATURE CITED

1. JENSEN, H. J. 1967. Do saprozoic nematodes have a significant role in epidemiology of plant diseases? Plant Dis. Rep. 51:98–102.
2. JENSEN, HAROLD J., and S. R. SIEMER. 1969. Protection of fungus spores from certain biocides following ingestion by saprozoic nematodes. J. Nematol. 1:12–13. (Abstr.)