Passage of Verticillium albo-atrum Propagules Through the Alimentary Canal of the Bulb Mite

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ABSTRACT

Specimens of the bulb mite, *Rhizoglyphus echinopus* (Acarina: Acaridae), reared on laboratory cultures of *Verticillium albo-atrum* (microsclerotial strain) in pectate-guanidine agar, produced fecal pellets containing conidia and fragments of microsclerotia. Fecal pellets collected aseptically were used to inoculate agar plates. New *V. albo-atrum* colonies developed from 76 percent of fecal pellets containing both kinds of propagules, while 92 percent of pellets containing only conidia tested positive for that fungus.

Treatments of fecal pellets with dilute sodium hypochlorite solutions and ultraviolet radiation killed conidia in pellets containing both conidia and microsclerotia. Development of *V. albo-atrum* from treated pellets indicated that the microsclerotial fragments were viable. Mites fed on laboratory preparations of pure microsclerotia produced fecal pellets free of conidia and composed almost entirely of microsclerotial fragments. New *V. albo-atrum* colonies developed from about 23 per cent of these pellets.

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Verticillium albo-atrum Reinke & Berthold (microsclerotial strain), the causal agent of Verticillium wilt of cotton (*Gossypium hirsutum* L.), attacks a wide variety of herbaceous and woody plants, including many of agricultural importance (8). This soilborne fungal pathogen has been partly responsible for diminishing cotton yields in the San Joaquin Valley of California during recent years (14).

Verticillium albo-atrum invades the host plant through the roots and moves systemically through the xylem vessels. When the host dies, the fungus produces microsclerotia in the xylem and adjacent plant tissues. In cotton, these resistant propagules are especially evident in the vascular region of the stem. Susceptible hosts in subsequent generations are infected by microsclerotia which have become free in the soil upon the decomposition of infested plant debris.

Saprophytic invertebrates, such as annelid worms, collembolans, fly larvae, psocopterans, and mites play an important role in decomposition processes (13). Most of these soil inhabitants are generalized feeders, and commonly ingest fungal hyphae and spores along with dead plant tissues (3, 9). Dobbs and Hinson (2) stressed the importance of invertebrates in the dispersal of fungal spores in the soil. Demonstration of the viability of fungal propagules in the feces of saprophytic invertebrates, however, has received only limited attention (6, 7, 10, 11, 12,15).

I have observed that numerous invertebrates invade and colonize decomposing cotton stems under field conditions. In the early stages of decay, these animals were most abundant in the outer vascular tissues of the stem, in tissues which often contained abundant *V. albo-atrum* microsclerotia. Microscopic examinations revealed whole or fragmented microsclerotia in the alimentary canal and fecal material of many of these animals. The present study was designed to determine whether fragments of *Verticillium* microsclerotia were viable after passage through the alimentary canal of the bulb mite, *Rhizoglyphus echinopus* Fumouze & Robin. The biology of this cosmopolitan species, commonly found in decomposing cotton debris in the San Joaquin Valley, is described by Garman (4). The study also provided information on the passage of *V. albo-atrum* conidia through this mite species.

MATERIALS AND METHODS.—Bulb mites from field soils in the San Joaquin Valley were reared in the laboratory on mature *V. albo-atrum* colonies in pectate-guanidine agar (PGN agar) (5). Mites reared on this fungus readily ingested hyphae, conidia, microsclerotia, and the agar substrate; and could be maintained for many generations without difficulty. The original source of the fungus was infested cotton stems from the San Joaquin Valley.

Prior to inoculation tests, 15-20 mites were removed from the stock cultures and allowed to feed on fresh *Verticillium* colonies in agar for 5 hours or longer. They were then removed with a curved needle and rinsed by passage through three baths of about 20 ml of sterile water in each of three small dishes. A small amount of wetting agent (1:1,000 aqueous dilution of Tergitol NPX, Sigma Chemical Co., St. Louis) was added to the first dish to permit submergence of the mites. Transfers from dish to dish were made with a pipette. In the third dish, where the mites were retained for up to 1 hour, each deposited from one to three fecal pellets. Since the pellets

were deposited in sterile water rather than on a solid substrate, the possibility of surface contamination was negligible. The pellets were passed through two additional sterile water rinses before being transferred individually with a sterile needle to the agar culture media. The integrity of the pellets, which appeared to be

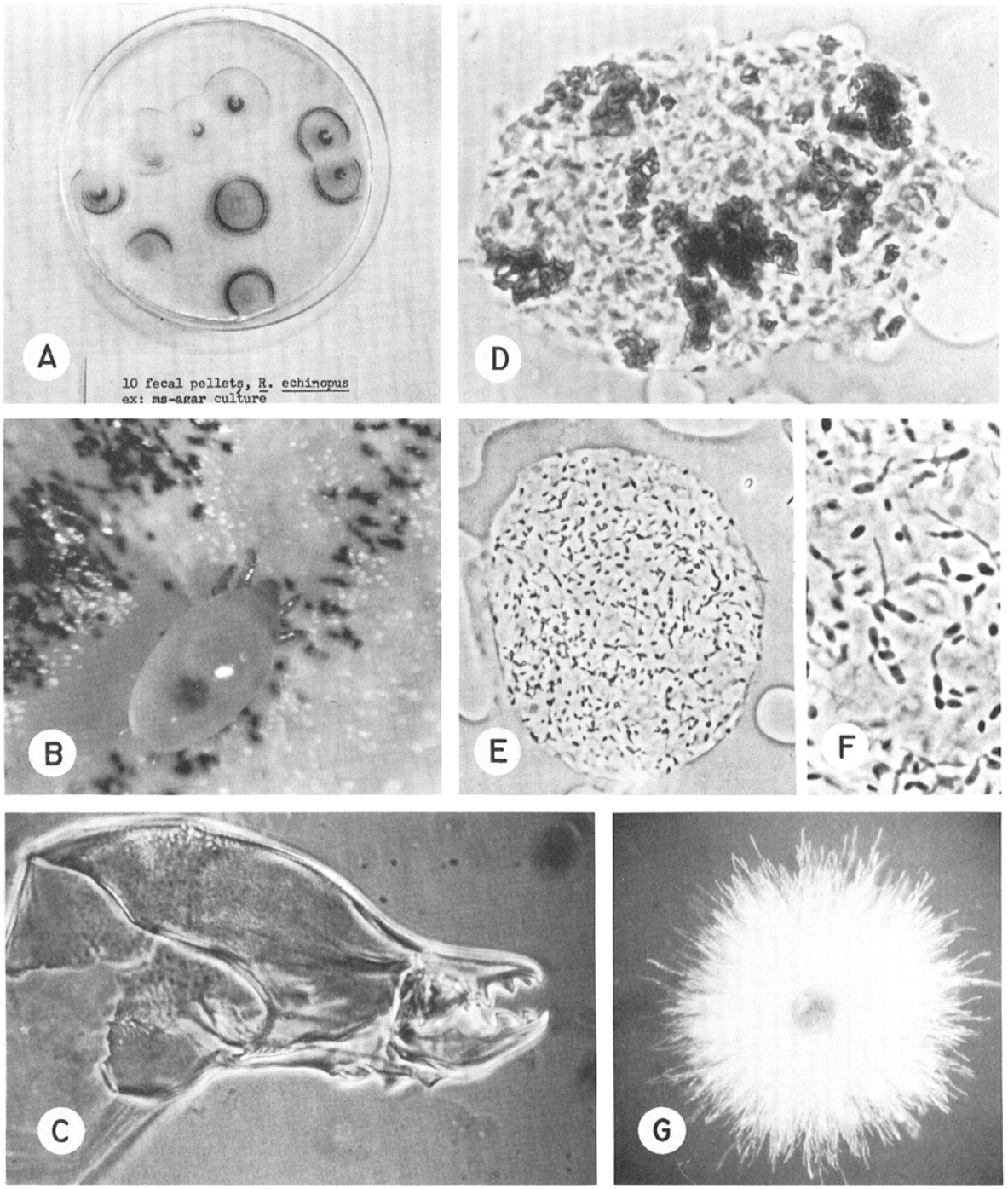

Fig. 1.-(A to G) Passage of *Verticillium albo-atrum* (microsclerotial strain) through the alimentary canal of the bulb mite, *Rhizoglyphus echinopus*. **A)** *Verticillium* colonies in pectate-guanidine agar. Each colony originated from a single mite fecal pellet. Dark areas are due to microsclerotia within each colony. **B)** Adult female bulb mite feeding on *Verticillium* in agar. **C)** A chelicera of the bulb mite. **D)** Fecal pellet of the bulb mite showing microsclerotial fragments and conidia. **E)** Fecal pellet on agar media showing conidia. Microsclerotial fragments are lacking. **F)** Enlargement of germinating conidia within a fecal pellet on agar media. **G)** *Verticillium* germinating from propagules within a bulb mite fecal pellet.

maintained by an enclosing membrane, allowed easy manipulation.

For treatments in dilute sodium hypochlorite solutions, groups of pellets from the last sterile-water bath were transferred to the treatment dish for periods ranging between 10 and 60 seconds. After exposure to the bleach, the pellets were rinsed in two successive sterile-water baths. Treatments with ultraviolet radiation were effected after the pellets were placed on the surface of the agar media. The emission of the mercury vapor, short-wave ultraviolet (UV) lamp, set at 13 cm from the agar surface, was principally at 253.7 nanometers.

The inoculated plates were incubated under room conditions for 10 days. *Verticillium* colonies of this age or older on PGN agar form numerous dark microsclerotia (Fig. 1-A, B). If present, these structures permit positive visual identification of this species.

Microscopic examination of fecal pellets from mites fed on *Verticillium* colonies revealed conidia as well as microsclerotial fragments (Fig. 1-D). Development of new colonies from these pellets, therefore, could have resulted from germination of microsclerotia or conidia or both. No fungal hyphae were observed in the fecal material.

To determine whether the conidia were viable, mites were fed on segments of *Verticillium* colonies containing hyphae and conidia, but not microsclerotia. Mites thus reared produced fecal pellets in which the only distinct structures were conidia (Fig. 1-E). These pellets were used to inoculate agar plates as described above.

Since pellets containing only conidia produced *Verticillium* colonies, it remained necessary to determine whether microsclerotial fragments were also viable. To do this, either the conidia in pellets containing both kinds of propagules had to be killed without killing the microsclerotia, or mites had to be fed on laboratory preparations of pure microsclerotia. Both methods were used. For the selective killing, treatments with dilute sodium hypochlorite or with UV radiation sufficient to prevent germination of conidia in pellets containing only conidia were applied to pellets containing both kinds of propagules. Development of *Verticillium* from treated pellets of the latter kind presumably resulted from germination of microsclerotial fragments.

Preparations of pure microsclerotia were obtained from cellophane-covered cultures of *Verticillium* in potato-dextrose agar. Mites fed on pure microsclerotia produced pellets free of conidia and composed almost entirely of microsclerotial fragments.

RESULTS.—In this study, 82.7% of 392 untreated fecal pellets from mites fed on *Verticillium* in agar served as a source of viable propagules when used to inoculate a culture medium. Whereas 92.1% of 165 pellets containing only conidia produced new fungal colonies, only 75.8% of 227 pellets containing both conidia and microsclerotia exhibited viable propagules (Tables 1 and 2). The cause of this difference is not clear. Microscopic examination of pellets containing conidia and placed on the agar media revealed that a high proportion of these propagules were able to germinate (Fig. 1-E, F).

Tests designed to kill conidia in pellets containing conidia and microsclerotial fragments indicated that these fragments also were viable (Tables 1 and 2). Treatment of pellets with a 1:100 dilution of commercial bleach for 10 seconds or longer was sufficient to kill all or most conidia (Table 1). Similarly, treatment with a 1:200 bleach solution for 20 seconds or longer (Table 1), or exposure to ultraviolet radiation for 3 minutes or longer (Table 2), prevented development of *Verticillium* from pellets containing only conidia. Pellets containing microsclerotial fragments exposed to these treatments gave rise to new *Verticillium* colonies (Tables 1 and 2), indicating that these fragments were viable.

Fecal pellets from mites fed on microsclerotia also gave

TABLE 1. Effects of treatments with dilute sodium hypochlorite on growth of *Verticillium albo-atrum* colonies from propagules in fecal pellets of bulb mites fed on *V. albo-atrum* colonies on pectate-guanidine agar

Treatment[a]	Exposure time (sec)	Pellets with conidia and microsclerotial fragments		Pellets with conidia only	
		Pellets (no.)	Growth (%)	Pellets (no.)	Growth (%)
1974 Trials:					
None	. . .	148	70.3	50	92.0
0.05% NaOCl	30	45	53.3	25	0.0
	60	15	66.7	15	0.0
0.026% NaOCl	30	15	86.7	15	0.0
1975 Trials:					
None	. . .	52	88.5	67	91.0
0.05% NaOCl	10	24	66.7	59	6.8
	20	17	52.9	18	0.0
	30	18	50.0	33	6.1
	40	18	50.0	6	0.0
	50	12	50.0	6	0.0
	60	12	16.7	10	0.0
0.026% NaOCl	10	25	100.0	51	51.0
	20	20	100.0	18	5.6
	30	10	100.0	10	0.0
	40	10	100.0	10	0.0

[a]0.05% = 1:100 aqueous dilution of commercial bleach (5.25% by weight); 0.026% = 1:200 dilution.

TABLE 2. Effects of ultraviolet radiation on growth of *Verticillium albo-atrum* colonies from propagules in fecal pellets of bulb mites fed on *V. albo-atrum* colonies on pectate-guanidine agar

Ultraviolet exposure time[a] (seconds)	Pellets with conidia and microsclerotial fragments		Pellets with conidia only	
	Pellets (no.)	Growth (%)	Pellets (no.)	Growth (%)
None	27	81.5	48	93.8
10	5	80.0	41	97.6
20	5	60.0	11	72.7
30	10	70.0	33	78.8
40	5	80.0	5	100.0
60	16	68.8	31	71.0
120	10	60.0	26	38.5
180	10	50.0	25	4.0
240	26	80.8	20	0.0
300	11	63.6	20	0.0

[a]Exposure to short-wave ultraviolet radiation from a 1.3 W mercury vapor lamp with emission at 253.7 nanometers at a distance of 13 cm.

TABLE 3. Development of *Verticillium albo-atrum* colonies on pectate-guanidine agar from individual microsclerotia and from fecal pellets of bulb mites fed on microsclerotia

Inoculum	Inoculum units (no.)	Verticillium colonies (%)
1974 Trials:		
Microsclerotia	90	56.7
Fecal pellets	62	0.0
1975 Trials:		
Microsclerotia	162	85.8
Fecal pellets:		
No treatment	304	13.5
1:100 NaOCl, 20-60 secs.	137	23.4

rise to new *Verticillium* colonies (Table 3). The germination, however, was much lower than pellets from mites fed on this fungus in agar. The apparent lack of viable propagules in fecal material of mites in the 1974 trials (Table 3) may have been due to the weakened condition of the microsclerotia prior to ingestion. Although the 1975 trials were made with more vigorous microsclerotia (Table 3), they were contaminated with *Penicillium* sp. Of 162 microsclerotia, 9.9% gave rise to *Penicillium* sp. rather than *Verticillium* colonies. Fecal pellets of mites fed on these microsclerotia gave rise to *Penicillium* sp. in 38.8% and *Verticillium* in 13.5% of 304 pellets (Table 3). To eliminate *Penicillium* sp. conidia, fecal pellets from mites fed on the 1975 preparation of microsclerotia were treated in sodium hypochlorite solutions for periods ranging between 20 and 60 seconds. Of 137 treated pellets, 23.4% gave rise to *Verticillium* (Table 3), while only 0.7% gave rise to *Penicillium* sp.

DISCUSSION.—Adult females of *R. echinopus* (Fig. 1-B) range between 750 and 900 μm in body length. All stages have powerful chewing chelicerae which are clearly capable of fragmenting microsclerotia and plant debris (Fig. 1-C). Fecal pellets of adult mites average about 90 μm in length (Fig. 1-D, E). *Verticillium* microsclerotia are elongate, variable in size, with a mean length of about 55μm. Fragments of microsclerotia in the fecal material measured up to about 17 × 27μm (Fig. 1-D). The larger fragments appeared to contain as many as 12 to 15 cells.

Since fewer colonies of *Verticillium* grew from fecal pellets of mites fed on pure microsclerotia than from intact microsclerotia (Table 3), it appeared that these fragments were weakened or killed by passage through the alimentary canal of this mite species. It is possible that in order for fragments to remain viable upon passage through these mites, they had to be comprised of a certain minimum number of cells. The outer cells may have protected the inner cells from the action of digestive enzymes. In addition, not every cell comprising microsclerotia has the ability to germinate (1). Thus, some pellets, although containing microsclerotial fragments, may not have included cells initially capable of germination.

The slightly elongate conidia, which measured 4 to 7μm long, passed easily through the digestive tract. Such passage appeared to have no adverse effect on these propagules. This is noteworthy in view of the transitory nature of these structures.

The data presented provide conclusive evidence that *Verticillium* conidia and at least certain cells in microsclerotial fragments can pass through the digestive tract of the bulb mite in a viable state. Although these findings do not indicate that the bulb mite plays a role in the epidemiology of Verticillium wilt, passage of viable propagules through this mite in vitro suggests the possibility of similar relationships under field conditions.

Further investigations are needed to clarify the role of this mite and other saprophytic invertebrates in accelerating decomposition processes and in freeing microsclerotia from infested cotton debris.

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