Mode of Transmission of *Corynebacterium insidiosum* by *Ditylenchus dipsaci*

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Transmission of *Corynebacterium insidiosum* (McCull.) Jensen (cause of bacterial wilt in alfalfa) by the stem and bulb nematode *Ditylenchus dipsaci* (Kühn) was demonstrated by Hawn (3). Disease development in plants where *D. dipsaci* was the vector was comparable to that in the controls where wilt was induced by the root-ball soak method devised by Cormack, Peake and Downey (1). Furthermore, the development of wilt was stimulated by this complex because the nematode itself lowered bacterial wilt resistance in alfalfa (4).

Since a 1963 study (3) showed that the lumen of the buccal stylet was too narrow to permit ingestion of *C. insidiosum*, the possibility that the bacterium was carried on the cuticle of *D. dipsaci* needed further investigation.

This paper reports the results of additional work performed to elucidate bacterial wilt transmission by *D. dipsaci*.

MATERIALS AND METHODS

CULTURE METHOD: Three treatments were used: (i) Living specimens of *D. dipsaci* were taken from infected alfalfa buds and transferred to tap water. Four of these nematodes were then placed singly on the surface of Burkholder's agar in separate petri dishes to assess bacterial contamination. (ii) Twelve nematodes were transferred from tap water to 3% hydrogen peroxide for 5 min and then placed, four per dish, on Burkholder's agar. (iii) Twelve nematodes were removed from tap water and treated with 3% hydrogen peroxide for 5 min, washed once in sterile distilled water, and placed in a suspension of *C. insidiosum* for 10 min. Each nematode was returned to sterile distilled water with a clean microneedle and, after 30 min, transferred aseptically to Burkholder's agar, four per dish.

All treatments were incubated at room temperature (21 to 23 C) for 14 days. Smears of the bacterial colonies were made, Gram-stained (2), and examined microscopically.

HISTOLOGY METHOD: Stem nematodes were taken from stem galls on wilt-free alfalfa. The nematodes were transferred individually to a water suspension of *C. insidiosum*, rinsed in sterile distilled water for 10 min, and removed by sterile microneedle to a drop of sterile water on a clean microscope slide. Ten such transfers were made to each drop of water. Each nematode was then pierced with a clean needle to allow the contents of the body to escape. The liquid was evaporated from each slide by gentle heat. The adhesive effect of the nematode protoplasm held the specimens *in situ* for staining. The release of body contents prevented the uptake of carbomethyl violet. Furthermore, because the Lugol's iodine used in the staining procedure imparted an amber color to the cuticle, microscopic examination of the features of the cuticle and detection of Gram-positive bacteria was facilitated.

Nyfeldt's modification (2) of Gram's method of differential staining was used with some further modification in time schedule.

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The procedure was as follows: The slide was warmed to remove all free moisture, cooled to room temperature, immersed 30 sec in carbomethyl violet, and then rinsed with tap water to remove excess carbomethyl violet. Next, the slide was immersed 30 sec in Lugol's iodine followed by 30 sec in 95% ethanol. Each slide was treated with safranin for 30 sec, rinsed with tap water to remove excess safranin, gently blotted dry, and warmed to remove any remaining water. A drop of immersion oil was placed over the specimen and covered with a glass cover slip, which was then sealed with Z® (Bennett's, 65 West First South Street, Salt Lake City, UT 84110). Specimens were examined under oil immersion at 1200×.

RESULTS

CULTURE METHOD: Treatment i.—There was a profusion of Gram-negative bacterial colonies in all dishes. These colonies developed along the 'tracks' made by the nematodes.

Treatment ii.—No bacterial growth.

Treatment iii.—Typical blue-pigmented, circular, raised, butyrous colonies of *C. insidiosum* developed in two of the three plates.

HISTOLOGY METHOD: Gram-positive bacteria, morphologically similar to *C. insidiosum*, were observed adhering to the cuticles of stem nematodes that had been exposed to the wilt bacteria (Fig. 1).

CONCLUSIONS

Both the nutrient culture and histological phases of this experiment demonstrated contamination of *D. dipsaci* by immersion in an aqueous suspension of *C. insidiosum*. The presence of numerous wilt bacteria on the nematode cuticles after immersion in clean water demonstrated how *C. insidiosum* could be transmitted from wilt-infected to healthy alfalfa by stem nematodes.

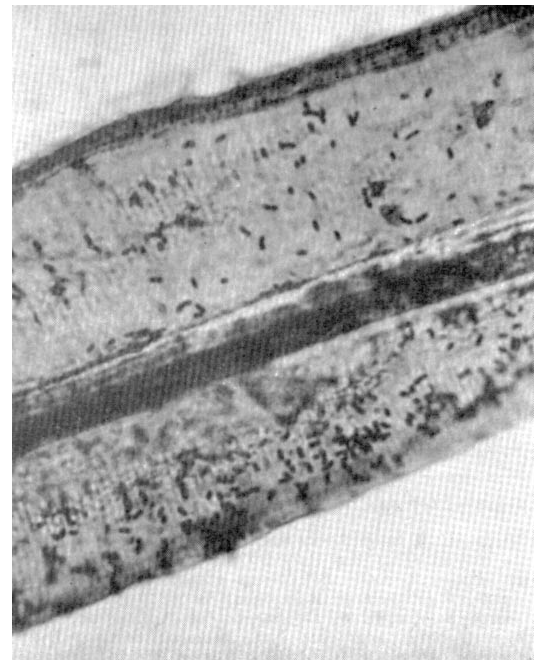

FIG. 1. *Corynebacterium insidiosum* adhering to the cuticles of two specimens of *Ditylenchus dipsaci* after 10 min in a water suspension of the bacteria and a 30 min distilled water rinse.

LITERATURE CITED

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