New Technique for Studying a Bacterium/Nematode Interaction in Alfalfa

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In 1963, Hawn (2) proved *Ditylenchus dipsaci* (Kühn) to be a vector of the alfalfa wilt bacterium, *Corynebacterium insidiosum* (McCull) Jensen. Rate of wilt development and severity were comparable to results obtained with the root-ball-soak method described by Cormack *et al.* (1) in 1957.

Subsequent evaluation of the role of the stem nematode in breaking resistance was hampered by the lack of a good technique for determining (i) the time between first appearance of nematode infection and the breaking of resistance, (ii) whether the nematode predisposes alfalfa to wilt, and (iii) whether both organisms must be present at the site of infection. To provide answers to these questions the following technique was based on one developed by Pawlowski in 1963 (3) and used by Pawlowski and Hawn in 1964 (4).

Sow alfalfa in 15-cm pots of steam-sterilized soil. One week after emergence, thin to 3/pot, and leave undisturbed until the upper tap root is about 1 cm diam. Dig the plants, wash, and remove the top growth and lateral roots. Bisect the crown and upper 5 cm of root of each plant with a sharp, clean scalpel (Fig. 1A). Transplant the 'twin-crowned' plant to a 15-cm plastic pot (Fig. 1B). Separate the two parts of the crown and set an 8-cm-deep Plexiglas (Rohm and Haas Company, Philadelphia, Pennsylvania) partition between them with its bottom resting on the inner shoulder of the pot and the ends of the strip fitting snugly against the upper walls of the pot (Fig. 1C). Add sterilized soil and pack it up to the base of the crowns.

The Plexiglas divider effectively separates the two sides of the crown and prevents mixing of organisms during inoculation and incubation. Place the plants in a growth chamber at 16 C and 16 hr of light (3,000 f.c.) per day until the cut root tissue has healed and both crowns of each plant have good top growth (Fig. 1D). The plants are then ready for inoculation with *D. dipsaci* and *C. insidiosum*.

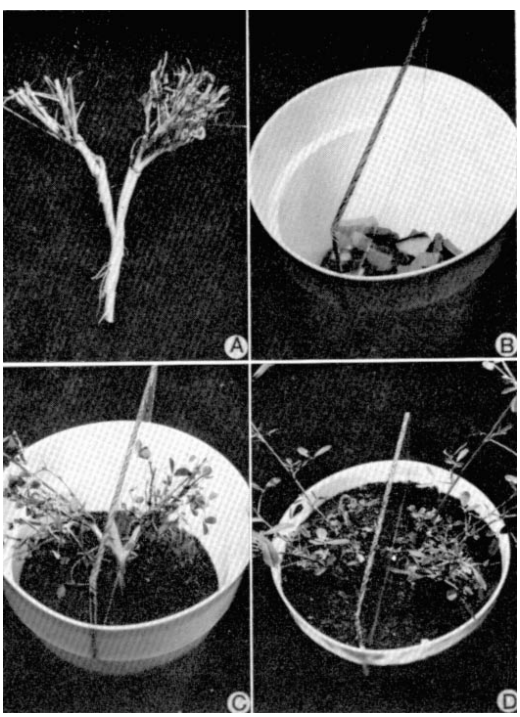

FIG. 1. Isolation of twinned alfalfa crowns. **A.** Split alfalfa plant ready for transplanting; **B.** Plastic pot fitted with Plexiglas divider; **C.** Plastic pot containing bisected plant with the Plexiglas divider in position; **D.** Healthy twin-crowned plant.

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In predisposition studies the nematode-infected crown can be removed before *C. insidiosum* inoculum is applied to the remaining crown.

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