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Hydraulic Lift Increases Herbivory by *Diaprepes abbreviatus* Larvae and Persistence of *Steinernema riobrave* in Dry Soil[1]

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Abstract: Citrus seedlings were grown in double pots that separated the root systems into discrete lower and upper zones to test the hypothesis that hydraulic lift affects persistence and efficacy of entomopathogenic nematodes. Three treatments were established: (i) both pots were irrigated at water potential ≤ −15 kPa (no drought); (ii) only the bottom pot was irrigated (partial drought); (iii) neither pot was irrigated (complete drought). *Steinernema riobrave* infective juveniles (IJ) were added to the soil in the top pots of all treatments. During 27 days, the water potential in soil in the top pots of both the partial and complete drought treatments declined to ca. −160 kPa. A greater number of nematodes ($P \leq 0.01$) persisted in soil as motile IJ under conditions of partial drought (143/pot) than under no drought (6.1/pot) or complete drought (4.4/pot). A second experiment was initiated with the same treatments as the first, except that only half of the 20 replicates in each moisture regime were inoculated with nematodes. After 15 days, all top pots were irrigated and two larvae of the insect *Diaprepes abbreviatus* were added to all of the top pots in each treatment. Irrigation regimes were reinstituted until water potential in the top pots under partial and complete drought had again declined to ca. −150 kPa and the experiment was terminated. In the absence of nematodes, the damage to tap roots caused by *D. abbreviatus* feeding under partial drought and complete drought was 80% and 32%, respectively, of that under no drought. Numbers of motile IJ in soil were greater under conditions of partial drought (736/pot) than under complete drought (2.0/pot) or no drought (7.2/pot). Survival of *D. abbreviatus* and insect damage to roots were reduced by the presence of *S. riobrave* to a greater extent under partial drought as compared to other treatments. Hydraulic lift from the lower to the upper rhizosphere appears to have modulated the effect of dry soil conditions on feeding behavior of *D. abbreviatus* and created favorable conditions for persistence and efficacy of the entomopathogenic nematode.

Key words: biological control, citrus, *Diaprepes abbreviatus,* drought, hydraulic lift, soil moisture, *Steinernema riobrave,* water potential.

The entomopathogenic nematode *Steinernema riobrave* Cabanillas, Poinar and Raulston is one of several species used for management of curculionid larvae (root weevils) that attack roots of citrus trees in Florida. *Diaprepes abbreviatus* L. is the most economically important of several weevil species that infest more than 50,000 ha of the approximately 300,000 ha of citrus in the state. Commercially formulated *S. riobrave* applied at rates > 100 infective juveniles (IJ)/cm^2 soil have been shown to reduce population densities of *D. abbreviatus* larvae in the field by as much as 80 to 95% within 3 to 4 weeks after treatment (Duncan et al., 1996b; Duncan and McCoy, 1996). However, recovery of IJ applied to soil declines to non-detectable levels in a matter of weeks, and insecticidal efficacy of the nematode is not apparent for more than 2 weeks following application (Duncan and McCoy, 1996; McCoy et al., 2000). Given the ephemeral biological control achieved by inundative treatment with *S. riobrave,* it is important to understand the optimum soil conditions to achieve maximum effectiveness.

Factors regulating *S. riobrave* population densities in the field are poorly understood although effects of some physical properties including soil moisture have received some study. In laboratory microcosms, persistence of *S. riobrave* was favored by relatively low water potentials (−100 to −0.1 Mpa) compared to lower or higher levels of moisture (Duncan et al., 1996a; Gouge et al., 2000). *Steinernema riobrave* also persisted longer within host cadavers in dry soil (−100 Mpa) than did three other species of entomopathogenic nematodes (Koppenhofer et al., 1997). Although soil water potential in the field is reduced by plant transpiration, hydraulic lift via roots may also provide an important source of water to rhizosphere inhabitants in dry soils (Caldwell et al., 1998; Duncan and El Morshedy, 1996). In a vertical-double-pot system, population density of the plant-parasitic nematode *Tylenchulus semipenetrans* Cobb was found to increase rapidly in dry soil in the upper root zone of citrus if deeper roots were well watered but to decline profoundly if the lower roots were also in dry soil (Duncan and El Morshedy, 1996). It was proposed that water moving along a deficit gradient through the root xylem from the lower to the upper root zone, in quantities too small to be detected by potentiometers, created conditions favorable for the nematode.

Soil moisture in the rooting zone of citrus and other crops in the field is frequently characterized by humid subsoils overlain by surface soil horizons that fluctuate from dry to humid between precipitation events. Under such conditions, the water potential experienced by rhizosphere-inhabiting organisms in dry surface soils may be fundamentally different in the presence or absence of hydraulic lift. The experiments reported herein describe effects of soil moisture on feeding behavior of *D. abbreviatus* larvae, and on the persistence and insecticidal efficacy of *S. riobrave,* under conditions that could permit or prevent hydraulic lift.

Materials and Methods

Persistence of S. riobrave: Nine-month-old rough lemon seedlings were grown with each root system contained

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in vertical double-pots containing autoclaved Astatula sand (96:2:2 sand:silt:clay) (Duncan and El Morshedy, 1996). Briefly, the root systems of the seedlings were allowed to grow through the bottom of a 500-cm^3 plastic pot and into a second lower pot. A 4-cm section of tap root exposed to air between the two pots was coated with lanolin and covered by rubber tubing to prevent desiccation. A potentiometer (Watermark; Irrometer Company, Inc., Riverside, CA) was installed 5 cm deep in each upper pot. Thirty days after roots were transplanted in the bottom pots, 6,000 third-stage infective juveniles (IJ) of *S. riobrave,* freshly harvested from larvae of *D. abbreviatus,* were added in 5 ml water to the soil surface of each top pot.

Three treatments were initiated immediately following addition of the nematodes. In the first treatment (no drought), both pots were irrigated with 50 ml tap water when the soil water potential in the top pot declined below −15 kPa. Irrigation water was added slowly in several increments to ensure that nematodes were not washed from the upper pots. The amount of irrigation was sufficient to increase the water potential to 2 kPa in the upper horizon of the top pot, but not great enough to drain from the bottom of the pot. The second treatment (partial drought) received no water in the top pot, but bottom pots were irrigated twice weekly. In the third treatment (complete drought), neither pot was irrigated. Treatments were replicated 10 times and arranged in a randomized block design in a greenhouse with mean daily temperatures of 24 to 29 °C.

Potentiometers were monitored daily and the experiment was terminated after 23 days, when the average soil water potential in the partial and complete drought treatments declined to ca. −160 kPa. The plant stems were cut at the soil line and discarded. The tap root between the two pots was cut, and the soil and roots were removed from the top pot. The soil in the top pot was separated from the root system, and *S. riobrave* was recovered by extracting all of the soil using the sugar centrifugation method of Jenkins (1964). Soil and nematodes adhering to the inner wall of the top pot were rinsed into a 100-ml test tube and allowed to settle for 24 hours before aspirating all but 25 ml of the water. Nematodes from bulk soil and from the tube walls were enumerated separately. Nematodes were classified as dead only if the internal body structure was disorganized. If the internal structure of the IJ appeared to have normal integrity, the nematode was classified as either moving or non-motile. The experiment was repeated once.

Interactions between S. riobrave and D. abbreviatus: A second experiment was initiated to study the behavior and interaction of both *S. riobrave* and *D. abbreviatus* in dry soil. Rough lemon seedlings and *S. riobrave* were used to establish the treatments described previously, except that each irrigation regime contained 10 replicates each with and without nematodes. Thus, the experimental design was a 3 × 2 factorial with three soil moisture regimes and two levels of nematodes. When soil moisture in both drought treatments declined to ca. −140 kPa, all pots were carefully rehydrated and two fifth-instar larvae of *D. abbreviatus* were buried 2 cm deep in the top pot of each treatment. The previous irrigation treatments were reestablished and maintained until soil water potential in both drought treatments again declined to ca. −165 kPa, when the experiment was terminated. After examination of the soil in the top pots to recover living and dead insect larvae, nematodes were recovered from the bulk soil and pot walls as previously described. The fibrous roots in the top pot were separated from the tap root, and both were dried at 70 °C and weighed. The area of channels in the cortex of each tap root caused by insect feeding was estimated as the sum of the products of the length and average width of each channel. Insect larvae and cadavers were maintained on moistened filter paper in petri dishes for 10 days to ascertain the rates of IJ infection and nematode population recycling.

Nematode counts from both experiments were transformed ($\log \chi + 1$) to equalize variances prior to analysis of variance. Tukey's Honestly Significant Difference Test ($P = 0.05$) was used for mean separation.

Results and Discussion

Persistence of S. riobrave: Both trials of the first experiment produced essentially the same results, and only those of the second trial are reported. Soil water potential declined at approximately the same rate under both partial drought and complete drought (Fig. 1A), as reported previously (Duncan and El Morshedy, 1996). Partial drought favored the persistence of *S. riobrave* ($P = 0.05$); nearly 24 times as many motile nematodes persisted under partial drought compared to no drought or complete drought (Fig. 2A). Despite increased survival under partial drought, less than 5% of the original inoculum in any treatment persisted as motile IJ during 23 days in the soil, which conforms to estimates of survival rates for this nematode in the field (Duncan and McCoy, 1996; Duncan et al., 1996b). Nematodes were recovered from 8 of 30 pot walls, but in numbers < 5% of those recovered from the bulk soil (data not shown).

Soil water potential measured with potentiometers did not differ between partial and complete drought; thus, it is likely that different IJ survival rates in the two treatments were due to undetected variation in water potential within the soil under partial drought. We hypothesize that, under partial drought, hydraulic lift from the lower pot produced a water film at the rhizoplane in the upper pot that provided a suitable environment for the IJ while remaining below the detection limit of the potentiometers. Compared to soils with

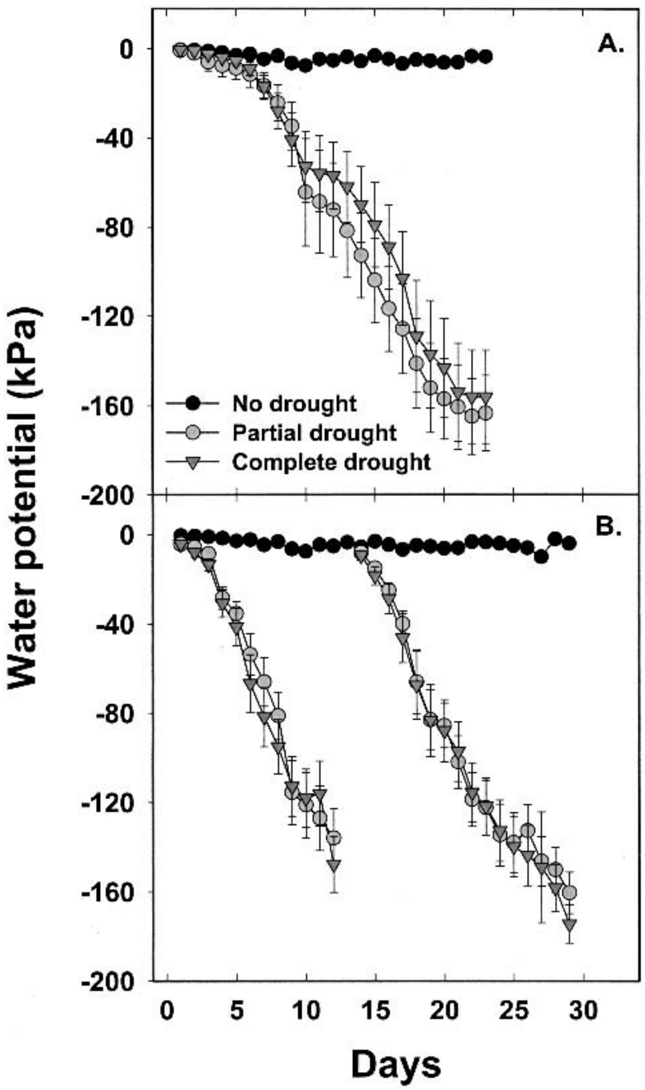

FIG. 1. Soil water potential in top pots of double pot systems in which both pots were irrigated at water potential ≤ −15 kPa (no drought), only the bottom pot was irrigated (partial drought), or neither pot was irrigated (complete drought). A) Experiment 1 to determine relative persistence of entomopathogenic nematodes in moist and dry soil. B) Experiment 2 to determine the behavior of entomopathogenic nematodes and weevil larvae in moist and dry soil.

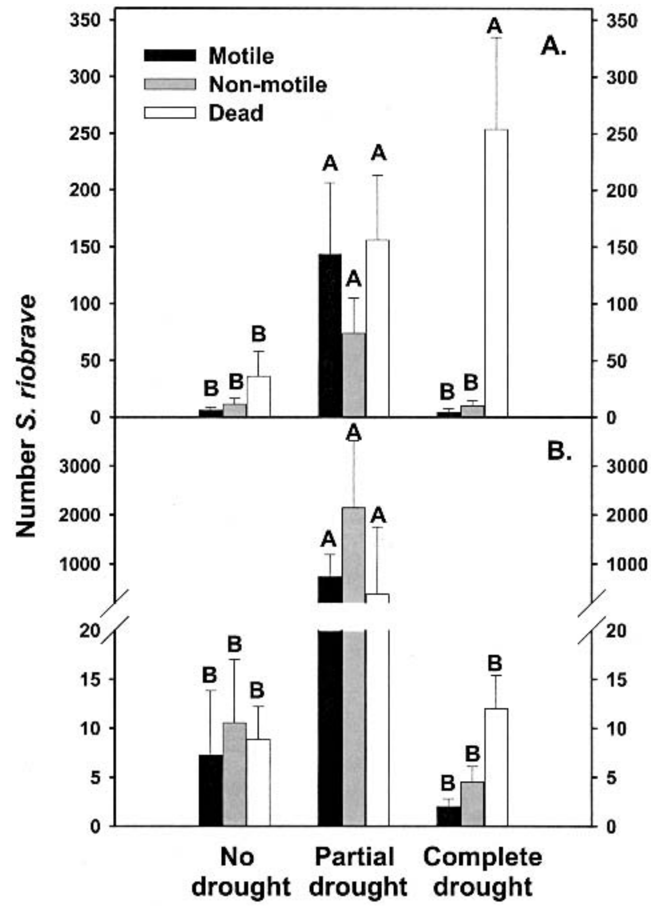

FIG. 2. Numbers of motile, non-motile, and obviously dead *Steinernema riobrave* recovered from the top pots of double pot systems following a single soil-drying cycle (A) and following a soil-drying cycle, addition of larvae of *Diaprepes abbreviatus,* and then implementation of a second drying cycle (B). Bars of the same type with the same letters do not differ ($P = 0.05$) according to Tukey's Honestly Significant Difference Test. Thin bars represent standard errors of means.

greater amounts of silt and clay, the large pore spaces in this sandy soil should result in air gaps between the root surface and the soil that will impede transfer of water from the root into the dry soil (Caldwell et al., 1998). A thin boundary of water between fine roots and dry soil could result in an environment suitable for IJ survival. This view is consistent with reports (Duncan et al., 1996a; Kung et al., 1991) that these nematodes persist better at relatively low water potential (partial drought) than at very low (complete drought) or very high (no drought) ones.

Interaction between S. riobrave and D. abbreviatus: As in the first experiment, soil water potential declined similarly under both partial and complete drought (Fig. 1B). Survival of IJ was also highest under partial drought in the second experiment (Fig. 2B). More motile nematodes were recovered from bulk soil in the second compared to the first experiment because some reproduction of the nematode in the *D. abbreviatus* larvae occurred prior to the termination of the experiment. Higher survival of IJ under partial drought resulted in greater efficacy against *D. abbreviatus* larvae (Fig. 3A). Analysis of variance revealed a significant effect of moisture regime ($P = 0.003$) and nematodes ($P = 0.006$) against *D. abbreviatus* larvae with no interaction ($P = 0.52$). Live larvae were reduced by 56%, 45%, and 17%, by *S. riobrave* under partial drought, complete drought, and no drought, respectively, compared to pots not inoculated with nematodes. During incubation after termination of the experiment, 41% of the larvae originally added to pots under partial drought produced a new generation of IJ, compared to 5% and 10% under no drought and complete drought, respectively (data not shown). Damage to the tap roots in the form of feeding channels was reduced by 82% by IJ

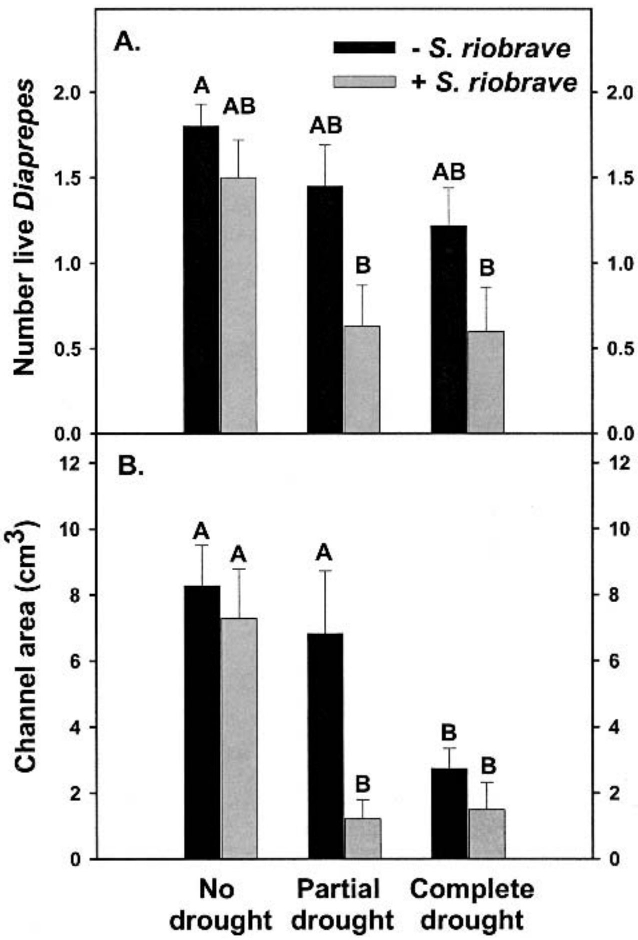

Fig. 3. Numbers of live larvae of *Diaprepes abbreviatus* (A) and cumulative area of channels in root cortex caused by insect feeding (B) in the top pots of double pot systems that were treated or not treated with *Steinernema riobrave*. Bars with the same letters do not differ ($P = 0.05$) according to Tukey's Honestly Significant Difference Test. Thin bars represent standard errors of means.

under partial drought, compared to 45% under complete drought and 12% under no drought (Fig. 3B). Both moisture regime ($P = 0.001$) and nematodes ($P = 0.01$) affected channel area with no significant indication of interaction ($P = 0.11$).

Insect larvae appeared to be relatively inactive under complete drought. In the absence of nematodes, numbers of live insect larvae under complete drought were 39% fewer than under no drought, but the feeding damage was reduced by 68%. In contrast, in the absence of nematodes, numbers of live insect larvae and amount of feeding damage were reduced (ns) by only 20% and 18%, respectively, under partial drought compared to no drought. Duncan and El Morshedy (1996) found that water content in citrus fibrous roots was 25%, 65%, and 79% after 23 days of complete, partial, and no drought, respectively. Because soil water potential was similar between the partial-drought and complete-drought treatments, water content of the root systems may have caused the differences observed in the insect feeding behavior in these two treatments.

Adequate soil moisture is a critical consideration for successful establishment of exogenously applied entomopathogenic nematodes that are harmed by rapid desiccation and prolonged exposure to ultraviolet radiation (Downing 1994; Gaugler and Boush, 1978). *Steinernema riobrave* IJ that remain near the soil surface following application persist less well than IJ that move more deeply into the soil, either actively or passively in irrigation water (Duncan and McCoy, 1996). However, following application of IJs, normal drying of the surface soil between irrigation cycles may not be detrimental to the survival of these nematodes. Indeed, these experiments suggest that if crop plants have roots in humid deep soil horizons, the roots in the dry shallow soils may provide a mechanism to prolong persistence and efficacy of entomopathogenic nematodes in the soil.

The data from this and a previous study (Duncan and El Morshedy, 1996) indicate that the behaviors of subterranean herbivores and nematode parasites of plants and insects are fundamentally different in dry soil containing plant roots, depending on whether the complete root system is contained only within dry soil or is apportioned between dry and humid soil. The latter conditions occur commonly in nature where surface soils tend to dry more rapidly due to transpiration and evaporation than do deeper layers of the soil profile. Our findings confirm that the role of plant root systems and hydraulic lift are important factors to consider when studying the fate of rhizosphere organisms in dry soils.

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