

Population Changes of *Tylenchulus semipenetrans* Under Localized Versus Uniform Drought in the Citrus Root Zone¹

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Abstract: Population development of *Tylenchulus semipenetrans* in dry soil was investigated in a greenhouse study. Citrus seedlings were grown in sandy soil in vertical tubes with upper and lower sections. Nematode population densities in the upper tubes were measured at 16, 23, and 37 days post-treatment. Three treatments consisted of i) irrigating both tubes when soil water potential reached -15 kPa (non-drought), ii) irrigating only the bottom tube (local drought), and iii) no irrigation (uniform drought). Soil water potential in the upper tubes did not differ under local and uniform drought during the first 16 days post-treatment, when it approached -125 kPa. Thereafter, the water potential of soil under uniform drought continued to decrease, while that under local drought stabilized at approximately -150 kPa. Treatments had no consistent effects on female *T. semipenetrans* counts from soil or roots. However, after 37 days, numbers of eggs, juvenile, and male nematodes per gram of root under local drought were more than 2.4-fold greater than those under non-drought or uniform drought. Numbers of juvenile and male nematodes in soil were 6.5 times higher under local drought than under non-drought after 37 days. Nematodes did not survive in soil under uniform drought. Most of the eggs recovered on each date, from roots under local and non-drought, hatched within 35 days. Sixteen days of uniform drought reduced cumulative egg hatch to 51%, and almost no eggs hatched after 23 and 37 days of uniform drought. Thus, the response of *T. semipenetrans* to dry soil is fundamentally different, depending on whether all or part of the rhizosphere experiences drought. These data and field observations suggest that hydraulic lift via the root xylem may prolong the activity of some nematodes and possibly other rhizosphere-inhabiting organisms in dry soil.

Key words: carbohydrate, citrus, citrus nematode, ecology, drought, hydraulic lift, soil moisture, survival, *Tylenchulus semipenetrans*.

The citrus root system can extend below 6 meters in the deep sands of Florida, but a major portion of roots lie just beneath the soil surface (4). During the dry season from October to May in Florida, surface roots outside of areas wetted by low-volume irrigation systems encounter frequent periods of water deficit. Soil moisture under these conditions can be lower than the permanent wilting point for up to several weeks (3). Population densities of *Tylenchulus semipenetrans* Cobb can decline rapidly on non-irrigated trees under drought (8,20,23). However, during peri-

ods of drought in irrigated orchards, it is not uncommon to find that highest population densities of *T. semipenetrans* are outside of the zone of soil wetted by irrigation (Joe Noling, pers. comm.; L. Duncan, unpubl.).

Vilardebo (23) suggested that female *T. semipenetrans* and the eggs, juveniles, and males in the gelatinous egg matrix were protected from drought stress by water extracted from the fibrous root cortex by the female nematodes. In contrast, juvenile and male nematodes free in soil might perish during the drought cycle, reducing the overall population density. This is consistent with observations of population change under uniform drought, but not for localized dry conditions associated with low-volume irrigation.

The responses of *T. semipenetrans* to water deficit have been studied in some detail. While juvenile and male nematodes survived water potentials to -1500 kPa in vitro, *T. semipenetrans* had the least capacity to enter anhydrobiosis of several plant-

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parasitic nematodes studied (19,20). In soils with moderately high clay content, *T. semipenetrans* population development was favored by dry (-60 kPa before irrigation) rather than wet (-10 kPa) conditions (21), possibly in response to low O_2 availability (22). An opposite relationship was noted in sandy soil, where nematode population density was highest in wet soil (21). Under conditions favorable for plant growth, moderate levels of clay (21) and organic matter (15) favor population growth of the nematode. Thus, the general response of the nematode to soil moisture suggests a preference for reduced water potentials, but a poor ability to survive severe drought. Soil moisture fluctuates to extremes less in heavier soils than in sandy soils. While "dry" conditions in heavier soils are optimum for *T. semipenetrans*, rapid fluctuations from wet to very dry conditions in sandy soils may adversely influence citrus nematodes (21).

The studies cited above were conducted using citrus seedlings growing in pots. The entire root systems of the seedlings were subjected to reasonably uniform water potentials. In nature, it is more common that roots in the surface soil horizons experience dry conditions more frequently and for longer duration than roots deeper in the soil. Hydraulic lift of water deep in soil can support surface roots of some plant species and possibly associated rhizosphere organisms during periods of drought (2). Hydraulic lift occurs when surface soil water potential is lower than the water potential in the root xylem, thereby causing water to flow from lower soil horizons through the xylem until effluxing into the drier surface soil. Surface soil water potential under these conditions exhibits diurnal change. During the day, when atmospheric water potential drops, soil water potential decreases in all horizons due to transpiration. As transpiration declines at night, hydraulic lift serves to rewet the dry shallow soil.

If hydraulic lift occurs in citrus, the response of *T. semipenetrans* to water deficit

in the entire rhizosphere (uniform drought) should differ from that where only part of the rhizosphere experiences water deficit (local drought). In particular, survival of juveniles and males free in soil should be expected to differ under the two conditions.

The purpose of the present study was two-fold. The responses of different life stages of *T. semipenetrans* to increasing water deficit were studied to test the hypothesis of Vilardebo that nematode survival during drought is primarily due to survival of root-inhabiting stages of *T. semipenetrans*. Also, conditions of uniform and localized soil dryness were created to determine whether the nematode responds to those conditions in fundamentally different ways.

MATERIALS AND METHODS

Experimental units: Eighty plastic seedling cones (Stuewe, Corvallis, OR) were each filled with 100 cm^3 of Candler fine sand (96% sand, 2% silt, 2% clay), and a single 6-week-old Volkamer lemon (*Citrus volkameriana* Tan and Pasq.) seedling was planted in each cone. The cones were supported in racks with the bottoms of each cone resting on methyl bromide-treated Candler sand in a soil bin in the greenhouse. The tap roots of the seedlings grew approximately 20 cm through and beyond a hole in the bottom of each cone by 6 weeks following transplanting. These lower portions of the root system were carefully removed from the soil bin and plants were removed from the cones. Root systems were transplanted into two 500-cm^3 cones, vertically separated by approximately 4 cm of tap root. The two cones were filled with Candler fine sand mixed with Metromix 500 (Scotts-Sierra Horticultural Products, Marysville, OH) potting mixture (equal parts by volume; 9.0% Metromix by weight) and moistened to approximately 19% (water per soil dry weight) to favor nematode infection of roots (15). Eighty plants were then placed

into a rack that supported both cones vertically. The 4-cm length of tap root that separated the two cones was coated with lanolin and covered with black polytubing. Plants were maintained on a greenhouse bench at 20 to 33 °C. Plants were fertilized weekly (20:10:20, N:P:K), and foliar arthropod pests were managed with weekly applications of Safer Soap (Safer, Newton, MA) and occasional oil sprays.

Ten days after establishing the double cone system, Watermark ceramic potentiometer blocks (Irrometer, Riverside, CA) were buried 10 cm deep in the top cone of 15 plants. The following day and again 1 week later, the top cone was inoculated with a total of 200,000 eggs and 180,000 juveniles and males of *T. semipenetrans* in aqueous suspension from rough lemon roots from a mature citrus orchard (14). Nematode populations were established for 7 months before initiating the treatments. Two-thirds of the length of plant stems were pruned 5 months post-inoculation to manage plant size.

Experimental treatments: Three experimental treatments were assigned randomly to plants on 11 February 1995. Five potentiometer blocks per treatment provided soil moisture estimates in the top tubes throughout the experiment. Both the upper and lower cones of the "non-drought" treatment were watered when the average potentiometer reading indicated soil moisture deficit of -15 kPa. The top cones of two other treatments received no irrigation beginning 11 February. Only the bottom cone of the second treatment was watered when the "non-drought" treatment received water to create "local drought" in the top cone. Neither cone was watered in the third treatment to create "uniform drought" in the top cone. The degree of wilt for each plant was established periodically using a 0–8 scale: no visible wilt (0), loss of turgor (1), definite wilt of $<10\%$ of leaves (2), curling of $<10\%$ of leaves (3), curling of 10% to 50% of leaves (4), curling of $>50\%$ of leaves (5), $<50\%$ of leaves browning (6), $>50\%$ of

leaf browning (7), and 100% browning with leaf loss (8). Twenty-four replicate plants were established for each treatment. Eight additional plants were subjected to uniform drought for the duration of the experiment, after which they were irrigated, to estimate plant survival under uniform drought.

Experimental measurements: The effects of local vs. uniform drought on roots and nematodes were measured 16, 23, and 37 days post-treatment. Variables were measured only in the top cones. Eight randomly selected plants per treatment were evaluated at each harvest. Plant tops were cut at the soil line and fresh and dry weights determined. The tap root between the two cones was cut and the root systems removed from the cones. Fifty cubic centimeters soil remaining in the top cone was processed on Baermann funnels for 48 hours.

Root systems were shaken free of loose soil. Fibrous roots were separated from the tap roots, cut into 2-cm lengths, and weighed. A 2-g aliquot of roots was processed (1) to extract all life stages of the nematodes on the roots. Another 2-g aliquot of roots was dried (40 °C) for 24 hours and hand separated from soil and organic matter, which were weighed separately. An aliquot of these cleaned roots was ashed at 500 °C and the mineral weight determined. Fresh and dry weights of roots were then estimated, based on the proportions of organic matter and mineral content of these aliquots. Fifty milligrams of the cleaned roots were processed to determine concentrations of starch and ketone sugars in the fibrous roots (8).

The remaining uncleaned roots were shaken vigorously in water in plastic bags, which were passed repeatedly through a sieve (44 μm) to collect eggs free of juveniles and males. Egg suspensions were incubated in water (0.5 cm deep) in dishes at 25 °C and periodically examined for up to 35 days to determine cumulative hatch.

Data were subjected to analysis of variance for treatments and time. Where ap-

appropriate, means were separated using Duncan's multiple-range test. Nematode data were transformed to $\log_e(x + 1)$ before analysis to equalize variances, but untransformed means are reported.

RESULTS

Soil and plant variables: Soil water potential decreased at a similar rate under local and uniform drought during the first 16 to 17 days of the experiment when suction reached approximately -125 kPa (Fig. 1A). Thereafter, the water deficit in-

creased at a slower rate under local drought compared to uniform drought. By day 30 post-treatment, average water potential was -130 kPa under local drought and < -191 kPa under uniform drought. Three and five replicates under uniform drought had soil moisture readings too low to be detected by the tensiometers on days 23 and 37, post-treatment, respectively. Average water potential in the non-drought treatment did not decrease below -20 kPa at any time during the experiment.

Plants under uniform drought exhibited increasing levels of wilt and death of leaves during the course of the experiment (Fig. 1B). In contrast, plants under local drought wilted slightly initially, but were indistinguishable from control plants in this regard during the final week of the experiment. Half of the plants under uniform drought survived, following irrigation that began 37 days post-treatment.

Fibrous root mass increased during the experiment under non-drought and local drought conditions (Fig. 2A). The water content of roots differed ($P = 0.01$) between all treatments from the earliest measurement date (Fig. 2B). By the end of the experiment, roots under uniform drought consisted of $< 10\%$ water, compared to 62% and 78% for those under local drought and non-drought, respectively.

Between days 23 and 37 post-treatment, starch concentrations in fibrous roots increased by 87% ($P = 0.01$) and 45% ($P = 0.05$) under localized and non-drought conditions, respectively. Starch concentration never differed between roots in these two treatments (Fig. 2C). In contrast, starches in roots under uniform drought declined ($P = 0.01$) to low levels during the course of the experiment. Compared to non-drought controls, ketone sugars in fibrous roots appeared to increase during the initial 16 days of drought, particularly under uniform drought ($P = 0.05$, Fig. 2D). By the end of the experiment, concentrations of these sugars were not different under local and non-drought. As with

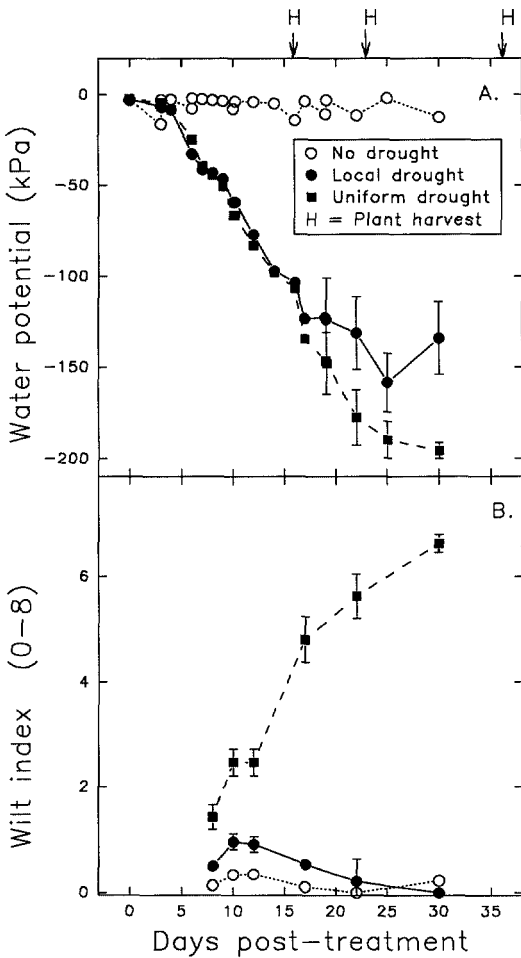


FIG. 1. The influence of localized and uniform drought on soil water potential in the upper tubes of vertical, double-tube systems containing the root systems of Volkamer lemon seedlings (A) and wilt of the seedlings (B) during 37 days in the greenhouse. Wilt was rated from slight (0) to plant death (8).

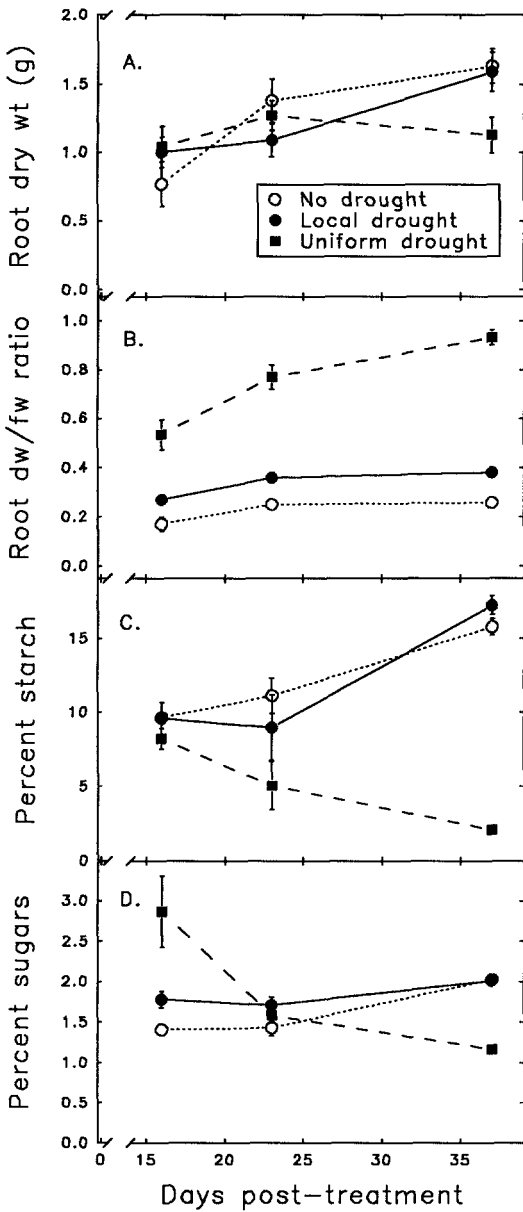


FIG. 2. The influence of localized and uniform drought on dry weight (A), dry weight to fresh weight ratio (B), starch concentration (C), and ketone sugar concentration (D) of fibrous roots of Volkamer lemon seedlings.

starch, ketone sugar concentrations in roots under uniform drought declined between days 16 to 37.

Nematode variables: Populations densities of female *T. semipenetans* on roots differed ($P = 0.05$) among treatments only at 23

days post-treatment, when they were highest under local drought (Fig. 3A). An increase in root mass between days 16 and 23 under non-drought conditions contributed to this difference by reducing the numbers of non-drought females per gram of root on that date. Numbers of fe-

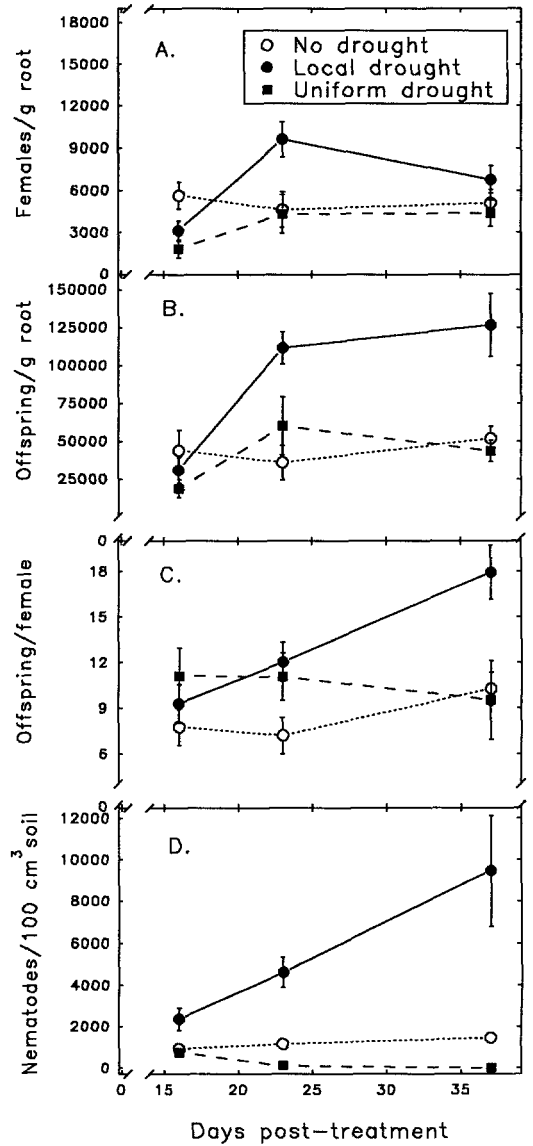


FIG. 3. The influence of localized and uniform drought on numbers of *Tylenchulus semipenetans* females per gram (dry weight) of root (A); eggs, juveniles, and males per gram (dry weight) of root (B); eggs, juveniles, and males from roots per female (C); and juvenile and male nematodes free in soil (D).

male nematodes per pot increased ($P = 0.01$) by more than 3-fold under local drought and more than doubled ($P = 0.05$) under non-drought conditions (data not shown).

Although population densities of females were not greatly affected by soil water relations, those of nematode offspring (eggs, juveniles, and males) on roots increased in response to local drought, compared to the other two treatments (Fig. 3B). The difference was clear by day 23 post-treatment ($P = 0.05$) and increased by day 37 ($P = 0.01$). Consequently, the numbers of offspring per female collected from roots under local drought were nearly 75% greater ($P = 0.01$) at the end of the experiment than under uniform and non-drought conditions (Fig. 3C). Increased numbers of offspring per female under both local and non-drought conditions between days 23 and 37 corresponded to the increased starch concentration in roots.

Numbers of nematodes per 100 cm³ soil also increased ($P = 0.01$) under local drought (Fig. 3D), compared to other treatments. The trend was significant on all dates and, by the end of the experiment, soil population densities were nearly 6.5-fold higher under local drought than under non-drought conditions. Few nematodes were recovered from soil under uniform drought, beginning on day 23 post-treatment. The respective numbers of nematodes per 100 cm³ soil in local vs. uniform drought on days 16, 23, and 37 were 2,372 vs. 761, 4,633 vs. 116, and 9,461 vs. 7.

The relatively high recovery of nematodes from roots compared to soil under uniform drought is explained by cumulative egg hatch (Fig. 4). The hatch rate of eggs under uniform drought collected 16 days post-treatment was lower than for the other two treatments (Fig. 4A). By days 23 and 37 post-treatment, only trace levels of eggs under uniform drought hatched. Consequently, most nematodes recovered from roots under uniform drought were probably dead or non-viable, whereas

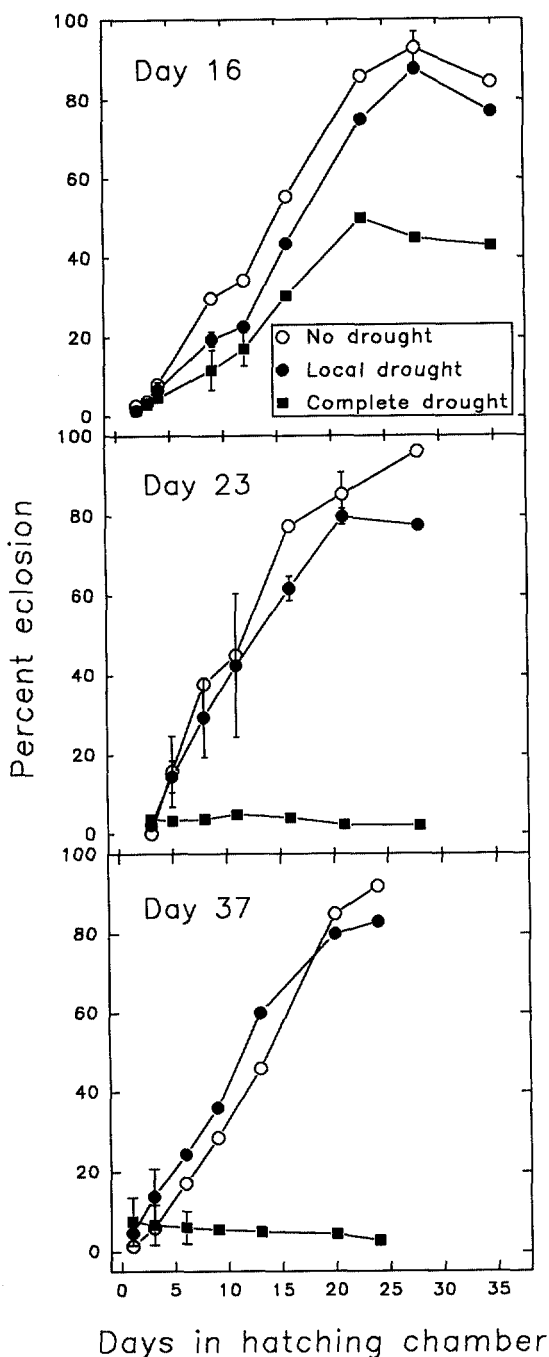


FIG. 4. The effects of different durations (16, 23, and 37 days) of localized and uniform drought on cumulative percentage hatch of *Tylenchulus semipenetrans* eggs during up to 35 days in hatching chambers.

dead nematodes in soil would not be recovered from Baermann funnels. By contrast, 80 to 95% of the eggs produced un-

der local or non-drought conditions hatched between 24 to 36 days in the incubation chambers.

DISCUSSION

Tylenchulus semipenetrans responded differently to the same soil water deficit depending on whether the drought-affected area encompassed all or part of the rhizosphere. During 2 weeks without direct supplemental irrigation, population density of the nematode under local drought began to increase rapidly, whereas density under uniform drought began a period of pronounced decline. Both conditions occur in the citrus rhizosphere in nature, particularly in non-irrigated orchards, where surface roots may experience soil water deficit and water may or may not be available to roots in the deeper soil horizons. The results of this study indirectly support Caldwell's (2) suggestion that hydraulic lift may prolong the activity of rhizosphere-inhabiting organisms in dry soil.

The mechanism by which local drought favored population growth of this nematode is unknown. Physical factors in the environment or aspects of host-plant physiology may be involved. Presumably, hydraulic lift from lower pots supplied roots in upper pots with water necessary for maintenance and growth in the local drought treatment (12). The fact that numbers of female nematodes tripled during 37 days of local drought suggests that soil water potential, at least at the rhizoplane, was adequate for normal nematode movement along root surfaces in the dry areas. If water potential under local drought is higher at the rhizoplane than in bulk soil, it may have affected population growth by restricting nematode migration to areas concentrated along root tissues. Increased oxygen availability should favor nematode population development under local drought compared to non-drought conditions (22). Indeed, with the incorporation of 9% organic matter in the soil, the irrigation schedule of the non-drought

treatment could have been based on a somewhat lower water potential, without causing water deficit in the plants. Therefore, differences between the population densities under local and non-drought could almost certainly be reduced under different experimental conditions.

A correlation between numbers of offspring per female (under both local and non-drought) and starch concentration in roots has been reported previously (8,9). However, there was no direct evidence in this study that availability of nutrients in the roots affected population densities differently among the treatments. Since non-infected controls were not used in the experiment, and population densities differed among treatments, it is not possible to know precisely how water deficit alone affected the allocation of storage carbon in the roots. Kosola and Eissenstat (12) found that considerably less labeled carbon flowed to citrus roots under local drought compared to non-drought conditions. Nevertheless, concentrations of starches and ketone sugars did not differ noticeably among those treatments in this study. Thus, while root metabolic processes (and possibly defenses) may be compromised under local drought, roots nonetheless maintained reserves of non-structural carbohydrate that were adequate for nematode population growth. Alternatively, nematodes may exert sufficient sink strength to change normal patterns of carbon allocation in the plant during drought. Non-structural carbohydrate availability differed only under uniform drought. However, given the severe negative effect of uniform drought on water in roots and on nematodes free in the soil, it seems likely that water rather than nutrient deficit reduced nematode numbers in that treatment.

Population differences under local and non-drought conditions may also have resulted from different rates of nematode mortality. Population densities of nematodes recovered from soil under local drought were more than six times higher than those under non-drought. The com-

parable difference from roots was only 2.4-fold. The difference in these ratios suggests that survival rates may be higher in the drier soil.

The effect of soil water potential on egg hatch was similar to that noted for *Meloidogyne incognita* from cotton (10). Hatch of *T. semipenetrans* eggs was reduced following exposure to < -100 kPa suction and was almost completely inhibited when suction approached -175 kPa. Egg hatch of *M. incognita* in the absence of a host plant was reduced following 24-hour exposure to -200 kPa suction, and exposure to -300 kPa resulted in complete hatch inhibition.

Soil moisture explained the major proportion of the seasonal variation in population density of *T. semipenetrans* in sandy soil in a Florida citrus orchard, compared to other climatic factors and concentrations of root carbohydrate, lignins, and phenolic compounds (9). Results of the present study support the existence of a causal, inverse relationship between soil moisture and nematode density, even in those sandy soils. Moreover, seasonal patterns of *T. semipenetrans* population development appear to be more pronounced in the tropics and subtropics (9,16–18) than in Mediterranean and semi-arid regions (5,11), for which heavy seasonal rains may be responsible. Similarly, population densities of this nematode on citrus tend to be significantly higher in drier climates (5,7, 13,24) than in the humid tropics and subtropics (6,9,16). Results of this study suggest that moisture availability in surface soils of these regions could be a major determinant of the population differences.

In conclusion, roots in dry soil maintained high levels of *T. semipenetrans* when water was available from other portions of the root system. Adverse effects of drought were not exhibited by populations of juveniles and males free in the soil. These results indicate the need to consider the potential role of hydraulic lift to understand the fate of this nematode in dry soil. Other possible causes of the population differences noted in this study, such as

differential mortality, require further investigation to understand the level of resource transfer from the root to populations of the parasite under different conditions of soil moisture.

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