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Location of *Heterodera glycines*-induced Syncytia in Soybean as Affected by Soil Water Regimes[1]

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Abstract: Locations of syncytia induced by the soybean cyst nematode (SCN), *Heterodera glycines* race 3, were compared in roots of 'Essex', a susceptible soybean (*Glycine max* (L.) Merr.) cultivar, at three soil water regimes. The plants were grown in wet (−5 to −20 kPa), moderately wet (−30 to −50 kPa), and moderately dry (−60 to −80 kPa) autoclaved Captina silt loam soil (Typic Fragiudult). In the moderately dry soil, syncytia were found only in the stele, but in moderately wet and wet soils, syncytia occurred primarily in the cortex and occasionally in the stele. The location of syncytia in the cortical tissue of roots growing in wet and moderately wet soils may account for the tolerance of susceptible soybean cultivars grown under well-irrigated conditions where there is less interference with water transport through roots. Cell-wall perforations and dense cytoplasm were characteristic of syncytial cells observed in root tissues of all treatments.
Key words: *Glycine max*, *Heterodera glycines*, histology, nematode, soil water, soybean, soybean cyst nematode, syncytia.

Infection by soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is one of the most critical disease problems of soybean (*Glycine max* (L.) Merr.) in the Midsouth of the United States, where 20% of the total yield loss caused by soybean diseases is attributed to *H. glycines* (10).

Soybean damage by SCN is related to the population density of the nematode, susceptibility of the cultivar, presence of other microorganisms, and environmental factors, such as temperature, oxygen, growing season duration, soil fertility, and soil physical properties (14). The life cycle of SCN takes 3 to 4 weeks, depending on the temperature and water status of the soil (5).

Recent observations indicate that the response of field-grown soybean to SCN depends on the water status of the soil and the level of SCN infestation (6). There is little or no effect of *H. glycines* on soybean seedlings when rainfall is abundant during the growing season in Arkansas. However, as soil water is depleted, severe plant damage by *H. glycines* occurs. Soybean plants grown on soils with high SCN population densities, but with adequate irrigation, maintained optimum rates of growth (6). Yields of soybean grown under such conditions were similar to yields of soybean grown in irrigated noninfested fields. However, when soybean plants were grown on soil containing high SCN population densities under nonirrigated or drought conditions, significant yield losses occurred (6).

Although syncytia in susceptible hosts have been studied extensively (4,8,9), detailed information on the effect of soil water regime on syncytial development in roots of susceptible hosts is lacking. The objective of this study was to determine the locations of syncytia in susceptible soybean roots under different soil water levels.

MATERIALS AND METHODS

The SCN-susceptible soybean cultivar, Essex, was grown in a Captina silt loam soil (Typic Fragiudult) (23% sand, 68% silt, and 9% clay). The soil was collected in bulk from 0 to 15 cm depth, air dried, screened through a 2-mm-d pore (9 mesh) sieve to remove coarse materials, autoclaved twice at 121 C for 1 hour at 103 kPa (13), and then placed in 10-cm-d growth containers in which water regimes categorized as wet (−5 to −20 kPa), moderately wet (−30 to −50 kPa), and moderately dry (−60 to −80 kPa) were maintained. All soil water treatments were monitored with microten-

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siometers during the experiments. These ranges in soil water potential were chosen to coincide with the irrigation threshold recommendations of −50 kPa at the 30-cm soil depth for soybean plants grown on silt loams in Arkansas (11). Three seeds were planted in each pot, and the seedlings were thinned at the cotyledon stage (VC) to one plant (2). Each pot was inoculated with approximately 500 second-stage juveniles (J2) of SCN race 3. At the time of inoculation, the moderately dry soil water treatment was kept moderately wet for 2 days to facilitate nematode penetration of the roots and then water was withheld until it equilibrated to the assigned water regime. Mean soil temperature during the study period was 25.5 C.

Sample preparation: Sixteen days after inoculation, roots were washed free of soil and examined with a light microscope to locate infected regions. Segments 2 mm long were then excised for processing. The segments were fixed for 2 hours at room temperature in modified Karnovsky's fixative (2% glutaraldehyde and 2% paraformaldehyhde in 0.05 M cacodylate buffer, pH 7.2), rinsed twice in the buffer, postfixed in 1% osmium tetroxide for 2 hours, washed in distilled water for 1 to 2 minutes, and prestained overnight in 0.5% uranyl acetate at 4 C. Tissues were dehydrated in an ethanol series and two changes of propylene oxide (15 minutes each) and then were infiltrated with Spurr's resin (12). Each root segment was placed in a capsule containing freshly prepared Spurr's resin. The tissues were positioned to facilitate transverse sectioning and were polymerized overnight at 70 C.

Tissue sectioning: For light microscopy, 1.0–2.0-μm-thick serial sections were cut with a glass knife near a mature female nematode, attached to glass slides, and stained with 1% toluidine blue in 30% ethanol. Sections were examined with a phase-contrast light microscope to determine the location of syncytia and their orientation in the tissue for each of the soil water treatments. At least 10 roots were examined for each treatment. The experiment was repeated twice.

Results

Syncytia were found in roots of soybean grown in all three soil water regimes. However, the locations of the syncytia differed among the soil water treatments. Syncytia in root tissues of the wet and moderately wet soils were located in the cortex, with only small portions of these syncytia extending into the stele (Figs. 1A–D). In the wet soil treatments no syncytia were located primarily in the stele. The dense portion of the syncytium (in contrast to other parts of the cell) may indicate a feeding point for SCN (Fig. 1A,B). Syncytia were confined to tissues of the stele in the moderately dry soil treatment (Fig. 2A,B). The cortex of roots in this treatment appeared disrupted, and part of the cortex sloughed from many of the roots observed.

Differences in sex ratio related to water availability were not observed, but only nematodes associated with the syncytia studied were examined.

Discussion

The site of syncytium development is of primary importance in determining the relative tolerance of a host plant to the SCN (8). Under drought conditions, overall root water potential decreases (3), but conducting tissues may maintain higher water potential because of their role in water and nutrient transport. This suggests two possibilities: i) the nematode senses that in the roots from the moderately dry soil adequate water and nutrients are not available in the cortical cells but are in the stele or ii) the cortical cells in the roots from the moderately dry soil do not produce feeding stimuli in the same manner as the stelar cells.

Although syncytia were formed in root tissues of all soil water treatments, the differences in syncytium location may help explain why soybean plants grown under

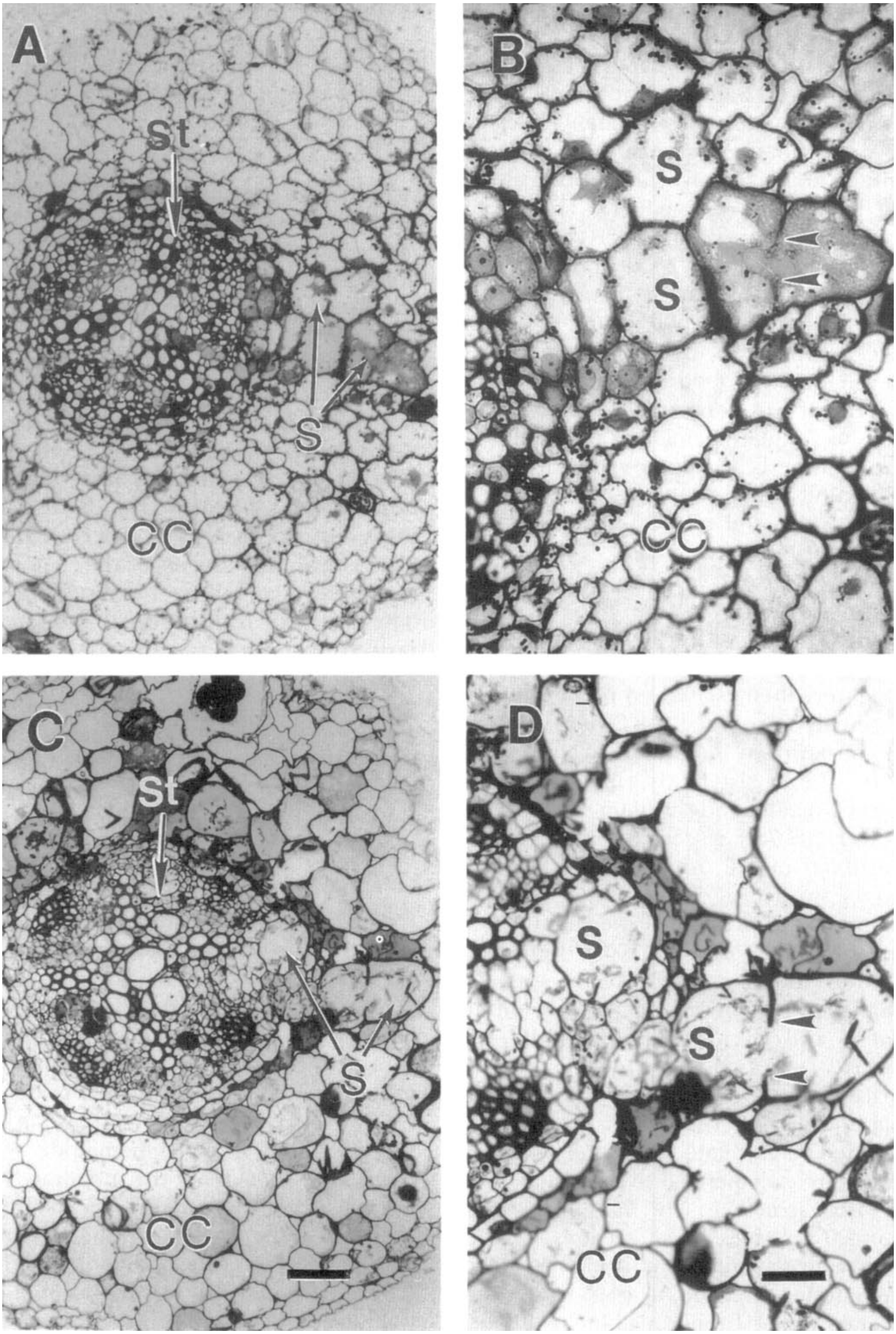

FIG. 1. Light micrographs of syncytia (S) in susceptible soybean roots under wet and moderately wet soil conditions. A) Transverse section of soybean root tissue grown in wet soil (−5 to −20 kPa) showing syncytium located in the cortex (CC). St = stele. B) Higher magnification of Figure 1A showing cell-wall perforation (arrowheads) and dense cytoplasm of syncytium-component cells. CC = cortex. C) Transverse section of soybean grown in moderately wet soil (−30 to −50 kPa) showing main body of syncytium in the periphery of cortex (CC)–stele (St) junction. D) Higher magnification of Fig. 1C showing syncytium in the cortex and in the stele. CC = cortex. Scale bars: A same scale as C = 64 μm; B same scale as D = 32 μm.

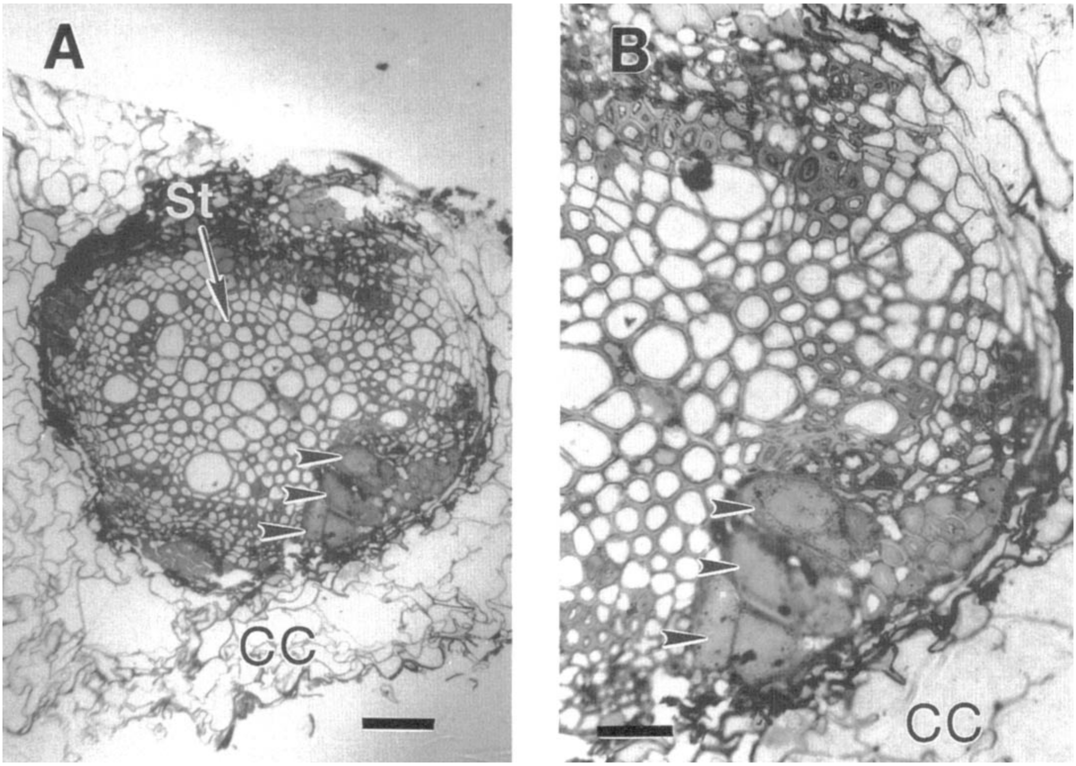

FIG. 2. Light micrographs of syncytium in soybean roots that developed in moderately dry soil (−60 to −80 kPa). A) Transverse section of soybean root showing syncytium (arrowheads) exclusively inside the stele (St). Note the shrunken and disrupted cortex (CC). B) Higher magnification of Figure 2A showing syncytium (arrowheads). The syncytium has characteristic dense cytoplasm. CC = cortex. Scale bars: A = 64 μm; B = 32 μm.

adequate moisture conditions seem more tolerant to the SCN than drought-stressed soybean plants. In common lespedeza (*Lespedeza striata* L.) cv. 'Kobe', a plant tolerant to SCN, syncytia were located primarily in the cortical region of the root and advanced centripetally in the stele (9). The location of syncytia and their expansion in lespedeza were similar to the occurrence of syncytia in the soybean roots grown on wet and moderately wet soils in our experiments.

The cortex of soybean roots consists of parenchymatous cells containing starch, sugar, and other minerals (1). At high soil water content, roots maintain their integrity (i.e., the cells maintain high turgor pressure). Starch and minerals stored in the cortical cells may be utilized by the SCN as syncytia are formed. In another study, SCN population density in soybean roots decreased significantly when soil water potential increased (7). At high soil water content, the number of SCN in the roots decreased, possibly because of lower oxygen levels. The lower oxygen level may also influence the location of the syncytia. This study indicates that water availability in a susceptible soybean cultivar causes the formation of syncytia outside of the vascular tissues. Syncytia in cortical tissues are less likely to interfere with water and nutrient transport in the soybean plant.

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