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Enhanced Hatching of *Globodera tabacum solanacearum* Juveniles by Root Exudates of Flue-cured Tobacco[1]

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Abstract: Stimulation of hatching of a tobacco cyst nematode (*Globodera tabacum solanacearum*) by root exudates from resistant NC 567 and susceptible K 326 cultivars of flue-cured tobacco, *Nicotiana tabacum*, was investigated. Root exudates were collected by soaking seedlings in deionized water for 2 hours at 22 °C in the dark. Fifteen mature and uniformly sized cysts were exposed at 15, 20, or 25 °C to undiluted root exudate, root exudate diluted 1:1 or 1:3 with deionized water, or deionized water alone. Hatched juveniles were counted and removed at weekly intervals during 42 and 53 days of exposure in experiments conducted in 1994 and 1995, respectively. Root exudates from both susceptible cultivar K 326 and resistant cultivar NC 567 stimulated more hatching than deionized water at 25 °C in 1994, and at all three tested temperatures in 1995. In 1994, dilution of root exudates 1:3 reduced stimulation of hatching at 25 °C compared to undiluted exudate. Hatching at 25 °C was similarly stimulated by exposure to undiluted root exudate and exudate diluted 1:1. In 1995, both dilutions reduced stimulation of hatching by root exudates at all the temperatures.

Key words: flue-cured tobacco, *Globodera tabacum solanacearum*, hatching, *Nicotiana tabacum*, resistance, root exudate, temperature, tobacco cyst nematode.

Tobacco cyst nematode, *Globodera tabacum solanacearum* (Miller and Gray) Behrens, is one of the most serious pathogens of flue-cured tobacco, *Nicotiana tabacum* (L.), in Virginia (Miller and Gray, 1972). The average yield suppression caused by *G. t. solanacearum* has been estimated at 15%, and complete crop failures also have been recorded (Komm et al., 1983). An estimated one quarter of the total hectarage of flue-cured tobacco in Virginia is infested by the tobacco cyst nematode (C. S. Johnson, unpubl.). Tactics for *G. t. solanacearum* management include crop rotation, nematicides, and host resistance. The limited availability of effective nematicides, the relatively high overwintering survival rate of the nematode, and the limited choices in rotation crops suggest that host resistance could play a more important role in improving management of tobacco cyst nematodes.

Various degrees of resistance to *G. t. solanacearum* exist in wild *Nicotiana* species (Baalawy and Fox, 1971; Hayes et al., 1997; Herrero et al., 1996). Most widely used commercial cultivars are susceptible to this nematode, but resistant cultivars have been identified (Johnson et al., 1989). However, significant yield losses continue to occur with resistant cultivars in the presence of large cyst nematode populations. Consequently, the main management tactic used is the application of contact nematicides, at an average cost of US $139/ha (Johnson et al., 1989).

Juveniles of *Globodera* spp. have long been known to hatch in response to exudates from host roots (Triffitt, 1930). Hatching of *G. t. solanacearum* increased following exposure of cysts to tobacco root exudate in combination with soil microorganisms (Fox and Webber, 1970). Hatching of tobacco cyst nematode is also stimulated by exudates from roots of tobacco and horsenettle (LaMondia, 1995). The efficiency with which host root exudates stimulate hatching has been correlated with resistance to potato cyst nematodes in several investigations (Arntzen et al., 1993, 1994; Farrer and Phillips, 1983; Turner and Stone, 1981). Poor stimulation of hatching activity also has

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been suggested as a component of host tolerance (Evans, 1983; Arntzen et al., 1994).

The mechanisms of resistance to *G. t. solanacearum* in flue-cured tobacco are unknown. Greater understanding of the mechanisms of resistance could facilitate the development of improved cultivars. This research was undertaken to compare hatching of *G. t. solanacearum* after exposure to root exudates from a resistant (NC 567) and a susceptible (K 326) flue-cured tobacco cultivar. The influence of root exudate concentration and temperature on egg hatching also was evaluated.

Materials and Methods

Hatching experiments were carried out in 1994 and 1995. Procedures in each of these experiments were similar.

Root exudate preparation: Ten-week-old seedlings of flue-cured tobacco cultivars K 326 (susceptible to *G. t. solanacearum*) and NC 567 (resistant) were collected from outdoor plant beds. After removal of plants from soil, root systems were washed, blotted dry, separated from above-ground plant parts, and weighed. Fifty-five grams of roots from each plant were placed in a 1,000-ml beaker. Deionized water was added to each beaker to a volume five times the weight of the root sample. Undiluted root exudate was collected by decanting beakers over a 45-µm-pore sieve (325 mesh) after 2 hours of soaking at 22 °C in the dark. Root exudate was diluted by mixing undiluted root exudate with equal (1:1) or triple (1:3) volumes of deionized water. All root exudate was kept at 4 °C throughout these experiments. The experiment was carried out in June 1994 and repeated in June 1995.

Hatching chamber: Hatching was monitored in chambers made by cutting off the tapered end of a polyethylene BEEM capsule and severing the cap from the capsule. Individual caps were perforated with an 8-mm-diam. hole. A piece of 20- × 20-mm nylon screen (150-µm-pore openings) was placed on one of the ends of each capsule before replacing the punctured cap on the end of the capsule. The other end of each capsule was left open. Hatching chambers were created by placing one capsule (with the open end on the top) on the bottom of a 20- × 150-mm test tube.

Preparation of cysts: Cysts of *G. t. solanacearum* were propagated on flue-cured tobacco cultivar Coker 319 in the greenhouse. In 1994, cysts were collected in March and stored in tap water for 12 weeks at 4 °C. Cysts used in 1995 were harvested in June, stored in tap water at 4 °C, and exposed to root exudate 1 week after harvesting. Cysts were extracted from soil by thoroughly mixing infested soil with water in a plastic bucket and allowing it to settle for 15 seconds. The supernatant was poured through an 850-µm-pore sieve nested over a 250-µm-pore sieve. Cysts retained on the finer sieve were collected for the hatching experiments. Cysts were crushed manually to determine average egg content. Eggs from crushed cysts were stained with acid-fuchsin, suspended in tap water, and counted under low magnification (Barker, 1985). Each cyst contained an initial average of 185 eggs in 1994, and 219 eggs and 26 well-developed second-stage juveniles in 1995.

Exposure of cysts to root exudate: Fifteen uniformly sized cysts were placed in each hatching chamber at the start of each experiment and then immersed in 1.5 ml of root exudate solution in each hatching chamber. Root exudate was renewed weekly in each hatching chamber throughout the period of the experiments. Deionized water was used as a control.

Treatments, experimental design, and analysis: Root exudate treatments were organized in a complete factorial design of cultivars and dilutions with four replications. Three dilutions (undiluted, 1:1, and 1:3) of root exudate from two cultivars (K 326 and NC 567) were included in the experiments. A deionized water treatment was used as a control for the two cultivars. Hatching chambers were maintained in the dark at each of three temperatures (15, 20, and 25 °C). Numbers of hatched juveniles were recorded on a weekly basis. Juveniles were removed from

the hatching chambers after counting. Experiments lasted for 42 and 53 days in 1994 and 1995, respectively. Results were evaluated by analysis of variance for the number of hatched juveniles at each of the individual sampling dates (SAS Institute, Cary, NC). Duncan's multiple range test was performed on all analyses to separate means.

Results

Increased hatching ($P \leq 0.05$) associated with exposure to root exudate from the susceptible cultivar K 326 was observed from 21 to 42 days of exposure in 1994 (Fig. 1). Root exudate from resistant cultivar NC 567 induced more ($P \leq 0.05$) hatching than that

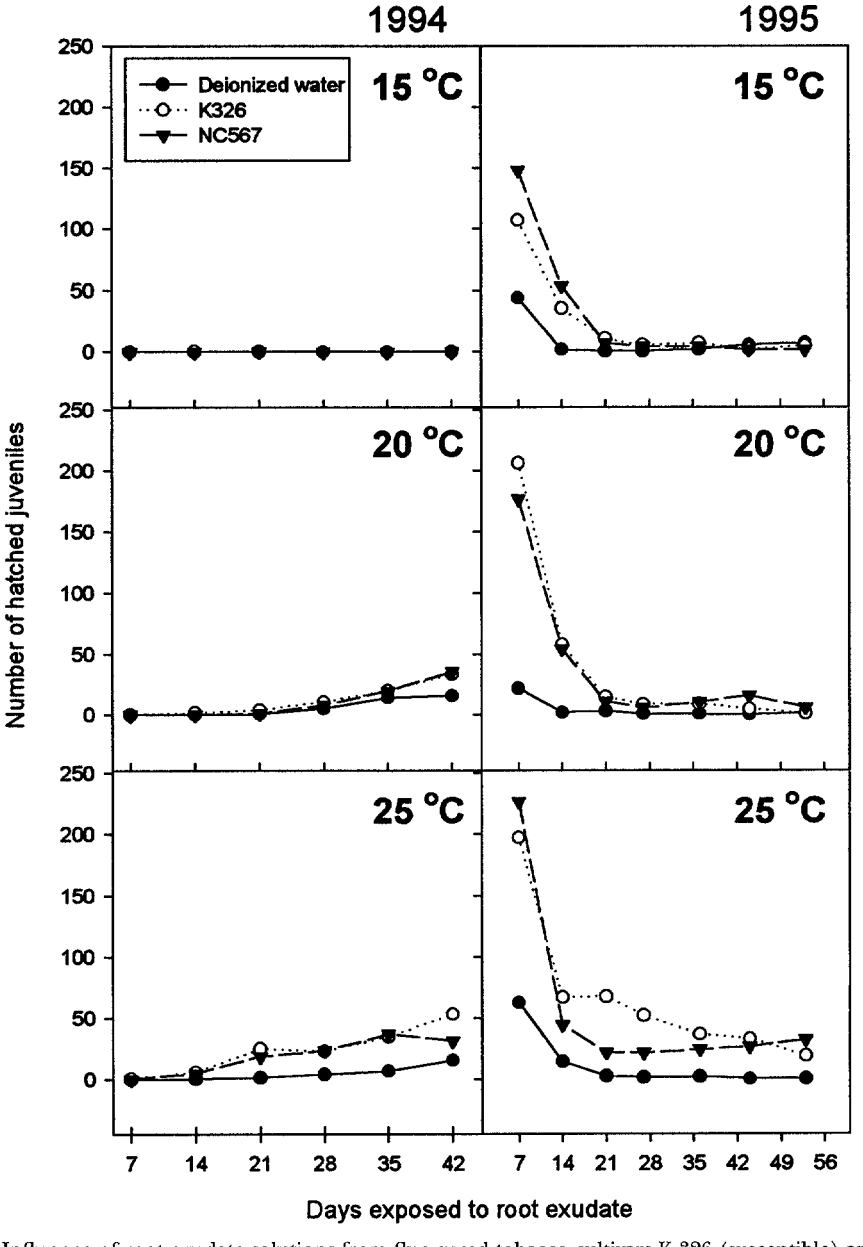

Fig. 1. Influence of root exudate solutions from flue-cured tobacco cultivars K 326 (susceptible) and NC 567 (resistant) on hatching of *Globodera tabacum solanacearum* at 15, 20, and 25 °C.

in deionized water at 28 and 35 days of exposure. In 1995, root exudate from K 326 also induced greater hatching ($P \leq 0.05$) than deionized water at 25 °C. More ($P \leq 0.05$) hatched juveniles were recorded after 7, 36, 44, and 53 days of exposure to root exudate from NC 567 than in deionized water at 25 °C. No differences were observed in stimulation of hatching by root exudates from the resistant and susceptible cultivars in 1994. Similar results were observed in 1995, except that root exudate from K 326 induced greater hatching ($P \leq 0.05$) than that from NC 567 on day 21 at 25 °C.

Hatching associated with the 1:1 dilution of root exudate in 1994 was similar to that observed with undiluted root exudate at 20 °C and 25 °C (Fig. 2). Dilution of root exu-

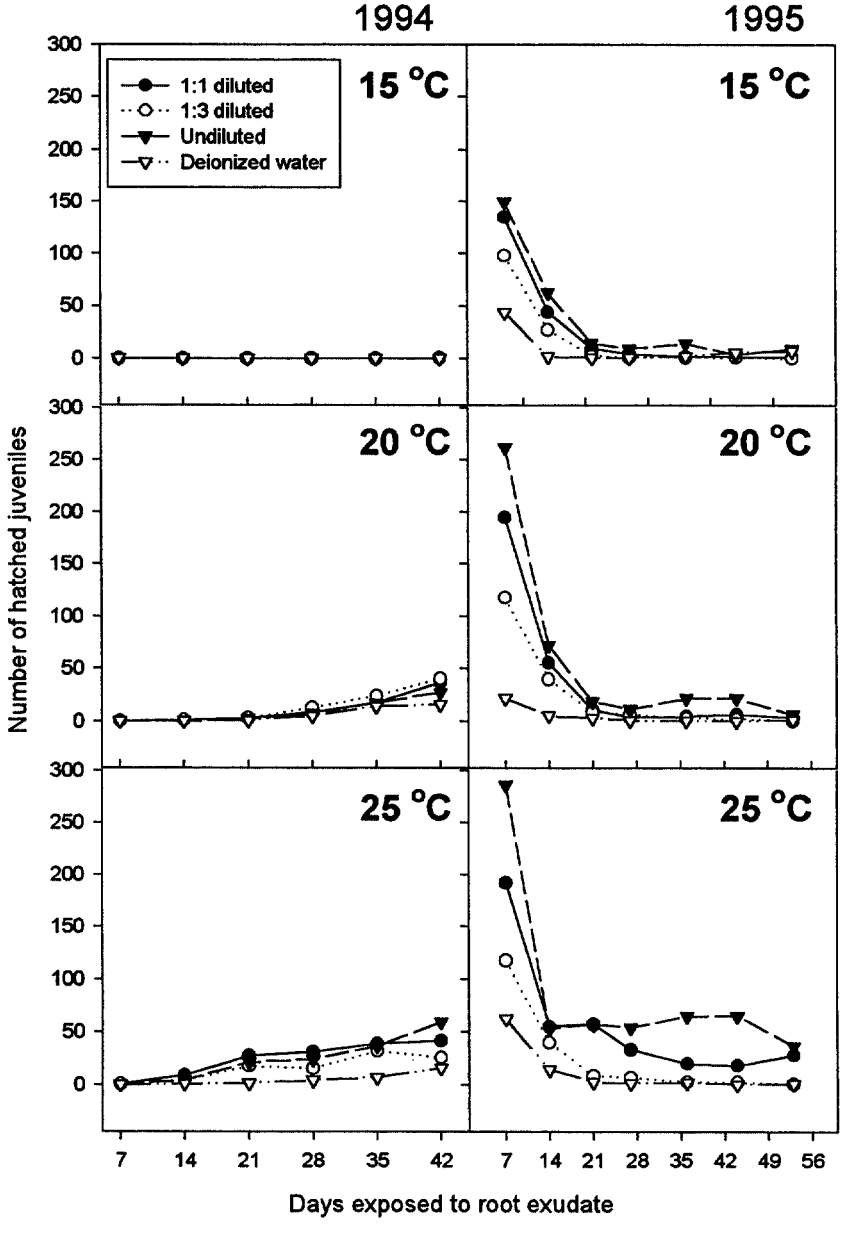

FIG. 2. Numbers of juveniles of *Globodera tabacum solanacearum* hatched in flue-cured tobacco root exudate, undiluted or diluted 1:1 or 1:3, or deionized water at 15, 20, and 25 °C.

date 1:1 did not reduce hatch stimulation ($P \leq 0.05$) in 1995 compared to the undiluted root exudate on day 21 but had lower ($P \leq 0.05$) hatching than undiluted root exudate at sample dates 27 and 36 at 15 °C, at 21 and 36 days at 20 °C, and at 36 and 44 days of exposure at 25 °C. The 1:3 dilution of root exudates reduced hatching ($P \leq 0.05$) compared to the 1:1 dilution and undiluted root exudate in 1994 after 28 and 42 days of exposure at 25 °C, respectively. Hatching at the 1:3 dilution was higher ($P \leq 0.05$) than that in deionized water only at 35 and 42 days at 20 °C and after 35 days of exposure at 25 °C. In 1995, the 1:3 dilution significantly reduced hatching compared to undiluted root exudate at 7, 14, and 27 days of exposure at 20 °C. More hatching ($P \leq 0.05$) was observed in undiluted root exudate than in the 1:3 dilution from 14 to 44 days of exposure at 15 °C and after 36 days of exposure at 25 °C. Differences ($P \leq 0.05$) in hatching between 1:3 dilution and deionized water were found in 1995 at 7, 14, and 44 days of exposure at 15 °C and at 7, 14, and 27 days of exposure at 20 °C, but only at 14 days of exposure at 25 °C. No interaction between cultivar and dilution was detected in either experiment, except on sampling day 27 at 20 °C in 1995. In that case, the 1:3 dilution of root exudate from NC 567 reduced hatching, whereas similar dilution of root exudate from K 326 had no effect. Very few juveniles hatched at 15 °C in 1994, despite exposure to root exudates from the two cultivars (Fig. 3). More hatching appeared to occur at 20 °C and 25 °C, although apparent differences in hatching between 15 °C and 20 °C did not persist beyond the first 2 weeks. No stimulation of hatching by root exudates from ei-

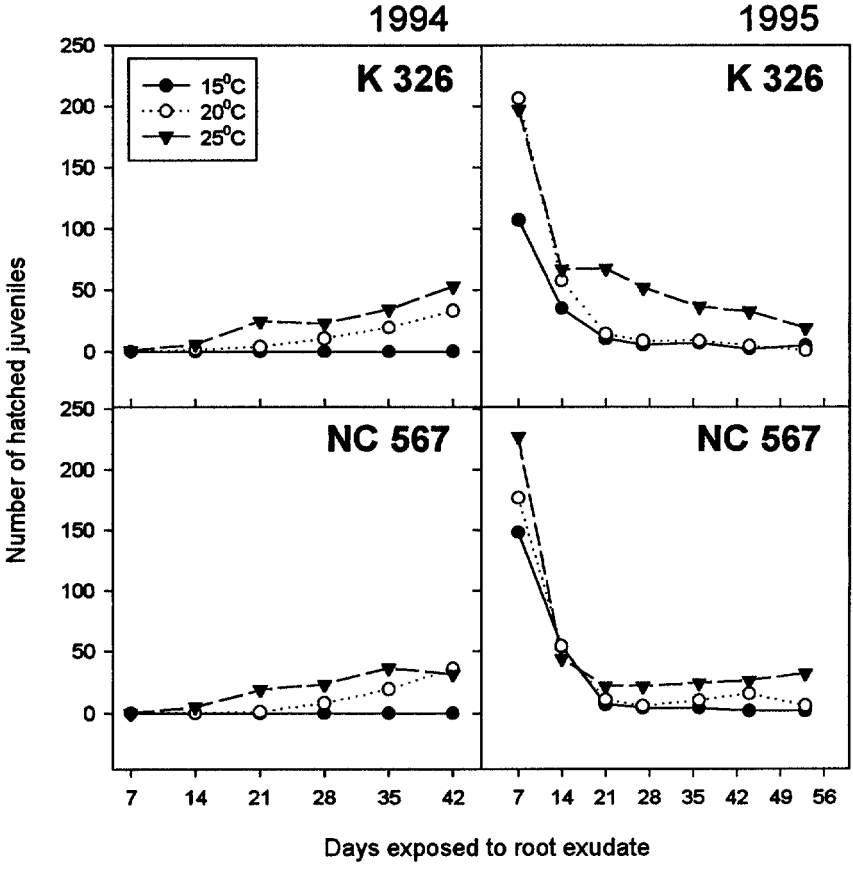

FIG. 3. Hatching by *Globodera tabacum solanacearum* at 15, 20, and 25 °C in root exudate solutions from flue-cured tobacco cultivars K 326 (susceptible) and NC 567 (resistant).

ther cultivar was observed at 15 °C or 20 °C in 1994 (Fig. 1). In 1995, more hatching ($P \leq 0.05$) was observed after exposure to root exudate from K 326 compared to deionized water during the first 4 weeks at 15 °C and 20 °C. Root exudates from NC 567 stimulated greater hatching ($P \leq 0.05$) for the first 21 and 27 days after exposure at 15 °C and 20 °C, respectively.

Discussion

The hatching response by *G. t. solanacearum* to tobacco root exudates was similar to that reported for *G. t. tabacum* but relatively small compared to that of potato cyst nematodes to exudates from potato roots (Arntzen et al. 1993; LaMondia 1995). As in the study with *G. t. tabacum,* the influence of host root exudates on hatch of *G. t. solanacearum* declined with increasing dilution of those exudates.

We did not find a correlation between hatching stimulation by tobacco root exudates and resistance to *G. t. solanacearum,* as has been reported for potato cyst nematodes (Arntzen et al., 1993, 1994; Farrer and Philips, 1983; Turner and Stone 1981). Our observations of similar hatching in response to exudates from tobacco cultivars resistant or susceptible to *G. t. solanacearum* also agree with those from a study of resistance to *G. t. tabacum* (LaMondia, 1988). The results from these experiments suggest that resistance to tobacco cyst nematodes does not involve a reduction in the stimulatory influence of host root exudates on nematode hatching. Nematodes hatch and penetrate resistant tobacco germplasm but develop at a slower rate or fail to reach reproductive maturity (Baalawy and Fox, 1971; LaMondia, 1988). Screening methods to identify germplasm resistant to *G. t. solanacearum* must, therefore, continue to focus on enumerating inhibited nematode development.

While tobacco root exudates exert an important stimulatory influence on hatching by tobacco cyst nematodes, other factors need to be carefully considered. Development and reproduction of these nematodes are greatly influenced by soil temperature (Adams et al., 1982). Little or no hatching occurred in our experiments at temperatures below 20 °C, regardless of the presence of host root exudates. Significant variation also occurred in the amount of hatching among repeat experiments in this work with *G. t. solanacearum,* as well as that with *G. t. tabacum* (LaMondia, 1995). Diapause induced by endogenous factors or environmental conditions during nematode egg production could account for this variability (LaMondia, 1995). These considerations and the smaller hatching rates observed for cyst nematode species parasitizing tobacco vs. potatoes could indicate differences in the relative importance of host root exudates in stimulating hatch of *G. t. solanacearum* and *G. t. tabacum* versus *G. rostochiensis* and *G. pallida.* Hatching stimulation by host root exudates may play a more important role in the life cycle of *G. rostochiensis* and *G. pallida,* which have a single generation in each growing season compared to species like *G. t. solanacearum* and *G. t. tabacum* that complete multiple generations in a growing season. Recognizing these differences is important in designing improved nematode management practices, particularly those involving cultural practices and host resistance.

In summary, hatching rates for *G. t. sclanacearum* were relatively low and were similarly stimulated by exposure to root exudates from a resistant (NC 567) and susceptible (K 326) cultivar of flue-cured tobacco. Resistance evaluation should continue to focus on identifying tobacco genotypes that restrict nematode development within host roots.

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