

RESEARCH/INVESTIGACIÓN

STIMULATING HATCH OF TOBACCO CYST NEMATODE, *GLOBODERA TABACUM TABACUM*, BY HYDROPONICALLY OBTAINED WEEDY *SOLANUM* SPP. ROOT EXUDATES

A. Adams^{1*}, J. LaMondia², R. Cowles², B. Nicholson¹, and T. Mione¹

¹Central Connecticut State University, Biological Sciences, New Britain, CT, USA; ²Connecticut Agricultural Experiment Station (CAES) Valley Laboratories, Windsor, CT, USA; *Corresponding author: aadams@ccsu.edu

ABSTRACT

Adams, A. A., J. LaMondia, R. Cowles, B. Nicholson, and T. Mione. 2020. Stimulating hatch of tobacco cyst nematode, *Globodera tabacum tabacum*, by hydroponically obtained weedy *Solanum* spp. root exudates. *Nematropica* 50:160-169.

Root infestation by the tobacco cyst nematode (TCN) is a significant problem in Solanaceous crops in North America and Europe. Some related Solanaceae that are typically regarded as weeds, are known or suspected hosts of TCN. Cyst nematode eggs usually hatch when chemicals produced by the roots of their specific host plants are detected. The purpose of this experiment was to use root exudates obtained from hydroponically grown plants to identify weedy species of *Solanum* that may stimulate the hatching of TCN. A hatching assay was conducted using three concentrations of root exudate from nine accessions of hydroponically grown *Solanum* spp. All plants were grown under *in vitro* conditions. Linear regression and ANOVA support that hydroponically obtained root exudates from accessions of *S. dulcamara*, *S. ptychanthum*, and *S. physalifolium* elicited a dose-dependent hatching response. *Solanum* spp. growing in or around agricultural fields may act as reservoir hosts, or some accessions may be used as trap crops for TCN management. Hydroponically produced exudate may be a more efficient alternative to field grown plants for nematological studies.

Key words: *Globodera tabacum*, hatching assay, hatching factor, hatching response, hydroponic, root exudate, *Solanum*, TCN, tobacco cyst nematode, weeds

RESUMEN

Adams, A. A., J. LaMondia, R. Cowles, B. Nicholson, y T. Mione. 2020. Estimulación de la eclosión del nematodo del quiste del tabaco (TCN, siglas en inglés), *Globodera tabacum tabacum*, con exudados radicales de la maleza *Solanum* spp. obtenidos por hidroponía. *Nematropica* 50:160-169.

La infestación de raíces por el nematodo del quiste del tabaco (TCN, siglas en inglés) es un problema significativo en cultivos de Solanáceas en Norte América y Europa. Algunas Solanaceae relacionadas que normalmente se consideran malezas, son hospederas conocidas o sospechosas de TCN. Los huevos de los nematodos formadores de quistes usualmente eclosionan cuando detectan químicos producidos por las raíces de sus hospederos específicos. El propósito de este experimento fue utilizar exudados radicales obtenidos de plantas cultivadas en hidroponía, para identificar especies de malezas de *Solanum* que puedan estimular la eclosión del TCN. Se estableció un ensayo de eclosión con tres concentraciones de exudados radicales de nueve accesiones de *Solanum* spp. cultivadas en hidroponía. Todas las plantas se cultivaron bajo condiciones *in vitro*. Los análisis de regresión lineal y ANOVA respaldaron que los exudados radicales

de las accesiones de *S. dulcamara*, *S. ptychanthum*, y *S. physalifolium* obtenidos por hidroponia desencadenaron una respuesta dependiente de la dosis en la eclosión. Las plantas de *Solanum* spp. que crecen dentro o en los alrededores de campos agrícolas pueden actuar como reservorios, o algunas accesiones pueden ser utilizadas como cultivos trampa para el manejo de TCN. Los exudados producidos por hidroponia pueden ser una alternativa más eficiente a las plantas cultivadas en campo para estudios nematológicos.

Key words: *Globodera tabacum*, ensayo de eclosión, factor de eclosión, respuesta de eclosión, hidroponia, exudado radical, *Solanum*, TCN, nematodo del quiste del tabaco, malezas

INTRODUCTION

The tobacco cyst nematode (TCN), *Globodera tabacum* (Lownsbery and Lownsbery, 1954) Behrens, 1975, is an obligate root parasite of plants in the Solanaceae (nightshade family), that feeds on the roots of many crop plants within the family, including tobacco (*Nicotiana tabacum* L.), tomato (*Solanum lycopersicum* L.), eggplant (*S. melongena* L.), and pepper (*Capsicum annuum* L.) (Lownsbery, 1953; Miller and Gray, 1968; Harrison and Miller, 1969). Of the three described subspecies, *G. tabacum tabacum* is found in Connecticut, where it is a serious concern of tobacco farmers, resulting in stunted growth, reduction in yield, reduced leaf quality, and increased severity of fusarium wilt in tobacco (LaMondia, 1995a, 1999, 2002, 2015a)

Cyst nematode embryogenesis occurs inside the deceased female, followed by four juvenile stages (J1 – J4), and then an adult reproductive stage (Williamson and Kumar, 2006; Thapa, *et al.*, 2017), which occurs within the host roots. Tobacco cyst nematodes may produce more than one, and up to three generations in a growing season, especially in warmer soils (LaMondia, 1995a, 2008; Wang *et al.*, 1997; Ambrogioni *et al.*, 2000), with an egg-to-egg generation time of approximately five to six weeks (LaMondia, 2008). Cysts persist in the soil for many years due to their desiccation and freezing tolerance (CABI, 2019).

Hatching is stimulated by chemical cues leaching through the soil from roots of potential host plants (Palomares-Rius *et al.*, 2013; Thapa, *et al.*, 2017), although spontaneous hatch in water does also occur in smaller quantities (Fenwick and Widdowson, 1958). Both host and non-host plants may stimulate hatching, but nematodes are unable to complete their life cycle in non-hosts. Susceptibility is further confirmed through microscopic examination of stained roots

(LaMondia, 1988, 1996) or recovery of new generations of cysts from soil (Harrison and Miller, 1969; LaMondia, 1988, 1995a, 1996). LaMondia (1991, 1999) determined that host resistance is genetically conveyed.

The purpose of this experiment was to identify species or accessions of weedy *Solanum* that may induce the hatching of TCN using root exudates obtained from hydroponically grown plants. Identifying weeds capable of stimulating hatch would be of use in TCN management. Traditional cyst nematode experiments have used root diffusate prepared from soil-grown plants, which necessitates removing soil from the roots before soaking (Ellenby and Perry, 1976; LaMondia, 1995b; Wang *et al.*, 1997). Hydroponically obtained root exudates have been used for potato cyst nematode (PCN) [*G. rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 and *G. pallida* (Stone, 1973) Behrens, 1975] hatching assays (Whitehead, 1977; Scholte, 2000; Navarre and Suszkiw, 2013). There appears to be a lack of similar studies in the literature with TCN (Whitehead, 1977; Navarre and Suszkiw, 2013). Obtaining viable hydroponic exudates for hatching assays offers a more efficient and cleaner methodology for cyst nematode studies.

MATERIALS AND METHODS

Soil containing field cysts was collected from six infested *Solanum ptychanthum* (Dunal) microplots maintained at CAES in Windsor, CT (LaMondia, 2002) in October of 2017, and pooled before air-drying. Cysts were extracted from the soil using a modified Fenwick can (LaMondia and Brodie, 1987), followed by hand-selection of cysts of similar size, color, and fullness. Those that were too small, misshapen, ruptured, shriveled, or contained visible empty airspace were discarded (LaMondia, *et al.*, 1986).

Test plants selected were restricted to *Solanum* species that may be grown in Connecticut River Valley soil, and species without highly aggressive thorns or spines that may make them undesirable for agricultural use. The following species (Table 1) were used to identify which may be producing hatching factors that stimulate the emergence of second-stage juvenile TCN: Carolina horsenettle (*S. carolinense* L.), bittersweet nightshade (*S. dulcamara* L.), hairy nightshade [*S. physalifolium* Rusby var. *nitidibaccatum* (Bitter)

Edmonds], eastern black nightshade (*S. ptychanthum* Dunal), Jerusalem cherry (*S. pseudocapsicum* L.), and sticky nightshade (*S. sisymbriifolium* Lam). Two different, locally growing accessions of *S. ptychanthum* and three different accessions of *S. pseudocapsicum* were used. One accession of *S. ptychanthum* (Windsor) was used as a positive control for the production of a hydroponic root exudate after a previous assay confirmed that this species was a preferred host of TCN (LaMondia, 1995b). ‘Winmalt’ winter barley

Table 1. Seed stock used for this experiment and their sources. Collection locations of parent plants are given where the information was available.

Species	Accession Number or Cultivar Name	Source or Collector/ Collection Location, Year
<i>Solanum carolinense</i> L.	Pulaski	T. Dewlen, Potato field, 41.091397, -86.591464, Pulaski County, IN, 2016
<i>Solanum dulcamara</i> L.	Bristol	A. Adams, Residential, 41.690835, -72.912966, Bristol, CT, 2015
<i>Solanum physalifolium</i> Rusby var. <i>nitidibaccatum</i> (Bitter) Edmonds	Mercer	R. Boydston and T. Anderson, USDA ARS Mercer, 2011
<i>Solanum ptychanthum</i> Dunal	New Britain	T. Mione, CCSU Campus, 41.690907, -72.766860 New Britain, CT, 2015
<i>Solanum ptychanthum</i> Dunal	Windsor	J. LaMondia, CAES Valley Laboratory, Windsor, CT, 2016
<i>Solanum pseudocapsicum</i> L.	PI 420412, ‘38’	USDA National Plant Germplasm System, Spain, 1975
<i>Solanum pseudocapsicum</i> L.	Griff 16422, ‘Red Giant’	USDA National Plant Germplasm System, France, 2006
<i>Solanum pseudocapsicum</i> L.	PI 478485	USDA National Plant Germplasm System, Bolivia, 1981
<i>Solanum sisymbriifolium</i> Lam.	PI 38121, ‘PBL 73’	USDA National Plant Germplasm System, India, 1973
<i>Solanum sisymbriifolium</i> Lam.	Medaryville	T. Dewlen, Vegetable garden, 41.077698, -86.890357, Medaryville, IN, 2016
<i>Hordeum vulgare</i> L.	‘Winmalt’	J. LaMondia, CAES Valley Laboratory, Windsor, CT 2016

(*Hordeum vulgare* L.) was used as a negative control. These confirmed hatch-inducing (*S. ptychanthum* from Windsor) and non-inducing species (barley) were used to assess the usefulness of a hydroponic system for obtaining root exudates for hatching studies. All plants were grown *in vivo* hydroponically using stone wool (Grodan A-OK Starter Plugs, Milton, ON; Pagliarulo, 2000) at room temperature averaging approximately 19°C. A photoperiod of 12 hr of light:12 hr of dark was used. Light sources consisted of a combination of incandescent and fluorescent lamps, with some filtered sunlight through a nearby window with intensity ranging from approximately 2200-7500 lux, as measured by a hand-held light meter (Model #850070, Sper Scientific, Scottsdale, AZ). Plant cultures were maintained in deep-water culture hydroponic chambers containing approximately 1500 ml buffered nutrient solution. Once a week, liquid in each growth chamber was replenished with nutrient concentrate (Botanicare Pure Blend Pro Gro Nutrient Solution, Vancouver, WA) diluted with distilled water according to manufacturer instructions per the age of the plants and buffered to a pH of 5.8 to 6.2 with Bloom City Professional Grade pH Up Growing Supplement (Pagliarulo, 2000). Distilled water was added to the chambers once a week to make up for liquid lost to evaporation. The pH of the hydroponic chambers was also monitored weekly and adjusted with buffer as needed. For each of the ten accessions, there were five plants per hydroponic chamber, and four replicates per accession. Plants were grown for eight to ten weeks before root exudate was collected, bottled undiluted or serially diluted with distilled water to result in 1:1, 1:10, and 1:100 parts exudate to distilled water, and stored at 4°C until the start of the hatching assay. The roots of all plants were harvested, pooled by accession, and weighed. Voucher specimens of laboratory-grown *Solanum* spp. were deposited at the CCSU Orville Bissett Herbarium, Biology Department, Central Connecticut State University, New Britain, CT.

The hatching assay was performed in 24-well plates according to LaMondia (1995b) with five rehydrated cysts per well, and two wells equaling a replicate to compromise between counting efficiency and reduced risk of loss/rupture. One milliliter of assigned treatment (including distilled water) was added to each of the ten wells containing cysts of the corresponding plate, with one treatment per plate to prevent cross-

contamination. Hatching plates were incubated in the dark at 25°C, and second-stage juvenile (J2) nematodes were counted at 40X weekly for five weeks to determine cumulative hatch/replicate. The corresponding liquid for each treatment was replaced after counting, including wells for which there was no hatch. To determine the percentage of J2 that hatched, cysts were individually crushed and dissected at 60X to count the viable remaining eggs (LaMondia, *et al.*, 1986; EPPO, 2013). Cyst viability was calculated as the number of cumulative J2 hatched per replicate plus the number of viable eggs remaining inside cysts. One hundred replicates were used to calculate expected mean cyst viability (approximately 1,000 cysts total for this measure). All treatments were analyzed as percent hatch based on this expected mean viability.

Data were analyzed using Statistix 9.0 (Analytical Software, 2009) and Minitab 17 (2010) after inverse normal transformation of the percent hatched to standardize the variance, and \log^{10} transformation of exudate concentrations. *Solanum* with multiple accessions were tested using analysis of variance (ANOVA) to determine whether the data could be pooled or eliminated by being redundant. This allowed us to conduct an ANOVA with a factorial design on different species/accessions, dilutions, and the interactions between. A least squares regression analysis was performed to evaluate for dose-dependent response, followed by a homogeneity of slopes test to compare both the slopes and intercepts of species exhibiting dose responses. A Bartlett's test was used to compare variances. These tests were analogous to a post hoc Dunnett's Multiple Comparisons test. Pearson correlations were used to determine if there was a relationship between hatching and root mass or hatching and plant age at collection. An alpha level of 0.05 was used for all statistical tests for determination of significance.

RESULTS

There was no significant difference in transformed percent hatched between the three different accessions of *S. pseudocapsicum* [F(2, 36) = 2.87, $P = 0.070$], or any interaction between accession exudate dilution (log-transformed) [F(4, 36) = 0.590, $P = 0.669$]. Accession Griff 16422 was excluded based on greater similarity between the other two *S. pseudocapsicum* accessions (Table 1).

Instead of pooling the two remaining accessions, PI 420412 was randomly selected by coin toss as representative for the species over PI 478485. The two accessions of *S. ptychanthum* were significantly different [$F(1, 24) = 19.07, P < 0.001$]; therefore, these two accessions were analyzed separately in all subsequent tests.

Out of all cysts that were dissected, the overall mean initial egg viability was 162.8 ± 54.5 . There were highly significant differences in hatching between species or accessions of species [$F(7, 96) = 9.78, P < 0.001$]. Figure 1 shows that the highest mean percentage hatch of J2 was in undiluted *S. dulcamara* ($M = 51 \pm 18.9$). The lowest mean percentage hatch was in 10% diluted root exudate from *S. pseudocapsicum* accession PI 420412 ($M = 5.3 \pm 5.0$) and undiluted exudate from *S. sisymbriifolium* accession PI 381291 ($M = 5.6 \pm 2.2$). Hatch in undiluted or 10% diluted exudates of these two aforementioned accessions were all lower than the lowest hatch (1% dilution) of the negative control, barley ($M = 7.3 \pm 4.9$). In distilled water (not shown), mean percentage hatch was 11.0 ± 7.2 .

The overall influence of diluting the exudates

was highly significant [$F(2, 96) = 5.62, P = 0.005$, Figure 2]. There was a highly significantly different dose response between species [$F(14, 96) = 3.49, P < 0.001$]. The first homogeneity of slopes test indicated that out of the seven species or accessions analyzed, four were significantly different from barley: *S. carolinense*, *S. dulcamara*, *S. physalifolium*, and *S. ptychanthum* from Windsor; however, *S. carolinense* did not display a significant dose-response (Table 2). There was no significant response to dilution in barley [$F(1, 13) = 0.080, P = 0.782$, adjusted $R^2 = 0\%$].

A second homogeneity of slopes test indicated that root exudate concentration significantly predicted hatch in *S. ptychanthum* from Windsor [$F(1, 13) = 26.46, p < 0.001$, adjusted $R^2 = 65\%$], *S. dulcamara* [$F(1, 13) = 21.76, p < 0.001$, adjusted $R^2 = 60\%$], and *S. physalifolium* [$F(1, 13) = 5.18, p = 0.040$, adjusted $R^2 = 23\%$]. Figure 2 emphasizes the dose-dependent responses for *S. dulcamara*, *S. physalifolium*, and *S. ptychanthum* from Windsor. The slopes of these three exhibited homogeneous variances ($P = 0.530$), with no difference in slope ($P = 0.147$) or intercept ($P = 0.304$).

There was no correlation between percent

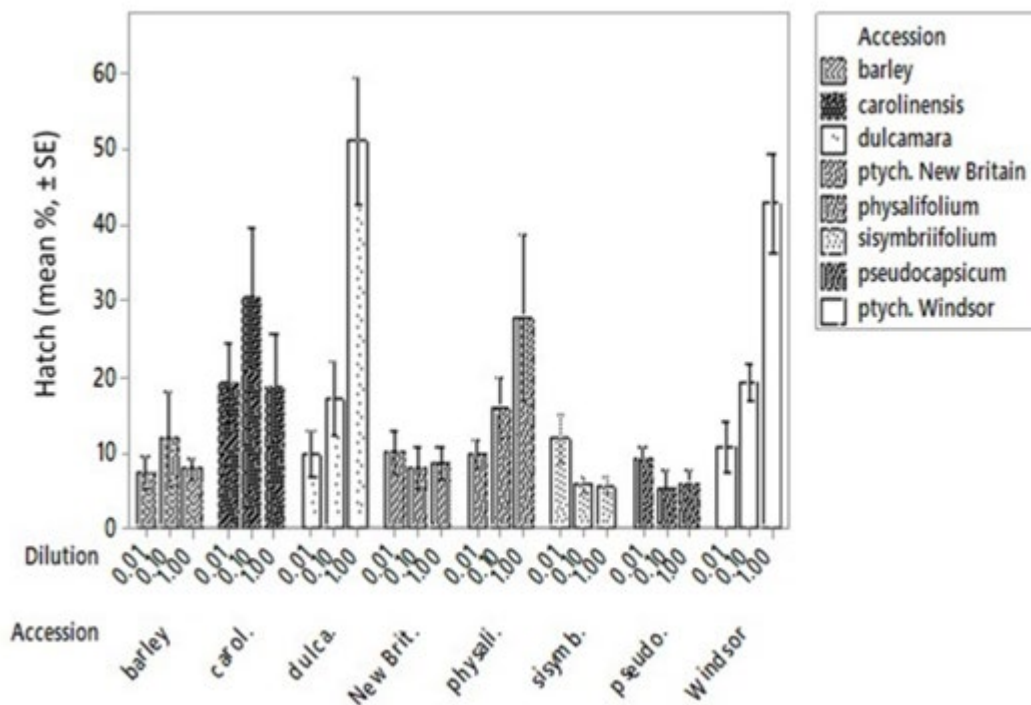


Figure 1. Comparison of the percent hatch (mean \pm SE) of tobacco cyst nematodes (*Globodera tabacum*) second-stage juveniles (J2) in three concentrations of hydroponic *Solanum* spp. and barley (*Hordeum vulgare*) root exudate.

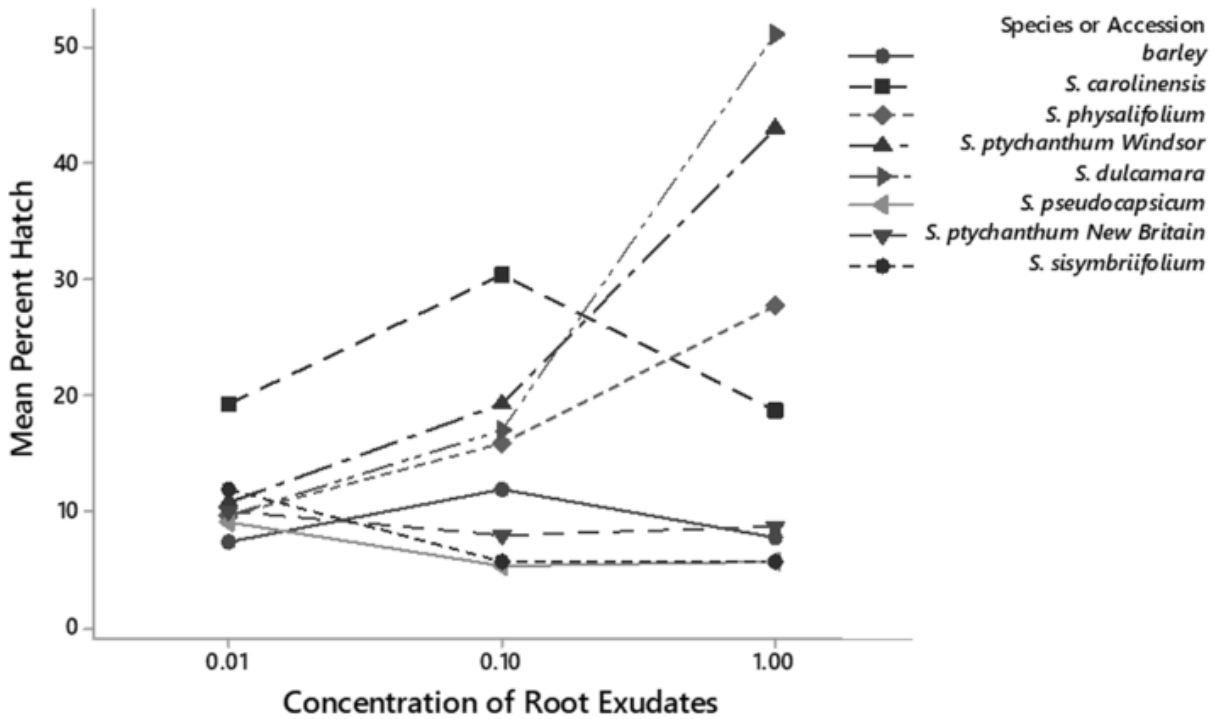


Figure 2. Comparison of dose-dependent responses of mean percent hatch of tobacco cyst nematodes (*Globodera tabacum*) second-stage juveniles (J2) with increasing concentrations of *Solanum* spp. and barley (*Hordeum vulgare*) root exudate.

Table 2. Results of the homogeneity of slopes test on linear regressions of *Solanum* spp. compared to barley (*Hordeum vulgare*): there were homogenous dose-dependent responses for *S. dulcamara*, *S. physalifolium*, *S. ptychanthum* from Windsor, with homogeneous variances ($P = 0.530$), no differences in slopes ($P = 0.147$) or intercepts ($P = 0.304$).

Species, Accession	Significantly Different from Barley	P-Value ^y	Significant Dose-Response	Slope	Intercept
<i>H. vulgare</i> (barley)	-	0.782	No	0.041	-1.428
<i>S. carolinense</i>	Yes ^z	0.894	No	-0.024	-0.870
<i>S. dulcamara</i>	Yes	< 0.001	Yes	0.711	-0.073
<i>S. physalifolium</i>	Yes	0.040	Yes	0.327	-0.687
<i>S. pseudocapsicum</i>	No	0.179	No	-0.159	-1.754
<i>S. ptychanthum</i> , New Britain	No	0.761	No	-0.042	-1.494
<i>S. ptychanthum</i> , Windsor	Yes	< 0.001	Yes	0.576	-0.231
<i>S. sisymbriifolium</i>	No	0.088	No	-0.177	-1.676

^y n = 15

^z*S. carolinense* was significantly different from barley in elevation, but not in slope.

hatch and plant age [$r(38) = -0.135$, $P = 0.405$] nor between root mass and increase in hatch [$r(38) = -0.132$, $P = 0.418$].

DISCUSSION

There were two objectives of this study. The

first was to identify Solanaceous weeds that may produce TCN hatch-stimulating compounds, for which evidence of a dose-dependent response indicated that some accessions stimulated hatch. The second objective was to determine whether an inexpensive, small hydroponic growth system could be used to cultivate plants for root exudate

collection. The observed increased percentage of hatch in the positive control, *S. ptychanthum* from Windsor, combined with the apparent lack of a dose response plus low hatching rate in the negative control, indicates that hydroponics is a viable source of root exudates for cyst nematode research. Once the nutrient solution and ambient conditions are optimized for intended species, a hydroponic system may benefit cyst nematode research by: simplifying the preparation of stock exudate solutions, facilitating the indefinite maintenance of plant clones, cross-breeding for resistance (LaMondia, 1999), and having the potential for culturing nematodes without risk of soil contamination (Yoshiga and Umezaki, 2015). As hatching rates have been demonstrated to increase in PCN when cultivated on plants exposed to a longer photoperiod, and when diapause time was decreased (Salazar and Ritter, 1993), TCN may also exhibit the same response to elongated photoperiods, which may be controlled throughout the year within an enclosed hydroponic system. A hydroponic method may enhance TCN for use as a model organism for PCN (APHIS, 2016).

Linear regression analysis and the homogeneity of slopes tests confirmed a decrease in TCN hatching response as concentration decreased for three accessions: *S. dulcamara*, *S. ptychanthum* Windsor, and *S. physalifolium*. Whitehead (1977) and Navarre and Suszkiw (2013) used hydroponically grown potato and other members of the Solanaceae to identify several compounds in root exudates that do, or do not act as hatching factors for PCN, although since potato is a poor host for TCN (Lownsbery, 1953; Miller and Gray, 1968), the exudate probably differs in composition.

The lack of dose-dependent effect in *S. carolinensis* could be due to hatching factors not being high enough to observe an effect, as Harrison and Miller (1969) identified this species as being a preferred host of TCN. A whitefly (*Trialeurodes vaporariorum* Westwood) infestation that was observed on *S. carolinensis* plants four weeks after exudate collection may be responsible for the conflicting results observed in our study, if the initial infestation was already underway at the time of collection, as this may have affected the chemical composition of the root exudates. Hoysted *et al.* (2018) demonstrated that foliar feeding can reduce the release of *G. pallida* nematode hatching factors in the rhizosphere. If

above-ground predation can affect the susceptibility to below-ground root parasites, this also raises the question of whether a lack of vigor, the presence of other pathogens, or insect or mite feeding, could make resistant cultivars or accessions more susceptible to cyst nematode infestation.

Almost 60 different species or cultivars of plants in Solanaceae have been investigated for host status by Lownsbery (1953) and Harrison and Miller (1969), some with conflicting results. In both studies ‘California Wonder’ pepper and ‘Katahdin’ potato were identified as poor hosts but their results for ‘Black Beauty’ eggplant did not concur. Taxonomic confusion or misidentification may explain further disagreement between results for the weedy species, *S. sarrachoides* (possibly *S. physalifolium*, as was used in this study) and *S. nigrum* (likely *S. ptychanthum* as in LaMondia (1995b)) and here. Although both *S. dulcamara* and *S. nigrum* were preferred hosts in the earlier experiments, little was published regarding the origins of the plants called *S. nigrum*, and it is now assumed that specimens growing in the northeastern United States are all actually *S. ptychanthum* (Ogg *et al.*, 1981; Schilling, 1981). *Solanum nigrum*, an Old-World native that has become naturalized in the United States (Schilling, 1981), has been found in the Mid-Atlantic states, and elsewhere in New England, but is considered as not present in Connecticut (Kartesz, 2015), where both of the accessions that we used were collected. It has been suggested that *S. ptychanthum* should be used for all plants collected in Connecticut (E. Schilling, University of Tennessee, Knoxville, pers. comm.). Taxonomic confusion is not the only possible explanation for differing results between studies.

In our experiment, the differences in chemical composition of solutions between hydroponic chambers were not determined, so it is also unknown whether the different species or accessions of plants significantly affected the background chemistry of the hydroponic growth chamber solution in other ways, which may have either stimulated or inhibited hatch. There may also be genetic accessional differences between populations of plants that affect hatch stimulation or host susceptibility, environmental differences between plant cultures affecting hatching potential, or differences between populations of TCN in their sensitivity to hatching factors or virulence. The

accession of *S. sisymbriifolium* used in our experiment did not stimulate hatch as expected, considering the plant's current use as a trap crop (Scholte, 2000; Timmermans *et al.*, 2006; Dias *et al.*, 2012). A preliminary hatching assay conducted in 2017 used a different accession of *S. sisymbriifolium* (Medaryville, IN), which did appear to stimulate hatch from root cuttings grown hydroponically. *Solanum ptychanthum* from Windsor stimulated hatch, but not the *S. ptychanthum* accession from New Britain.

These results may suggest some important implications for TCN management. Host-pathogen relationships depend on genetic and environmental factors of both organisms. Because of this potential variation there is no guarantee that all resistant species or cultivars of plants will be so to all TCN populations. Both native or invasive *Solanum* spp. growing in or around agricultural fields may be reservoir hosts for TCN (Sullivan *et al.*, 2007; Mimee *et al.*, 2014). Plants that stimulate hatch may indicate susceptibility to the pathogen. Plants that stimulate hatch may be also used as triggers to induce hatching before planting crops (Whitehead, 1977; Timmermans *et al.*, 2006; Dias *et al.*, 2012), but a screening should be performed for the production of hatching stimulants before a new plant accession is used as a source of root exudate or for TCN management.

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