RESEARCH

Interactions of Selected Potato Cultivars and Populations of *Meloidogyne hapla* Adapted to the Midwest U.S. Soils

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ABSTRACT

Meloidogyne hapla Chitwood is among the most serious nematode pests in temperate vegetable crops grown in rotation with potato (*Solanum tuberosum* L.), a crop with known resistance to potato cyst. While the types of agronomic trait improvements, crops, and pests are limited by resources, preserving improved crops in a rotation system requires understanding their performance against nontarget pests such as *M. hapla*. In greenhouse experiments, the interactions of four populations of *M. hapla* (*Mh* 1, *Mh* 2, *Mh* 3, and *Mh* 4) and six potato cultivars and lines, including 'Boulder' and 'Missaukee' with the *H1* gene, were tested. The potato cultivars showed a 12 to 33% degree of suitability to the populations of *M. hapla* when compared with tomato (*Solanum lycopersicum* L. [syn. *Lycopersicon esculentum* Mill.), the host in which the populations were cultured, indicating that buildup of the populations of *M. hapla* potentially may be a problem. However, the responses varied by *M. hapla* population and potato cultivar interactions, suggesting that the management challenges will be site specific. Of the four *M. hapla* populations, *Mh* 3, an isolate from sandy soil under extended methyl bromide and other pesticide application, was the most pathogenic. The study provides critical data for developing agro-biologically integrated approaches to managing nematode parasitic variability.

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Abbreviations: *Mh*, *Meloidogyne hapla*.

While varying regionally, root-feeding plant-parasitic nematodes are among the economically important pests in potato (*Solanum tuberosum* L.) and other vegetable cropping systems. In temperate climates of North America, the northern root-knot (*Meloidogyne hapla* Chitwood) is a serious pest of vegetable crops (Bird et al., 2004). Although potato is a host, *M. hapla* is not considered a serious pest in the Midwest. However, potatoes are grown in cropping systems that include vegetable crops that are very sensitive to *M. hapla* (Bird et al., 2004).

With the increasing environmental and consumer health awareness driven restrictions on broad-spectrum pesticides and nematicides, developing nematode-resistant crops is a needed alternative management technology (Project GREEEN, 2010). However, resource limitation is a major factor in developing nematode resistance management. For example, potato cyst nematodes (*Globodera* spp.) are the most important pest of potatoes worldwide, attracting the most resources toward developing resistance and other management strategies (Bates et al., 2002). When potato cultivars with resistance to cyst and other target nematodes are grown with rotation crops typical in the Midwest United States, however, two challenges arise: (i) preventing

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nontarget nematodes from becoming serious pests of the potato cultivars and (ii) protecting the rotation crops from build up of nontarget nematodes such as *M. hapla*. Little is known about the status of the Midwest U.S. potato cultivars against *M. hapla*.

In addition to broad host range within field and vegetable crops and no commercially available resistant cultivars, *M. hapla* continues to be a problem because of parasitic (genetic) variability (look the same but act differently) (Melakeberhan et al., 2007). Moreover, the production soils and production practices where populations of *M. hapla*, potatoes, and the rotation crops are grown are highly heterogeneous, and we have limited understanding of the role of agro-ecological factors on *M. hapla*–host interactions (Melakeberhan et al., 2007). To develop potentially sustainable management strategies, therefore, an integrated understanding of the interactions of populations of *M. hapla* and the crops in a rotation system will be needed.

The overall project goal is to develop resistance management for nematodes through mapping their ecological distribution in production soils and understanding their parasitic variability in selected Michigan vegetables. The objective of this study was to screen the status of selected potato cultivars and breeding lines with known nematode resistance (*H1* gene) against Michigan populations of *M. hapla* present in potato production soils. The working hypothesis is the potato cultivars will not be suitable hosts for *M. hapla* populations present in the production systems. The hypothesis may be true or false for some or all of the selected potato cultivars and populations of *M. hapla*. True for all will mean that the potato cultivars can be grown with little concern about the presence of *M. hapla*, and false for all will require supplementary management strategies that suppress *M. hapla*. Partially true for some of the cultivars and/or populations of *M. hapla* will mean that the problem will probably be site specific, providing the proof-of-concept that growers can use when selecting cultivars.

MATERIALS AND METHODS

Experimental Organisms and Soil Type

Using steam-sterilized Newaygo (frigid Alfic Haplorthods) sandy loam soils (87:8:5 sand:silt:clay texture and pH 7.3), the study included four populations of *M. hapla,* six potato cultivars and breeding lines adapted to Midwest conditions, and the tomato (*Solanum lycopersicum* L.) cultivar Rutgers (Tables 1 and 2). Rutgers, in which the populations of *M. hapla* were maintained, was included as a susceptible control for nematode viability and relative reference of host suitability because there are no isolines available. Sandy loam soil was selected because it is the most common soil type for potato and other vegetable production where most of the populations of *M. hapla* (*Mh*) are found. The *Mh* 1, *Mh* 2, and *Mh* 3 populations were collected from mineral soils of similar texture as the experimental soil and *Mh* 4 was collected from Houghton muck (mesic Typic Haplosapritis) soil (Table 1). The populations were selected for this study because they had exhibited parasitic variability relative to their habitats (Melakeberhan et al., 2007).

Table 1. Production systems and soil textures from where the experimental populations of *Meloidogyne hapla* (*Mh*) came and percent differentiation of the inocula cohorts in the two experiments. Data are means of four replications per experiment.

Nematode sources†		Differentiation‡	
Population	Crop	Soil texture	Percent§
Mh 1	Nursery¶	Sandy	23.5 b#
Mh 2	Nursery	Sandy loam	26.6 ab
Mh 3	Nursery	Sandy	22.6 b
Mh 4	Celery	Muck	29.9 a

† Nematode sources were as described in Melakeberhan et al. (2007).

‡ Inoculum embryogenesis was measured as differentiated (juvenile visible) and undifferentiated (Zuckerman, 1985).

§ Results from Exp. 1 and 2 were combined because there is no difference between runs or significant interaction between experiment and *M. hapla* populations.

¶ Nursery refers to plant propagation site.

Means followed by the same letters are not statistically different from each other ($p < 0.05$). Numbers are rounded off to the closest decimal.

Table 2. Potato cultivars and lines, source of experimental plants, and agronomic and other traits used in the two experiments.

Potato cultivars, plant source, and traits			
Cultivar If and or line	Source	Agronomic†	Resistance to nematodes
Snowden	Tuber	Chip processing	Susceptible‡
Missaukee	Tuber	Table, round white *H1* gene	
Boulder	Tuber	Table, round white *H1* gene	
Michigan Purple	Tuber	Table, specialty	Susceptible‡
Jacqueline Lee	Tuber	Table, yellow	Susceptible‡
Kalkaska	Tuber	Chip processing	Susceptible‡
Rutgers§	Seed	Control	Susceptible

† Detailed varietal descriptions are available at the Michigan State University Potato Breeding and Genetics Program (2011).

‡ The cultivars' status against *M. hapla* and other nematodes was unknown.

§ Tomato cultivar Rutgers was included as a susceptible control for nematode viability.

The potato cultivars were selected for their chip processing, table specialty, and color (Table 2). In addition, the cultivars Boulder and Missaukee have the *H1* gene, which confers resistance to the golden cyst nematode, *Globodera rostochiensis* Mulvey and Stone (Douches et al., 2010). All but 'Snowden' were developed from the MSU Potato Breeding Program (Michigan State University Potato Breeding and Genetics Program, 2011).

Nematode Inoculum Preparation and Inoculations

Meloidogyne hapla eggs were obtained following the bleach (5% NaOCl) method described by Hussey and Barker (1973). Stages of embryogenesis were determined as differentiated or undifferentiated by randomly looking at 100 eggs four times per sample (Zuckerman, 1985). Inocula in both experiments had similar stages of embryogenesis but differed by *M. hapla* population (Table 1). *Meloidogyne hapla* 4 had significantly more differentiation than *Mh* 2 and *Mh* 3. Inoculum concentrations

were estimated from four 1-mL suspensions and were pipetted into three to four 1-cm diameter holes around each plant (Mennan et al., 2006).

Experiments

Using the four populations of *M. hapla*, two consecutive greenhouse experiments tested the reaction of Snowden, Missaukee, Boulder, MI Purple, Jacqueline Lee, and Kalkaska potato cultivars and the Rutgers tomato used as a susceptible control for nematode viability. Nematodes were inoculated at 9000 and 8500 eggs per 500 cc of soil contained in white Styrofoam cups in Exp. 1 and 2, respectively. Uninfected controls received tap water. Each treatment was replicated four times and each experiment consisted of 140 (7 cultivars × 5 *M. hapla* treatments × 4 replications) experimental units. At the time of nematode inoculation, potato plants, generated from tubers, were 30 d old and tomato seedlings, from emergence, were 11 d old. The experiments were terminated 30 d after nematode inoculation.

Greenhouse conditions were set at 25 ± 2°C with diurnal cycles of 8 h dark and 16 h day with photosynthetically active radiation of 300 to 500 μmol m^{-2} s^{-1} at canopy level. Plants were fertilized weekly with Scotts' Professional 20–20–20 (N–P–K) commercial mix (Scotts-Sierra Horticultural Products Company) and watered daily as needed. At the experimental temperatures, approximately 450 degree days (base 10°C) were accumulated, enough for *M. hapla* to complete a life cycle (Insera et al., 1982; Melakeberhan et al., 2010).

Measurements

At the end of each experiment, roots and tubers were carefully separated from soil and washed free of soils (Fig. 1) and fresh root weight was measured. An approximately 2- to 4-mm layer of each tuber was carefully peeled using fine scalpels and weighed. To maximize detection of infection levels, whole root system and tuber peels per treatment were stained in acid fuchsin (Hussey 1985). Stained roots and tuber peels were kept at 4°C until counted. The numbers and developmental stages of nematodes in roots and tubers were categorized as infective and early and late swollen second-stage juveniles, third- and fourth-stage juveniles, and adults (Melakeberhan and Dey, 2003; Agrios, 1997). Staining the whole root system was designed to minimize sampling errors and to maximize detection of infection levels. The staining process is prone to dislodging eggs that may have been in egg masses in root tissue. Therefore, presence or absence of eggs in the samples was noted as 1 and 0, respectively.

Data Analyses

The effects of cultivars and nematode population on nematode numbers were analyzed using two-way ANOVA. The assumption of normality of the residuals was tested by examining normal probability plots and stem-and-leaf plots of the residuals. The homogeneity of variances assumption was assessed visually by examining the side-by-side box plots and checked using Levene's test for equal variances. When the residuals were found to be right skewed, for example, for nematode population density, the data were ln(x) transformed. The back-transformed means of the studied variables for each cultivar, *M. hapla* population, and their interactions are presented in the results. The data analysis was conducted in PROC MIXED (SAS Institute Inc, 2009). The probability of eggs present in stained roots was analyzed using logistic regression model in PROC GLIMMIX (SAS Institute Inc., 2009). The statistical tests were conducted using the probability of Type I error of 0.05. When the interactions between the studied factors were found to be statistically significant, we examined the interactions using cell means plots and slicing tests in PROC MIXED (SAS Institute Inc, 2009). Multiple comparisons among the means were conducted using t tests when respective factor, interaction, or slicing effects were found to be statistically significant at 0.05 levels.

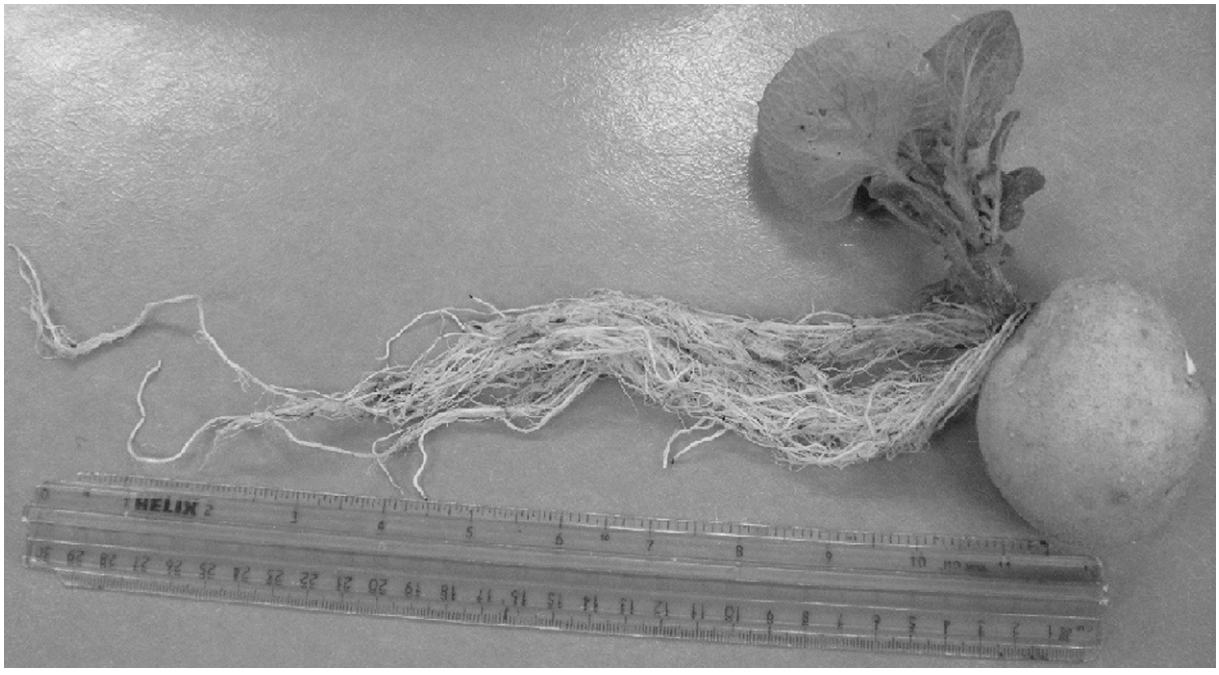

Figure 1. Size of root and shoot growth during the experimental period.

RESULTS

Few nematodes in potato tuber peels and males in roots were detected in either experiment (data not shown). Measurements from both experiments were combined because they were not statistically different between the experiments (Table 3; Fig. 2). Total numbers of nematodes includes adult females (the most of all stages) and infective second-stage and third- and fourth-stage juveniles. Across all cultivars, significantly fewer nematodes were recovered in Snowden roots followed by Boulder, Kalkaska, and Michigan Purple than in Missaukee and Jacqueline Lee. Overall, the potato cultivars were 12 to 33% as suitable as hosts as Rutgers was to the populations of *M. hapla*, with *Mh* 3 infecting the cultivars the most and *Mh* 2 the least (Fig. 2). Eggs were present in samples from all cultivars and populations of *M. hapla* (Fig. 3). Presence of eggs was lower in Rutgers and Kalkaska than in the other cultivars and lower in *Mh* 1 and *Mh* 2 than *Mh* 3 and *Mh* 4.

Across nematode populations and cultivars, the total numbers of nematodes recovered in roots were significantly different at different combinations of potato cultivars and populations of *M. hapla* (Table 3). *Meloidogyne hapla* 1, *Mh* 2, and *Mh* 3 infected Rutgers significantly more than *Mh* 4. *Meloidogyne hapla* 3 infected Snowden, Missaukee, Boulder, and MI Purple more than *Mh* 1 and *Mh* 2, Kalkaska more than *Mh* 2 and *Mh* 4, and Jacqueline Lee more than *Mh* 2. *Meloidogyne hapla* 4 infected Snowden,

Table 3. Total numbers of third- to adult-stages per gram fresh root weight in Exp. 1 and 2 at different combinations of cultivar *Meloidogyne hapla* populations.

	Meloidogyne hapla populations†			
Cultivars‡	*Mh* 1	*Mh* 2	*Mh* 3	*Mh* 4
Snowden	22.0 dBC§	14.6 dD	55.7 cA	40.5 bAB
Missaukee	41.7 bcD	70.1 bBC	135.6 bA	103.5 aAB
Boulder	36.2 cdBC	25.0 cdC	142.6 abA	54.6 bB
Kalkaska	58.6 bcAB	22.4 dC	104.6 bA	47.0 bB
Michigan Purple	48.4 bcB	15.8 dC	97.5 bcA	63.4 abAB
Jacqueline Lee	68.3 bAB	42.1 bcB	122.7 bA	73.0 abAB
Tomato (control)	304.9 aA	317.4 aA	327.1 aA	113.3 aB

† Results from Exp. 1 and 2 were combined because there is no significant interaction between runs and cultivars and *M. hapla* populations.

‡ Tomato cultivar Rutgers was included as a standard susceptible.

§ Different lowercase letters within a column, and uppercase letters within rows mark the means that are statistically different from each other ($p < 0.05$). Numbers are rounded off to the closest decimal.

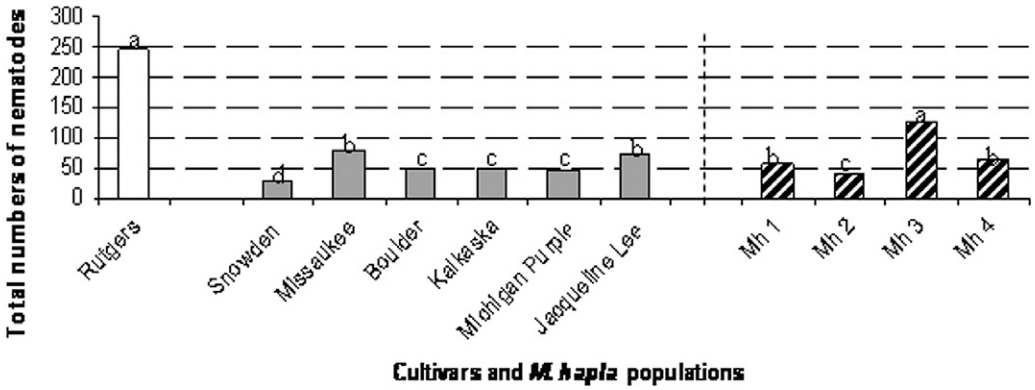

Figure 2. Mean numbers of total nematodes per gram fresh root weight in Rutgers tomato (susceptible control, blank bar) compared with six potato cultivars (solid bars) and those of *Meloidogyne hapla* (*Mh*) populations (cross bars) across all cultivars in Exp. 1 and 2. Bars with different letters among cultivars and nematodes are statistically different at $p = 0.05$. Numbers are rounded off to the closest decimal.

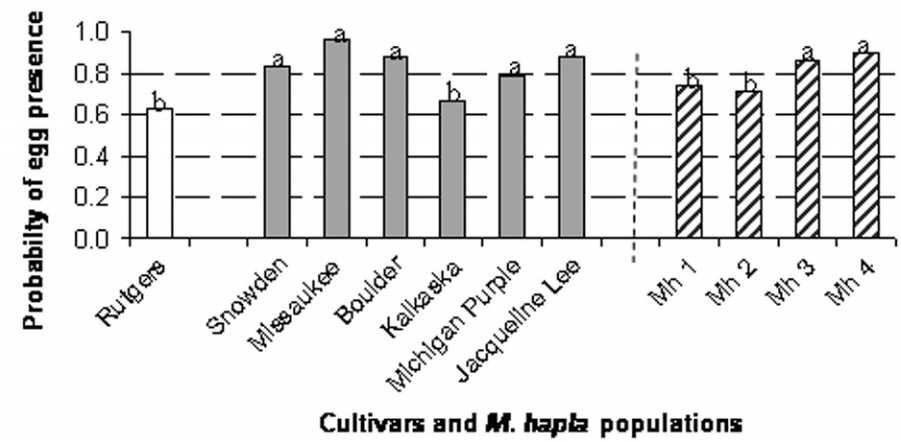

Figure 3. The probability of presence of eggs in root samples of Rutgers tomato (susceptible control, blank bar) compared with the six potato cultivars (solid bars) and those of *Meloidogyne hapla* (*Mh*) populations (crossed bars) across all cultivars at the end of the study. Bars with different letters within cultivars and within nematodes are statistically different at $p = 0.05$.

Kalkaska, and MI Purple more than *Mh* 2 and Missaukee more than *Mh* 1. *Meloidogyne hapla* 1 infected Kalkaska and MI Purple more than *Mh* 2.

Meloidogyne hapla 1 infected Jacqueline Lee the most and Snowden the least, *Mh* 2 infected Snowden the least and Missaukee the most, *Mh* 3 infected Boulder the most and Snowden the least, and *Mh* 4 infected Missaukee the most and Snowden the least (Table 3).

DISCUSSION

The greenhouse study shows that the selected potato cultivars are suitable hosts for *M. hapla* populations present in the potato production systems, proving false the working hypothesis that the potato cultivars will not be suitable hosts for the *M. hapla* populations. This suggests that the presence of *M. hapla* may be a problem for the potatoes and rotation crops that are suitable hosts to *M. hapla*, indicating the need for supplementary management strategies that suppress *M. hapla*. The interaction effects of potato cultivar and nematode population and the varying degrees of suitability to the populations of *M. hapla*, however, suggest that the problem is probably site specific, providing growers with management options of which cultivars may be least suitable for the nematode in question in their environment and breeders and researchers with information they can use when selecting the next generation of cultivars for ecosystems in which the two organisms inhabit.

The duration of the experiments was designed to test infection levels within the completion of a life cycle relative to tomato, a standard susceptible and nematode viability control. This assumes that nematode reproduction will be unimpeded thereafter, which is supported by the presence of infective second-stage juveniles and eggs. Although the population density may vary by cultivar, the study shows that the populations of *M. hapla* will reproduce in these potato cultivars. Whether or not the observed infections will reach economic threshold levels in these potato cultivars or vegetable crops in a rotation is unknown and will require repeated season-long analyses beyond the scope of the current study. All factors being equal, however, it is probable that the economic threshold level will vary by the crop and the cultivar in use. Accurate economic threshold level determination of the *M. hapla* populations in the potato cultivars or susceptible rotation crops will require analyses beyond the traditional comparison of nematode population densities at preplant and at harvest (Barker et al., 1985). This, in turn, will require analyses of all nematode developmental stages corresponding to the numbers of generations over the growing season and the prevailing soil and environmental conditions (Melakeberhan and Avendaño, 2008). This single life cycle study provides a basis for determining the numbers of generations that may occur during a growing season.

The populations of *M. hapla* were collected from muck and a range of mineral soils where nursery crops were propagated or where celery (*Apium graveolens* L.) and or potato crops were grown. While there were differences in the stages of embryogenesis at the time of inoculation (Table 1) and the numbers of samples where eggs were observed at harvest (Fig. 3), the differences were not consistent. Therefore, differences in total population densities at the end of the study (Fig. 2) are more likely to be due to differences in pathogenicity among the populations than in the inoculum cohort. Of the four populations tested across carrot (*Daucus carota* L.) and celery (H. Melakeberhan, unpublished data, 2011) and these potato cultivars, *Mh* 3 infected all three crops the most. *Meloidogyne hapla* 3, which came from high methyl bromide use production and high soil pH, was reported to be most pathogenic in earlier studies as well (Melakeberhan et al., 2007). This suggests that differences in production practices may influence a population's pathogenicity.

The development of Missaukee and Boulder lines, both with the *H1* gene conferring resistance to the golden nematode, represents significant breakthroughs. Knowing that these cultivars are suitable hosts for *M. hapla* is helpful for the best use of these cultivars. For example, multitaxa infestations are common and if *M. hapla* infection reaches damage threshold levels they may suffer yield loss, and/or the buildup of population density becomes a problem for the rotation crop. Such an outcome, even if Missaukee and Boulder control cyst nematodes, could potentially lead to the cultivars' not being preferred by growers. With the knowledge that Missaukee and Boulder are suitable hosts to *M. hapla*, however, growers can design practices that suppress *M. hapla* and exploit the full potential of the cyst nematode resistant cultivars.

In the absence of *M. hapla* resistance in potatoes and the rotation crops, the differing degrees of suitability of the potato cultivars are significant. We recognize that the same level of reproduction in potato and tomato hosts does not mean the same economic threshold level. However, knowing whether or not the potato cultivars are as suitable as tomato, the host in which the populations of *M. hapla* were cultured, is an important indicator of relative nematode reproduction and potential impact on a rotation system that may include susceptible hosts. For example, a cultivar that is 12% as suitable a host as Rutgers, the nematode viability control host, should allow less buildup of an *M. hapla* population than a cultivar that is 33% as suitable a host as Rutgers. Compared with celery and carrots, however, the levels of host suitability observed here are low (H. Melakeberhan, unpublished data, 2011). Whether or not the recovery of few nematodes in tubers indicates differential reaction within plant parts (Brown et al., 2009) and the levels of host suitability observed here will result in yield loss are yet to be determined.

Moreover, the presence or absence of tolerance to *M hapla* in these cultivars is unknown. Overall, the study provides critical data for developing agro-biologically integrated approaches to managing nematode parasitic variability.

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