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Relationship of Resistance to *Meloidogyne chitwoodi* (race 2) and *M. hapla* in Alfalfa

Per H. McCord

Abstract: In the Pacific Northwest, alfalfa (*Medicago sativa*) is host to two species of root-knot nematodes, including race 2 of the Columbia root-knot nematode (*Meloidogyne chitwoodi*) and the northern root-knot nematode (*Meloidogyne hapla*). In addition to the damage caused to alfalfa itself by *M. hapla*, alfalfa's host status to both species leaves large numbers of nematodes available to damage rotation crops, of which potato is the most important. A nematode-resistant alfalfa germplasm release, W12SR2W1, was challenged with both nematode species, to determine the correlation, if any, of resistance to nematode reproduction. Thirty genotypes were screened in replicated tests with *M. chitwoodi* race 2 or *M. hapla*, and the reproductive factor (RF) was calculated. The distribution of natural log-transformed RF values was skewed for both nematode species, but more particularly for *M. chitwoodi* race 2, where more than half the genotypes screened were non-hosts. Approximately 30 percent of genotypes were non-hosts or very poor hosts of *M. hapla*, but RF values for *M. hapla* on susceptible genotypes were generally much higher than RF values for genotypes susceptible to *M. chitwoodi* race 2. The Spearman rank correlation was positive (0.52) and significant (p-value = 0.003), indicating there is some relationship between resistance to these two species of root-knot nematode in alfalfa. However the relationship is not strong enough to suggest genetic loci for resistance are identical, or closely linked. Breeding for resistance or immunity will require screening with each species separately, or with different DNA markers if marker-assisted breeding is pursued. A number of genotypes were identified which are non-hosts to both species. These plants will be intercrossed to develop a non-host germplasm.

Key words: alfalfa, host status, *Meloidogyne chitwoodi*, *Meloidogyne hapla*, pest resistance.

In the Pacific Northwest, three species of root-knot nematode (*Meloidogyne*) are present. These include the Columbia root-knot nematode (*M. chitwoodi* Golden, O'Bannon, Santo, and Finley), the northern root-knot nematode (*M. hapla* Chitwood), and the barley root-knot nematode (*M. naasi* Franklin). While root-knot nematodes are rare on non-irrigated soils in this area (Smiley et al., 2004), they can be present and cause significant economic damage to crops grown on irrigated lands (Faulkner and McElroy, 1964; Ingham et al., 2000). In terms of value of production, two of the most important irrigated crops in this region are alfalfa and potatoes (NASS, 2012), and both are damaged by *M. chitwoodi* and/or *M. hapla*. *M. hapla* is damaging to alfalfa seedlings (Inserra et al., 1980), and also contributes to yield losses and shortened stand life (Noling and Ferris, 1985). While *M. chitwoodi* appears to cause little damage to alfalfa itself, both *M. chitwoodi* and *M. hapla* can reproduce on alfalfa and cause damage to successive potato crops. Total yield of potato is rarely affected, but both *M. chitwoodi* and *M. hapla* cause unsightly blemishes on potato tubers (Santo and O'Bannon, 1981), which must be culled, reducing the marketable yield (USDA-AMS, 2008). Other crops grown in the region are also impacted by one or both species, including sugarbeet (Griffin et al., 1982), carrot (Bélair, 1992; Wesemael and Moens, 2008), onion, and peppermint (Faulkner and McElroy, 1964).

Chemical control by fumigation is expensive, and in the case of alfalfa, it is cost-prohibitive. The development of alfalfa varieties that are non-hosts to these nematodes could lower the costs of production for alfalfa, and if these varieties were used in a rotation before a susceptible crop (such as potato), could eliminate the need for fumigation. Fortunately, genetic resistance to these pests does exist. Mojtahedi et al. (1989) screened 50 alfalfa cultivars and germplasms with race 2 of *M. chitwoodi*, and identified two germplasm sources, Nevada Synthetic XX and W12SR2W1, with high levels of resistance. When 100 individual seedlings of W12SR2W1 were challenged with race 2, an estimated 63 were either poor hosts or non-hosts of the nematode, with 19 of these being non-hosts. In addition, Mojtahedi et al. reported that 50 to 66% of W12SR2W1 seedlings were also non-hosts to *M. hapla*. This suggests that resistance in alfalfa to these two species of root-knot nematodes could be related. This phenomenon is not new. For example, the *Mi* gene in tomato provides resistance to several different species of root-knot nematode (Thomason and Smith, 1957; Winstead and Barham, 1957).

The purpose of this experiment was to determine, through replicated screening of identical (clonal) plants, whether or not resistance to race 2 of *M. chitwoodi* and *M. hapla* is truly correlated. A high correlation would indicate that resistance to two different nematode species is conditioned by the same locus, or at least linked loci.

Materials and Methods

All experiments were conducted in the greenhouses of the USDA-ARS Vegetable and Forage Crops Research Unit at Prosser, WA. Genotypes were selected at random from W12SR2W1. Genotypes were propagated by stem cuttings, and rooted cuttings were transplanted into

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USDA-ARS, Vegetable and Forage Crops Research Unit, Prosser, WA 99350.
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Email: per.mccord@ars.usda.gov
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550 mL cone-shaped plastic pots containing methyl bromide-fumigated soil (nominally 84% sand, 10% silt, and 6% clay). Each plant was inoculated with 1300 eggs of either *M. chitwoodi* race 2 (isolate WAMC27) or *M. hapla*, which had been maintained on tomato (cv. 'Rutgers'). New tomato plants were inoculated at the same time to verify the viability of the nematode eggs. Eggs were extracted from the roots of all plants beginning at 55 days after inoculation by first rinsing the roots to remove soil, shaking the roots in a 10% solution of household bleach for several minutes, capturing the eggs on a 500-mesh screen, and washing them into a glass bottle for counting. The concentration of eggs was estimated by counting in a counting chamber under 40X or 100X magnification. The total number of eggs recovered was estimated by multiplying the concentration of eggs per ml by the total volume of wash solution recovered. This figure was divided by 1300 (the number of eggs used for inoculation) to determine the reproductive factor (RF). At least three plants were screened per genotype and species of nematode. Prior to statistical analysis, the RF values were transformed by taking the natural logarithm of the original value plus one, due to the presence of genotypes with an RF of zero. From the initial dataset of 35 genotypes with at least 3 replications, five were excluded whose data were highly variable (e.g. two replicates with RF >10, one replicate with RF = 0). Histograms, scatter plots, and correlation analyses were performed using SAS software, version 9.2 (SAS, Cary, NC). All analyses and plots were calculated using the genotype means.

Results and Discussion

The histogram for the distribution of transformed RF values for *M. chitwoodi* race 2 was highly skewed, with the vast majority of genotypes with an RF near zero (Fig. 1). Indeed, of the 30 genotypes tested, 16 had an RF of zero. For *M. hapla*, a large portion of genotypes (approximately 30%) also had low RF values, but a significant number also supported large numbers of nematodes (Fig. 2). For example, individuals with a transformed RF of 3 or greater (approximately 55%) supported greater than 19-fold reproduction of *M. hapla*. The Pearson product-moment correlation of transformed RF values for the two species was non-significant at α=0.05. The Spearman rank correlation was 0.52, and was significant (p-value = 0.003). Due to the skewed data distributions, the non-parametric Spearman correlation is more appropriate. This suggests there is a positive relationship between RF values for the two species, possibly due to genetic linkage between the two traits. However, this relationship is not biologically significant enough to allow breeding for resistance to both nematodes based on screening for only one of the species. As can be seen in the scatterplot in Figure 3, many individuals had a transformed RF of zero or near zero for *M. chitwoodi* race 2, but much larger transformed RF values for *M. hapla*. Phenotypic screening for resistance to root-knot nematodes is laborious and time-consuming, and it would be ideal to have a molecular marker to select upon instead. In this case, a marker (or markers) will be required for each species.

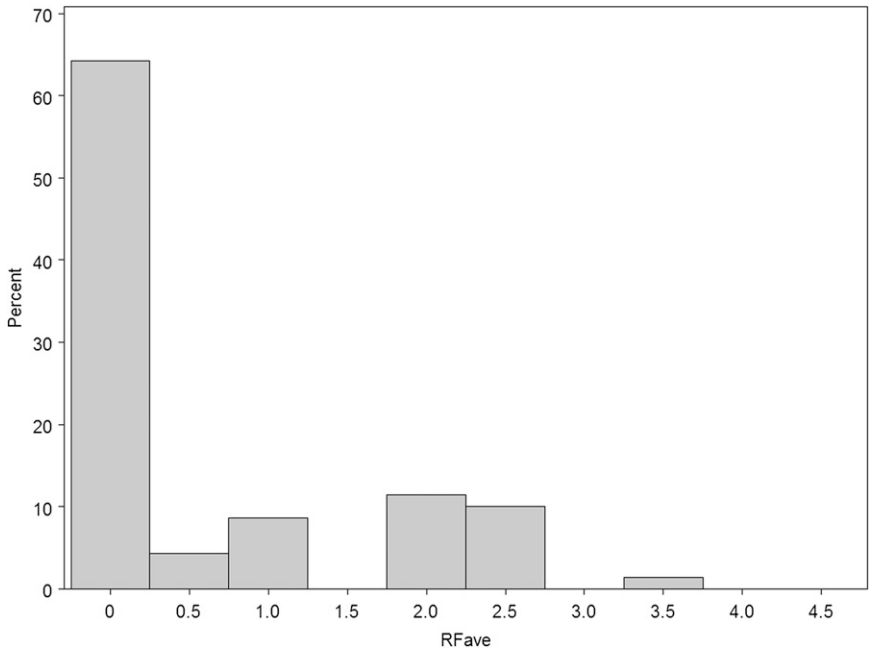

Fig. 1. The distribution of transformed (ln(X +1)) RF values for *M. chitwoodi* race 2.

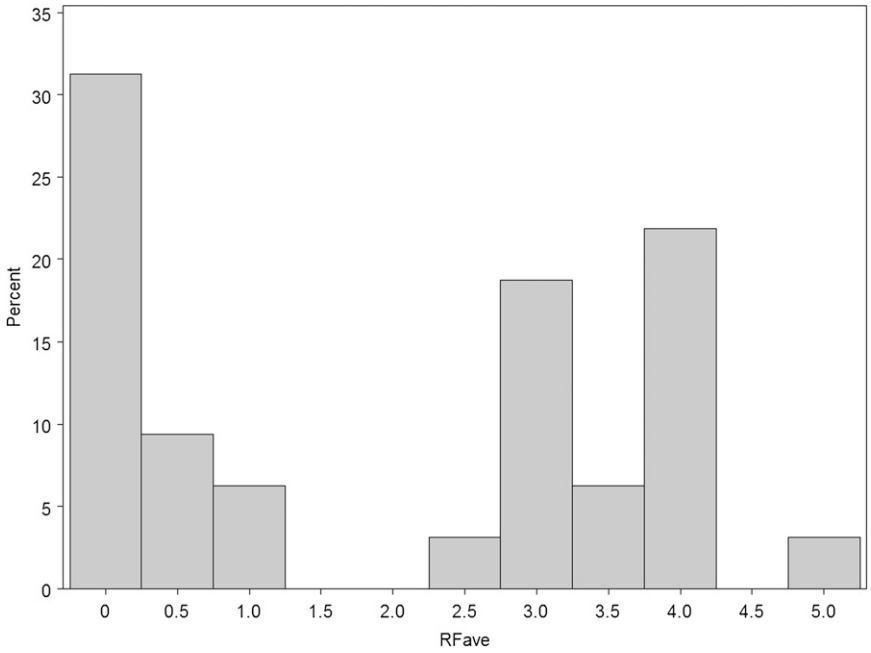

Fig. 2. The distribution of transformed (ln(X +1)) RF values for *M.hapla*.

It can also be seen from Figure 3 that a small number of individuals were recovered which are non-hosts (transformed RF = 0) to both species. Although resistance in alfalfa to *M. chitwoodi* and *M. hapla* is apparently conditioned by different loci which are not closely linked, the existence of these individual genotypes which are non-hosts to both species is encouraging, and indicates it is possible to generate entire synthetic populations that do not support root-knot nematode reproduction. Biparental crosses between these non-host plants and highly susceptible genotypes have been or are being made, and will be used in further experiments to determine the inheritance and approximate position of resistance loci. These studies will advance the development of molecular markers for root-knot nematode resistance in alfalfa, which will speed the development of superior varieties that are immune to damage themselves from these species, and suppress nematode populations to protect successive crops.

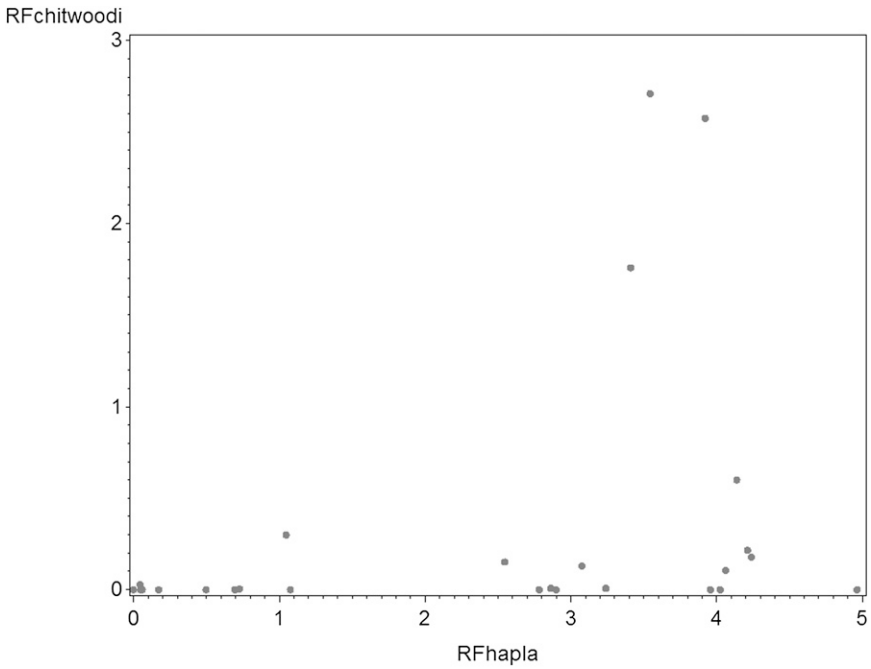

Fig. 3. Scatter plot of transformed RF values for *M.chitwoodi* race 2 (y axis) and *M. hapla* (x axis).

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