Effect of *Mi-1.2* gene in Natal Host Plants on Behavior and Biology of the Tomato Psyllid *Bactericerca cockerelli* (Sulc) (Hemiptera: Psyllidae)[1]

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Abstract The *Mi-1.2* gene has been isolated from wild varieties of tomato, *Solanum peruvianum* (Mill), and incorporated into near isogenic commercial varieties of tomato, *Solanum lycopersicon*. Plants containing the gene confer resistance to tomato psyllid, *Bactericerca cockerelli* (Sulc), as well as species of aphids, whiteflies, and nematodes. Considering bias in host preference and oviposition responses based on early adult experience is well documented in the literature; the objective of this study was to determine if the presence or absence of the *Mi-1.2* in the rearing host influenced subsequent host selection, oviposition, and development time. The results presented here show that the host that psyllids developed on did not affect subsequent selection of host for oviposition, but incidence of oviposition was higher on plants that did not contain the *Mi-1.2* gene [Moneymaker (*mi-1.2*)] compared to plants with the *Mi-1.2* gene [Motelle (*Mi-1.2*)]. Psyllid nymph survival and development time did not differ between plant varieties. Consequently, plants containing the gene may be useful components to IPM programs to control the tomato psyllid.

Key Words *Bactericerca cockerelli*, tomato psyllid, *Solanum lycopersicon*, tomato, plant resistance, host selection, preference, antixenosis, antibiosis

Plant resistance is an important component of an IPM program, and insect resistance has been introduced into numerous crops (Smith 1989). The resistance gene *Mi-1.2* confers resistance to several species of root-knot nematode (*Meloidogyne* spp.) (Milligan et al. 1998), potato aphids (*Macrosiphum euphorbiae* Thomas) (Rossi et al. 1998), and two biotypes of whiteflies [*Bemisia tabaci* Gennadius B- and Q-biotypes] (Nombela et al. 2003). Tomato plants, *Solanum lycopersicon* (Mill), that contain the *Mi-1.2* gene also confers resistance to the tomato psyllid, *Bactericerca [Paratrioza] cockerelli* (Sulc), a serious pest of tomato in the central and western half of North America (Casteel et al. 2006). Significant losses in commercial tomato crops in recent years resulted from a new outbreak of a genetically-distinct population of *B. cockerelli* adapted for North American west coastal environments (Liu et al. 2006). This pest is rapidly increasing in economic scope and importance.

Resistance to the tomato psyllid conferred by the *Mi-1.2* gene is from antixenosis (host selection) and antibiosis (survival) (Casteel et al. 2006). *Mi-1.2* also has been

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shown to affect whitefly host selection, as well as whitefly pupae production and fecundity (Nombela et al. 2000, 2003). Feeding, longevity, fecundity, and developmental rate are similarly affected in the potato aphid (Kaloshian et al. 1997, Rossi et al. 1998). Whereas the precise mechanisms affected by the *Mi-1.2* gene are still unclear, it appears highly specific to each pest (Milligan et al. 1998, Nombela et al. 2003, Rossi et al. 1998).

In previous research (Casteel et al. 2006), tomato psyllids were reared on potato (*Solanum tuberosum* L.), a relative of tomato and alternate host of the tomato psyllid. No variety of potato contains *Mi-1.2* resistance. When tomato psyllids develop on plants lacking the *Mi-1.2* gene (*mi-1.2*), there is the possibility that these psyllids would prefer oviposition hosts that also lacked this resistance gene. Bias in host preference and oviposition responses based on early adult experience is well documented in the literature (Hoffmann 1985, Hoffmann 1988, Jaenike 1983, 1988). Rausher (1983) observed that experience on a given host affected host preference in the beetle *Deloyala guttata* (Oliver). Juvenile experience also has been shown to affect future host preference in stick insects (Cassidy 1978) and beetles (Phillips 1977). Experiments with the pea aphid, *Acrythosiphon pisum* (Harris) have demonstrated significantly greater fitness on the plant genotypes from which the aphids were collected (Via 1989). Considering the large body of literature supporting this phenomenon, the objective of this study was to determine if the presence or absence of the *Mi-1.2* in the rearing host influenced subsequent host selection, oviposition, and development time of the tomato psyllid.

Materials and Methods

Plants and insects. Two lines of near isogenic tomato, the susceptible cultivar Moneymaker (*mi-1.2*) and the resistant cultivar Motelle (*Mi-1.2*) were used in our bioassays. These lines were selected due to previously demonstrated aphid and whitefly resistance (Kaloshian et al. 1997, Nombela et al. 2000). The plants were grown in a greenhouse (24-26°C) in 473-ml pots of UC soil mix (Matkin and Chandler 1957) and fertilized once each week with Miracle-Gro® nutrient solution (Scott's Co., Marysville, OH) and once with 5 g per plant of Osmocote® slow-release nutrient (Scott's Co., Marysville, OH). Fluorescent lights and blue grow lights (L16:D8) were used to supplement the natural lighting.

Two colonies of tomato psyllids were reared in an environmental growth chamber (25 ± 2°C; 16:8 h L:D) on greenhouse-grown tomatoes (*S. lycopersicon*). One colony was maintained on the tomato variety Moneymaker (*mi-1.2*) and the other on Motelle (*Mi-1.2*). Psyllids were reared through 4 generations on both cultivars of tomato before initiation of the experiments. Adults used in all tests were 2-3 days old, based on their light teneral coloration.

Host selection. Adult tomato psyllids were collected from both laboratory-reared colonies approx. 2-3 d after molting to limit variability in psyllid reproductive status, and insure they have mated. In general, oviposition does not occur before approx. 3 d (Knowlton and James 1931). Using an aspirator and stereomicroscope, females were selected based on the presence of an ovipositor. Female adults from both colonies were then held in growth chamber conditions (24-25°C RH 35%) in separate tube cages until initiation of experiments (~1-3 h).

To test for an association between the *Mi-1.2* gene in natal host plants and resistance levels, psyllids from each colony were confined in separate tube cages. Tube cages confined an intact single leaflet of the susceptible Moneymaker (*mi-1.2*) at one end and an intact single leaflet of the resistant Motelle (*Mi-1.2*) at the other end. Four adult female psyllids from the Moneymaker colony or from the Motelle colony were transferred to the center of a tube cage made from two stiff plastic tubes on either end (3.81 cm diam, 8.89 cm long) and a smaller central stiff plastic tube (2.86 cm diam, 5.4 cm long), essentially creating one long tube with a narrow middle. Split foam plugs (2.89 cm diam, 1.3 cm thick) were used to seal both ends and cages rested horizontally on a platform, positioning them at leaf level. Five cages were prepared for psyllids from each colony. Cages were located on the most apical, fully expanded leaf near the apical meristem of 5-wk-old tomato plants. Plants were randomly chosen from the population and arranged in a completely randomized design within an environmental growth chamber during the duration of the experiment (24-25°C; L16:D8; RH 35%).

Psyllid positions within tube cages were recorded 15 min after the adults were introduced and then hourly for the next 4 h. Thereafter, positions of the 4 psyllids were recorded at 8, 12, and 24 h. Insects were allowed to feed and oviposit for a total of 48 h. Cages were dismantled after 48 h, and final adult positions were recorded. The number of psyllid eggs on each cultivar was counted using a stereomicroscope. There were four insects per each cage and five cages for each treatment. The experiment was repeated two times ($n = 10$).

Nymphal development and survival. Adult tomato psyllids were collected from the two laboratory-reared colonies to examine the effect of the *Mi-1.2* gene in natal plants on psyllid nymphal development and survival. Eight adults of equal sex ratio were then placed within individual tube cages, made from a stiff plastic tube (3.81 cm diam, 8.89 cm long) and sealed with a metal screen at one end and with a split foam plug at the other (2.89 cm diam, 1.3 cm thick). Each cage contained an intact single leaflet of the most apical internode of either Motelle or Moneymaker. Cages were wrapped with stiff wire and anchored in the soil of each pot to position it at leaf level. Plants were arranged in a completely randomized design within a growth chamber (24 –25°C; L16:D8; RH 35%). Four adult females and four adult male psyllids were placed in each cage and females were allowed to oviposit for 6-10 h and were then removed. The number of eggs was counted using a stereomicroscope. Each leaf within a cage contained 5-20 eggs. Eggs were removed on counts with >20. Development and survival were recorded daily for 27 d. Nymphal instar status was determined by size, development of wing pads and coloration, classifying nymphs into two groups: 1st to 2nd and 3rd to 5th instars (Pletsh 1947, Rowe and Knowlton 1935). This classification was used to calculate the days to adulthood, by monitoring daily and determining the number in each nymph group and the number of adults that emerged. Adults were removed after emergence. The experiment was repeated 8 times for each of the two plant lines ($n = 8$).

Data analysis. Data were analyzed using procedures of SAS Institute (2002). Host selection data were analyzed using 2 × 4 contingency tables (PROC FREQ). The Pearson Chi-square statistic was used to test for significant differences between observed and expected frequencies ($P < 0.05$). In instances where chi-square may not have been valid (due to a large number of cells having counts <5), Fisher's exact test (two-tail) was used. Factorial ANOVA was performed to determine if there were interactions between natal host plant and host selected in regards to mean number of eggs deposited. Tomato psyllid survival, mean number of eggs and development were analyzed using a two fixed factor and two level analysis of variance (ANOVA) with means being compared using least significant difference (LSD) and *t*-tests ($P < 0.05$) (PROC GLM procedure; SAS Institute 2002). Survival data were transformed using square root transformation procedure (SQRT) to meet the assumptions of normality.

Results

Host selection. Within 3 h, all psyllids had settled and selected a host plant within the assay tubes; there was no change in their location for the duration of the experiment. There were no significant differences between host plant selected or host plant reared for each time point recorded, so only the data from the final time point (48 h) are presented. There was no significant difference between the numbers of tomato psyllids selecting the resistant Motelle (*Mi-1.2*) versus the susceptible Moneymaker (*mi-1.2*) (Fig. 1). The genotype of the tomatoes used as the natal plants did not affect subsequent host selection (Fig. 1). No significant interaction was found between the two treatments over the duration of the experiment.

The genotype of the natal plant host also did not influence egg deposition in choice tests (Fig. 2). For example, approx. 9 eggs per leaflet were deposited on Moneymaker leaves when insects were reared on either Moneymaker or Motelle plants. A similar observation was made with Motelle, where the number of eggs deposited was not significantly different between natal host genotypes (Fig. 2). Regardless of the natal plant (*Mi-1.2* or *mi-1.2*), oviposition events were twice as frequent on Moneymaker (*mi-1.2*) plants as Motelle (*Mi-1.2*) plants ($F = 4.89$; df = 1; $P = 0.0399$) (Fig. 2). Host

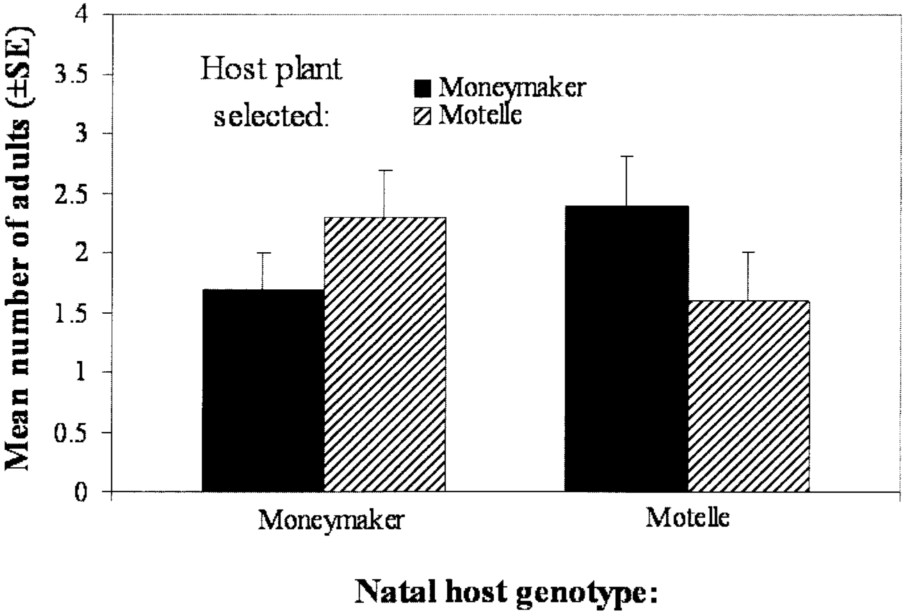

Fig. 1. Mean (±SE) number of tomato psyllids on Moneymaker (*mi-1.2*) or Motelle (*Mi-1.2*) as a function of previous natal exposure to these genotypes in a host selection choice test (N = 10). Treatments were not significantly different between host plants insect chose (ANOVA, $P > 0.05$).

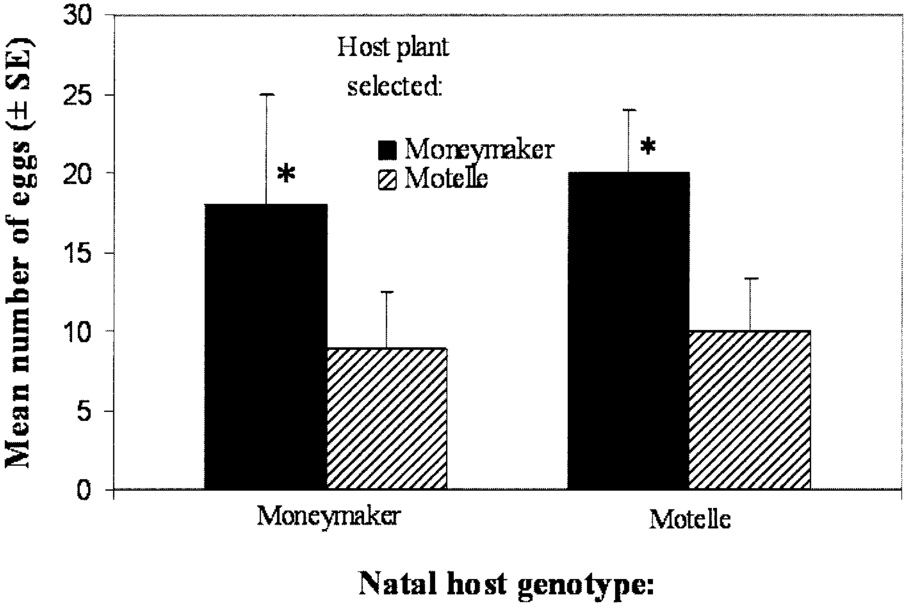

Fig. 2. Mean (±SE) number of tomato psyllid eggs deposited in 48 h on Moneymaker (*mi-1.2*) or Motelle (*Mi-1.2*) as a function of previous natal exposure to these genotypes. (N = 10). *Significantly different means (±SE) (ANOVA, $P < 0.05$).

plant selected was significant ($F = 5.67$; df = 1; $P = 0.0300$), but there was no significant interaction between the natal host plant and host type selected ($F = 1.63$; df = 1; $P = 0.2196$).

Nymphal development and survival. There was no significant difference in cumulative psyllid survival from egg eclosion to adult among the treatments, i.e., host plant confined on ($F = 1.27$; df = 1; $P = 0.2702$), natal host plant experience ($F = 1.56$; df = 1; $P = 0.221$), or from the interaction of the two treatments ($F = 2.32$; df = 1; $P = 0.1391$).

No significant difference in tomato psyllid nymph development time was observed between insects developing on Motelle and Moneymaker from insects previously reared on either plant type (Table 1). In all treatments, the psyllids developed from egg eclosion to adult in a mean of 21 d.

Discussion

Positive as well as negative effects of previous host exposure on subsequent host-insect interactions have been reported in the literature. For example, negative effects have been seen in *Drosophila* feeding and oviposition host selection experiments where insects avoid natal host plants (Hoffmann and Turelli 1985). Hoffmann (1985) conducted laboratory experiments examining oviposition selection site and adult distribution in large containers and tested olfactory responses in a wind tunnel. Negative association or no effect of prior plant exposure were observed in these

Table 1. Cumulative mean (±SE) percent survival and mean (±SE) number of days from egg eclosion to adult of tomato psyllids on Moneymaker (*mi-1.2*) or Motelle (*Mi-1.2*) as a function of previous natal exposure to these genotypes

Natal host	Host confined to	Survival % ±SE	n	Mean days to adult ±SE	n
mi-1.2	*mi-1.2*	16.4 ± 6.3	8	21.3 ± 0.35	13
mi-1.2	*Mi-1.2*	18.0 ± 6.8	8	21.3 ± 0.29	18
Mi-1.2	*mi-1.2*	32.6 ± 8.9	8	21.2 ± 0.35	15
Mi-1.2	*Mi-1.2*	18.0 ± 11.4	8	21.1 ± 0.39	17

No values were significantly different; survival data was SQRT transformed (ANOVA, $P > 0.05$).

experiments (Hoffmann 1985, Hoffmann and Turelli 1985). No effect of previous host plant exposure is consistent with our results in regards to the *Mi-1.2* gene.

In contrast, other research demonstrates a positive effect of host exposure subsequent responses to the host (de Boer 2004, Jaenike 1983, Jermy et al. 1968). Hoffmann (1985) showed positive effects for oviposition in small cages with *Drosophila,* where adults oviposited more on plants to which they were previously exposed. Positive effects for oviposition in small cages in *Drosophila* also were observed by Jaenike (1983, 1985) in the laboratory and in the field. Food preference for plants that insects were previously exposed also has been demonstrated in lepidopteran larvae (de Boer 2004, Jermy et al. 1968).

Tomato psyllid females respond to the presence of the resistance gene *Mi-1.2* in a host leaf when reared on tomato by laying fewer eggs on a *Mi-1.2* leaf than a *mi-1.2* leaf. Suitability of oviposition host to tomato psyllid in this study appears to result from the presence or absence of the *Mi-1.2* gene, because Motelle and Moneymaker are near isogenic. This inhibition of oviposition is possibly due to a host plant chemical associated with the tissue or with damage to tissue containing the *Mi-1.2* gene. Females may use tactile or volatile cues to determine whether the leaf is a suitable oviposition host.

In previous studies (Casteel et al. 2006) where psyllids were reared on potatoes, female psyllids selected and oviposited on tomatoes lacking the *Mi-1.2* gene 3 times more frequently than *Mi-1.2* plants. Our results corroborate with the *mii-1.2* plants having 2-fold higher levels of eggs deposited per leaf surface. The advantage for the psyllids that were reared on potato was that nymphal survival on tomatoes without the *Mi-1.2* gene are 2-fold greater than plants with *Mi-1.2*.

Whereas no difference in survival or host selection was observed in females reared on the two cultivars of tomato in this study, female psyllids reared on either *Mi-1.2* or *mi-1.2* tomato can distinguish between plants that contain the *Mi-1.2* gene and those that do not (Casteel et al. 2006). Further research is required to determine if this oviposition response may also have an adaptive advantage.

Whereas the presence or absence of the *Mi-1.2* gene in the rearing tomato cultivar had no effect on subsequent adult psyllid-host interactions, experience of *B. cockerelli* with hosts of different species clearly affected succeeding host interactions. Casteel et al. (2006) revealed strong psyllid host-selection preference and significant difference in survival when exposed to the cultivars of tomato with and without the *Mi-1.2* gene. In those choice experiments, there were no significant differences in oviposition responses (Casteel et al. 2006). Survival and host selection were not affected by the *Mi-1.2* gene in insects reared on tomato, although oviposition was affected. This difference could be due to habituation, or decreasing response to a stimulus after exposure over a period of time, resulting in changes in specificity of host finding cues (Quinn et al. 1974). Natal host plant cues might be more familiar to psyllids resulting in less caution in choice, whereas non natal host cues are less familiar requiring more caution before choosing to use it as a host. After contacting the plant the insect would be more discriminating, using other tactile or chemical cues. In the context of the *Mi-1.2* gene, the decision to deposit fewer eggs was made after initial host selection decision making.

Tomatoes used in this experiment were 5-wk-old. The *Mi-1.2*-mediated resistance gene to tomato psyllids may be developmentally regulated, as in the other pests. *Mi-1.2*-mediated resistance against aphids is not functional until approximately the fifth week after germination, 6-8 wk old plants were used with aphids to insure activation of the resistance mechanism (Kaloshian et al. 1995, Martinez de Ilarduya et al. 2001). Resistance to whiteflies was demonstrated in plants that were 2 months old (Nombela et al. 2003), and with younger plants this was not observed (Pascual et al., 2000). This is in contrast to nematode resistance of *Mi-1.2*, where resistance is functional very early in plant development (Kaloshian et al. 1995, Martinez de Ilarduya et al. 2001). Further research with tomato psyllids and plants of differing developmental ages are needed to distinguish resistance regulation or actual loss of resistance.

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