

Screening for Resistance Against the Sugarbeet Root Maggot, *Tetanops myopaeformis* (Diptera: Ulidiidae), Using a Greenhouse Bioassay

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Abstract

The sugarbeet root maggot, *Tetanops myopaeformis* (von Röder) (Diptera: Ulidiidae), is a major pest of sugar beet *Beta vulgaris* L. (Carophyllales: Amaranthaceae) in the United States and Canada. Larval feeding on roots can reduce both stand and yield. Current management practices are heavily reliant on chemical control. However, the carbamate and organophosphate insecticides that are commonly used against *T. myopaeformis* are being phased out of use. Host plant resistance against this pest shows promise, but difficulties with maintaining *T. myopaeformis* in culture have largely limited such studies to the field. A primary objective of this study was to develop protocols for rearing a laboratory colony of *T. myopaeformis* that would expedite assays aimed at screening for host plant resistance. Third (final) instar larvae were collected from the field and reared to the adult stage. These laboratory-reared adults laid eggs and ultimately produced a second generation of third-instar larvae in the lab. Adult flies reared from field-collected larvae were used to examine the modality of resistance of a known resistant variety by performing no-choice and paired-choice experiments alongside a susceptible variety in the greenhouse. Paired-choice tests showed no difference in oviposition rates between the two varieties, whereas no-choice tests showed significantly greater feeding damage and abundance of larvae on the susceptible variety. For the resistant variety examined here, we observed evidence of antibiosis, not antixenosis, as the putative modality of resistance. Our laboratory and greenhouse protocols can be used to expedite the process of developing varieties with resistance to this key pest of sugar beet.

Key words: host plant resistance, antibiosis, antixenosis, *Beta vulgaris*

Sugar beet (*Beta vulgaris* L. [Caryophyllales: Amaranthaceae]) is an economically important crop in the United States that accounts for about 55% of U.S. sugar production (McConnell 2017). Since 2015, approximately 56% (257,000 hectares) of the sugar beet acreage in the United States has been grown in the Red River Valley of North Dakota and Minnesota (USDA-NASS 2017), while Idaho has ranked third among U.S. states for sugar beet production with an average of approximately 69,200 hectares planted each year (USDA-NASS 2017).

Sugarbeet root maggot, [*Tetanops myopaeformis* (von Röder) (Diptera: Ulidiidae)], is a major pest of sugar beet in the United States and Canada. These insects can be found nearly anywhere in North America that sugar beet is grown and have been reported in Alberta and Manitoba, Canada, as well as in the U.S. states of Washington, Oregon, California, Colorado, Idaho, Montana, Minnesota, North Dakota, Nebraska, Utah, and Wyoming (Mahrt and Blickenstaff 1979, Lange 1987, Hein 1995). Although *T. myopaeformis* is widespread, it is a serious pest of sugar beet only in the Red River Valley

(Boetel et al. 2009) and in certain parts of Idaho (Bechinski et al. 1989, Wenninger et al. 2018).

T. myopaeformis is native to North America (von Röder 1881, Hawley 1922, Jones et al. 1952). American colonists brought *B. vulgaris* to North America (Harveson et al. 2009), which ultimately resulted in a new encounter between *T. myopaeformis* and sugar beet. Hosts that support larval development include different cultivated forms of *B. vulgaris* as well as spinach (*Spinacia oleracea* L. [Caryophyllales: Amaranthaceae]), spear saltbush (*Atriplex patula* L. [Caryophyllales: Amaranthaceae]), and garden orache (*Atriplex hortensis* L. [Caryophyllales: Amaranthaceae]) (Mahrt and Blickenstaff 1979, Msangosoko 2012). Eggs have been found on common lambsquarters (*Chenopodium album* L. [Caryophyllales: Amaranthaceae]), redroot pigweed (*Amaranthus retroflexus* L. [Caryophyllales: Amaranthaceae]), prostrate pigweed (*Amaranthus blitoides* Watson [Caryophyllales: Amaranthaceae]), black nightshade (*Solanum nigrum* L. [Solanales: Solanaceae]), and curly dock (*Rumex crispus* L. [Caryophyllales: Polygonaceae]) (Hawley 1922,

Mahrt and Blickenstaff 1979, Msangosoko 2012); however, these have been deemed unsuitable hosts (Mahrt and Blickenstaff 1979). All known hosts are not native to North America, so the natural host plant of *T. myopaeformis* remains unknown (Mahrt and Blickenstaff 1979).

T. myopaeformis overwinter as third-instar larvae and move toward the surface to pupate when soil temperatures rise in the spring (Harper 1962). Adults emerge starting in late spring, then mate and lay eggs near the base of young sugar beet seedlings (Harper 1962). Male flies may live for an average of 6 d, whereas females live an average of 14 d (Ure 1966). Females lay 1 to 31 eggs at a time and individuals have been reported to lay an average of 120 eggs during their lifespan (Harper 1962, Wenninger et al. 2018). Eggs begin hatching within 3 to 5 d after being laid and progress through three larval instars as they feed on the roots of sugar beet plants (Gojmerac 1956, Ure 1966). After reaching the third instar, usually during late summer, larvae tunnel deeper into the soil to begin diapause (Harper 1962). The winter diapause period is obligatory for successful maggot pupation in the spring and typically lasts 6 mo (Chirumamilla et al. 2008). However, flies have been observed to successfully pupate after 4 mo in diapause (S. D. Eigenbrode, personal communication).

T. myopaeformis larvae feed by rasping the root surface with their mouth hooks and consuming beet juices (Anderson et al. 1975, Wenninger et al. 2018). Feeding damage on older roots produces black scarring on the root surface and reduces yield. However, yield reductions can be even more severe if *T. myopaeformis* feeding severs the tap root of sugar beet seedlings, resulting in stand reduction. Such reductions in stand can be especially severe if beets are planted later than usual (Campbell et al. 1998). Yield losses can range from 10 to 100% in areas where *T. myopaeformis* pressure is high (Cooke 1993).

Cultural and biological control practices have been pursued for management of *T. myopaeformis*. However, these control methods are not as effective as chemical control in areas of high pest pressure (Campbell et al. 2000, Majumdar et al. 2008, Dunkel et al. 2010, Wenninger et al. 2018); thus, insecticides remain the cornerstone for management of this pest. The two groups of insecticides most commonly used against *T. myopaeformis*, carbamates and organophosphates, are being phased out of use (Anonymous 2010), and application of these pesticides is opposed by advocates for farm workers, child-safety, and the environment (Donley 2016). Overreliance on carbamate and organophosphate insecticides has likely contributed to the development of resistance to these chemicals in *T. myopaeformis* (Boetel et al. 2015). With chemical options for control of heavy infestations of *T. myopaeformis* decreasing, research into alternative approaches to manage this pest is sorely needed.

Following the first report on genetic factors for resistance to *T. myopaeformis* feeding in sugar beet (Theurer et al. 1982), work on the development of resistant sugar beet varieties has increased. Several varieties have been registered that exhibit lower *T. myopaeformis* feeding damage relative to susceptible varieties (Campbell et al. 2000, 2011; Campbell 2015). However, varieties with resistance to the *T. myopaeformis* are not widely used partly because yield potential and pathogen resistance may be lower than in other commercial varieties.

The production and testing of resistant lines of sugar beet are challenging in part because screening of germplasm is limited to field assays that rely on natural infestation. Greenhouse assays would expedite the process of screening sugar beet germplasm with putative resistance; however, *T. myopaeformis* thus far has not been

successfully reared beyond the second instar in laboratory cultures (Ure 1966). Here, we describe a protocol for rearing *T. myopaeformis* in a laboratory setting to generate mature, third-instar larvae. Further, we compare oviposition, larval development, and feeding damage between a resistant and susceptible variety in no-choice and paired choice experiments. The results presented here contribute to our understanding of the modality of resistance for the resistant variety tested and outline a greenhouse protocol for screening germplasm with putative resistance to *T. myopaeformis*.

Materials and Methods

Collection and Rearing of Flies

Third-instar maggots were collected in the Red River Valley of North Dakota in portions of commercial sugar beet fields in which insecticides were not used. Collections were performed in July during the 2013, 2014, and 2015 growing seasons, after maggots had reached maturity. *T. myopaeformis* typically reach the third instar and stop feeding by late June to early July and tunnel deeper in the soil as temperatures decrease at the end of the summer (Hein 1995). Maggots were then transported to the University of Idaho Kimberly Research and Extension Center, in Kimberly, ID.

Maggots were stored together in plastic vials filled with soil and held in an environmental chamber (Percival Scientific, Inc., Perry, IA) until needed for use in experiments. The environmental chamber was held at 4°C with 70% RH and no light. Maggots were held at 4°C for a minimum of 6 mo to allow the insects to go through the diapause period needed for pupation (Harper 1962). An organic potting soil (“The EarthWorks”; Brandtastic Soil, LLC., Twin Falls, ID) was used for storing maggots and for all experiments. The soil mix was comprised of equal parts peat moss, composted cow manure, perlite, and coconut coir. Potting soil was autoclaved at 121°C and 137,895 Pa for 30 min to reduce mortality of maggots from soil-borne pathogens. After autoclaving soil, deionized water was added to achieve gravimetric soil moisture content of 30 to 40%. Rearing containers consisted of clear plastic 30-ml cups (Sovereign Drinkware, Federalsburg, MD) partially filled with soil into which a maggot was gently placed using sterilized forceps and then covered with a ca. 6-mm layer of soil. After fitting each cup with a lid (Dixie, Easton, PA), cups were transferred to clear, plastic trays. Each tray was then placed inside a two-gallon plastic freezer bag. A 3 × 5 × 0.5 cm cutting of a sponge moistened with ca. 3 ml of deionized water was added to each bag to help maintain a humid environment within the bag and reduce loss of soil moisture.

To obtain adult flies for use in experiments, cups with larvae were transferred to an environmental chamber that was maintained on a 16:8 L:D cycle at 24°C and 80% RH during photophase and 21°C and 70% RH during scotophase. Each cup was inspected daily for insect pupation and for emergence of adult flies, and the sex of each fly was determined at eclosion based on morphology (Gojmerac 1956). All flies used in experiments were 0–5 d post eclosion. Emerged flies that were not immediately used in experiments were offered honey water inside their rearing cup (1:9 honey:water) on a 5-mm cutting of an 8-mm diameter cotton dental roll (TIDI Products, Neenah, WI). Flies were kept in their individual rearing cups until they could be placed into rearing cages.

Laboratory Rearing of Third-Instar Larvae

For these assays, sugar beet variety BTS 27RR20 (BetaSeed, Inc., Shakopee, MN) was used; this variety exhibits strong resistance to rhizomania, Fusarium root rot, and beet curly top virus and produces high yields of estimated recoverable sucrose, but is susceptible to

T. myopaeformis feeding (BetaSeed, Inc. 2016). Two beet seeds were planted in pots that measured 10 × 10 cm wide by 9 cm tall. After beets had germinated, they were thinned to one plant per pot. Beets were watered every 3 d until 3 wk after emergence at which point they were watered every other day. Plants were grown to the two- to four-leaf stage before use in experiments. All plants were housed in a greenhouse maintained between 25°C and 32°C throughout both night and day periods. Artificial lights were used in the greenhouse to maintain a 16:8 L:D cycle.

An individual fly of each sex was placed on each plant, housed in a mesh cage (Fig. 1). Flies were caged on plants by placing an insect-rearing sleeve (MegaView Science, Taiwan) over each plant. Rearing sleeves were supported over the plant using two lengths of galvanized steel wire with a diameter of 1.63 mm (L G Sourcing, Wilkesboro, NC); each wire was curved into a parabolic shape and each end of the wire was inserted into the soil on opposite corners of the plant pot. Flies were provided with a small glass vial filled with honey water (see Collection and Rearing of Flies, the previous section) that was placed into the soil of each plant with a cotton dental roll inside but protruding just out of the top of the vial. Sleeves were secured around the base of the plant pot using a rubber band. Rearing cups containing individual flies were placed inside a sleeve cage and opened slightly to allow flies to exit. Once flies had exited the cups, rearing cups and lids were carefully removed. After the plants were infested, they were placed on a greenhouse bench and watered every 3 d by opening sleeve cages slightly and pouring water from a plastic pitcher directly on the soil surface until soil was saturated.

After 14 d of exposure to flies, the soil within each plant pot was inspected for the presence of maggots, and plants were inspected for evidence of root maggot feeding. Third-instar maggots were collected into a vial with sterilized potting soil at 40% soil moisture



Fig. 1. Insect cage with sugar beet used for rearing and no-choice experiments.

and moved into cold storage at 4°C. Larval instars were determined based on morphological descriptions given by Gojmerac (1956) and Bjerke et al. (1992). Any maggots that had not reached the third instar were deposited into soil on new plants of the same variety at the two- to four-leaf stage. After another week, soil was inspected again, and third-instar larvae were collected and moved into cold storage.

No-Choice Experiments (Adults)

Two genotypes were used in these experiments: F1010, which is susceptible to *T. myopaeformis* feeding damage (Campbell 1990), and F1024, a hybrid cross of F1016 and 19961009H2, which are, respectively, resistant to *T. myopaeformis* (Campbell et al. 2000) and to multiple pathogens (Panella et al. 2008). F1010 and F1024 seeds used in these experiments were supplied by Dr. Larry Campbell of the United States Department of Agriculture-Agricultural Research Service. Field trials have shown F1024 to be resistant to *T. myopaeformis* feeding damage and to be generally larger and more vigorous than F1016 (Campbell et al. 2011). Plants were grown from seed in the greenhouse under the same conditions described previously for growing BTS 27RR20. Plants were used in experiments when they reached the two- to four-leaf stage. F1010 plants germinated slightly faster than F1024 plants, so planting of the latter variety ca. 4 d before the former facilitated synchronizing the growth stage of the two varieties.

Individual plants were caged using sleeve cages and infested with a male/female pair of flies using the same methods as described previously under Laboratory Rearing of Third-Instar Larvae (Fig. 1). Plants were arranged on the greenhouse bench in a randomized complete block design, with 15 replicates of each plant genotype. After 14 d, the soil from each pot was inspected for the presence of root maggots. Plants were gently washed to avoid secondary root destruction and rated for feeding damage.

A noncontinuous rating scale from 0 to 3 was used in these experiments. A damage rating of 0 indicated that there were no observable scars or evidence of feeding on the sugar beet by maggots (Fig. 2A). A rating of 1 indicated that there was evidence of feeding damage in the secondary roots with no observable damage to the primary or tap root (Fig. 2B). A score of 2 indicated evidence of between one and three black feeding scars on the tap root (Fig. 2C). Finally, a rating of 3 indicated four or more feeding scars or a complete severing of the tap root by feeding (Fig. 2D).

Paired-Choice Experiments

F1010 and F1024 plants were grown from seed in the greenhouse, as described previously under No-Choice Experiments (Adults). Two potted plants, one of each variety, were placed next to each other and caged together using two sleeve cages (Fig. 3). Two vials of honey water were placed side-by-side in the middle of the cage, with one vial in each plant pot.

Cages were infested with one female and one male fly and cages were arranged on a greenhouse bench in a randomized complete block design with 55 initial replicates. After 5 d in the greenhouse, surviving flies were collected and plants were transferred to a refrigerator set at 4°C to delay egg hatch during the 2 to 4 d over which soil samples were examined (see the following paragraphs). Replicates in which the female was found dead at the end of the 5-d period and no eggs were present were not included in analyses ($n = 31$).

Using a stereomicroscope, the top 2.5 cm layer of soil from each pot was inspected for the presence of root maggot eggs. Any remaining eggs were then sampled from the same top 2.5 cm of soil in



Fig. 2. Examples of plants representing each discrete damage rating. A) Rating of 0, in which no feeding damage is observed; B) Rating of 1, with evidence of small amounts of feeding on secondary roots but no damage to tap root; C) Rating of 2, with feeding scars visible on the tap root; D) Rating of 3, in which the tap root is completely severed by feeding.

each pot using salt-water flotation (Dryden et al. 2005). Each soil sample was poured into a small plastic container that was filled with saturated salt water. Eggs that floated to the surface were collected. The total number of eggs recovered from each pot using both the direct observation approach and the salt-water flotation approach were counted. Eggs were collected from the soil and salt water using a small, wet paintbrush. Collected eggs were used immediately in no-choice experiments.

No-Choice Experiments (Eggs)

F1010 and F1024 plants were planted in the greenhouse and grown to the two- to four-leaf stage, as described previously under No-Choice Experiments (Adults). Five replications of each variety were used. Twenty eggs were placed at the base of each plant. Plants were watered immediately prior to infestation to ensure a moist environment that reduced the likelihood of egg desiccation. Plants were all placed in a large cage to inhibit colonization by nontarget greenhouse pests (e.g., aphids, fungus gnats, and spider mites) and watered every 3 d. Plants were labeled and arranged randomly within the cage. After 2 wk, root maggots in the soil were counted and damage ratings were recorded on each plant as described previously. Third-instar maggots were collected and placed in cold storage, whereas first- and second-instar maggots were placed on new plants of the same variety and re-inspected later for presence of third-instar maggots, as described previously under Laboratory Rearing of Third-Instar Larvae to be used in future rearing experiments.

Data Analysis

All analyses were carried out using SAS version 9.4 (SAS Institute 2015). For all data, the univariate procedure was used to determine whether distributions were Gaussian (PROC UNIVARIATE). For laboratory-reared adults, the number of days to eclosion was compared between sexes using Analysis of Variance (PROC ANOVA). For this analysis of variance, equal variance was tested for each population using the General Linear Model procedure (PROC GLM) alongside a homogeneity of variance test. Adult emergence data were examined using the General Linear Mixed Model procedure (PROC GLIMMIX) with a Gaussian distribution and Tukey's range test to compare emergence data between sexes and among years that maggots were collected. Year of maggot collection was used as an independent variable. The number of maggots observed in no-choice experiments was compared between varieties using a Wilcoxon Two-Sample Test (PROC NPAR1WAY). The number of eggs collected in paired-choice experiments was compared between varieties using PROC GLIMMIX with a Poisson distribution and block as a random factor.

Results

Laboratory Rearing of Third-Instar Larvae

Over the course of all experiments, 274 adult flies were successfully reared from field-collected larvae. There was no significant difference in adult emergence time based on sex ($F_{1,258} = 1.63$, $P = 0.202$;

Fig. 4) or the year that flies were collected ($F_{2,258} = 1.19, P = 0.306$). However, there was a significant sex by year interaction effect ($F_{2,258} = 7.41, P < 0.001$; Table 1). Males collected in 2013 emerged significantly later than females. Emergence times were shorter for females only during 2013; the number of days to adult emergence did not differ between sexes for maggots collected during 2014 or 2015 (Table 1). Emergence percentages tended to be lower for maggots that had been held in cold storage for longer periods of time (Table 2), though these data were not subjected to statistical analysis. Ten of the flies died within the soil of the rearing cup and were not found until rearing cups were emptied. These flies had deteriorated to the point that they could not be identified to sex.



Fig. 3. Insect cage with sugar beet used in paired-choice experiments.

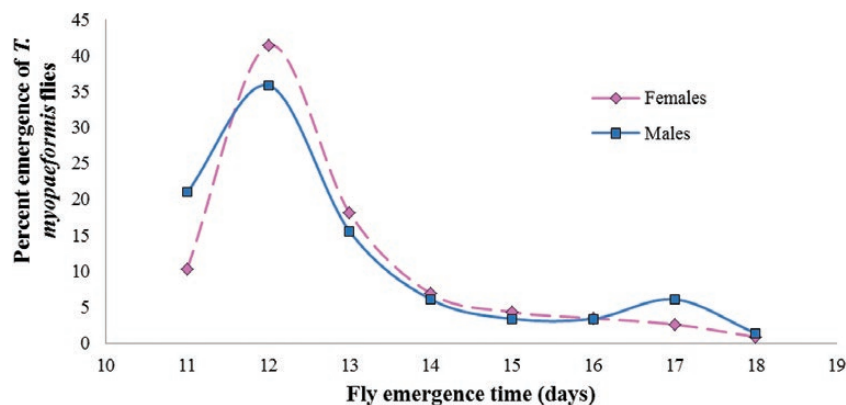


Fig. 4. Time to emergence for adult *T. myopaeformis* after postdiapause maggots were placed in rearing chamber. $n = 116$ females; $n = 148$ males; $n = 264$ total.

Two weeks after adults were placed on plants, *T. myopaeformis* maggots were observed in the first, second, and third instar. Most feeding occurred on secondary roots at the bottom of pots on BTS 27RR20 plants. Most larvae were second instars and measured ca. 2–4 mm in length. Second instars that were placed on new plants advanced to the third instar after one additional week of feeding. None of the third instars that were collected during these experiments and placed in cold storage survived after transfer from cold to warm conditions following a 6-mo diapause period.

No-Choice Experiments (Adults)

Significantly more maggots were found on F1010 plants (mean \pm SEM: 30.8 ± 8.9 ; range: 0–101) relative to F1024 plants (1.2 ± 1.2 ; range: 0–17) ($Z = 3.16, P < 0.0008$). Maggots on F1010 roots ranged from the first to the third instar, and nearly all exhibited white coloration and moved when gently prodded with a small paint brush. Maggots were found on only one F1024 plant; all were first instar, exhibited dark coloration, and did not move when touched, indicating that they were dead or moribund. The mean \pm SEM damage rating for F1010 plants was 1.0 ± 0.3 . No damage was evident on any F1024 plants. On eight of the nine F1010 plants on which maggots were observed, there was evidence of feeding on the secondary roots at the bottom of the plant pot. The taproots of two F1010 plants were completely severed by feeding.

Paired-Choice Experiments (Adults)

In total, 55 replications of paired-choice assays were performed; however, 31 of the replications were not included in analyses because the female died before the end of the 5-d assay and no eggs were found. For the remaining 24 replications, the female survived to the end of the assay and/or eggs were recovered from the soil. Overall, only 9% of the female flies used in these experiments survived the 5 d, whereas 29% of the male flies survived the 5 d. There was no difference in the number of eggs recovered from the soil around F1010 plants (7.8 ± 2.6 eggs) relative to F1024 plants (5.5 ± 1.5 eggs; $F_{2,3} = 0.00, P = 0.999$).

No-Choice Experiments (Eggs)

A mean of 3.0 ± 1.7 larvae was collected from all plants. The mean number of larvae found on F1010 plants was 5.4 ± 1.8 , whereas the mean found on F1024 plants was 0.6 ± 0.4 . Maggots found in soil around F1010 were first and second instar, exhibited bright coloration, and moved when gently prodded with a small paint brush. Maggots found in soil around F1024 were all first instar, exhibited dark coloration, and did not move when touched, indicating

that they were dead or moribund. The mean \pm SEM damage rating for F1010 plants was 1.0 ± 0.32 . Damage was not evident on any F1024 plants. Due to the small sample size, data were not statistically analyzed.

Discussion

Our bioassay successfully produced third-instar *T. myopaeformis* larvae under laboratory conditions. Previous efforts to rear *T. myopaeformis* did not yield third-instar larvae (Ure 1966). However, completion of the entire life cycle of *T. myopaeformis* in the current study was not a success. None of the third-instar larvae reared survived the artificial 6-mo diapause period. Mortality may have been caused by a variety of factors. Although soil moisture content of 30–40% has proved effective for pupal development (MacRae and Armstrong 2000), optimal soil moisture for overwintering larvae has not been studied. Maggots were washed and stored in soil that had been autoclaved, so any entomopathogenic pathogens that might have contributed to mortality likely originated from within the maggots themselves. Bacterial endosymbionts associated with *T. myopaeformis* include *Serratia liquefaciens* (Grimes and Hennerty) (Enterobacteriales: Enterobacteriaceae), *Serratia marcescens* Bizio (Enterobacteriales: Enterobacteriaceae), and *Stenotrophomonas maltophilia* (Hugh) (Xanthomonadales: Xanthomonadaceae) (Iverson et al. 1984), and it is likely that a healthy microbiome also is required for survival of maggots. Further experiments on endosymbionts as well as storage conditions and handling of greenhouse-reared third-instar larvae will be required to clarify mortality factors for third-instar larvae. Completion of the *T. myopaeformis* life cycle in the laboratory will enable year-round greenhouse studies on host plant resistance.

No-choice and paired-choice experiments confirmed that F1024 exhibits resistance to *T. myopaeformis* with antibiosis as its modality. Antixenosis was not observed as a modality of resistance against adult *T. myopaeformis* flies. There was no evidence of egg-laying preference between varieties; thus, *T. myopaeformis* females appear

to be choosing indiscriminately between these two hosts for oviposition sites. This suggests that F1024 plants are not producing compounds that deter flies from laying eggs. Similar results were reported by Tabari et al. (2017) for a lepidopteran pest of rice in which oviposition often was not reduced on rice genotypes that exhibited antibiosis against larvae.

Smigocki et al. (2006) showed that *T. myopaeformis* maggots tended to aggregate along the roots of susceptible F1010 plants and dispersed from moderately resistant F1016 plants, reportedly due to the latter variety's unpalatability. It is possible that in F1016, a parent of F1024, secondary metabolites produced in response to mechanical feeding damage may be repelling larvae within the soil (Smigocki et al. 2006). This could mean that both antixenosis and antibiosis could be affecting maggot survival on F1024, though this hypothesis remains to be tested. Whether or not antixenosis against larvae affects their survival on F1024, no evidence was found in the current study for antixenosis against female oviposition.

For the no-choice egg experiments, the extremely low number of maggots found on F1024 plants and the poor apparent health of these maggots suggests that F1024 plants exhibit antibiosis against *T. myopaeformis* larvae. The small sample size precluded statistical analyses; however, results were similar to those of no-choice experiments using adults. That is, scarcely any larvae survived on F1024 relative to F1010 plants. Egg collection directly from soil proved to be a time-consuming and difficult task, in part, because soil particles readily stuck to eggs and obscured them from vision. We do not know if both methods of egg collection (direct observation and salt-water floatation) were necessary for efficiently collecting eggs from soil; however, it is possible that salt-water floatation would be sufficient on its own. Challenges with egg collection underscore the value of screening germplasm for resistance by inoculation with adult insects rather than eggs or larvae when possible.

F1016, one of the parents of F1024, exhibits moderate resistance to *T. myopaeformis* feeding (Campbell et al. 2011). In both F1016 and F1010 plants, the gene *BvSTI* codes for a serine protease inhibitor (Puthoff and Smigocki 2007, Smigocki et al. 2013), a type of protein that is upregulated by mechanical or feeding damage (Smigocki et al. 2007, Savić and Smigocki 2012). Serine proteases have been found to be the predominant type of midgut proteases in Lepidoptera and Diptera (Smigocki et al. 2013) and have a functional role in the gut of *T. myopaeformis* larvae (Wilhite et al. 2000). The *BvSTI* gene shows higher activity in the roots of F1016 relative to F1010 plants (Savić and Smigocki 2012), and could explain the mechanism of resistance in F1024 if a similar pattern of expression occurs. Diversion of resources toward production of secondary compounds, regulated by *BvSTI*, might also explain why F1010 grew at faster rates than F1024 plants. Feeding by first instar larvae during no-choice experiments could have caused an upregulation of serine protease inhibitors which in turn would have caused other larvae feeding on the root to suffer digestive damage or to disperse from the

Table 1. Mean time to adult emergence compared by sex and year that larvae were collected

Year	Sex	Mean \pm SEM emergence time (days)
2013	Male	15.8 \pm 0.66 a
	Female	12.8 \pm 0.64 b
2014	Male	14.6 \pm 0.96 ab
	Female	14.1 \pm 1.08 ab
2015	Male	12.9 \pm 0.37 b
	Female	14.1 \pm 0.45 ab

Means that do not share a letter are statistically different ($\alpha = 0.05$).

Table 2. Emergence rates of *T. myopaeformis* used in all experiments

Year collected	Initial no. maggots	No. flies emerged				Percent emergence		
		Male	Female	Unknown	Total	Male	Female	Total
2013	180	32	34	0	66	48.5	51.5	36.7
2014	90	15	12	10	37	55.6	44.4	41.1
2015	256	101	70	0	171	59.1	40.9	66.8
2013–2015 ^a	516	148	116	0	274	56.1	43.9	53.1

^aSummary of data from all three collection years.

root (Smigocki et al. 2006). Fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) larvae that were fed transgenic *Nicotiana benthamiana* (Domin) (Solanales: Solanaceae) leaves with high *BvSTI* expression experienced delayed onset to pupation and were smaller and lighter in color than larvae that were fed nontransgenic leaves, indicating that *BvSTI* expression may increase resistance of these crops to insect feeding (Smigocki et al. 2013).

Several studies on emergence rates of *T. myopaeformis* flies reared under laboratory conditions have been performed (Ure 1966, Whitfield and Grace 1985, Chirumamilla et al. 2008). Adult emergence rates and sex ratios in our experiments were similar to those reported by Chirumamilla et al. (2008). Moreover, reduction in adult emergence rates for maggots stored for longer durations (Chirumamilla et al. 2008) also was observed during the current study. This reduction may be caused by the decrease of internal lipids over time in storage, which is more pronounced in smaller maggots (Chirumamilla et al. 2010). The delayed emergence timing of male flies collected in 2013 as a function of storage duration observed in the current study might be attributed to lower lipid reserves of male maggots given that female flies are larger than male flies. Because there was no significant difference in male and female emergence times overall based on sex, no evidence for protandry in *T. myopaeformis* was observed in the current study. Females are capable of mating and laying eggs more than once during their lifespan so we may not expect protandry to occur within this species.

The bioassays used in these experiments are an effective way to conduct greenhouse screening of sugar beet varieties with putative resistance against *T. myopaeformis*. These methods may provide a useful protocol for screening of biological control agents as well. Differences in damage ratings observed on F1010 and F1024 plants during our no-choice experiments were similar to differences in these varieties observed in field trials in North Dakota (Campbell 2015). This underscores the potential utility of our greenhouse trials in expediting the process of screening resistant germplasm. It is important to note that field studies in areas with high populations of *T. myopaeformis* are essential for conclusions to be drawn on whether or not germplasm that displays resistance in the greenhouse performs well under field conditions.

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