
A Sugarbeet Root Maggot (*Tetanops myopaeformis* Röder) Bioassay Using *Beta vulgaris* L. Seedlings and *In vitro* Propagated Transformed Hairy Roots

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ABSTRACT

A bioassay was developed to study interactions between sugarbeet (*Beta vulgaris* L.) roots and sugarbeet root maggot larvae (SBRM, *Tetanops myopaeformis* Röder: Ulidiidae). Sugarbeet root material included seedlings (2 to 3-wk old) and *in vitro* propagated hairy root cultures of SBRM susceptible (F1010) and moderately resistant (F1016) germplasm. Second-instar SBRM aggregated in clusters on roots of F1010 but not the F1016 seedlings. Feeding damage, including rasping marks, tunnels and severed roots, was more prominent on the F1010 roots. When *in vitro* propagated hairy roots of the corresponding genotypes were used in the bioassay, larval tracks were

visualized due to growth of bacteria in the trail of the larva. Dense circular and roaming tracks surrounded the F1016 roots in contrast to tracks that were confined to the area primarily along the lengths of the F1010 roots. Feeding on hairy roots stained in 0.01% safranin or crystal blue was correlated with dye-stained frass and intestinal tracts of first-instars. When F1010 hairy roots were used to bioassay the effects of potential toxins, more than 50% of the larvae died within 24 h, compared to 100% survival for the control. These bioassays have potential as rapid methods for pre-screening sugarbeet accessions and genetically modified germplasm for SBRM resistance as well as for evaluating the effects of potential toxins on the larvae.

Additional key words: insect resistance, hairy root cultures, *Agrobacterium rhizogenes*

The sugarbeet root maggot (SBRM), *Tetanops myopaeformis* Röder (Diptera), is a major insect pest of sugarbeet (*Beta vulgaris* L.) in the north-central and western United States and Canada. Root maggot infestations have spread to over two-thirds of the 1.5 million beet-producing acres in the United States. In early summer, newly emerged SBRM larvae begin feeding on tap and feeder roots of the sugarbeet plant (Yun et al., 1986). This feeding continues throughout the growing season, causing significant crop damage either by severing seedling tap-roots or badly scarring the surface of larger roots. Losses inflicted by SBRM can range from 10% to 100% (Blickenstaff et al., 1981; Campbell et al., 1998; Cooke, 1993). Control of SBRM has relied on a handful of chemical insecticides that have been in use for over 30 years. Possible loss of these chemicals because of environmental safety concerns and the potential for development of resistance to the insecticide has serious implications for the profitability of sugarbeet production in the future.

Conventional breeding methods have produced two moderately resistant sugarbeet lines, however, germplasm with a high level of SBRM resistance is still lacking (Campbell et al., 2000; Theurer et al., 1982). Recent advances in molecular biology have the potential to yield a large number of newly developed insect control agents and germplasm that could lead to useful strategies for SBRM management. Even though herbivory by root feeders is recognized as a serious agricultural problem in many economically important crops, including sugarbeet, studies on host resistance mechanisms in roots are rare. Most studies of host interactions with plant parasitic arthropods have focused on

aboveground plant organs, primarily due to the limitations imposed by the complexity of the rhizosphere environment (Wu et al., 1999).

Development of efficient insect bioassays is imperative to rapid screening of resistance resources. This, in turn, assists with and leads to the design of effective tools for controlling insect pests. The inability of the SBRM larvae to complete their life cycle in a laboratory environment has hindered the development of an efficient laboratory bioassay for SBRM. The need to collect larvae from infested fields, either for immediate applications or for cold storage to be used in future studies, limits the scope and the number of experiments that can be executed. Compounding problems associated with cold-stored larvae include low frequencies of larval pupation, fly emergence, fly mating and the ensuing egg laying on caged sugarbeet plants. Problems are also encountered with stored SBRM eggs that exhibit variable frequencies of egg hatch and viability of newly hatched larvae. The need to utilize taproots of field- or greenhouse-grown plants in root maggot feeding assays have also played a negative role in the development of a successful SBRM bioassay.

In this paper, we report on the development of a relatively rapid SBRM bioassay using sugarbeet seedlings and their respective *in vitro* propagated hairy root cultures. We demonstrate larval feeding and distinguishable feeding behavior on a susceptible F1010 and moderately resistant F1016 germplasm.

MATERIALS AND METHODS

Sugarbeet germplasm

Sugarbeet germplasm with resistance to the sugarbeet root maggot, F1016 (PI608437), and a susceptible, F1010 (PI535818), germplasm line (Campbell, 1990; Campbell et al., 2000), were utilized in this study. Thiram 42S (Gustafson, LLC, Plano, TX) coated seeds were imbibed in water, planted in soil in 7-inch pots, and maintained in a growth chamber at 27°C with a 16 h photoperiod (270 $\mu\text{mol}/\text{m}^2\text{s}$).

Sugarbeet hairy root cultures

Hairy roots were obtained by using *Agrobacterium*-mediated petiole transformation (Kifle et al., 1999). Petioles of 2-month-old plants were surface-sterilized in 20% (v/v) commercial bleach (5.25% w/v sodium hypochlorite) and 0.01% (w/v) sodium dodecyl sulfate solution for 15 min and then washed four times with sterile water. Petioles were submerged in an overnight culture of *A. rhizogenes* strain 15834 for 10 min, blotted dry, transferred to solid 1/2 strength B5 (1/2 B5)

medium (Gamborg et al., 1968), and co-cultivated at 25°C for 2 d in total darkness. Petioles were then washed in a solution containing 500 mg/l Cefotaxime (Sigma, St. Louis, MO) and 1000 mg/l carbenicillin (Sigma, St. Louis, MO), blotted dry, transferred to solid 1/2 B5 medium containing 400 mg/l Cefotaxime and 500 mg/l carbenicillin and maintained at above conditions. Approximately 18 d after infection, individual hairy roots were excised and 1-cm explants were cultured on 1/2 B5 medium with 200 mg/l Cefotaxime and 300 mg/l carbenicillin. Hairy root cultures were sub-cultured every 2 wk and antibiotics were eliminated from the culture medium after 4 passages. After the fifth subculture, roots were maintained in liquid 1/2 B5 medium at 25°C under a 16 h diurnal photoperiod provided by fluorescent lights (cool-white, 30 $\mu\text{mol}/\text{m}^2\text{s}$) on a gyratory shaker at 120 rpm.

SBRM larvae

SBRM, *T. myopaeformis*, second-instars, diapausing larvae and pupae were collected from commercial sugarbeet fields near St. Thomas, ND (Pembina County) and stored at 4°C. For fly emergence, larvae and pupae were moved to room temperature. Flies were allowed to mate and lay eggs on caged sugarbeet plants. Eggs were collected and stored for 2 to 4 wk at 4°C on “muck” plates (Petri dishes filled with black, dyed plaster of Paris, covered with black velvet and moistened with water) (Mahrt and Blickenstaff, 1979). To obtain first-instars, eggs were incubated at 25°C and newly hatched larvae emerged after 24 to 48 h. In some experiments, eggs were surface-disinfected in 4% (v/v) commercial bleach (0.2% hypochlorite) for 5 min and washed in phosphate-buffered saline (10 mM Na₂HP0₄, 10 mM NaCl, pH 7.2; PBS) and then sterile water.

SBRM bioassay

Sugarbeet seedlings (2 to 3-wk old) were removed from soil, washed with tap water to remove residual soil particles and gently blotted on paper towels. Washed seedlings were placed on water-moistened Whatman (No. 3) filter paper, nylon membrane or on 0.8% agar in 150 x 15 mm Petri plates. A total of 60 (4 plates) F1016 and 117 (8 plates) F1010 seedlings were infested with second-instar SBRM at a rate of 3 larvae per seedling. Two experiments were run, each with 2 and 4 plates of F1016 and F1010 seedlings, respectively. Plates were sealed with Parafilm (Pechiney, Chicago, IL) and incubated in a growth chamber at 25°C in the dark. First-instars were used to infest 161 (16 plates) F1016 and 49 (5 plates) F1010 seedlings at a rate of 2-6 larvae per seedling in reps of 2-3 plates. Seven experiments were run over a 5

month period whenever newly hatched first-instars were available.

When *in vitro* propagated hairy roots were used in the bioassay, 2 to 3 hairy root explants (about 2 - 3 cm in length) were placed on 1/2 B5 medium in a 60 x 10 mm Petri plate. Ten newly hatched larvae per plate were placed directly on the hairy root explants. Hairy root explants from two independently derived F1016 and two F1010 lines were infested with the larvae. Experiments were carried out twice in replicates of 2-6 plates.

To visualize feeding by first-instars, hairy root explants were stained in 0.01% saffranin or crystal violet for 5 min, blotted dry and 3 roots were placed on water-moistened Whatman filter paper (No. 3), wet nylon membrane or 1/2 B5 medium in a 60 x 10 mm Petri plate. Benomyl (10 mg/l, Sigma, St. Louis, MO), Cefotaxime (300 mg/l) and carbenicillin (400 mg/l) were added to the 1/2 B5 medium to reduce the growth of microbial contaminants.

Bioassay of plant extracts

F1010 hairy roots were cut into 1 cm lengths and dipped in aqueous suspensions of insecticidal leaf extracts (10 mg extract/ml) from five disease resistant transgenic (N.t. 1, 2, 3, 4 and 5) plants or the extract from untransformed (N.t. 6) *Nicotiana tabacum* plants (Mujer and Smigocki, 2001; Smigocki and Wilson, 2004). Extracts were from five independent transformants (N.t.1, 2, 3, 4 and 5) carrying a *CYP72A2* gene known to have insecticidal activity capable of killing tobacco hornworm larvae (Mujer and Smigocki, 2001; Smigocki and Wilson, 2004). Each extract-coated root was placed in a well of a 12-well Costar plate (Corning, Inc., Corning, NY) that was lined with Whatman filter paper (No. 1) saturated with 0.16 ml of the extract or water (water control). The treatments were replicated in three wells and each well was infested with 10 newly hatched first-instars. Plates were sealed and incubated at 25°C in total darkness. Behavioral responses and mortality of SBRM larvae were recorded daily using a dissecting microscope. The experiment was conducted using a completely random design with 7 treatments and 3 samples per treatment. Percentages were transformed using the arcsin transformation described by Steel and Torrie (1980) prior to analysis. Means were compared using Fisher's protected LSD (Carmer and Walker, 1985) with $\alpha = 0.10$. Due to the exploratory nature of this experiment, it seemed that a probability level of 0.10 was more appropriate than the frequently used 0.05 and 0.01 levels. The Contrast and Estimate functions of the SAS (SAS Institute, Cary, NC) GLM Procedure were used to determine if the mean of the five extracts from the transgenic plants was significantly different than the extract from the untransformed plant or the water control and to estimate the magnitude

of these differences.

RESULTS

SBRM feeding on F1010 and F1016 seedlings

Sugarbeet seedlings of the susceptible F1010 and moderately resistant F1016 germplasm were used to demonstrate distinct germplasm-specific feeding patterns of SBRM larvae. When second-instars were placed on F1016 roots, most of the larvae dispersed away from the roots (Fig. 1A and 1B). On similarly infested F1010 roots, a majority of the larvae remained on the roots in small clumps or aggregates (Figs. 1C, 1D and 1E). Within 2 h following infestation, feeding zones were observed on F1010 roots and hypocotyls (Fig. 1F). After 24 to 48 h, prominent rasping marks and discolored lesions were visible. Damage to the roots was similar to that generally observed on taproots in SBRM-infested sugarbeet fields. Holes and tunnels inflicted by burrowing and rasping second-instars were visible on taproot explants of greenhouse-grown plants used in the bioassay (Fig. 1G). Integrity of the taproot explants was disrupted and fragments of root tissues were found lying near the explants. When the bioassays were done on filter paper or agar media, larvae were observed to move under the paper and to burrow through the paper or agar especially when they were placed on taproots of the resistant F1016 explants. Using nylon membranes as a support for the roots prevented the larvae from feeding on or tunneling through the membrane or agar (Fig. 1E). Similar feeding behavior was observed when first-instars were used to infest the F1010 and F1016 seedlings. However, since the larvae were not readily visible to the naked eye, it was difficult to locate larvae that were concealed by the seedlings, even when fewer seedlings per plate (i.e. 10 vs. 15 for second-instars) were used in the bioassay. Similarly, bioassay of two additional SBRM resistant accessions revealed the same feeding behavior of second-instars as that observed on the F1016 seedlings. The larvae dispersed away from the roots of an SBRM resistant breeding line (designated UT-8) that is in the parentage of F1016 (Fig. 1H) and PI179180 (Campbell, 2005), a globe-shaped red beet accession from the USDA Beta collection (Fig. 1I).

SBRM bioassay on F1010 and F1016 hairy roots

Axenic hairy root cultures were established from the F1010 and F1016 germplasm and used as a source of root tissues in the SBRM bioassay with first-instars. These experiments were carried out on agar media as newly hatched larvae were prone to rapid dehydration and death when placed on moistened paper or nylon membranes. First-instars are barely visible to the naked eye, therefore, the pattern of larval movement and

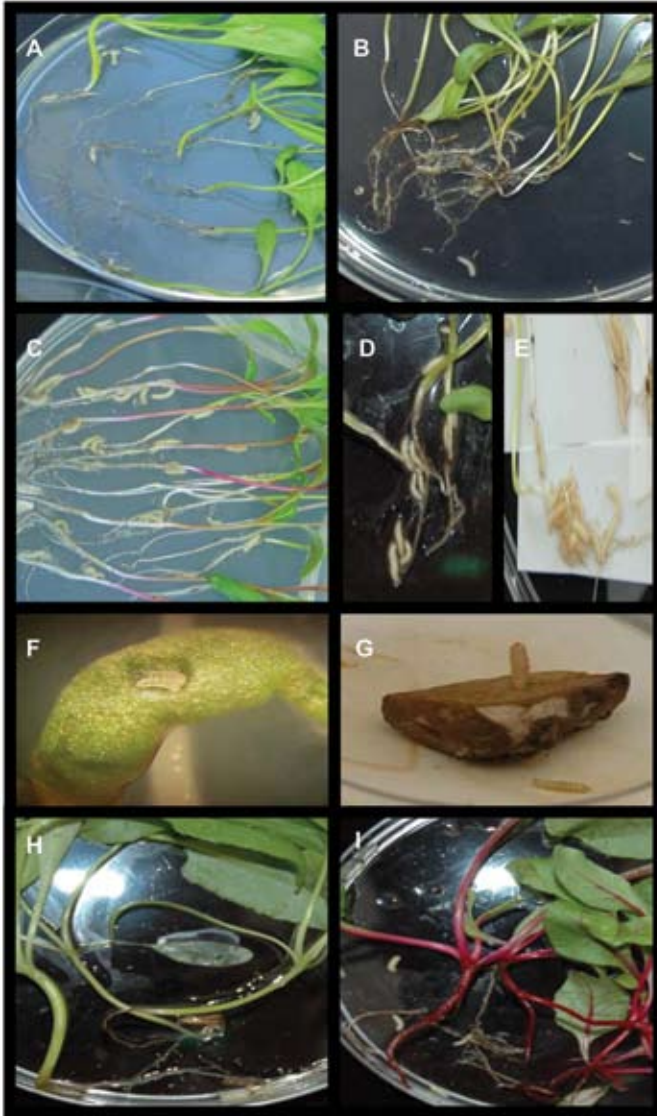


Figure 1: SBRM bioassay using 17-day-old sugarbeet seedlings. Second-instar SBRM feeding on the moderately resistant F1016 (A and B) and the susceptible F1010 (C – E) seedlings. (F) Damage inflicted by two second-instar SBRM feeding on a hypocotyl of an F1010 seedling. (G) Second-instar SBRM burrowing into an F1010 taproot explant (about 1.5 cm in length) of a 1-year-old, greenhouse-grown sugarbeet plant. (H and I) UT-8 and PI179180, respectively, infested with second-instar SBRM.

feeding was tracked by the residual trail of contamination left behind by the non-sterile larvae as they moved on antibiotic- and fungicide-free medium. Feeding on the moderately resistant F1016 hairy roots was characterized by dense circular tracks as the larvae roamed the surface of the agar (Figs. 2A and B). In comparison, the trail of contamination on F1010 hairy roots tended to be confined to the area immediately surrounding the roots, depicting movement of larvae along the lengths of the hairy roots (Figs. 2C and D). The single line of contamination, with little divergence from the path, suggests the movement of a single larva between the F1010 root pieces (Fig. 2C). The pattern of larval movement was similar on two independently derived hairy root cultures of each genotype. Larval feeding on hairy roots that were stained with saffranin dye was evidenced by the presence of red dye in larval frass (Fig. 2E). Similarly, blue dye was observed in larval midguts when the hairy roots were stained with crystal violet (Fig. 2F). When dye-stained F1010 hairy roots were infested with second-instar SBRM, severed root hairs were observed after 24 to 48 h (Figs. 2G and H).

F1010 hairy root cultures, dipped in aqueous suspensions of insecticidal leaf extracts, were used to determine if this technique might provide a method to obtain initial information on the effects of potential toxins on SBRM larvae. After 24 h, less than 50% of the larvae exposed to *N. tabacum* leaf extracts were alive, compared to 100% survival for the water controls (Table 1). All six extracts had a detrimental effect on the larvae, and the 28% differential in survival between the average

Table 1. SBRM mortality in bioassay of insecticidal plant extracts prepared from transgenic (N.t. 1, 2, 3, 4 and 5) and control untransformed (N.t. 6) *N. tabacum* leaves, and water.

Extract	Larval mortality	
	24 h	48 h
	----- % -----	
N.t. 1	70a*	94a
N.t. 2	74a	82ab
N.t. 3	41a	84ab
N.t. 4	62a	82ab
N.t. 5	48a	82ab
N.t. 6	31a	64b
Water control	0b	29c

* Treatment means within a column followed by the same letter are not significantly different, based upon LSD ($P = 0.10$).



Figure 2: SBRM bioassay using sugarbeet hairy roots of susceptible F1010 and moderately resistant F1016 germplasm. F1016 (A and B) and F1010 (C and D) hairy root explants (2 – 3 cm) infested with first-instar SBRM at 48 h. Arrows point to tracks of contamination that correspond to the movement of larvae. (E) Saffranin-stained F1010 hairy root infested with first-instar SBRM at 48 h. Arrow points to saffranin-stained frass. (F) Dye-stained midgut (arrow) of first-instar SBRM after feeding on hairy roots stained with crystal violet. (G and H) Saffranin-stained root explant from an F1010 hairy root culture, respectively. Arrow points to dye-stained roots severed by second-instar SBRM.

of the leaf extracts from the 5 transgenic plants (59%) and the extract from the untransformed plants (31%) was not significant ($P = 0.21$), 24 h after first-instars were placed on the cultures. After 48 h exposure, mortality was significantly lower for first-instars exposed to water than mortality after exposure to the six plant extracts. Extracts from one of the transgenic plants (N.t. 1) significantly increased mortality, compared to an extract from untransformed plants (N.t. 6); however, as a group, the extracts from the five transformed plants (85% average mortality) did not appear to be superior to the extract from the untransformed plants (64%; difference = 21%, $P = 0.13$). The 29% mortality after 48 h for the water control treatment suggests that treatment differences may be difficult to assess in longer experiments unless conditions can be adjusted to reduce background mortality. The ability to efficiently mass produce early-generation larvae would permit more replication and, perhaps along with other refinements, increase the precision of these bioassays.

DISCUSSION

The relative inability to rear SBRM larvae in the laboratory and a need to utilize taproots of greenhouse- or field-grown sugarbeet have collectively hindered the development of an efficient bioassay that would facilitate the screening of new germplasm and other agents for SBRM management (Campbell, 2005; Theurer et al., 1982). In this report, we established a bioassay to study the interactions between sugarbeet roots and SBRM larvae that utilizes sugarbeet seedlings or their corresponding axenic hairy root cultures derived from SBRM-susceptible F1010 or moderately resistant F1016 germplasm. We observed differences in feeding patterns when SBRM larvae were allowed to feed on these tissues. In general, feeding damage on the susceptible roots was more prominent than on the moderately resistant roots. Feeding patterns on F1010 seedlings were characterized by aggregation of the larvae on the roots that was not observed on the F1016 roots (Figs. 1A-E). On both the seedlings and hairy roots, larvae tended to move away from the resistant roots suggesting that the larvae may have been searching for a more palatable food source (Figs. 1A and 1B; Figs. 2A and 2B). The mechanism responsible for the moderate level of SBRM resistance in the F1016 germplasm has not been deciphered (Campbell et al., 2000), but our studies suggest that the resistance mechanism in the F1016 germplasm may be mediated, at least in part, by secondary metabolite(s), many of which are known to repel and/or inhibit insect feeding. Cloning of genes that are preferentially modulated in response to SBRM infestations in F1016 and F1010 should provide clues to how the plant protects itself from

the insect (Puthoff and Smigocki, 2005).

The demonstration that 2 to 3 wk old F1010 and F1016 seedlings or *in vitro* cultured hairy roots can be used as hosts for SBRM will facilitate further studies of host-pest interactions. Screening of sugarbeet accessions using the seedling bioassay will aid in selection of much-needed SBRM resistant germplasm for sugarbeet breeding programs. A preliminary screen of three additional SBRM-resistant accessions, UT-8, PI179180, and 02N0024 (an advanced SBRM-resistant breeding line selected from a cross between F1016 and a *Cercospora* leaf spot resistant breeding line from USDA, Fort Collins, CO) showed a similar larval feeding pattern as on the F1016 seedlings (Fig. 1; data not shown), thus supporting the potential of the seedling bioassay as a tool for relatively rapid screening of germplasm for SBRM resistance. On the other hand, the hairy root bioassay is an effective and rapid approach for *in vitro* testing of toxic compounds for their effect on SBRM. Many secondary metabolites have been implicated in plant defense against diseases and insects, but it has been difficult to investigate the effects in roots. The use of hairy root co-cultures with insects facilitates such studies (Askani and Beiderbeck, 1991; Webb et al., 1994; Wu et al., 1999). Using aseptic co-cultures of sugarbeet hairy roots, we demonstrated that *N. tabacum* extracts derived from transgenic insect-resistant as well as control untransformed plants were lethal to SBRM and could potentially be explored as a measure for SBRM control (Table 1). In addition, hairy root cultures can be transformed with known or newly discovered resistance genes to aid in the rapid evaluation of the genes' effects on insect feeding (Down et al., 1996; Zhang et al., 2004). Expression of the snowdrop lectin gene in potato hairy roots was used to study resistance to aphids prior to initiation of lengthy and laborious experiments to regenerate transgenic potato plants carrying the lectin transgene (Down et al., 1996). Similarly, *Hyoscyamus niger* hairy root cultures were engineered with genes for *in vitro* synthesis of insecticidal and pharmacological secondary metabolites (Zhang et al., 2004). Introduction of candidate genes that are prescreened using the sugarbeet hairy root bioassay could potentially lead to the subsequent introduction of engineered plants into sugarbeet breeding programs and, ultimately, future development of transgenic insect-resistant germplasm for management of this key insect pest of sugarbeet.

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LITERATURE CITED

- Askani, A. and R. Beiderbeck. 1991. *In vitro* propagation of *Dactylophaera vitifolii* Shimer (Homoptera Phylloxeridae) on shoot and root cultures of *Vitis* hybrid. *Vitis* 30:223-232.
- Blickenstaff, C.C., R.E. Peckenpaugh, D. Traveller, and J.D. Stallings. 1981. Insecticide tests for control of the sugarbeet root maggot, 1968-1978. U.S. Dept. Agric. Sci. and Educ. Admin. ARR-W-18. 75 pp.
- Campbell, L.G. 1990. Registration of F1010 sugarbeet germplasm. *Crop Sci.* 30:429-430.
- Campbell, L.G. 2005. Sugarbeet root maggot. In E.Biancardi, L.G. Campbell, G.N. Skaracis, and M. DeBiaggi (eds.), *Genetics and Breeding of Sugar Beet*. Science Publishers, Enfield, NH. pp. 113-114.
- Campbell, L.G., A.W. Anderson, R. J. Dregseth, and L.J. Smith. 1998. Association between sugarbeet root yield and sugarbeet root maggot (Diptera: Otitidae) damage. *J. Econ. Entomol.* 91: 522-527.
- Campbell, L.G., A.W. Anderson, and R.J. Dregseth. 2000. Registration of F1015 and F1016 sugarbeet germplasms with resistance to the sugarbeet root maggot. *Crop Sci.* 40:867-868.
- Carmer, S.G. and W.M. Walker. 1985. Pairwise multiple comparisons of treatment means in agronomic research. *J. Agron. Edu.* 14: 19-26.
- Cooke, D. A. 1993. Pests. In: D. A. Cooke & R. K. Scott (eds.), *The Sugar Beet Crop*. Chapman and Hall; London, pp. 428-483.
- Down, R.E., A.M.R. Gatehouse, W.D.O. Hamilton, and J.A. Gatehouse. 1996. Snowdrop lectin inhibits development and decreases fecundity of the glasshouse potato aphid (*Aulacorthum solani*) when administered *in vitro* and via transgenic plants both in laboratory and glasshouse trials. *J. Insect Physiol.* 42:1035-1045.

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- Gamborg, U.L., R. A. Mille, and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.* 50: 48-51.
- Kifle, S., M. Shao, C. Jung, and D. Cai. 1999. An improved transformation protocol for studying gene expression in hairy roots of sugar beet (*Beta vulgaris* L.). *Plant Cell Rep.* 18:514-519.
- Mahrt, C. G. and C. C. Blickenstaff. 1979. Host plants of the sugarbeet root maggot, *Tetanops myopaeformis*. *Ann. Entomol. Soc. Am.* 72:627-631.
- Mujer, C.V. and A. C. Smigocki. 2001. Cytokinin- and wound-inducible cytochrome P450 from *Nicotiana plumbaginifolia*. *Physiol. Plant.* 111:172-181.
- Puthoff, D. and A.C. Smigocki. 2005. Sugar beet (*Beta vulgaris* L.) genes regulated by sugarbeet root maggot (*Tetanopsis myopaeformis*) infections. *Proc. Am. Soc. Sugar Beet Technol.* 33:214-219.
- Smigocki, A. C. and D. Wilson. 2004. Pest and pathogen resistance enhanced by heterologous suppression of a cytochrome P450 gene *CYP72A2*. *Biotech. Lett.* 26: 1809-1814.
- Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics; a biometrical approach. McGraw-Hill Book Company, New York.
- Theurer, J.C., C.C. Blickenstaff, G.G. Mahrt, and D.L. Doney. 1982. Breeding for resistance to the sugarbeet root maggot. *Crop Sci.* 22: 641-645.
- Webb, S.E., M.L. Kok-Yokomi, D.J. Gray, and C.M. Benton. 1994. *In vitro* rearing of grapevine aphid (Homoptera Aphididae) on micropropagated shoot cultures of bunch grape and muscadine plants. *Ann. Entomol. Soc. Am.* 87:879-885.
- Wu, H., J. Wittkamper, and H. Flores. 1999. Root herbivory *in vitro*: interactions between roots and aphids grown in aseptic coculture. *In Vitro Cell Dev. Biol. – Plants.* 35:259-264.

- Yun, Y. M. 1986. Part II. Major insects and arthropods. *In*: E. D. Whitney & J. E. Duffus (eds.), *Compendium of Beet Diseases and Insects*. APS Press; St. Paul, MN, pp. 40-50.
- Zhang, L., R. Ding, Y. Chai, M. Bonfill, E. Moyano, K.-M. Oksman-Caldentey, T. Xu, Y. Pi, Z. Wang, H. Zhang, G. Kai, Z. Liao, X. Sun, and K. Tang. 2004. Engineering tropane biosynthetic pathway in *Hyoscyamus niger* hairy root cultures. *Proc. Natl. Acad. Sci.* 101:6786-6791.