AN *IN VITRO* SUGARBEET ROOT MAGGOT (*TETANOPS MYOPAEFORMIS*) FEEDING ASSAY

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INTRODUCTION

The sugarbeet root maggot (SBRM, *Tetanops myopaeformis* Röder) is considered the most serious pest of sugarbeet (*Beta vulgaris* L.) in the United States and Canada. SBRM infestations have spread to over two-thirds of the 1.5 million U.S. beet-producing acres and all of the Canadian growing areas. In most areas, larval feeding on tap and feeder roots begins in early to mid-June and continues throughout the growing season. Feeding injury causes significant crop damage that includes severed seedling taproots or badly scarred root surfaces (Yun 1986). Damaged roots are predisposed to secondary infections inflicted by opportunistic microbial pathogens that further contribute to reductions in sugar yields (Campbell et al., 1998; Cooke 1993). Although moderately resistant lines have been released, sugarbeet germplasm with high resistance to SBRM is lacking (Campbell et al., 2000). Current control measures rely primarily on chemical insecticides but alternative controls are being sought as the potential for buildup of increased resistance to these pesticides is anticipated.

Development of efficient insect bioassays is imperative for rapid screening of resistance resources in order to design effective approaches for control of insect pests. The inability to completely rear the insect in the laboratory and a need to utilize mature sugarbeet taproots have hindered the development of an efficient bioassay for SBRM larvae. Axenic plant tissues have been used in insect feeding studies to rear some insects *in vitro* and to study plant-insect interactions (Kimmons et al., 1990; Wu et al., 1999). By transforming sugarbeet petioles with *Agrobacterium rhizogenes*, we generated hairy root cultures of sugarbeet lines that are either susceptible or moderately resistant to SBRM (Campbell et al., 1990; Campbell et al., 2000; Smigocki et al. 2005). Using these sugarbeet hairy root cultures as well as the corresponding susceptible and resistant seedlings, we developed an *in vitro* SBRM bioassay.

MATERIALS AND METHODS

Sugarbeet germplasm

Sugarbeet (*Beta vulgaris* L.) germplasm with resistance to the sugarbeet root maggot, F1016, and a susceptible germplasm, F1010, were utilized in this study (Campbell 1990; Campbell et al., 2000). Seeds were imbibed in water, planted into soil in 7-inch pots and maintained in a growth chamber at 27°C with a 16 h day (270 $\mu ml/m^2s$):8 h night photoperiod.

Sugarbeet hairy root cultures

Sugarbeet hairy root cultures were obtained by *Agrobacterium*-mediated petiole transformation (Kifle et al. 1999; Smigocki et al., 2005). Hairy roots were propagated in liquid ½ strength B5 (½B5) medium (Gamborg et al., 1968) at 25°C under a 16 h diurnal photoperiod provided by fluorescent lights (cool-white, 30 $\mu mol/m^2s$) on a gyratory shaker at 120 rpm.

Sugarbeet root maggot larvae

Sugarbeet root maggot, *T. myopaeformis*, larvae (first-instars) were obtained from eggs of laboratory-reared flies (first-instar) or from soil samples from infested fields (second-instar). Eggs were stored for upto 7 weeks at 4°C on Muck plates (petri dishes filled with black, dyed plaster of Paris, kept moist, and covered with black velvet). To induce egg hatch, Muck plates were incubated at 25°C for 24 to 48 h (Mahrt and Blickenstaff, 1979). 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 Na_2HP0_4, 10 mM NaCl, pH 7.2; PBS) and sterile water before being placed on plant tissues.

Sugarbeet root maggot bioassay

Sources of root material included 3 week-old seedlings and *in vitro* propagated hairy root cultures. Sugarbeet seedlings were gently dislodged from the soil and carefully washed with tap water to remove any residual soil particles. Up to 15 seedlings were placed on water-moistened Whatman (no. 3) filter paper or on 0.8% agar in 150x15 mm Petri dishes and five second-instar SBRM were placed on each seedling. Plates were sealed with Parafilm (Pechiney, Chicago, IL) and incubated in a growth chamber at 25°C in total darkness.

In vitro propagated hairy roots were gently blotted on filter paper and 2 - 3 roots (each 3 cm long) were placed on ½ B5 medium in a 60 x 10 mm Petri plate. Five newly hatched first-instars were added to each plate and plates were incubated at 25°C in the dark.

In some experiments, Benomyl (10 mg/l, Sigma, St. Louis, MO), Cefotaxime (100 or 300 mg/l) and carbenicillin (200 or 500 mg/l) were added to the medium. Plates were incubated for 6 days and the degree of microbial contamination was recorded.

RESULTS

Cold storage and antimicrobial effects on SBRM eggs and larvae

SBRM eggs stored at 4°C for almost 2 weeks exhibited a 90% hatch rate on the Muck plates when moved to 25°C (Table 1). Storing the eggs for 21-25 days at 4°C reduced that rate by more than 25% (i.e., 1148 of 1740 eggs hatched). After almost 6 weeks of storage at 4°C, the eggs were no longer viable as newly emerged larvae were not detected. Placement of the eggs directly on plant tissues induced microbial growth and reduced the hatch rate significantly (data not shown). Surface-disinfection of the eggs prior to being placed on plant tissues reduced the hatch rate by more than 90%.

Table 1. Influence of cold storage on SBRM hatch rate. SBRM eggs were collected from laboratory-reared flies between June and December 2004. Eggs were stored at 4°C for the indicated length of time prior to being moved to 25°C.

Length of cold storage (days)	Total number of eggs	Hatch rate (%)
7-13	1790	90
21-25	1740	66
40-49	840	0

To reduce contamination from newly emerged, non-sterile first-instars, the fungicide Benomyl and antibiotics Cefotaxime and carbenicillin were added to the medium. Adding all three antimicrobials did not appear to reduce survival of larvae because more than 90% were still alive after 6 days (Table 2). Bacterial contamination was low (i.e., covering less than 10% of the plate surface) and no visible fungal growth was observe. When no anti-microbial compounds were added or when only the fungicide was used, high levels of contamination were observed and survival rates were reduced to about 30% (Table 2).

Table 2. Survival of newly hatched SBRM larvae on media containing a fungicide (Benomyl) and antibiotics (carbenicillin and Cefotaxime). Larvae were placed on F1010 hairy roots for 6 days.

Benomyl (mg/l)	Carbenicillin (mg/l)	Cefotaxime (mg/l)	% of live SBRM	Contamination[1]	
				Fungal	Bacterial
0	0	0	33 (4/12)[2]	++	++
10	0	0	39 (11/28)	-	++
10	200	100	92 (22/24)	-	+
10	500	300	90 (18/20)	-	+

[1] –, no fungal or bacterial contamination on agar plates; +, low contamination, covering less than 10% of the plate; ++, high contamination, covering more than 50% of the plate.
[2] Number of live SBRM per total number of SBRM.

Screening germplasm for resistance

Sugarbeet seedlings or hairy root tissues of susceptible and moderately resistant germplasm were successfully utilized to demonstrate the distinct germplasm-specific feeding patterns of SBRM larvae. Second-instar larvae aggregated around the roots and hypocotyls of the F1010 seedlings, whereas on F1016 seedlings the majority of the larvae were dispersed away from these tissues, burrowed into the medium, or roamed its surface (Figure 1A and B). Infestation of hairy roots derived from the corresponding genotypes

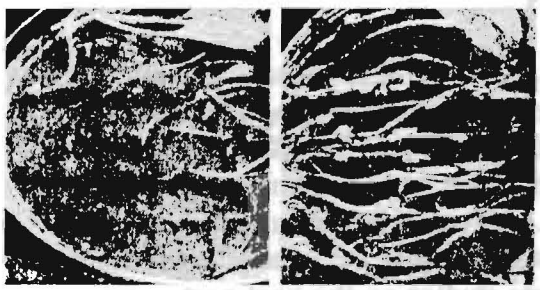

Figure 1: SBRM bioassay using 3-week-old sugarbeet seedlings. A) F1016 (moderately resistant) and B) F1010 seedlings infested with second-instar SBRM.

similarly revealed a preference of first-instars for the susceptible F1010 germplasm (Figure 2). Since first-instars are barely visible to the naked eye, their movement on hairy roots was tracked by the residual trail of contamination that grew on medium lacking a fungicide and antibiotics after the non-sterile larvae crawled on the plates.

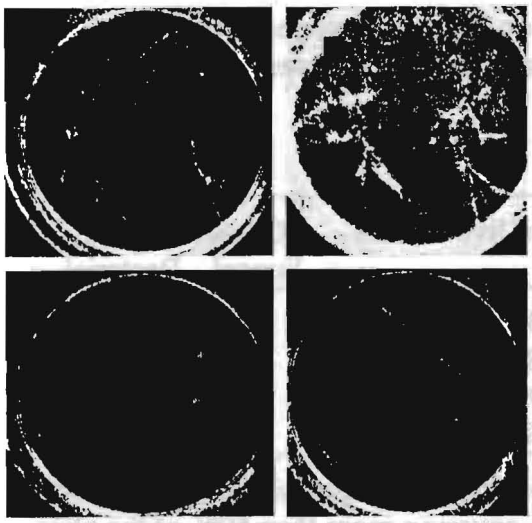

Figure 2: SBRM bioassay using hairy root cultures of susceptible F1010 and moderately resistant F1016 sugarbeet lines. A, B) F1016 hairy roots infested with first-instar SBRM. C, D) SBRM-infested F1010 hairy roots.

Dense circular, swirling and roaming trails away from F1016 roots were observed 48 h after infestation (Figure 2A and B). In contrast, the trail of contamination on F1010 hairy roots was primarily confined to the area immediately surrounding the roots, thus depicting larval mobility along lengths of the roots (Figure 2C and D). The single line of contamination directly between two roots with little divergence from the path and no swirling tracks suggests movement by a single larva between the F1010 root pieces (Figure 2C).

DISCUSSION

An *in vitro* system was established to study interactions between sugarbeet roots and SBRM larvae. Sources of root material included sugarbeet seedlings and their corresponding axenic hairy root cultures that were derived from SBRM-susceptible and moderately resistant lines. Differences in feeding behavior were documented when SBRM larvae were allowed to feed on these tissues. Larvae either aggregated on susceptible tissues or wandered away from the resistant tissues due to as of yet unknown resistance mechanism. This bioassay should facilitate studies on the host-pest interactions and lead to the elucidation of the resistance mechanism. In addition, the assay will make possible the *in vitro* evaluation of resistance and testing of potential resistance compounds and synergistic effects of genes and compounds. Hairy root cultures established with known or newly discovered resistance genes will aid in the rapid evaluation of the genes' effects on SBRM thus pre-selecting potential candidate genes for further analysis at the whole-plant level. This could potentially lead to the subsequent introduction of the engineered plants into sugarbeet breeding programs and, ultimately future development of transgenic insect protected sugarbeet germplasm events for management of this key insect of sugarbeet.

Acknowledgments: ACS would like to thank Kaylene Klingenstein and Michael Whitmore, students at Eleanor Roosevelt High School, for their assistance with the *A. rhizogenes* transformation experiments and Dr. David P. Puthoff of MPPL, ARS for the pictures of the infested hairy root cultures.

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