

# Morphological Characteristics of Sugarbeets and Their Relationship to the Expression of Resistance to the Sugarbeet Root Maggot

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## INTRODUCTION

The sugarbeet root maggot, *Tetanops myopaeformis* (Roder), is one of the most serious insect pests affecting sugarbeets in the western United States and Canada (12). Theurer et al. (9) estimate about 38% of the U. S. Sugarbeet acreage is subject to damage with an average loss of about 2% of the yield, or 530,000 tons.

Recently Theurer et al. (9) reported moderate to high levels of resistance to the sugarbeet root maggot (SBRM) in several beet breeding populations. The purpose of this study was to determine some of the morphological characteristics of resistant and susceptible beets and their relationship to the expression of resistance and their effect on the biology of the SBRM. Knowledge of these characters will help in selecting for resistance and provide a better understanding of the mechanisms involved in resistance.

## METHODS AND MATERIALS

### External Morphological Studies

Test plants were two stable inbreds: L29, designated resistant, and L89, designated susceptible, based on previous field and laboratory studies; and several entries selected in a recurrent breeding program specifically showing low and high damage to the SBRM. Flats were seeded and seedlings were transplanted after emergence to clay pots (15.2 cm diam. x 16 cm deep) or paper pots (10.2 cm x 15 cm deep) containing a soil mix of 49% vermiculite 40% washed mortar sand, 20% peat, and 300 g Morgro® 6-10-4 fertilizer/cement mixer load.

To test the effect of plant age on resistance and

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morphology, four entries were planted on four dates one week apart. After transplanting and establishment of the youngest seedlings each plant was infested with about 25 eggs placed near the plant base just under the soil surface. Plants from each planting date were in a different growth stage at infestation as measured by true leaf number (Table 1). Entries were completely randomized in pairs (infested next to uninfested) on greenhouse benches and replicated 10 times. Greenhouse plants were grown under 16-hr photophase maintained with high intensity metal halide lamps and a temperature regime of about 27°C daylight and 16°C dark.

Two months after infestation the soil was sifted, larvae were recovered, measured, and weighed, and the damage to the plant assessed (2). Larvae were stored at 5°C for four months to break diapause and adult emergence determined. The following plant characters were measured: leaf area, taproot length, fibrous root growth, root penetrometer readings, and plant dry weight. Leaf area was measured on a LI-COR® area meter (Lambda Instrument Co). Fibrous root growth was determined on a visual scale of 1 = very slight to 8 = very extensive. Relative epidermal strength or resistance was determined with a hand-held Chatillion® penetrometer (John Chatillion and Sons Mfg.).

#### Internal Morphological Studies

To study possible internal morphological differences between resistant and susceptible beets and their reaction to SBRM feeding or artificial damage, root samples from several tests were prepared for microscopic examination. Samples were taken from the plant age study, from a field test under heavy natural infestation, and from several greenhouse studies in which roots were artificially damaged. Considerable variation was found in tissue composition in both the plant age and field test, probably due to the difference in degree of damage and uncontrolled environmental conditions. Therefore, we artificially damaged roots under a controlled environment in the greenhouse. Plants in the 4- to 6-leaf stage were artificially

damaged by removing a section from the side of the taproot about 2 mm wide, 5 mm long, and 1 mm deep. Damaged roots were replanted and allowed to heal for 1 to 4 weeks. Samples from all tests were fixed in Karnovsky's fixative (5% gluteraldehyde, 4% paraformaldehyde, pH 6.5 at 5°C), dehydrated by passing through a graded series of ethyl and tertiary butyl alcohol, and imbedded in hard Paraplast®. Serial sections were cut at 12µm with a rotary microtome and affixed to slides. Slides were stained, mounted in synthetic resin, covered, and dried (1,6).

Slides were examined with a compound light microscope and the percentage tissue composition determined by the point-counting stereological method of Weibel et al. (10). Tissue types determined were as follows: Periderm, cortex, cambium, central vascular core (Stele), vascular tissue, and the formation of wound periderm. Four slides with at least four sections each were examined and integrated for replicate means. Tests were replicated from 6 to 15 times.

#### RESULTS AND DISCUSSION

##### External Morphology and Maggot Development

Few significant differences were found among plant entries for larval survival, size, plant damage, or adult emergence on any of the four planting dates. The data are summarized by averaging over planting dates and plant entries in Table 1.

Plant entries separated as expected with resistant entries having significantly fewer larvae and significantly lower damage ratings. Although adult emergence from larvae developing on resistant entries was about 16% less than on susceptible entries, the difference was not significant. The number of larvae increased with plant age as indicated by a rank correlation between planting dates and entry means which was inversely correlated and highly significant ( $r = -0.62$ ,  $P < .01$ ,  $n = 16$ ). Both the length and weight of larvae also showed significant increases on the older plants. Damage ratings, however, remained about the same or showed a slight decrease with age. Lower

Table 1. Effects of plant age and resistance in sugarbeets on development of SBRM larvae.

Plant entry	No. leaves	Larvae			Damage rating	% adult emerg.
		No.	Length mm	Weight mg		
Planting dates combined						
40G3+a (R)		3.5a	7.2	16.4	0.8a	55
L29 (R)		4.7a	6.6	14.7	1.0ab	50
L89 (S)		6.7b	6.9	14.8	1.4bc	68
40H2 (S)		5.8b	6.7	14.3	1.8c	68
		*	ns	ns	**	ns
Plant entries combined						
Planting date						
1	10+	7.0	8.2c <sup>1/</sup>	21.6b	1.2	71
2	6-8	6.2	7.3bc	17.7b	1.4	59
3	4-6	3.7	5.5a	9.9a	1.0	-
4	2-4	4.0	6.4ab	11.0a	1.5	56
		ns	**	**	ns	ns

<sup>1/</sup> Means not followed by the same letter are significantly different  $P < .05$  when compared by Duncan's multiple range test. \*\* = significant at  $P < .01$ , \* = significant at  $P < .05$ , ns = nonsignificant.

damage would be somewhat expected as larger plants have more tissue available for feeding spread over a wider area which would reduce crowding effects and apparent damage. The data show no clearcut differences in resistance due to plant age.

Resistant and susceptible entries showed no consistency in reaction within planting dates. Therefore, the response of external plant characters to egg infestation are summarized by entry averaged over planting dates in Figure 1.

Leaf area was somewhat variable, although differences among entries were not significant (Figure 1). Infestation caused a decrease in all entries with 40G3+a suffering the least reduction and 40H2 suffering the largest and only significant reduction. The decrease in 40H2 was essentially to the level of uninfested plants in the other entries. The difference in leaf area between entries among the uninfested plant is probably an indication of a basic difference in photosynthate partitioning between leaves and roots.

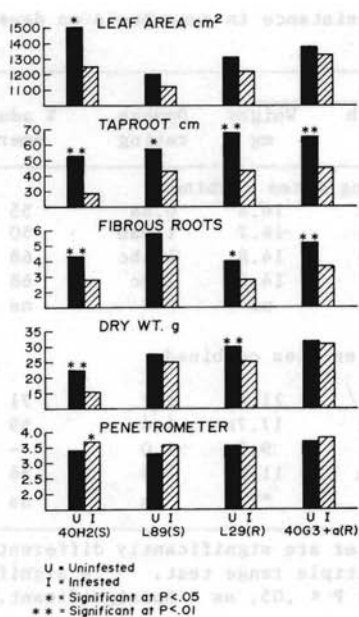


Figure 1. Comparison of resistant and susceptible sugarbeets and changes caused by infestation with sugarbeet root maggot eggs.

Some significant differences were found in tap root and fibrous root growth (Figure 1). Resistant entries had the longest taproots in the uninfested plants, while the susceptible entry L89 had the largest amount of fibrous root growth. Infestation (in 7 of 8 comparisons) significantly reduced both taproot and fibrous root growth. In both cases, 40H2 had the largest reductions but L89, the other susceptible entry, had the least. The resistant entries had longer taproots to start with (uninfested plants) and L89 suffered the least reductions. Therefore, the effect of maggot feeding was probably less severe on these three entries than on 40H2.

Significant differences were found among entries for dry weight (Figure 1). In the infested plants, 40H2 weighed significantly less, while 40G3+a had the heaviest plants. Infestation reduced dry weight but was not consistent for resistant or susceptible entries. In addition, the relationship between plant weight and larval numbers was not significantly correlated as might be expected. The resistant entry 40G3+a, which averaged the

least number of larvae (Table 1), had the highest dry weight in each planting date and for planting dates combined.

Penetrometer readings were variable and were not significantly different among entries (Figure 1). Infestation caused little change in the resistant entries; however, both susceptible entries had a 9% increase in readings. Possibly, penetrometer readings would be worthy of further study.

#### Internal Morphology

Entries in the age study showed few consistent differences in internal morphological characteristics for any particular planting date. The data, therefore, are illustrated averaging over planting dates and plant entries in Figures 2 and 3. The resistant entry L29 had significantly more periderm in the uninfested plants and although L29 showed a significant decrease due to infestation, 40G3+a actually had an increase (Figure 3). Percentage

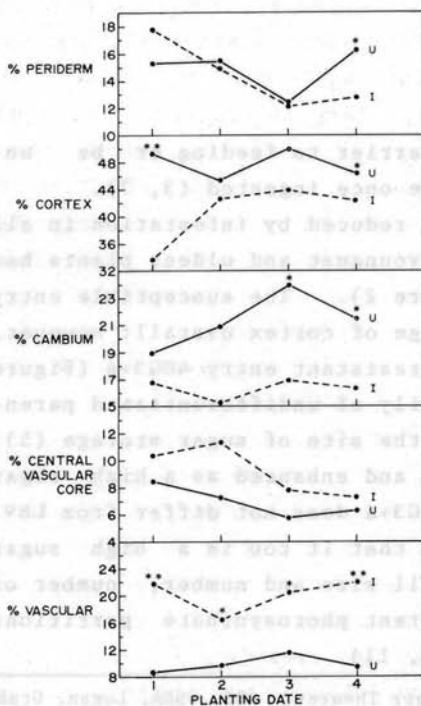


Figure 2. Percent tissue composition of resistant and susceptible beets from the plant age study with planting dates combined and infested with eggs.

periderm was significantly reduced by infestation in the youngest plants with no difference in the intermediate-aged plants and an increase in the oldest plants (Figure 2). Periderm is a highly lignified and suberized material

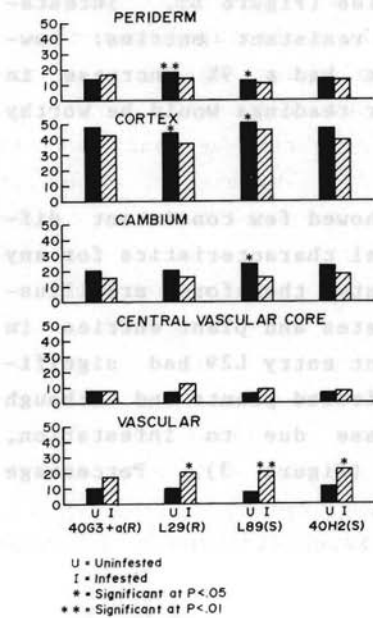


Figure 3. Percent tissue composition of resistant and susceptible beets from the plant age study with entries combined and infested with eggs.

which may form a physical barrier to feeding or be unpalatable and/or indigestible once ingested (3, 7).

The amount of cortex was reduced by infestation in all planting dates but only the youngest and oldest plants had significant reductions (Figure 2). The susceptible entry L89 had the highest percentage of cortex overall; however, it did not differ from the resistant entry 40G3+a (Figure 3). Cortex consists primarily of undifferentiated parenchyma cells and in beets is the site of sugar storage (5). L89 was originally selected and enhanced as a high sugar content beet<sup>1</sup>. Because 40G3+a does not differ from L89, this could be an indication that it too is a high sugar content beet. However, cell size and number, number of vascular rings and the inherent photosynthate partitions also effect sugar content (8, 11).

<sup>1</sup>/ Personal communication with Clair Theurer, ARS, USDA, Logan, Utah.

Sugarbeet roots have an anomalous type of secondary growth in which a series of supernumerary cambia arise outside the normal vascular core and produce several increments of vascular tissue and storage parenchyma cells (5). Because of this unusual growth, the percentage cambial tissue was determined. The percentage cambium was reduced by infestation in all planting dates, significantly in the two youngest plantings (Figure 2). Each of the four entries also showed a reduction but only L89 had a significant reduction (Figure 3). With each reduction in cambium, there was a corresponding reduction in cortex and a corresponding increase in vascular tissue. A decrease in cortex might be expected but an increase in vascular tissue is hard to explain. The change might indicate a difference in partitioning of resources from one of general root growth and storage to an increase in vascular tissue. An increase in vascular tissue would "help" the plant to overcome the loss of water absorbing surface and fibrous roots caused by maggot feeding.

No significant differences were found among planting dates for vascular tissue in the central core (Figure 2). However, significant differences were found among entries with planting dates combined for vascular rings outside the central core (Figure 3). In each case the infested entries had a higher percentage vascular tissue than the non-infested plants. As already indicated, infestation caused an increase in both types of vascular tissue. The increases were not significant for planting dates or entries for the central core but they were significant in 7 of 8 comparisons for the vascular rings outside the central core (Figure 2 & 3).

The wound healing response to infestation was somewhat variable and although no significant differences were found among entries, the resistant entries (48%) had a much higher percentage of plants forming wound periderm than the susceptible entries (28%). Wound periderm formation may not by itself be an indicator of resistance. Wound periderm may be related to overall plant vigor and



the higher percentage of periderm found in the resistant entries. However, wound cork is highly lignified and suberized and may form a barrier to feeding on an old site once formed. In addition, closing of a wound would prevent loss of root contents and possibly help prevent other soil insects and wound entry organisms from attacking the plant.

#### Field Test

Damage in the field test was extremely high with most of the roots covered with feeding scars (Table 2). Many Table 2. Percent tissue composition and damage repair of resistant and susceptible beets grown under field conditions.

Entry	Periderm	Cortex	Cambium	Central vascular		Wound periderm	$\bar{x}$ damage rating
				core	Vascular		
40F6 (R)	12b <sup>1/</sup>	44a	16a	15b	14	38	2.71a
L29 (R)	13b	42a	15a	14b	16	50	3.00a
L89 (S)	6a	48b	16a	13b	16	20	4.63b
40F11 (S)	14b	41a	21b	6a	19	23	4.50b
F	**	**	**	**	ns	ns	*

<sup>1/</sup> Numbers not followed by the same letter are significantly different P < .05 as determined by a Duncan's multiple range test. \* = significant at P < .05, \*\* = significant at P < .01, ns = nonsignificant.

of the beets were stunted and misshapen and in very poor condition. The high amount of damage may have confounded the overall results. However, significant differences were found among entry means for percentage of periderm, cortex, cambium, central vascular core, and damage ratings. The differences between resistant and susceptible entries were not consistent with the exception of damage ratings. The largest differences were between the two susceptible entries, which differed significantly from one another in 4 cases. The wound periderm data may not be reliable since we had no way of determining the age of the wounded areas studied or if they were still being fed upon. Overall, the resistant beets averaged almost twice the percentage of wound periderm formation when compared to the susceptible entries. Most of the values obtained in this test fit well with the results of the age study.

Artificial Damage

Tissue composition showed considerable variation in those beets fed on by larvae in both the age and field study. Most of the variation is probably due to a difference in the degree of damage and uncontrolled environmental conditions. Therefore, we artificially damaged roots to the same degree under a controlled environment in the greenhouse. After the roots were damaged they were replanted and allowed to heal for 1, 2, or 4 weeks.

No differences were found in percent tissue composition between the three healing periods with the exception of wound healing. The data, except wound periderm, are summarized over the three healing periods in Table 3.

Table 3. Percentage tissue composition of resistant and susceptible beets artificially damaged.

Entry	Periderm	Cortex	Cambium	Central vascular core	Vascular
40J20 (R)	19	48	18	8	10
L29 (R)	17	49	15	9	10
25A2 (Parent)	17	48	17	9	10
L89 (S)	16	50	20	6	8
40HS (S)	16	49	18	7	10
F	ns	ns	ns	ns	ns

Little variation was found between entries for any of the tissues measured and none of the differences were significant. Damage to the roots was very slight and they are probably best compared to the uninfested plants from the plant age study even though the age study beets were considerably older when they were evaluated. The differences between the two tests are probably within the normal range of variation.

The wound healing response did show differences between plants allowed 1 week to heal and those allowed 2 or 4 weeks; the data are summarized in Table 4. With only one week to heal, none of the roots had actually formed a wound periderm. Wound periderm takes up to 7 days to form closing layers, 10 days to form phellogen (wound or cork cambium), and up to 14 days for formation of the wound

Table 4. Percentage of plants forming wound periderm in response to artificial damage.

	% forming wound periderm <sup>1/</sup>			
	1	2	4	$\bar{x}$
40H2 (S)	25	34 a <sup>2/</sup>	23 a	27 a
L89 (S)	40	56 abc	59 b	52 ab
25A2 Parent) <sup>3/</sup>	36	79 bc	-	58 ab
L29 (R)	54	92 c	88 c	78 b
40J20 (R)	60	89 c	97 c	82 b
	ns	*	**	*

<sup>1/</sup> 1 = one week to heal after damage, 2 = two weeks to heal after damage, and 4 = four weeks to heal after damage.

<sup>2/</sup> Numbers not followed by the same letter are significantly different at  $P < .05$  as determined by a Duncan's multiple range test. \* = significant at  $P < .05$ , \*\* = significant at  $P < .01$ , ns = nonsignificant.

<sup>3/</sup> Original broadbase parent from which the high damage selection 40H2 and the low damage selection 40J20 were first selected.

cork layers (4). Plants which had formed closing layers or a rudimentary phellogen were counted as having formed a wound periderm. At one week of healing no significant differences were found among entries; however, as in the previous tests, the resistant entries had a considerably higher percentage of plants forming wound periderm. In addition, the broadbase parent (25A2) was intermediate to the divergent high (40H2) and low damage selections (40J20).

With two and four weeks to heal, significant differences were found among entries. The resistant entries had the highest percentage of plants forming wound periderm and the parent was again intermediate between high and low damage selections. Also a considerable overall increase in wound healing was found when compared with one week of healing. No increase was found in the percentage of plants forming wound periderm between two and four weeks of healing.

Entry means for the three healing periods also differed significantly with resistant entries having the highest percentage of wound periderm.

Wound healing appeared to show some real differences between resistant and susceptible entries and appeared to

be influenced at least in part by plant age, the degree of damage, and the conditions under which the plants were tested.

#### CONCLUSIONS

Resistant beets allowed fewer SBRM larvae to develop and sustained less damage than susceptible beets. However, resistance is apparently not clearly related to plant age. Therefore, no clearcut advantage can be gained by using different aged plants to test for resistance.

Leaf area, taproot length, fibrous root growth, and plant dry weight all decreased due to SBRM infestation. The resistant entries tended to suffer less reduction so the effect of maggot feeding was less severe. Penetrometer readings remained the same in resistant entries and increased by 9% in the susceptible entries. Possibly, penetrometer readings are worth further study.

Comparisons of plant tissue composition of entries in the age, field, and artificial damage tests were somewhat variable but probably all within the normal range of variation. Overall, the resistant entries had a higher percentage of periderm. Periderm is a highly lignified and suberized tissue which may form a barrier to feeding or be unpalatable or indigestible. The susceptible entry L89 had the highest percentage of cortex, the site of sugar storage; however, it was followed closely by 40G3+a, a low damage selection. The percentage cambium was highest in the susceptible entries and appeared to be related to the amount of cortex and vascular tissue. Vascular tissue, both the central core and vascular rings, tended to be higher in the resistant entries. The amount of vascular tissue also increased due to infestation in all entries. Increased vascular tissue might indicate a change in the partitioning of resources from general plant growth and root storage to replace the loss of water absorbing surfaces caused by maggot feeding.

The resistant entries in all tests had a higher percentage of plants forming wound periderm. Some variability between tests appeared to be influenced at least

in part by plant age, degree of damage, and the conditions under which they were allowed to heal and grow. Wound periderm in itself may not be an indication of resistance and may be related to the higher amount of periderm in the resistant entries or overall plant vigor. However, formation of a wound periderm prevents loss of root contents and helps prevent attack by other insects and wound entry microorganisms.

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## INTRODUCTION

It is well known that the yield and quality of sugarbeet roots are affected by the cold temperature climates of the northern sugarbeet growing regions such as Michigan. Improvement of this crop could be facilitated through more efficient overwintering methods of seed production. Currently, sugarbeet seedlings are removed from the field in the fall and their roots, with crowns intact, are packed in crates and stored in a cold room (4°C). The following spring, these roots with their laterally induced buds are replanted in soil to promote flowering, cross pollination, and seed production. Although this process is effective, it reduces the amount of sugarbeet breeding field research that can be accomplished in the northern temperate zones.

Cold hardy temperate zone crop plants are able to withstand winter temperatures of -30°C or less, but in the spring and summer months, they are susceptible to cold and can be easily killed at temperatures near 0°C (1). Cold hardiness of these species is dependent on their genetically controlled acclimation to diverse freezing temperatures and their ability to express this trait.

Freezing ambient temperature appears to be the most important environmental parameter for inducing cold hardiness in cereals (2,3). Low, above-freezing temperatures impart cold hardiness in the fall as well as those plants acclimate as temperatures gradually fall below 10°C

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