THE ANATOMY OF SWEET POTATO PERIDERM AND ITS RELATIONSHIP TO WIREWORM, *DIABROTICA, SYSTENA* RESISTANCE [1,2]

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Abstract: Periderm thickness was positively correlated with field control of insects belonging to the wireworm, *Diabrotica* and *Systena* complex. The amount of recoverable compounds extracted from the periderm tissue was related to periderm thickness, but not correlated with levels of resistance found in the field.

Key Words: *Ipomoea batatas, Conoderus, Diabrotica* and *Systena* species, phellem, phellogen, phelloderm, root injury.

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Sweet potatoes [*Ipomoea batatas* (L.) Lam.], can be severely damaged by the wireworm, *Diabrotica, Systena* complex (WDS). This complex consists of seven species of beetles of which two are wireworms, (*Conoderus falli Lane, C. vespertinus* Fabricius), two are *Diabrotica,* [*D. balteata LeConte, D. undecimpunctata howardi* (Barber)], and three are *Systena* (*S. blanda* Mersheimer, *S. elongata* Fabricius, and *S. frontalis* Fabricius). The larvae of all these beetles cause root injury that cannot be easily differentiated at harvest time. The damage is usually characterized by shallow feeding holes, but when larvae of this complex penetrate into the vascular cambium, the holes can be considerably deepened by subsequent root growth. Control has been erratic or ineffective because of the nonpersistent nature of currently recommended pesticides for the WDS complex. Researchers have developed sweet potato lines and cultivars highly resistant to these pests offering an alternative to insecticides (Hamilton et al. 1985; Jones et al. 1983, 1984). Previous research indicated that an unidentified factor in the periderm of some sweet potatoes was responsible for resistance to soil insects (Cuthbert and Davis 1971). The periderm consists of three different types of tissue, the phellem, phellogen, and the phelloderm (Esau 1958, 1960). The purpose of this research was to study the relationships of periderm anatomy and chemistry to observed differences in reactions of the various genotypes to feeding by the WDS complex under field conditions.

MATERIALS AND METHODS

Tests were conducted in 1982 and 1984 (Test I and II). All plants were grown using commercial practices. Individual rows (61 m) contained plants from a single

1 COLEOPTERA: Elateridae, Chrysomelidae, respectively.

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entry (breeding lines or cultivars). Plants were spaced 30 cm apart within rows, and 1 m between rows. Six to ten roots were randomly sampled for each entry from 12 - 16 freshly dug hills. The samples were washed gently with tap water to remove soil, then air dried. Because of the volume of work in tissue preparation and microscopic analyses, three roots from the field samples for each entry per date were chosen for study. The roots were weighed and their diameters recorded.

Percent field control of the WDS complex in resistant and susceptible lines and cultivars was based on the formula:

$$\frac{b - a}{b} \times 100$$

where a = percent root damage in the test entry and b = percent root damage in the susceptible check (SC 1149-19). Average percent field control was calculated from 1975 - 1984 from 1 - 31 replicated trials per genotype. In each trial, plots contained 10 plants spaced as described above and replicated four times. All marketable roots were evaluated for WDS damage. Entries were considered resistant when field control of the WDS complex exceeded 70%.

In 1982 (Test I), 15 sweet potato breeding lines and four cultivars which differed in resistance to the WDS complex were planted on 6 June (Table 1).

Table 1. Resistance (percent field control of wireworm, *Diabrotica, Systena*) of 19 sweet potato entries and mean thickness of periderm and phellem tissue for several growth stages, Test I.

		Thickness† (μm)	
Entry	% control*	Periderm	Phellem
W-184	84.1	248.3	195.8
W-151	82.7	254.1	211.8
W-80-42	82.3	120.6	87.6
W-13	79.1	176.7	132.2
W-175	73.0	115.0	90.2
Regal	72.4	117.1	86.1
Resisto	72.3	141.2	108.2
W-177	58.5	211.9	83.4
W-51-19	48.6	122.1	92.0
MD-715	47.4	173.7	140.2
NC-835	42.8	123.3	96.8
NC-727	40.9	139.5	101.2
V5-58	38.0	135.6	105.5
V5-113	30.9	163.7	129.6
Jewel	26.1	122.6	92.4
Centennial	26.1	144.5	113.6
V2-394	10.4	162.9	122.0
V4-292	0.0	164.4	129.0
SC 1149-19	0.0	92.9	69.6
LSD (0.05)		21.0	18.3

* Average % field control was calculated for 1975 - 1984 from 1 - 31 replicated trials per genotype. Formula: plant entry (% root damaged) = a; susceptible (SC 1149-19) check injury (% root damaged) = b; b-a/b × 100 = % control.

† Four growth stages combined after stabilization (65, 88, 107, and 125 d after planting).

Roots were sampled for periderm analyses at five growth stages (44, 65, 88, 107, and 125 d after planting) (Table 2). Samples averaged 11.0 mm diameter and 12 g at early enlargement stage 44 d after planting and increased in size and weight until harvest (67 mm diameter and 327 g).

Table 2. Mean periderm and phellem thickness for 19 and 16 entries (Test I and II, respectively) influenced by root development.

Days after planting	$\bar{X}$ root diameter (mm)	$\bar{X}$ root weight (g)	Periderm Thickness (μm)	Periderm Cell layers	Periderm Cell thickness (μm)	Phellem Thickness (μm)	Phellem Cell layers	Phellem Cell thickness (μm)
			Test I					
44	11.9	12.1	78.6	3.4	22.7	74.6	3.2	23.6
65	37.0	67.1	138.2	5.4	25.4	105.5	3.7	28.6
88	47.9	148.5	158.6	6.1	25.9	121.9	4.1	29.6
107	66.1	344.1	150.1	6.3	23.5	114.0	4.1	27.1
125	67.9	327.5	145.4	5.8	25.1	119.1	4.3	27.4
LSD (0.05)	3.6	17.7	12.4	0.5	2.9	9.8	0.3	3.7
			Test II					
63	27.0	37.7	159.9	5.9	27.3	114.0	3.6	32.2
135	61.0	301.2	189.2	6.7	28.3	143.0	4.7	30.5
LSD (0.05)	2.2	26.6	11.0	0.4	NS*	13.4	0.3	NS*

* NS = nonsignificant.

In 1984 (Test II), 11 sweet potato breeding lines and five cultivars were planted on 30 May (Table 3). For periderm measurements, roots were sampled at two growth stages at early enlargement (63 d after planting when roots averaged 27 mm diameter, 37.7 g) and at harvest (135 d when roots averaged 61 mm diameter, 301.2 g) (Table 2). Harvests were made at 104 and 150 d after planting for periderm dry weight and extractable compound studies.

All histological analyses of periderm tissues were made on fresh root samples (5 mm^3) frozen directly onto the microtome platform using CO_2 gas. Sections (24 μm thick) were stained with Safranin (5% in 70% ethanol) for 3 - 5 min and gently floated onto slides (using a fine brush) and covered with cover slips. Each slide contained five to seven sections of fresh root and no less than five slides represented a plant entry (three roots/entry, per growth stage). Microscopic (100 ×) examinations were made of three tissues: phellem (cork), phellogen (cork cambium), phelloderm (inner tissue). Fifteen observations of cell sizes and tissue layers were recorded for each sample per plant entry in Test I and 30 observations for Test II.

Dry weight per unit area of sweet potato periderm was determined by sampling (from Test II plants) four uniform sections per root (415.5 mm^2) from five randomly dug fresh roots (20 samples) for each entry. At 104 and 150 d after planting periderm tissue for dry weight and analyses was carefully scraped from fresh roots. Periderm tissue is easily separated from the underlying cortical tissue. Also, microscopic examinations of cortical tissue, after scraping, revealed a marginal number of periderm (phellogen and phelloderm) cells left. The samples were placed in individual stainless planchettes, oven-dried at 50°C overnight and weighed.

Table 3. Resistance (percent field control of wireworm, *Diabrotica, Systena*) of 16 sweet entries and mean thickness of periderm, phellem, dry weight, and recoverable compounds, Test II.

				Periderm			
				Dry wt.		Recoverable compounds	
		Thickness (μm)†		mg · cm^{-2}		mg · g^{-1}DW¶	mg · cm^{-2}#
Plant entry	% control*	Periderm	Phellem	104‡	150§		
Sumor	96.7	151.0	102.4	1.15	1.98	359.3	0.70
L80-15	85.6	163.1	122.8	1.20	2.28	336.7	0.78
W-184	84.1	298.0	238.4	2.25	3.77	328.3	1.23
W-151	82.7	224.0	175.5	1.37	1.93	341.8	0.66
W-13	79.1	238.8	185.9	1.28	2.51	276.5	0.69
W-175	73.0	145.3	107.8	1.19	1.96	379.7	0.74
Regal	72.4	168.0	121.5	1.11	1.96	446.3	0.87
Resisto	72.3	168.4	123.5	1.37	2.86	286.7	0.83
MD-708	54.6	164.4	118.2	1.20	2.05	293.2	0.59
W-51-19	48.6	156.8	120.9	1.34	1.72	322.7	0.59
NC-727	40.9	167.6	109.8	1.37	2.42	300.0	0.74
V5-58	38.0	152.1	114.2	1.05	2.85	246.8	0.71
V5-113	30.9	192.1	144.6	1.33	2.18	324.7	0.71
Jewel	26.1	137.7	84.7	1.01	2.06	323.0	0.67
Centennial	26.1	160.9	114.8	1.03	2.20	393.8	0.86
SC 1149-19	0.0	104.4	75.9	0.85	1.83	317.0	0.59
LSD (0.05)		30.9	38.1	0.73	0.16	27.4	0.09

* Average percent field control was calculated for 1975 - 1984 from 1 - 31 replicated trials per genotype. Formula: plant entry, injury (% root damaged) = a; susceptible (SC 1149-19) check injury (% root damaged) = b; b-a/b × 100 = % control.

† Mean of two growth stages (63 and 135-d-old roots).

‡ Average dry weight of periderm for 104-d-old plants.

§ Average dry weight of periderm for 150-d-old plants.

¶ Average extractable compounds mg · g^{-1} dry weight (DW).

Average extractable compounds mg · cm^{-2} (mg · g^{-1} × mg · cm^{-2}/1000).

Recoverable compounds were extracted from 200 mg of dried periderm from five randomly dug fresh roots of each entry (Test II, 150 d after planting). Sequential extracts of the periderm were done with solvents (50% and 80% methanol, 100% ethanol, respectively), centrifuged and then combined. The combined extracts were condensed and transferred to pre-weighed planchettes for drying and weighing. The extracted tissues were also weighed. Weights of the recoverable compounds were determined for the periderm sample and then expressed as total soluble compounds per unit area of periderm for each entry.

Data in all tables were subjected to analyses of variance and mean separations were determined by LSD at the $P = 0.05$ (*) level. Correlation coefficients were assessed at the $P = 0.01$ (**) and $P = 0.05$ (*) levels.

RESULTS AND DISCUSSION

Generally, as the roots enlarged they reached a stage of growth where periderm thickness stablized (Test I). This stability occurred about 65 d after planting when roots were 30 - 40 mm in diameter (Table 2). Mean cell thickness after stabilization was 25.4 μm for periderm and 28.6 for phellem tissues. Phellogen and phelloderm tissues were consistently one to three cell layers thick throughout the season. The phellem averaged 3.88 $\pm$ 0.34 cell layers over all root developmental stages. Thickness of periderm and phellem varied between entries (Table 1). Entries with the thickest periderm and phellem tissues were W-151 and W-184. The thinnest periderm and phellem tissues were found in SC 1149-19. Significant correlations between periderm, phellem, number of cell layers and cell thickness were observed for all stages of root growth. Resistance (percent field control) was associated with phellem and periderm thickness early in root development, prior to the 65-d stability period, and as roots developed the correlation disappeared (Table 4).

Roots, in Test II, from 63-d-old plants had thinner periderm than those from older plants (Table 2). Mean cell thickness for 63-d-old roots was 27.3 μm and 32.2 μm for periderm and phellem tissue, respectively. In 135-d-old roots correlations existed (0.05 level) between percent field control for periderm and phellem thickness, however, in 63-d-old roots correlations existed between the 0.1 and 0.05 level for periderm tissues (Table 4). Periderm thickness of entries varied. Entries with thick periderm and phellem tissue were W-184, W-13, and W-151 (Table 3). The thinnest periderm and phellem tissues were observed in SC 1149-19. In roots from both sampling periods significant correlations occurred between periderm tissue (total thickness, cell layers, and cell thickness), dry weight and recoverable compounds and were significant in almost all cases. Neither recoverable compounds nor periderm dry weight (per unit area) were correlated with percent field control (Table 4) for roots sampled at 104 and 150 d after planting.

In general, the thickness of the periderm was associated with increases in the number of cell layers of the phellem tissue (Table 2).

Periderm measurements of entries common to both tests (beginning at stabilization) indicate strong environmental effects on periderm thickness (Test I and II averaged 151.7 μm, 178 μm, respectively) (Tables 1 and 3).

A thick periderm provides a degree of resistance during early stages of root development which appears to be a physical mechanism. This should be of value in the protection of roots against *Diabrotica* spp. because they normally feed on

Table 4. Correlation between percent field control of the wireworm, *Diabrotica, Systena* complex and skin thickness of sweet potato roots sampled at different stages of growth, Test I and II.

Days after planting	Periderm			Phellem			Dry† weight (mg)	Recoverable‡ compounds (mg)	
	Thickness (μm)	Cell layers	Cell thickness (μm)	Thickness (μm)	Cell layers	Cell thickness (μm)		mg · g^{-1}	mg · cm^{-2}
				Test I					
44	0.520*¶	0.296	0.668**	0.508*	0.132	0.630**			
65	0.509*	0.258	0.696**	0.561*	0.285	0.642**			
88	0.291	0.155	0.425	0.310	0.220	0.180			
107	0.219	0.134	0.276	0.227	0.133	0.313			
125	0.289	0.187	0.115	0.142	0.105	0.397			
				Test II					
63	0.483	0.355	0.491	0.416	0.318	0.471	–	–	–
104	–	–	–	–	–	–	0.473	–	–
135	0.526*	0.365	0.606*	0.539*	0.418*	0.565*	–	–	–
150	–	–	–	–	–	–	0.245	0.213	0.399

† Dry weight of periderm based on an area of 415.5 mm^2 for each plant entry.
‡ Weights of recoverable compounds determined for periderm samples (mg · g^{-1} dry weight and mg · cm^{-2}) for each plant entry.
¶ Correlation significant at the P = 0.05 (*) and 0.01 (**) level.

roots early in the season (Cuthbert 1967; Schalk and Jones 1982). This mechanism may continue to provide resistance at all growth stages in types like W-184 where the periderm is relatively thick at stabilization. In this situation the degree of resistance would be useful against the wireworm and *Systena* sp. which attack roots throughout or in the later part of the season (Cuthbert 1967). The physical barrier of the periderm is not the only mechanism of resistance to the WDS complex, as highly resistant cultivars (Regal, Resisto, and Sumor) have periderm thickness no different than the susceptible cultivar Centennial (Table 1 and 3). In this study we found that total soluble recoverable compounds were not correlated with resistance found in the field. However, feeding experiments, using the same genotypes that were intact or had their periderm removed showed differential responses among cultivars (Schalk et al. 1986). It is therefore speculated that chemical composition of the periderm, rather than total extractable compounds, plays a role in resistance to feeding.

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