Pre- and Postharvest Changes in Sweetpotato Root Surface Chemicals Modulating Insect Resistance

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Abstract. Levels of major root surface components for two sweetpotato [*Ipomoea batatas* (L.) Lam.] cultivars that differ in susceptibility to the sweetpotato weevil [*Cylas formicarius elegantulus* (Summers)] were determined. Analyses were made 30 days before harvest, at harvest, after curing, and after 2 months of storage during two seasons. Significant variation in the amounts of individual components, especially boehmeryl acetate, which is known to be an ovipositional stimulant for the weevil, was found before and after harvest, with season, and between cultivars. These results suggest that variation in field susceptibility of cultivars displaying moderate levels of resistance may be due in part to seasonal variation in the level of ovipositional stimulants.

Worldwide, the sweetpotato weevil (*Cylas* sp.) is the single most important insect pest of the sweetpotato. Due to its feeding habit, the weevil is difficult to control with insecticides. As a consequence, the development of weevil-resistant sweetpotato cultivars is seen as an essential component in the integrated management of the insect and has been a major objective of plant breeders for almost 50 years (Martin and Jones, 1986).

Relatively low levels of resistance to *Cylas formicarius elegantulus* (Cockerham and Deen, 1947; Jones et al., 1980; Mullen et al., 1980, 1981, 1982, 1985; Waddill and

Conover, 1978) have been identified in several lines of sweetpotato. However, despite a concerted effort to breed resistant cultivars, the results thus far have not been overly promising. Selected lines have failed to consistently display the same level of resistance over time and location (Cockerham and Deen, 1947; Mullen et al., 1980, 1982; Talekar, 1982; Waddill and Conover, 1978). In some instances, lines that have displayed significant levels of resistance for three consecutive years were susceptible the following year (N.S. Talekar, personal communication). Thus, breeding for resistance has yet to have a major impact on weevil control (Talekar, 1982).

Recent results of allelochemical control of resistance by our research group have shown that certain surface chemicals appear to be critical in conferring high levels of susceptibility to the weevil (Wilson et al., 1988). Boehmerol and boehmeryl acetate were identified as major components on the sur-

face of storage roots of cultivars that were susceptible to the sweetpotato weevil (Son et al., 1990b). The latter compound, a pentacyclic triterpene that contains an acetate moiety, acts as an ovipositional stimulant for the sweetpotato weevil (Wilson et al., 1989). The concentration of boehmeryl acetate correlates with differences in weevil susceptibility among cultivars (Son et al., 1990a).

An essential prerequisite in selection for or against specific allelochemicals involved in insect resistance is understanding the factors that determine their concentration. To test whether distinct changes in surface components occur during development and storage and between seasons, we monitored the surface chemistry of sweetpotato storage roots using two cultivars; one exhibiting high and one low levels of boehmeryl acetate.

'Centennial' and 'Resisto' sweetpotatoes were grown at the Univ. of Georgia horticulture farm in 1987 and 1988 using standard procedures (Barber et al., 1986). Previous tests had established that 'Centennial' is highly susceptible to the weevil, while 'Resisto' is moderately resistant (Nottingham et al., 1987). The storage roots of the two cultivars were sampled on similar dates in 1987 and 1988: 25 Sept. (=30 days before harvesting); 25 Oct. (1 day after harvesting); 1 Nov. (after curing for 7 days); 1 Dec. (after 2 months of storage). The roots used for analysis were U.S. no. 1 grade, having surface areas of 120 to 150 cm² and no surface damage. The roots were cured after being harvested and then were stored under recommended conditions (i.e., 13C, 85% relative humidity; Kushman and Wright, 1969) until analysis. At the individual sampling dates, three replications (three to five roots each) of each cultivar were tested.

The surface chemicals on the storage roots were extracted using methylene chloride and ultrasonication for 9 rein, as previously described (Son et al., 1990a). Analysis for surface triterpenes was by capillary gas chromatography after derivatization (Son et al., 1991). Extractions were made using glass-distilled methylene chloride (Burdick and

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Table 1. Effects of pre- and postharvest sampling date, season, and cultivar on the level of major methylene chloride extractable components from sweetpotato storage root periderm.

	Sampling date ¹	Unknown-1		Boehmerol		Unknown-2		Unknown-3		Boehmeryl acetate	
Cultivar		1987	1988	1987	1988	1987	1988	1987	1988	1987	1988
Concentration ($\mu\text{g}\cdot\text{cm}^{-2}$)											
Centennial (MS) ^y	1	0.64 ab ^x	0.25 a [*]	1.77 ab	1.07 a ^{NS}	2.42 ab	0.57 a ^{**}	0.95 a	0.50 a [*]	15.17 a	3.78 ab ^{**}
	2	0.85 a	0.31 a ^{NS}	1.82 a	1.13 a [*]	2.98 a	1.13 a ^{NS}	0.87 a	0.70 a ^{NS}	15.61 a	4.52 a ^{**}
	3	0.43 ab	0.40 a ^{NS}	1.07 b	0.91 a ^{NS}	1.35 b	1.25 a ^{NS}	0.50 b	0.68 a ^{NS}	7.25 b	3.19 b ^{**}
	4	0.39 b	0.07 b [*]	1.43 ab	0.92 a ^{NS}	1.22 b	0.31 a [*]	0.43 b	0.21 b ^{NS}	7.90 b	3.08 b ^{**}
Total mean		0.58	0.26	1.52	1.01	1.99	0.82	0.69	0.52	11.48	3.64
Resisto (MR) ^y	1	1.22 a	0.57 a ^{**}	T ^w	T	3.47 a	3.23 a ^{NS}	5.35 a	1.10 c [*]	1.41 a	0.28 a [*]
	2	0.86 b	0.25 bc [*]	T	T	2.60 ab	0.33 b ^{**}	5.21 a	1.70 bc [*]	1.54 a	T a ^{**}
	3	0.57 b	0.39 ab ^{NS}	T	T	1.81 b	0.51 b ^{**}	4.73 a	2.22 ab ^{**}	1.37 a	T a ^{**}
	4	0.53 b	0.07 c [*]	T	T	1.60 b	0.55 b ^{NS}	4.04 a	2.80 a ^{NS}	1.03 a	T a [*]
Total mean		0.80	0.32	T	T	2.37	1.46	4.83	1.95	1.34	0.07
Significance											
Centennial	Time	*		**		*		**		NS	
	Year	**		**		**		**		**	
Resisto	T $\times$ Y	NS		*		NS		NS		NS	
	Time	**		---		*		NS		**	
	Year	**		---		**		**		**	
	T $\times$ Y	*		---		NS		NS		**	

¹Date sampled: 1 = 30 days before harvest; 2 = one day after harvest; 3 = after curing for 7 days; and 4 = after 2 months' storage.

^yMS and MR; most susceptible and most resistant to the sweetpotato weevil (see Nottingham et al., 1987).

^xMean separation in columns by Duncan's multiple range test, $P = 0.05$.

^wT = Trace; present below $0.01 \mu\text{g}\cdot\text{cm}^{-2}$.

^{NS, **, *} Nonsignificant or significant at $P = 0.05$ or 0.01 , respectively.

Jackson, Richmond, Calif., distilled-in-glass grade); extracts were silylated with trimethylsilylimidazol (TMSI) and Tri Sil-Z (TMSI in dry pyridine, $1.5 \text{ meq}\cdot\text{ml}^{-1}$) (Pierce Chemical, Oakford, Ill.) Hexacosanol (Analabs, North Haven, Conn.) was used as an internal standard.

Of the five major components analyzed from the two cultivars (Table 1), two had previously been identified as pentacyclic triterpenes, i.e., boehmerol and boehmeryl acetate (Son et al., 1990b). The remaining compounds were tentatively identified based on GC/MS data (i.e., molecular ions, base peaks, and fragmentation patterns) as triterpenoids.

Previously (Son et al., 1990a), we established that boehmeryl acetate was the major component in the most susceptible (MS) line and unknown-3 the major component in the most resistant (MR) line to the sweetpotato weevil. Wilson et al. (1989) demonstrated that boehmeryl acetate acts as an ovipositional stimulant to the weevil.

Previously we found boehmeryl acetate to be absent in lines displaying moderate levels of weevil resistance (i.e., 'Resisto', 'Regal') (Son et al., 1990a). In this study, the compound was present, although at a low concentration in 'Resisto' throughout development and storage in 1987, but generally was not found in detectable quantities in 1988. There were significant differences in the amounts of boehmeryl acetate in 'Centennial' during the 2-year test and these levels were 5.3 to 11.1 times higher than for 'Resisto' in 1987. The levels of boehmeryl acetate in 'Centennial' in 1987 were significantly different from those in 1988 at each sampling date, being about three times higher in 1987 than in 1988. The concentration of boehmerol and boehmeryl acetate in 'Centennial' roots stored for 5 months was 0.66

and $3.67 \mu\text{g}\cdot\text{cm}^{-2}$, respectively (Son et al., 1990a). Boehmerol was below the detection limit in 'Resisto' during both years, a result consistent with analyses in 1986 (Son et al., 1990a). Unknown-3, the major component of 'Resisto', did not change in 1987, but was significantly lower in 1988.

Differences in the major periderm components of storage roots of the two cultivars exhibiting different levels of susceptibility to the sweetpotato weevil support the following generalizations: 1) there were no definitive trends in the levels of major periderm components in either cultivar at harvest or during storage, although the level of several components did decline during storage; 2) significant differences in the total amount of surface chemicals were found from year to year; and 3) the concentration of both of the identified components (boehmeryl acetate and boehmerol) monitored varied in a similar manner.

Based on variation in the level of resistance within a cultivar relative to growing season and location, and the absence of a reliable allelochemical source of resistance, Talekar (1987a, 1987b) suggested an allomone (deterrent) is absent because the insect seldom endangers the growth, reproduction, or survival of sweetpotatoes in the field. However, he postulated the possible existence of kairomones (stimulants) evolving through the convolution of the sweetpotato and the weevil because of the weevil's monophagous habit.

The importance of environment in susceptibility is suggested by a report that 'Resisto' and 'Regal' were susceptible to the weevil in southern Florida, a region with high weevil population pressure (Jansson et al., 1987). These cultivars have consistently displayed moderate levels of resistance to the weevil in South Carolina (Jones et al., 1983, 1985;

Mullen et al., 1985). These findings support the hypothesis that differences in oviposition between susceptible and resistant cultivars may be due to different levels of ovipositional stimulants (Son et al., 1990a; Wilson et al., 1989). Ovipositional stimulants appear to vary from year to year and location to location in their absolute concentrations. Recently, Wilson et al. (1990) demonstrated that oviposition by the weevil was stimulated by as little as $0.002 \mu\text{g}\cdot\text{cm}^{-2}$ of purified boehmeryl acetate. Other factors that are not yet identified are no doubt also important (e.g., inhibitors and/or additional stimulants) in oviposition (Ma and Schoonhoven, 1973; Schoni et al., 1987). For example, the identity and biological importance of unknown-3, which is found in significant concentration in 'Resisto' (moderately resistant), have not been established.

Wilson et al. (1989) tested four fractions from the crude periderm extract of 'Centennial' to determine the presence of oviposition stimulants. The methylene chloride-hexane fraction (1:3) that contained > 96% boehmeryl acetate displayed the highest oviposition stimulation, but was not significantly different from the other fractions with the exception of the hexane fraction. Thus, other components in the periderm also appear to be important in oviposition. Acceptance or rejection by the female weevil of a plant as a suitable host for oviposition is probably based on multiple stimuli, which maybe both stimulator and inhibitory.

In conclusion, we have demonstrated significant quantitative variation in the surface chemistry of sweetpotato storage roots due to stage of development and year. These differences may have a significant influence on the level of resistance displayed by a cultivar under differing environmental conditions. As a consequence, multiple forms of resistance

may be essential in new cultivars to obtain consistent levels of resistance in the field.

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