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Resistance to the Weevils *Cylas puncticollis* and *Cylas brunneus* Conferred by Sweetpotato Root Surface Compounds

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ABSTRACT: Seven resistant varieties of sweetpotato were compared with three susceptible varieties in field trials and laboratory bioassays and showed that resistance was an active process rather than an escape mechanism, as field resistant varieties also had reduced root damage and oviposition compared with susceptible varieties in the laboratory. Liquid chromatography−mass spectrometry (LC−MS) of root surface and epidermal extracts showed significant variation in the concentration of hexadecyl, heptadecyl, octadecyl, and quinic acid esters of caffeic and coumaric acid, with higher concentrations correlated with resistance. All compounds were synthesized to enable their positive identification. Octadecyl coumarate and octadecyl caffeate applied to the surface of susceptible varieties in laboratory bioassays reduced feeding and oviposition, as observed on roots of resistant varieties, and therefore are implicated in weevil resistance. Segregating populations from breeding programs can use these compounds to identify trait loci for resistance and enable the development of resistant varieties.

KEYWORDS: *hydroxycinnamic acid esters, resistance, sweetpotato varieties, weevil*

INTRODUCTION

Sweetpotato (*Ipomoea batatas* L.) contributes significantly to food security, nutrition, and income generation,[1,2] ranking as the seventh most important staple crop in the world and the fifth in developing countries after rice, wheat, maize, and cassava.[3] It is particularly important in sub-Saharan Africa. Damage caused by insects is a major constraint in sweetpotato production. Sweetpotato weevils, *Cylas* spp., are the most harmful insect pests of sweetpotato worldwide. They attack both vines and roots, resulting in an unacceptable odor and bitter taste, rendering the roots unfit for human and animal consumption.[4] They are capable of causing crop losses ranging up to 98%.[5,6] Cultural control measures, including rotation,[7] use of clean planting material, removal of volunteer plants and alternative hosts,[5] and rehilling of mounds to prevent or fill cracks, have been recommended for sweetpotato weevil management.[8]

There is already evidence that the variety New Kawogo is resistant to sweetpotato weevils.[9] Hydroxycinnamic acid esters in the latex were found previously to be biologically active against the weevil larvae, reducing feeding damage and oviposition.[10] However, the latex vessels sit below the root surface, which is the first point of contact for adult weevils, so latex chemistry may not directly influence adult weevils' behavior or be toxic to them. In the present study, we investigated whether these hydroxycinnamic acid esters occur on the root surface and in the periderm and if they are biologically active against adult weevils of both *Cylas* species of importance in Africa, namely, *Cylas puncticollis* and *Cylas brunneus*. The root surface is the point of first contact between the weevil and the crop and it is here where these compounds could mediate the interaction between the adult weevil and the plant by reducing feeding and oviposition. Identification of compounds that confer resistance would provide important phenotypic characters that could help identify quantitative trait loci for resistance and facilitate breeding for resistant varieties.

MATERIALS AND METHODS

Field Trials. Seven sweetpotato varieties from improved and local germplasm that have been reported to be resistant in previous field experiments in Uganda[9] were selected: HMA 519, ARA 230, LIR 302, APA 356, ARA 228, RAK 865, and New Kawogo. These were evaluated for resistance to weevils in a field trial in comparison with three susceptible varieties: NASPOT 1, Kakamega, and Tanzania. The trial was carried out at the National Semi-Arid Resources Research Institute (NaSARRI) (1°32′N, 3°27′E) following published protocols.[9] Basal damage was assessed by cutting the first 10−15 cm of two of the three vines per plot. The stems were then dissected with a knife and damage rated on a 1−5 score for both internal and external damage, where 1 = 0−20%, 2 = 21−40%, 3 = 41−60%, 4 = 61−80%, and 5 = 81−100% of stem damaged. The means of the internal and external damage scores for each variety were then used to evaluate the varieties for levels of damage using the stem base indicator.

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Bioassays. *C. puncticollis* and *C. brunneus* were reared in the laboratory on sweetpotato storage roots, in cages held at approximately 28 °C. Two-week-old gravid female adult weevils were introduced to 12-well feeding trays at a rate of two weevils per root core cut with a cork borer (no. 15). The lid was cemented in place with clay to prevent the weevils from escaping from the test arena and perforated to allow air circulation. The weevils were allowed to feed and oviposit for 48 h.

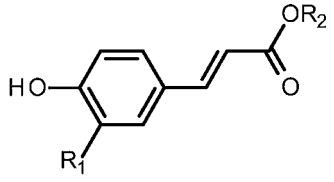
Data Collection and Statistical Analysis of Bioassay Data. The number of feeding punctures and fecal droppings on the root plug were counted for each treatment after 48 h using a similar procedure to that reported earlier.[10] The root periderm was gently removed with a scalpel and the number of eggs deposited was determined using a magnifying glass. Data were analyzed using one-way ANOVA (GenStat 13th ed., Genstat Procedure Library Release PL21.1).

Extraction of Root Surface Compounds. Freshly harvested roots of each of the above 10 varieties were extracted in analytical grade (95%) hexane (Fisher, Loughborough, UK) for analysis of the variation in surface chemistry between different varieties. Prior to the extraction, roots were weighed, and the surface area was estimated by wrapping them in graph paper and cutting away excess paper folds, leaving only paper that was in contact with the surface. The graph paper was then removed and laid flat, and squares remaining on the graph paper were counted to give the surface area of the root sample used. The surface area to weight relationship was plotted to allow root surface areas to be estimated according to their weight based on the measurement of three roots from each of the 10 varieties following procedures described earlier.[9]

Three roots from each variety were extracted individually by complete immersion of the roots in hexane (500 mL) for 1 min. This reduced the extraction of compounds from deeper layers of the root. More polar compounds were extracted by the same procedure but for 24 h in methanol. The crude extracts were evaporated to dryness in vacuo on a rotary evaporator and redissolved in a few mL of methanol. The supernatant was transferred into LC−MS vials, labeled, and stored in the refrigerator prior to LC−MS analysis.

Extraction of Freeze-Dried Plant Samples. Sweetpotato storage roots harvested from the field were washed under tap water. Two transverse root disks were cut from the middle of each root and freeze-dried (True-Ten Industrial Co.) for 72 h. The periderm and epidermal sections of the freeze-dried root disks were separated using the edge of a kitchen knife. The separated portions were powdered using a laboratory blender. Approximately 50 mg of the powdered material of each plant part was weighed using Mettler AT 201 and transferred into Eppendorf tubes. Methanol (1 mL) was added and allowed to extract for 24 h. The crude mixture was spun using a Mini-centrifuge (Costar) at 1300 rpm for 5 min and the supernatant collected for analysis.

Synthesis of Hydroxycinnamic Acid Esters. Compounds **1**−**5** (Figure 1) were prepared in high yield from the corresponding acids by acetylation, esterification, and deacetylation as reported earlier.[10] The hydroxyl group(s) of the three hydroxycinnamic acids were acetylated to give the acetoxy acids, and these were then coupled to one of three different alcohols (hexadecanol, hepatadecanol, and octadecanol) using *N*,*N*′-dicyclohexylcarbodiimide catalyzed by *N*,*N*-dimethylaminopyridine in dichloromethane. The acetoxy groups were then removed with potassium carbonate in methanol to give the esters, which precipitated out on acidification.



		R_1	R_2
1	Hexadecylcaffeic acid	OH	$-(CH_2)_{15}CH_3$
2	Hexadecylcoumaric acid	H	$-(CH_2)_{15}CH_3$
3	Heptadecylcaffeic acid	OH	$-(CH_2)_{16}CH_3$
4	Octadecylcaffeic acid	OH	$-(CH_2)_{17}CH_3$
5	Octadecylcoumaric acid	H	$-(CH_2)_{17}CH_3$

Figure 1. Compounds **1**−**5** identified in sweetpotato roots and associated with resistance to sweetpotato weevils.

Spectroscopic Analysis. Nuclear magnetic resonance (NMR) spectra were acquired in DMSO-d_6 at 30 °C on a JEOL Delta 2 500 MHz instrument. Standard pulse sequences and parameters were used to obtain 1D ^{1}H and 1D ^{13}C spectra. Chemical shift referencing was carried out using the internal solvent resonances at δ_H 2.50 and δ_C 39.5 (calibrated to TMS at 0.00 ppm). Heptadecylcaffeic acid has not previously been recorded in the literature in nature, so we report here the NMR data for this compound for the first time.

Heptadecylcaffeic acid, **3** (off white amorphous powder): PDA-UV (MeOH−H_2O) λ_{max} 325 nm; MS *m*/*z* 181.3, 163.5, 417.2 $[M + H]^+$; ^{1}H NMR δ 0.85 (t, *J* = 7.0, 17-CH_3), 1.23 (m, 3−16-CH_2), 1.61 (td, *J* = 6.7, 2-CH_2), 4.09 (t, *J* = 6.8, 1-CH_2), 6.24 (d, *J* = 15.9, H-7′), 6.76 (d, *J* = 8.0, H-5′), 6.99 (dd, *J* = 8.25, 2.2, H-6′), 7.04 (d, *J* = 1.8, H-2′), 7.46 (d, *J* = 15.9, H-8′); ^{13}C NMR δ 13.9, 22.1, 25.4, 28.7, 28.9, 28.96, 29.04 (9 × CH_2), 31.3, 63.7, 113.9, 114.8, 115.7, 121.3, 125.5, 145.0, 145.6, 148.4, 166.6.

Sample Analysis by Liquid Chromatography−Mass Spectrometry. All extracts applied to HPLC columns were first passed through 0.45 μm nylon Acrodisc filters. Chemical analysis of the filtered samples was carried out using a LC−MS detector (Agilent Technologies, 1200 series) interfaced with a single quad mass spectrometer using an electrospray ionization (ESI) source operating in positive mode under standard conditions and source voltages tuned for optimal transmission of rutin. Extracts were separated with a 150 mm ×4.0 mm i.d. Zorbax Eclipse C18 column with 5 μm particle size (Agilent Technologies, UK) operating under gradient conditions, with A = MeOH, B = H_2O, C = 1% HCO_2H in MeCN; A = 0%, B = 90% at *t* = 0 min; A = 90%, B = 0% at *t* = 20 min; A = 90%, B = 0% at *t* = 30 min; A = 0%, B = 90% at *t* = 31 min. Column temperature was 30 °C and flow rate 0.5 mL/min. Injection volume was 10 μL and data analysis was performed using Chemstation software (Agilent Technologies 1200 series).

Compounds were detected by their photodiode array ultraviolet (PDA-UV) and MS spectra, where fragmentation in ESI-MS(+) was characterized by the molecular ion of each compound accompanied by two fragments representing the two ion forms of the cinnamate moiety (e.g., coumarate and coumaroyl). For example, the molecular ion for hexadecyl-*p*-coumaric acid, **2**, was *m*/*z* 389 $[M + H]^+$, which is in agreement with a molecular weight of 388, with fragments at *m*/*z* 165 (coumaric acid) and *m*/*z* 147 (coumaroyl), consistent with losses of $C_{16}H_{33}$ and $[O-C_{16}H_{33}]^+$, respectively. Compound **1**: UV (LC−PDA) λ_{max} 325 nm; MS *m*/*z* 163.5, 181.3, 403.2 $[M + H]^+$. Compound **2**: UV (LC−PDA) λ_{max} 315 nm; MS *m*/*z* 165.2, 147.3, 389.4 $[M + H]^+$. Compound **3**: UV (LC−PDA) λ_{max} 325 nm; MS *m*/*z* 181.3, 163.5, 417.2 $[M + H]^+$. Compound **4**: UV (LC−PDA) λ_{max} 325 nm. MS *m*/*z* 181.3, 163.2, 433.1 $[M + H]^+$. Compound **5**: UV (LC−PDA) λ_{max} 315 nm; MS *m*/*z* 165.2, 147.3, 417.2 $[M + H]^+$. Compound **6**: UV (LC−PDA) λ_{max} 325 nm; MS *m*/*z* 355.4 $[M + H]^+$.

The integrated peak areas extracted from single ion chromatograms based on $[M + H]^+$ were quantitated against calibration curves of synthetic standards of **1**−**5** as described above and against 5-*O*-caffeoylquinic acid (**6**) (Sigma Aldrich, Dorset, UK) and analyzed by one-way ANOVA with GenStat (13th ed.). Means were generated and mean separation was done using least significant difference (LSD) at 5% level.

Toxicity and Deterrent Effects of Octadecylcaffeic and Octadecylcoumaric Acids to *C. puncticollis* and *C. brunneus*. Both octadecylcaffeic acid and octadecylcoumaric acid were tested in a choice bioassay. Twelve root cores of the susceptible variety NASPOT 1 in a 12-well tray were treated with three different concentrations of the compounds at 0.10, 0.01, and 0.001 mg/mL and the organic (carrier) solvent control (0.0 mg/mL) (three root cores per treatment

Table 1. External and Internal Stem Damage Index and Number of Larvae in Stems of Selected Sweetpotato Germplasm (mean ± SEM) after Artificial Infestation with *C. puncticollis* and *C. brunneus* at NaSARRI, Uganda, $N = 10$[a]

variety	root damage (%)	stem damage index		no. of larvae
		external	internal	
APA356	0.5 ± 0.3 a	1.5 ± 0.1 a	1.3 ± 0.1 a	0.1 ± 0.0 a
ARA228	0.0 ± 0.0 a	1.7 ± 0.1 a	1.5 ± 0.1 a	0.0 ± 0.0 a
ARA230	2.3 ± 0.5 b	2.2 ± 0.3 b	2.2 ± 0.3 b	0.9 ± 0.6 a
HMA519	1.4 ± 0.6	1.7 ± 0.2 a	1.6 ± 0.2 a	0.5 ± 0.2 a
LIR302	0.0 ± 0.0 a	1.6 ± 0.1 a	1.6 ± 0.1 a	0.5 ± 0.0 a
NASPOT 1	1.0 ± 0.4 a	2.8 ± 0.3 b	2.8 ± 0.2 b	3.1 ± 1.5 b
New Kawogo	0.0 ± 0.0 a	1.3 ± 0.2 a	1.2 ± 0.2 a	0.1 ± 0.3 a
RAK865	0.5 ± 0.4	1.4 ± 0.1 a	1.3 ± 0.1 a	0.1 ± 0.0 a
Kakamega	1.6 ± 0.4 ab	1.9 ± 0.1 ab	1.9 ± 0.1 ab	0.5 ± 0.3 a
Tanzania	1.9 ± 0.8 ab	2.5 ± 0.2 b	2.5 ± 0.2 b	2.7 ± 1.4 b
LSD	1.2	0.5	0.5	1.0
P	<0.001	<0.001	<0.001	<0.015

[a]Means followed by the same letters are not significantly different from each other ($P \leq 0.05$ Tukey's test).

or control) representing, when applied as a 100 μL aliquot, 2.0, 0.2, 0.02, and 0 μg/cm^2 on the surface. The carrier solvent used was acetone and was allowed to dry for 3 h prior to experiments. Onto each root core 2 adult (2-weeks-old) gravid females of either *C. puncticollis* or *C. brunneus* were introduced before covering the well plate with the lid. The lid was raised to about 1 cm above the wells to allow for free movement of the weevils between the periderms, using clay into which tiny holes were made to allow air circulation. The experiment was in a completely randomized design. Each experiment setup was replicated five times and the whole experiment repeated three times. The number of feeding holes, eggs laid, and fecal droppings produced in 24 h were recorded as an indication of the deterrent effect of compounds, where insects were allowed to choose between root cores treated with different concentrations of the pure compounds.

RESULTS AND DISCUSSION

Field Trials. Damage on internal and external stem bases of sweetpotato varieties differed significantly between clones (ANOVA, $P \leq 0.05$) (Table 1). Stem damage is an important parameter for determining weevil resistance in sweetpotato,[11] and there was significant ($r = 0.94$, $P \leq 0.004$) correlation between external stem damage and root damage in the field.

No-Choice Feeding and Oviposition Bioassays. The mean number of fecal droppings was significantly lower on the root cores of New Kawogo compared to the number on NASPOT 1, Tanzania, Kakamega, RAK 865, and ARA230 ($P \leq 0.001$), indicating that weevil feeding was reduced on this variety (Table 2). In line with this result, the number of feeding holes was significantly higher on NASPOT 1 root cores compared to all other varieties except RAK 865, Kakamega, and Tanzania, indicating that weevils were willing to feed on these varieties significantly more than others. The number of feeding holes was lowest on New Kawogo and LIR 302 (Table 2). Similarly, the mean number of eggs laid on the root cores of New Kawogo was significantly lower than all other varieties but was highest on NASPOT 1, Kakamega, Tanzania, and RAK 865 (Table 2) ($P \leq 0.0001$). A range of feeding responses by *Diabrotica* species feeding on sweetpotato from different cultivars have been reported,[12] suggesting a quantitative resistance to this insect that could be related to the effect reported here from *Cylas* spp. The fact that both studies presented adults with root surfaces and showed a similar effect supports this. Perhaps the most important result was that even when presented in a no-choice situation *C. puncticollis* lays significantly fewer eggs on the root cores of New Kawogo than on all other cultivars. LIR302 and HMA519, shown to be resistant in the field, also recorded significantly fewer eggs laid than on the most susceptible variety NASPOT 1.

Table 2. Number of Fecal Droppings, Feeding Holes, and Eggs (mean ± SEM) on the Root Plug of Different Varieties in a No-Choice Bioassay[a]

variety	*N*	fecal droppings	feeding holes	eggs laid
APA356	24	13.9 ± 1.8 ab	18.5 ± 1.4 b	10.2 ± 0.7 b
ARA230	24	20.5 ± 1.1 b	18.6 ± 1.0 b	10.7 ± 0.6 b
HMA519	24	14.7 ± 1.0 ab	15.0 ± 1.5 ab	9.1 ± 0.5 ab
LIR302	12	13.0 ± 1.8 ab	14.5 ± 2.8 ab	7.3 ± 1.3 a
NASPOT 1	24	23.6 ± 1.9	26.8 ± 2.0	13.1 ± 1.0 b
New Kawogo	24	11.6 ± 1.2 a	12.8 ± 1.6 a	5.6 ± 0.9 a
RAK865	24	20.4 ± 1.1 b	19.9 ± 5.6 b	12.2 ± 1.2 b
Kakamega	24	20.0 ± 1.2 b	20.6 ± 1.7 b	12.3 ± 1.1 b
Tanzania	24	23.6 ± 2.0 b	20.7 ± 1.9 b	12.2 ± 1.2 b
LSD		3.84	4.55	2.64
P		<0.001	<0.001	<0.001

[a]Means accompanied by same letters are not significantly different from each other ($P \leq 0.05$, Tukey's test).

Choice Feeding and Oviposition Bioassays. Similar levels of feeding and oviposition were recorded in choice bioasssays. Weevils feeding on NASPOT 1 produced significantly more fecal droppings than weevils on any other variety followed by Tanzania but produced the least on New Kawogo ($P \leq 0.0179$), indicating that weevils feed less on New Kawogo (Table 3). The number of feeding holes also differed significantly ($P \leq 0.015$) among varieties, with most on NASPOT 1 and with New Kawogo having the least feeding holes of all the varieties (Table 3). The number of eggs laid on the root cores differed significantly ($P \leq 0.033$) among sweetpotato varieties. The number of eggs laid was highest on NASPOT 1 and lowest on New Kawogo (Table 3). The variability in the mean number of fecal droppings, feeding holes, and eggs laid on the root cores of different varieties indicate that sweetpotato weevils have a preference for particular varieties when presented with a choice. Significantly fewer fecal droppings and feeding holes and lower oviposition

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Table 3. Numbers of Fecal Droppings, Feeding Hole, and Eggs (mean ± SEM) Laid on the Root Cores in a Multiple-Choice Bioassay[a]

variety	fecal droppings	feeding holes	eggs
LIR302	14.0 ± 3.2 a	12.0 ± 2.9 a	7.7 ± 1.0 a
NASPOT 1	26.7 ± 7.0 c	18.0 ± 4.5 c	11.7 ± 2.0 b
New Kawogo	10.0 ± 5.0 a	6.0 ± 4.6 a	6.3 ± 3.0 a
Tanzania	18.0 ± 1.2 b	16.3 ± 1.2 b	11.0 ± 0.3 b
LSD	4.65	2.192	3.1
P	<0.0179	<0.015	<0.033

[a]Means accompanied by the same letters are not significantly different from each other ($P \leq 0.05$, Tukey's test).

on New Kawogo indicated that this variety was less preferred than susceptible varieties. In both the choice and no-choice tests NASPOT 1, Tanzania, ARA230, and Kakamega were the most susceptible to damage and egg laying by weevils. Earlier reports by farmers that New Kawogo was resistant[10] are supported by these laboratory bioassays but also indicate that the mechanism is not based on escape but is an active mechanism, as proposed in earlier work.[11]

Phenolic Compounds on the Root Surface of sweetpotato Obtained from Methanol Extracts. Six hydroxycinnamic acid esters were identified from the sweetpotato root surface by comparison with five standards synthesized as described above and reported earlier[10] and 5-*O*-caffeoylquinic acid (chlorogenic acid) purchased commercially (Sigma Aldrich, Dorest, U.K.). These compounds were identified as hexadecylcaffeic acid, (**1**), hexadecylcoumaric acid (**2**), heptadecylcaffeic acid (**3**), octadecylcaffeic acid (**4**), octadecylcoumaric acid (**5**) (Figure 1), and 5-*O*-caffeoylquinic acid (chlorogenic acid) (**6**). The concentration of compounds varied significantly among the different varieties, but overall, total hydroxycinnamic acid esters were highest in the resistant variety New Kawogo. Individually, there was a higher concentration of hexadecylcaffeic acid on the surface of New Kawogo than all other varieties followed by ARA228 and then LIR302 (Figure 2). These three varieties were shown to be resistant in field and laboratory studies. The concentration of hexadecylcaffeic acid was lowest on the root surface of the susceptible variety Kakamega (Figure 2). Similarly, the concentrations of hexadecylcoumaric acid on the root surface differed significantly ($P < 0.005$) among the different varieties, with higher concentrations present on the surface of New Kawogo followed by another resistant variety ARA228 and was lowest on Kakamega (Figure 2).

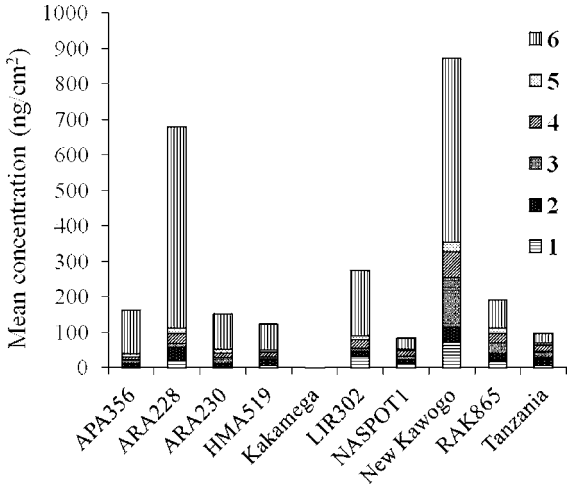

Figure 2. Combined concentration of hydroxycinnamic acid esters on the root surface of selected sweetpotato varieties (ng/cm^2).

The concentration of heptadecylcaffeic acid also differed significantly ($P \leq 0.003$) among the selected sweetpotato varieties. New Kawogo had a higher concentration compared to the other varieties (Figure 2). Similarly, the concentration of octadecylcaffeic acid and octadecylcoumaric acid differed significantly among the varieties ($P \leq 0.011$) with the highest concentration of both recorded on the root surface of New Kawogo while Kakamega recorded the lowest concentration of both compounds (Figure 2). Caffeoylquinic acid on the root surface also differed significantly ($P \leq 0.001$) among the selected sweetpotato varieties. ARA228 had the highest concentration on its root surface, followed by New Kawogo and LIR302. Caffeoylquinic acid on the root surface was lowest on Kakamega (Figure 2).

Hydroxycinnamic Acid Esters in the Periderm Extract of Different Sweetpotato Varieties. Total hydroxycinnamic acid ester concentrations also varied significantly in the root periderm ($p \leq 0.001$) among the sweetpotato varieties. The concentration of hexadecylcaffeic acid was highest in the periderm of New Kawogo compared to other varieties and was lowest in ARA230 and NASPOT 1 (Figure 3). Hexadecylcoumaric acid was lowest in the root periderm, although it differed significantly ($P \leq 0.01$) among the different varieties.

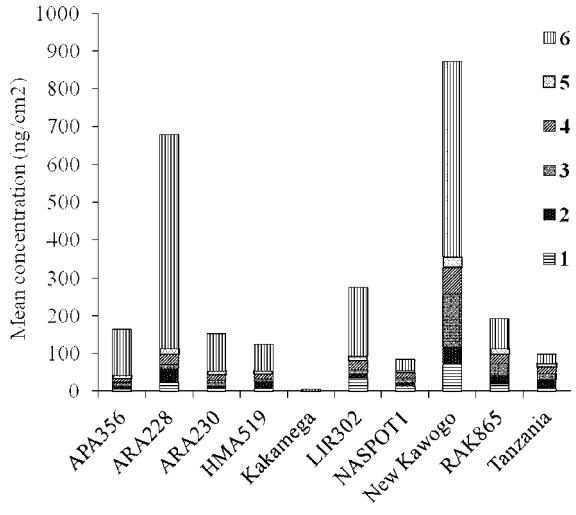

Figure 3. Combined concentration of hydroxycinnamic acid esters in the peridermal tissues of selected sweetpotato varieties (ng/g).

Heptadecylcaffeic acid was the most abundant hydroxycinnamic acid ester found in the root periderm. The concentration of heptadecylcaffeic acid was highest on New Kawogo followed by ARA228 and was lowest in NASPOT 1 and ARA230 (Figure 3) ($P \leq 0.01$). Similarly, octadecylcaffeic acid was highest on New Kawogo followed by ARA228 and was lowest in NASPOT 1 (Figure 3). The concentration of octadecylcoumaric acid in the periderm extracts also differed significantly ($P < 0.005$), with RAK865 having the highest concentration followed by New Kawogo. Octadecylcoumaric acid was lowest in the susceptible variety NASPOT 1 (Figure 3). Finally, New Kawogo had the highest concentration of caffeoylquinic acid

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followed by LIR302 and ARA228. Caffeoylquinic acid was lowest in NASPOT 1 and ARA230 (Figure 3).

Hydroxycinnamic Acid Esters in the Epidermal Extracts of Different Sweetpotato Varieties. Root epidermis from sweetpotato varieties showed significant variation in total hydroxycinnamic acid esters ($P \leq 0.045$). Varieties known to be susceptible in the field were associated with the lowest concentrations of hydroxycinnamic acid esters on the root epidermis. For example, the concentration of hexadecylcaffeic acid was significantly higher in ARA228, New Kawogo, and LIR302 than the susceptible varieties Tanzania, NASPOT 1, Kakamega, and ARA230 (Figure 4). Similarly, the

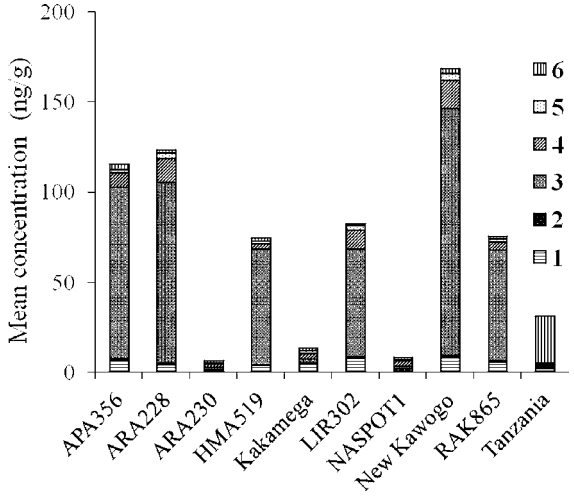

Figure 4. Combined concentration of hydroxycinnamic acid esters in the epidermal tissues of selected sweetpotato varieties (ng/g).

concentration of heptadecylcaffeic acid and octadecylcaffeic acid was significantly higher in New Kawogo, ARA228, and LIR302 than in the susceptible varieties ARA230 and Tanzania (Figure 4).

The caffeoylquinic acid concentration on the root epidermal tissues also differed significantly ($P \leq 0.036$) among the sweetpotato varieties. The amount of caffeoylquinic acid was highest in New Kawogo compared to the other varieties, while Tanzania had the lowest concentration (Figure 4).

Earlier work on sweetpotato latex showed that the concentration and variety of compounds in New Kawogo differed from those in Tanzania.[10] These results supported earlier suggestions that chemical differences in latex could mediate resistance to *Cylas* spp.[14] The present study showed that these chemical differences manifest themselves on the surface of the root and in the peridermal and epidermal tissue and so present a barrier to root colonization by adults. This mechanism was proposed earlier,[14] where susceptible roots were coated with latex and showed reduced oviposition and feeding. The following experiments report the results of bioassays that determine whether the hydroxycinnamic acid esters identified earlier in latex and here on root surfaces accounted for the biological activities reported for latex[14] and above in laboratory feeding trials of root cores.

Biological Activity of Hydroxycinnamic Acid Esters from Sweetpotato on *C. puncticollis* and *C. brunneus* Adults. Surface treatment of sweetpotato with two representative compounds from the root surface of sweetpotato, octadecylcaffeic acid and octadecylcoumaric acid, affected weevil feeding, as indicated by fecal droppings. This did not differ significantly ($F = 2.34$, $P \leq 0.149$) from *C. puncticollis* on root cores treated with octadecylcaffeic acid esters, although this may be due to weevils moving from one location to another during bioassays. Feeding punctures, however, differed significantly ($F = 8.42$, $P \leq 0.05$) when *C. brunneus* was made to feed on the root cores treated with octadecylcaffeic acid esters. The number of feeding punctures and eggs laid were significantly lower on root cores treated with octadecylcaffeic acid and octadecylcoumaric acid than on untreated root cores of the susceptible variety NASPOT 1. Treated periderms had significantly ($P \leq 0.05$) fewer feeding punctures than the controls. Weevil feeding decreased with increased concentration applied on the food (Figure 5).

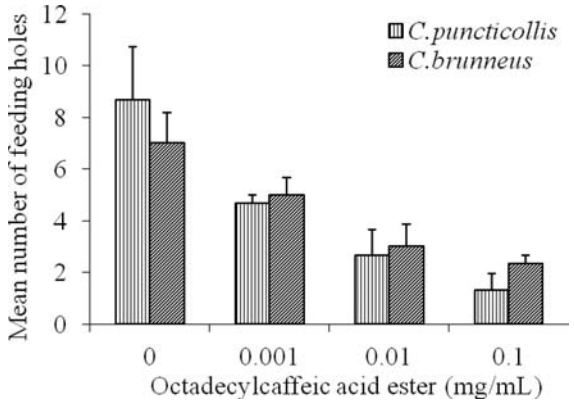

Figure 5. Mean number of *C. puncticollis* and *C. brunneus* feeding holes on root surface treated with octadecylcaffeic acid.

The mean numbers of droppings were lower on treated compared to untreated periderms (Figure 6). The number of

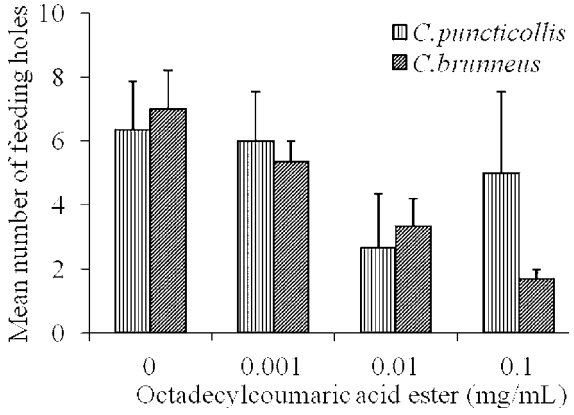

Figure 6. Mean number of *C. puncticollis* and *C. brunneus* feeding holes on root surface treated with octadecylcoumaric acid.

fecal droppings was significantly lower ($F = 28.3$, $P \leq 0.001$) when *C. brunneus* were allowed to feed on root cores treated with octadecylcaffeic acid. Conversely, the number of fecal droppings was not significantly different ($F = 2.45$, $P \leq 0.139$) on similarly treated root cores fed to *C. puncticollis* (Figures 7 and 8). This could indicate that the reduction in feeding seen on treated periderms may not necessarily correspond to numbers of weevil droppings in specific locations, as insects affected by the compounds may drop frass on any root periderms as it moves within the feeding arena.

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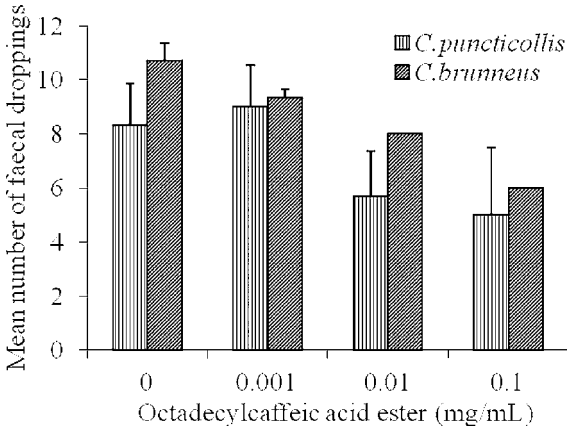

Figure 7. Mean number of *C. puncticollis* and *C. brunneus* droppings on root surface treated with octadecylcaffeic acid.

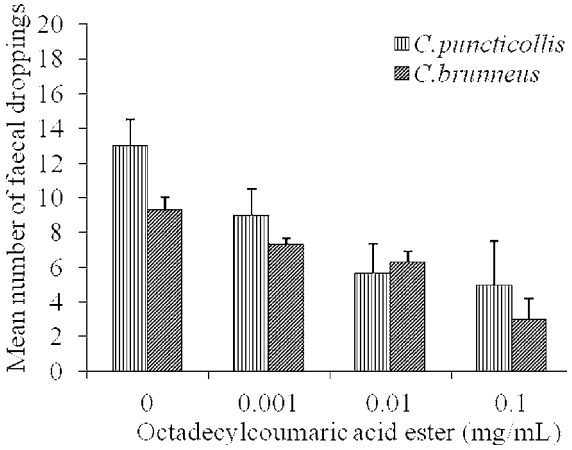

Figure 8. Mean number of *C. puncticollis* and *C. brunneus* droppings on root surface treated with octadecylcoumaric acid.

Root periderms treated with octadecylcaffeic acid esters recorded significantly lower ($F = 5.55$, $P \leq 0.023$; $F = 9.29$, $P \leq 0.006$) oviposition than the controls for *C. puncticollis* and *C. brunneus*, respectively (Figure 9), indicating that the compounds deterred oviposition. No oviposition by either *C. puncticollis* or *C. brunneus* was recorded on periderms treated with octadecylcaffeic acid at high concentration, indicating that the effect of this compound is dose-dependent (Figure 9). So,

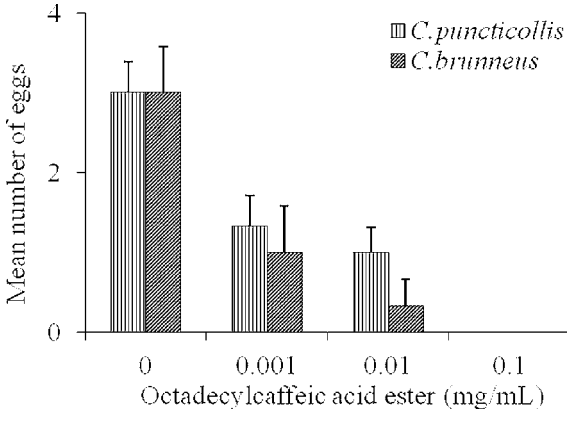

Figure 9. Mean number of *C. puncticollis* and *C. brunneus* eggs on sweetpotato roots treated with octadecylcaffeic acid.

higher concentrations on resistant roots account for lower insect damage. However, some eggs were laid by both *Cylas* species on the periderm treated with octadecylcoumaric acid at a similar concentration ($F = 94.81$, $P \leq 0.038$; $F = 9.29$, $P \leq 0.006$) (Figure 10), suggesting that the octadecylcaffeic ester

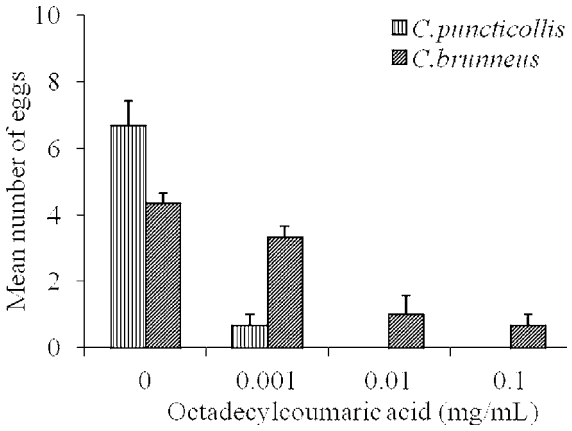

Figure 10. Mean number of *C. puncticollis* and *C. brunneus* eggs on sweetpotato roots treated with octadecylcoumaric acid.

was more biologically active than the octadecylcoumaroyl ester. Feeding punctures and oviposited eggs may be good measures of resistance to *Cylas* weevils and with chemical analysis could help identify quantitative trait loci for resistance.

The effect of octadecylcaffeic acid on oviposition was profound but more potent at higher concentrations. This indicates that differences in the concentration of these compounds at the root surface are likely to account for the reduced oviposition recorded on the resistant varieties New Kawogo, LIR302, and ARA228 and thus mediate resistance to the weevils. It is also notable that the compounds have similar activities but occur as several different structures, potentially reducing the chance of developing tolerance to their effects in the pest insect. The biological significance of this study is that the combined effect of the hydroxycinnamic acid esters can reduce feeding and oviposition by *C. puncticollis* and *C. brunneus* adults in a swee tpotato variety expressing higher concentrations of these compounds on the root surface.

Earlier work reported that hexadecyl, octadecyl, and eicosyl esters of coumaric acid were associated with *Cylas* resistance, but the compounds identified were not tested directly against weevils.[15] Elsewhere, caffeoyl and coumaroyl esters were shown to reduce development of larvae and so possibly contribute to resistance.[10] The present work adds significantly to this earlier work by showing that these hydroxycinnamic acid esters occur on the root surface and, when applied to roots of otherwise susceptible varieties, reduce feeding and egg laying by adults. The combined effects of these compounds on adults and larvae explain the resistance in these varieties. In addition, 5-*O*-caffeoylquinic acid was also associated with lower feeding and egg laying, so this compound may also contribute to the observed effects in field and laboratory bioassays and supports earlier reports that caffeoylquinic acids were associated with defense in sweetpotato.[16]

The varieties investigated in this study varied in their response to sweetpotato weevil under field and laboratory conditions and support earlier reports that herbivorous pests respond to different varieties in ways that strongly indicate varying levels of susceptibility or resistance.[12,13] Although there

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is no sweetpotato variety that has been reported to be completely immune to sweetpotato weevil, this study has shown that the levels of susceptibility vary widely across varieties and sufficiently to substantially reduce field damage under similar levels of infestation. Indeed, under no-choice conditions in the laboratory, the most resistant varieties, New Kawogo and LIR302, showed significantly reduced oviposition and feeding damage, yet in the field, they suffered no root damage at all, possibly owing to reduced pest pressure in field. It is also important to recognize that resistance is best deployed alongside other integrated pest management tools to reduce the chance of resistance breaking down. For example, hilling-up is useful for filling cracks in the soil that are otherwise an important access point for weevils and without them some plants can appear to be resistant in the field.[8]

The significant differences in response of *Cylas* species to sweetpotato were shown to be determined by the presence of higher concentrations of hydroxycinnamic acid esters on the root surface and in epidermal and peridermal tissues, since they occurred at higher concentrations on resistant roots and the application of these compounds to root plugs of susceptible varieties led to reduced oviposition and feeding as recorded on resistant varieties. Thus, the selection of sweetpotato varieties with higher levels of hydroxycinnamic acids, particularly in the surface root tissue and on the surface, could be selected for to optimize the development of resistance to sweetpotato weevils. This will entail screening of potentially hundreds of breeding progeny using freeze-dried samples of roots and analysis by LC−MS as described above.

We propose that resistance to *Cylas* spp. based on chemical analysis and biological assays could be studied in a mapping population of a cross between the resistant variety New Kawogo and a widely grown susceptible variety, such as Beauregard, an important variety in the United States for which detailed quantitative trait loci (QTLs) have been identified already for several traits, including dry-matter, starch, β-carotene and root-knot nematode resistance.[17,18] This would enable the identification of QTLs for weevil resistance and assist breeding for the good agronomic and culinary traits already established as well as weevil resistance.

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Notes

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