Biological Activities and Contents of Scopolin and Scopoletin in Sweetpotato Clones

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Abstract. Periderm and cortex tissues of 14 genetically diverse sweetpotato [Ipomoea batatas (L.) Lam.] clones were grown under low stress conditions and analyzed for their content of scopoletin (7-hydroxy-6-methoxycoumarin) and scopolin (7-glucosylscopoletin). A wide range of concentrations of both compounds was found in both tissues. The two compounds were tested in vitro for their biological activity (concentration–activity relationships) using several bioassays: germination of proso millet (Panicum miliaceum L.) seed; mycelial growth of the sweetpotato fungal pathogens Fusarium oxysporum Schlecht. f. sp. batatas (Wollenw.) Snyd. & Hans, F. solani (Sacc.) Mart., Lasiodiplodia theobromae (Pat.) Grif- fon & Maubl., and Rhizopus stolonifer (Ehr. ex Fr.) Lind; and growth and mortality of diamondback moth [Plutella xylostella (L.)] larvae on artificial diet. The glycoside scopoletin showed little activity, except moderate inhibition of F. oxysporum. The aglycone scopolin inhibited seed germination and larval growth; however, at much higher concentrations than were measured in the tissues. Mycelial growth of the four pathogenic fungi, however, was inhibited at concentrations occurring in some sweetpotato clones.

The phenolic compound scopolin was discovered by Gentner (1928), who observed strong blue fluorescence when germinating seeds or seedlings were observed under ultraviolet light. The compound was identified by Best (1944) as 7-hydroxy-6-methoxy coumarin. Scopolin and its glycoside scopoletin are ubiquitous in the plant kingdom. They occur in low concentrations in the vascular systems of healthy plants (Andreae, 1948; Chou and Waller, 1980; Parker, 1977; Santamour and Riedel, 1994). Scopolin accumulates around necrotic lesions of virus-infected plants and is subsequently transported to the vascular bundles (Best, 1936). Various experiments showed that scopoletin accumulates as a result of injury, and the amount produced is dependent on the type of injury and the amount of compound normally present in unaffected plants (constitutive levels) (Best, 1936). Martin (1957), observing exudation by root out, concluded that favorable culture criteria result in low release rates and that exudation rates of scopoletin provide a sensitive indicator of biotic and abiotic stresses. For example, scopoletin accumulates 10 times higher in tobacco plants infected with Pseudomonas solanacearum than in healthy plants (Se-

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is not clear (Ahl Goy, 1993). The glycoside scopolin—being inactive in most bioassays, including those reported here—may well be the common modality for transport and a nontoxic storage form of scopoletin. This would follow a general pattern for plant-produced toxic compounds, including phenolics (Parkas and Kiraly, 1962). Metabolism and some roles of scopolin in plant disease were discussed by the last-mentioned authors as well as by Goodman et al. (1967). Scopolin also inhibits plant growth and is released by living roots or dead tissues. Its allelopathic potential was shown by several researchers (Datta and Dasmahapatra, 1984; Eberhardt and Martin, 1957; Einhellig et al., 1970; Faye and Duke, 1977; Martin and Rademacher, 1960; Shimomura et al., 1982).

Our objectives were to determine the variability in scopolin and scopolin contents of the periderm and cortex tissues of 14 genetically diverse clones. The effects of scopoletin and scopolin on seed germination, insect larval growth and survival, and mycelial growth of four sweetpotato fungal pathogens were determined quantitatively using in vitro bioassays in order to establish potential biological activities for these defense chemicals.

Materials and Methods

Seed germination assays. The effect of scopolin and scopolin on proso millet seed germination was tested utilizing a previously described experiment (Peterson and Harrison, 1991). Scopolin and scopolin dissolved in methanol were added to filter papers in 10-cm petri dishes. After the methanol was fully evaporated, each dish received 5 mL purified water. The concentrations tested were 150, 300, 500, 700, and 900 µg mL−1 for scopolin and 200, 400, and 800 µg mL−1 for scopoletin. The dishes received 100 seed each and were incubated at 22 ± 0.5 °C for 42 h. The criterion for germination was radicle length equal to or greater than the diameter of the seed. The data for the 10 replicates per experiment were averaged and the four replications of the experiments served as replicates for analysis of variance in a completely random design. Sigmaoidal regression lines best fitted to the data were determined utilizing the Regression Wizard program of SigmaPlot (SPSS, Chicago).

Fungal assay procedures. The effect of scopolin and scopolin on four sweetpotato root pathogenic fungi, Fusarium oxysporum f.sp. batatas (FDB), Fusarium solani (FS), Lasiodiplodia theobromae (LT), and Rhizopus stolonifer (RS), was determined using a previously described bioassay experiment (Harrison et al., 2001). Test concentrations were 25, 50, 100, 200, 400 µg mL−1 potato dextrose agar medium (PDA) (BBL Potato Dextrose Agar, Beckton Dickinson Microbiology Sys-

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tions were pipetted into 35 × 10 mm petri dishes. After cooling, segments of mycelium 1-mm diameter were transferred to the center of the petri dishes, placed in an incubator in the dark at 25 °C and incubated for 60, 60, 40, and 18 h for FOB, FS, LT, and RS, respectively. At the end of the incubation period, the diameter of the fungal colonies was measured with a caliper. The experiments were replicated 5× and all experiments were repeated 4×. The experiments were arranged in a completely random design and treatment means from the four experiments were used as replicates in an analysis of variance (ANOVA) for a completely random design. Sigmoidal regression lines were generated as mentioned before.

Insect rearing. The diamondback moth colony originated from the Geneva 88 Colony, which was started in 1988 and has been maintained continuously since then (Shelton et al., 1991). A starter colony was maintained continuously in our laboratory since late 1997. The insects were reared on a Multiple Species Diet (Southland Products, Lake Village, Ark.), using techniques described by Shelton et al. (1991) and adapted for this study (Jackson and Peterson, 2000).

Insect bioassays. An aliquot of scopolin or scopoletin for each treatment concentration was weighed into a small polystyrene cup (hollow stopper) and dissolved into a minimum amount of purified methanol. The methanol was evaporated, leaving a film of the test chemical on the cup walls. This procedure facilitates dissolution of the chemicals. About 3 mL of warm (52 °C) diet was added to a cup and the material was stirred vigorously for 2 min. After cooling for at least 30 min, the cups were weighed to determine the actual amount of diet in the cup since it was impossible to pipet the diet accurately. The specific weight of the diet was determined gravimetrically 8×. Subsequently, 1.5 mL diet with test chemical was packed into the tip of a 1.5-mL microcentrifuge tube. Air was excluded as much as possible to slow down oxidation of diet components. Each tube received one neonatal larva, taken from a cohort. The tubes were closed with a lid that had a small hole in it to prevent condensation. Incubation was at 25 ± 0.5 °C, photo/scoto periods of 14:10 h, and 50% relative humidity. After 7 d, mortality was determined and live larvae were weighed. Each concentration treatment was replicated 10× and controls (concentration = 0) were replicated 20×. The nominal concentrations for scopoletin were: 625, 1250, 2500, 5000, and 10,000 µg·mL−1; and for scopolin were: 313, 625, 1250, and 2500 µg·mL−1. These numbers were corrected for the deviation of the diet volumes, which were 3.0 mL nominally. Scopecolin experiments were repeated 5×, and scopoletin experiments were repeated 3×.

Effects on mycelial growth of pathogenic fungi. In comparison with the controls, all concentration of scopoletin inhibited Fusarium solani and Rhizopus stolonifer (Fig. 2B), with concentrations of 50 µg·mL−1 and higher inhibited Fusarium solani and Lasiodiplodia theobromae (Fig. 2A–D). The IC50 values extrapolated from regression lines were 190, 100, 160, and 120 µg·mL−1 for F. solani, F. oxysporum, R. stolonifer, and L. theobromae, respectively. Fusarium solani and R. stolonifer were also inhibited by scopoletin (IC50 = 2000 µg·mL−1). Growth of the other three pathogens was not different from the controls at scopoletin concentrations up to 2000 µg·mL−1 (data not presented).

Effects on total live weight and survival of diamondback larvae. Total live weight, expressed as percent control, showed a steep decline at scopoletin concentrations >2500 µg·g−1 diet after 6 d (Fig. 3A). The extrapolated IC50 was 4100 µg·g−1 diet. Survival was negatively correlated with scopoletin concentrations. After 6 of exposure, 50% of the larvae died (LD50) when the concentration of scopoletin was >8300 µg·mL−1 diet (Fig. 3B). Scopolin was bioassayed at concentrations up to 2500 µg·g−1 diet, the highest concentration that remained in solution. This concentration is 25× higher than the highest concentration we found in sweetpotato storage roots. No effects were observed on larval survival or on total live weight after 6 d.

Concentrations of scopoletin and scopolin in periderm and cortex tissues of 14 sweetpotato clones. The concentrations of scopoletin ranged from 0 to 103 µg·g−1 dry weight of periderm and from 5.8 to 35.6 µg·g−1 dry weight of cortex tissue (Table 1). Scopolin concentrations ranged between 50.7 to 773 µg·g−1.
µg·g⁻¹ dry periderm and between 98.9 and 588 µg·g⁻¹ dry cortex tissue. In addition, periderm “densities” (mg·cm⁻²), thickness of the cortex, water content, and contents of scopolin and scopoletin on a fresh weight basis are listed in Table 1.

Discussion

Earlier research showed that insect resistance factors occurred primarily in the periderm and cortex tissues of sweetpotatoes (Peterson and Schalk, 1990; Schalk et al., 1986), Stele tissue which comprises most of the storage root played a minimal role, or in some cases none at all. In preliminary experiments with sweetpotato pathogenic fungi we observed the same pattern (unpublished data). We therefore limited the chemical analyses to periderm and cortex tissues. In addition to concentrations of defense chemicals, the thickness of the tissues in which these chemicals occur may play a role, since for example, insects that can survive on stele tissue would have to “consume” their way through the periderm and cortex. Also, when considering allelopathic suppression of weeds, the total amount of active chemicals released would be the most relevant parameter. For these reasons the thickness and moisture content of the cortex and the density of the periderm were measured (Table 1). The water content of the periderm is difficult to determine because the tissue dries rapidly when separated from the root. However, the thickness expressed as mg·cm⁻² dry periderm tissue (Table 1) allows the calculation of amount of chemical on a root surface area basis. The bioassays employed in the research reported here are similar to those used previously (Harrison et al., 2001; Jackson and Peterson, 2000; Peterson et al., 1998, 1999, 2002) allowing estimation of relative contributions the various chemical components may make in specific defense roles.

The chemical data presented in Table 1 show concentrations which may be expected under low stress conditions. It is not known what levels may be attained under conditions of specific high stresses. These parameters are under current investigation. If, however, constitutive levels reflect inducible ones (Best, 1936; Martin, 1957), Table 1 shows that large differences in inducible concentrations can be expected and breeding potentials may be favorable. For breeding purposes, data for the relative concentrations of defense chemicals are useful and the use of the same clones in our research allows comparisons of relative contributions these chemicals may make. The absence of scopoletin in the periderm of most clones suggest that this compound is synthesized in the cortex.

An additional complication arises when considering the roles of scopolin. The results

![Fig. 1](image1.png)

Fig. 1. Inhibition of prosomillet seed germination by scopoletin and scopolin in a petri dish bioassay. Vertical bars represent standard errors of the means.

![Fig. 2](image2.png)

Fig. 2. (A–D) Growth of sweetpotato root rotting fungi, Fusarium solani, Lasiodiplodia theobromae, and Rhizopus stolonifer, in response to scopoletin and Fusarium oxysporum in response to scopoletin and scopolin in potato dextrose agar medium in a petri dish bioassay. Vertical bars represent standard errors of the means.
Table 1. Content of scopolin and scopoletin in periderm and cortex tissue, density of periderm, and thickness and dry matter content of cortex tissues of 14 sweetpotato clones.

| Clone          | Densitya (mg·cm⁻²) | Scopolin content (µg·g⁻¹ DW) | Scopoletin content (µg·g⁻¹ DW) | Thickness (mm) | DMb (%) | Scopolin (µg·g⁻¹ DW) | Scopoletin (µg·g⁻¹ FW) |
|----------------|---------------------|-------------------------------|--------------------------------|----------------|---------|----------------------|------------------------|
| Beauregard     | 2.01                | 138.0                         | 0.0                            | 3.36           | 13.41   | 103.61               | 13.86                  |
| Carolina Bunch | 0.44                | 73.2                          | 0.0                            | 3.20           | 12.03   | 234.70               | 28.22                  |
| Excel          | 2.76                | 138.0                         | 0.0                            | 3.27           | 14.28   | 302.73               | 43.23                  |
| Jewel          | 2.13                | 82.2                          | 0.0                            | 3.82           | 16.57   | 143.97               | 23.96                  |
| Regal          | 2.08                | 773.0                         | 0.0                            | 3.65           | 32.87   | 98.93                | 32.43                  |
| SC1149-19      | 1.19                | 571.0                         | 0.0                            | 5.56           | 16.69   | 587.75               | 98.18                  |
| SC1149-19      | 1.97                | 50.7                          | 51.9                           | 4.34           | 17.56   | 96.42                | 16.95                  |
| Sulfur         | 1.58                | 93.3                          | 103.0                          | 3.47           | 19.46   | 93.10                | 18.14                  |
| Sumor          | 2.22                | 110.0                         | 0.0                            | 4.45           | 19.36   | 76.73                | 14.86                  |
| Tinian         | 2.59                | 353.0                         | 0.0                            | 2.96           | 18.82   | 446.20               | 97.86                  |
| W 274          | 0.39                | 179.0                         | 0.0                            | 4.68           | 21.48   | 109.05               | 21.19                  |
| TIS 80637      | 1.55                | 334.0                         | 0.0                            | 4.95           | 12.78   | 182.18               | 23.29                  |
| TIS 9101       | 2.48                | 77.6                          | 0.0                            | 4.13           | 23.47   | 157.70               | 37.03                  |
| TIS 70357      | 1.99                | 112.0                         | 80.6                           | 3.38           | 18.40   | 132.57               | 30.52                  |

LSD0.05 for comparing means within a column.

aPeriderm dry weight per cm² of root surface area.
bDry matter.

cLSD0.05 for comparing means within a column.

of bioassays reported here show little if any effect at relatively high concentrations. Snook et al. (1992) showed that glycosilated coumarins, including scopolin, did not inhibit the growth of Phytophthora parasitica var. nicotianae; the aglycones, however, were active. Most phenolics in plants occur in the water-soluble glycosidic or other conjugated form (Harborne, 1977) and are as such not harmful to the tissues in which they occur (Stenlid, 1970). The glycosidic linkage may be broken in the plant upon leaf senescence or prior to excretion (Harborne, 1977) by β-glucosidase activity produced by a fungal agent (Oku, 1959). This may explain our observation that scopolin was active against F. oxysporum.

Scopoletin inhibited proso-millet seed germination only slightly (IC₅₀ = 610 µg·mL⁻¹) as compared to resin glycosides from the periderm (IC₅₀ = 11 µg·mL⁻¹; Peterson and Harrison, 1991). It is not expected that scopolenin and its glycoside, when released from decaying sweetpotato tissue in the field, play significant roles in suppressing surrounding weeds.

Previous research showed that sweetpotatoes contain various chemical components that inhibit mycelial growth of the fungi used in this study (Harrison et al., 2001). The data show that the constitutive levels of scopoleten, on a fresh weight basis, are low (Table 1). However, the combined concentrations of scopolin and scopoletin exceed the IC₅₀ values for scopoletin (Fig 2) in a few clones. Considering that the reported concentrations are constitutive, it is possible that they are important in defense against fungal pathogens if stress induces the accumulation of higher levels.

The concentration–activity plot for total live weight of diamondback larvae exposed to scopoletin shows a strong negative correlation (Fig 3); however, the concentrations needed for effective antibiosis are above 2000 µg·mL⁻¹. It should be noted that sublethal dosages may have chronic negative effects, as was shown for sweetpotato resin glycosides (Jackson and Peterson, 2000).
In conclusion, constitutive levels of scopoletin and scopoletin in some sweetpotato germplasm sources were adequate to interfere in vitro with mycelial growth of F. oxysporum, F. solani, L. theobromiae, and R. stolonifer. Scopoletin inhibited germination of prosomillet seed and displayed antibiosis against diamondback larvae but at concentrations higher than the constitutive levels occurring in the 14 clones analyzed. We propose that the mass concentration and scopoletin concentrations may be useful as quantitative chemical markers for sweetpotato genotypes with disease resistance.

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