Biochemical Systematics and Ecology 41 (2012) 126–129

Contents lists available at SciVerse ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Banana slug antifeedant in the growing cane tips of Himalayan Berry *Rubus armeniacus*

William F. Wood*

Department of Chemistry, Humboldt State University, 1 Harpst St., Arcata, CA 95521, USA

ARTICLE INFO

Article history:
Received 14 October 2011
Accepted 29 December 2011
Available online 16 January 2012

Keywords:
Rubus armeniacus
Rosaceae
Himalayan blackberry
Ariolimax columbianus
Arionidae
Banana slug
2-Heptanol
Methyl salicylate

ABSTRACT

A GC–MS comparison of the volatile organic compounds in extracts from young versus mature leaves from first year canes of Himalayan blackberry *Rubus armeniacus* (Focke) showed young leaves to contain significantly higher concentrations of 2-heptanol and methyl salicylate. Tissue disruption of both types of blackberry leaves showed no increase in the concentration of these compounds. 2-Heptanol had significant antifeedant activity against the banana slug, *Ariolimax columbianus* (Gould), but methyl salicylate did not inhibit banana slug feeding at the concentration present in young leaves.

1. Introduction

The Himalayan blackberry (*Rubus armeniacus,* Focke; Synonyms; *Rubus discolor* Weihe & Nees, *Rubus procerus* P. J. Müll) is native to the Caucasus region in Eurasia (Caplan and Yeakley, 2006). The Himalayan blackberry has become naturalized in the Pacific Northwest from California north into British Columbia and is a serious invasive species in Hawaii, Europe, Australia, New Zealand, and South Africa. This blackberry is perennial, growing canes the first year that bear fruit the following year. First year canes grow vigorously and can reach 10 m in length with a cane base is 2–3 cm in diameter. The mature canes are rigid and covered with large thorns, but the growing cane leaf tips are pliable and appear succulent. Visual examination of these growing tips from Northern California plants showed no obvious herbivory by the indigenous banana slug (*Ariolimax columbianus* Gould) or phytophagous insects. Thus, volatile chemical constituents of the cane tip leaves were investigated and tested for antifeedant activity.

2. Materials and methods

2.1. Collection and extraction

Leaves of *R. armeniacus* were collected in Humboldt County, California (July 2011) and a voucher specimen (HSC 099891) was placed in the Humboldt State University Herbarium. The first 5 cm of the growing leaf tips from actively growing, first year canes were collected from 10 different plants. From each cane previously sampled, a sample of mature leaves was

* Tel.: +1 707 826 3109; fax: +1 707 826 3279.
E-mail address: wfw2@humboldt.edu.

0305-1978/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bse.2011.12.029

collected 1.0 m from the growing tip. Within 30 min of collection, the leaf samples were placed in 10 mL of CH_2Cl_2 and extracted for 5 days at room temp. To investigate if tissue disruption would change the composition of volatiles, a second set of samples were likewise collected, crushed and extracted.

2.2. Chemical analysis

Gas chromatograph-mass spectrometry (GC–MS) analyses were performed on the CH_2Cl_2 extracts in a splitless mode (0.5 min), using a Hewlett–Packard GCD Plus fitted with a 30 m × 0.25 μm cross-linked phenyl methyl silicone capillary column (HP-5MS). The gas chromatograph was programmed so the oven temperature was kept at 40 °C for 4 min, then increased to a final temperature of 325 °C at a rate of 30 °C/min and held at this temperature for 5 min. Impurities in control solvent samples were not reported. Initial identification of 2-heptanol and methyl salicylate was done by comparison to published electron impact – mass spectra (EI-MS) in the National Institute of Standards and Technology (NIST) 1998 computerized mass spectral library, and confirmed by comparison of spectra and retention times of commercially available standards. The concentration (μg/g leaf) for each of these compounds was determined by comparison of GC–MS peak areas of leaf extracts to solutions with known concentrations. The chirality of 2-heptanol was not determined.

2.3. Slug bioassays

In feeding experiments, the growing tip leaves from *R. armeniacus* canes were placed in a container with a single slug for 24 h. When cane tips had not been eaten during this period, a 1.0 cm^2 of iceberg lettuce (*Lactuca sativa*) was placed 1 cm in front of each slug to determine feeding activity. Feeding test results were not recorded if a slug did not eat this lettuce within 3 min. Twenty different adult free ranging slugs (at least 15 cm in length) were used for these tests.

Methyl salicylate and 2-heptanol were tested for banana slug antifeedant activity at the concentration in the growing leaf tips. For testing, a single slug was placed on a clean 20 cm by 20 cm glass plate. After 1 min, a 1.0 cm^2 piece of commercial iceberg lettuce was placed 1 cm in front of the slug's head. Slugs that did not start to eat this lettuce within 1 min were excluded from further testing. Then 1.0 μL CH_2Cl_2 of solution containing 165 μg methyl salicylate, 234 μg of (±)-2-heptanol or a combination of 165 μg methyl salicylate and 234 μg of (±)-2-heptanol was deposited on the surface of a 1.0 cm^2 piece of lettuce. The CH_2Cl_2 was allowed to evaporate and the treated lettuce was placed 1 cm in front of the slug. Antifeedant activity was rated positive if the slug investigated and rejected the sample. To exclude slugs that had ceased to feed, a second piece of untreated lettuce was offered to each. Only if the slug started eating the new untreated lettuce within 1 min were the previous test results recorded. A control experiment using 1.0 μL of CH_2Cl_2 was done to exclude lettuce rejection due to this solvent. Twenty different adult free ranging slugs (at least 15 cm in length) were used for each test so previous exposure to the test chemicals would not bias the results.

3. Results

3.1. Volatiles in young and mature leaves

There were major differences in the volatile components between the young leaves on the growing cane tips and mature leaves of *R. armeniacus*. GC–MS analysis showed the leaves on the cane tips to contain two major volatile compounds, 2-heptanol and methyl salicylate at much higher concentrations. Table 1 shows the difference in concentrations (μg/g leaf) between young and mature leaves. Mature leaves contain 52 μg of 2-heptanol per g of leaf and this increases 4 fold in young leaves to 234 μg/g leaf. Likewise, mature leaves contain 21 μg/g of methyl salicylate and this increases 16 times in young leaves to 165 μg/g leaf. Leaves crushed before extraction showed no significant difference in concentration of these compounds from uncrushed leaves (Table 1). Thus, these compounds do not arise from tissue disruption.

3.2. Slug bioassays

In the 24 h palatability test on young leaves from growing cane tips none of the 20 slugs ate the plant material. Because of the rejection in these trials, slug antifeedant activity was tested at the concentration of 2-heptanol and methyl salicylate found in young leaves (Table 2). At 234 μg 2-heptanol, 15 of 20 slugs rejected the treated lettuce. At 165 μg methyl salicylate, all 20 slugs

Table 1
Comparison of (±)-2-heptanol and methyl salicylate amounts in young and mature leaves.

Leaf type	(±)-2-Heptanol (μg/g leaf)	Methyl salicylate (μg/g leaf)
Young leaves	234 ± 68	165 ± 36
Mature leaves	53 ± 27	21 ± 10
Crushed young leaves	212 ± 62	143 ± 33
Crushed mature leaves	37 ± 10	20 ± 9