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Technical Note

Influence of Plant Material Handling Protocols on Terpenoid Profiles of One-Seed Juniper Saplings

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Abstract

Accurate estimation of one-seed juniper (*Juniperus monosperma* [Engelm.] Sarg.) intake by herbivores often requires harvesting, transporting, and storing plant material that is later used in pen experiments. Such manipulation could alter terpenoid profiles and modify herbivory levels significantly. We used gas chromatography mass spectrometry (GC/MS) to analyze the terpenoid profile of leaves from 10 short (0.5 m ± 0.05, mean ± SE) and 10 tall (1.14 m ± 0.06) one-seed juniper saplings subjected to 3 handling protocols: a) placed on dry ice after clipping and stored after 5 hours at −80°C for 3 weeks (Control); b) kept at ambient temperature for the first 24 hours and then frozen at −80°C for 3 weeks; or c) kept at ambient temperature for the first 24 hours, and then stored at 8°C for 3 weeks. Juniper saplings contained 51 terpenoids, 3 of which were unknown compounds. Fourteen terpenoids accounted for 95% of the total amount of volatiles. The most abundant compound was α-pinene, which accounted for 65% of total terpenoids present. Handling protocols were not associated with detectable differences in total terpenoid content (Means ± SE, Control: 21.68 ± 1.42 $mg \cdot g^{-1}$ dry matter [DM]; Frozen after 24 hours: 19.55 ± 1.08 $mg \cdot g^{-1}$ DM; Refrigerated after 24 hours: 18.80 ± 1.13 $mg \cdot g^{-1}$ DM). However, total terpenoid amount and concentration of a few major compounds tended to decrease with increasing storage temperature. Handling protocols induced detectable variations in a small number of minor terpenoids. We observed large among-plant variation in terpenoid profiles that was not fully explained on the basis of sapling size. This study suggests that the length of storage period of one-seed juniper branches should not exceed 3 weeks and that storage refrigeration temperatures should be kept below 8°C to prevent significant alterations in terpenoid profiles.

Resumen

Estimación precisa del consumo de *Juniperus monosperma* por herbívoros a menudo requiere cosecha, transporte y almacenaje de material de planta que luego es usado en experimentos a corral. Tal manipulación podría alterar el perfil de terpenos y modificar significativamente los niveles de herbivoría. Usamos cromatografía gaseosa junto a espectrometría de masa para analizar el perfil de terpenos de hojas de 20 renuevos de *Juniperus monosperma* pequeños (0.5 m ± 0.05; n = 10) y grandes (1.14 m ± 0.06; n = 10), sujetas a 3 protocolos de manipulación: a) puestas en hielo seco después de cosecha y almacenadas luego de 5 horas a −80°C por 3 semanas (Control); b) mantenidas a temperatura ambiente durante las primeras 24 horas, y luego congeladas a −80°C por 3 semanas; o c) mantenidas a temperatura ambiente durante las primeras 24 horas, y luego conservadas a 8°C por 3 semanas. Los renuevos de *Juniperus* presentaron 51 terpenos, 3 de los cuales fueron desconocidos. Catorce terpenos representaron el 95% de la cantidad total de volátiles. El compuesto más abundante fue α-pinene, representando el 65% del total de terpenos presentes. El contenido total de terpenos no difirió entre los tratamientos de manipulación (Control: 21.68 ± 1.42 $mg \cdot g^{-1}$ DM; Congelado luego de 24 horas: 19.55 ± 1.08 $mg \cdot g^{-1}$ DM; Refrigerado luego de 24 horas: 18.80 ± 1.13 $mg \cdot g^{-1}$ DM). A pesar de que la cantidad de terpenos totales y algunos terpenos mayores tendieron a disminuir al aumentar la temperatura de almacenaje, los protocolos de manipulación solo indujeron variaciones detectables en algunos terpenos menores. Detectamos gran variación en el perfil de terpenos entre plantas que no fue completamente explicado por el tamaño de los renuevos. Este estudio sugiere que el almacenaje de ramas de renuevo de *Juniperus* no debe exceder las 3 semanas y que la temperatura de refrigeración durante almacenaje debe mantenerse por debajo de los 8°C para prevenir alteraciones significativas en el perfil de terpenos.

Key Words: *Juniperus monosperma*, plant volatiles, gas chromatography, storage conditions, plant variation

INTRODUCTION

Terpenoids have been shown to deter herbivory in one-seed (*Juniperus monosperma* [Engelm.] Sarg.) and other juniper species (Riddle et al. 1996; Pritz et al. 1997; Dearing et al. 2000) as well as in a number of native woody invasive plants of western North America (Pfister 1999). This herbivore deterrence is a result of terpenoid toxicity, bitter flavor, and aversive odors, all of which are the result of distinct terpenoid mixtures that occur in specific plant tissues (Langenheim 1994). Terpenoid

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