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Phytochemical Induction by Herbivores Could Affect Quality of Essential Oils from Aromatic Plants

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Plant tissues may show chemical changes following herbivory. In aromatic plants such changes could affect the specific compounds on which commercial exploitation is based. This possibility was analyzed for *Mintosthachys mollis*, a member of the Lamiaceae native to Central Argentina with medicinal and aromatic uses in the region, and two types of insect herbivores: a leaf miner and a gall insect. Analysis of the essential oils of mined/undamaged leaves, as well as leaves from stems with and without galls, revealed changes in concentrations of the two main monoterpenes. A decrease in pulegone concentration was associated with both types of insect damage, whereas menthone increased significantly only in mined leaves. Inducible chemical changes in aromatic and medicinal plants may be common and widespread; their economic implications deserve investigation.

KEYWORDS: Phytochemical induction; plant−insect interactions; aromatic plants; Lamiaceae; leaf miner; gall; terpenoids; *Mintosthachys mollis;* herbivory

INTRODUCTION

Plants have evolved an enormous array of mechanical and chemical defenses against herbivores. Some ("constitutive") defenses are built into plants and so offer them permanent protection, whereas others ("inducible defenses") are produced only in response to tissue damage (*1*).

Plant tissues may show chemical changes following herbivory, and such changes seem to increase plant resistance to subsequent herbivore attack, which suggests they may be active plant defenses (*2*). Their defensive role can be further expanded by attraction of natural enemies of the herbivores (*3*). The substances involved are generically known as allelochemicals or secondary plant metabolites.

Interest in this phytochemical induction or "induced resistance" is based on two lines of reasoning: (1) from a biological perspective it is important that the plant can save energy by mobilizing a latent resistance capacity only when necessary; (2) agronomists may use this knowledge to mobilize resistance in crop plants, thereby reducing their susceptibility to pests (*4*). Cultivars could be selected that would react strongly to herbivore attack when necessary, without bearing the cost of producing allelochemicals constitutively when they may never be needed (*5*). Management plans involving induced resistance through vaccination-like techniques (employing less damaging or easily controlled species or strains of herbivores) could be used to protect plants against herbivorous insects and mites (*6*). Furthermore, ecologists are interested in the possibility of indirect interspecific interactions mediated by induced chemical changes (*7*−*9*).

Chemical changes induced by herbivores could be particularly important from a completely different point of view when aromatic or medicinal plants are considered. In this case, the change per se would matter, particularly if the specific compounds giving economic value to the plant are affected. Such effects might be magnified if they involve a systemic response, affecting parts of the plant other than those specifically damaged by the herbivore, as has been observed (*10*).

Despite their obvious importance, herbivore-induced changes in chemistry of aromatic plants have rarely been addressed (*11*), and we do not know of any study focusing on such changes from the perspective of essential oil quality for production. In the present paper, we analyze the response of *Mintosthachys mollis* (Kunth.) Griseb., a member of the Lamiaceae native to Central Argentina with medicinal and aromatic uses in the region, facing two different types of insect herbivores: the leaf miner *Chromatomyia platensis* (Brethes) (Diptera: Agromyzidae) and a cecydomid gall insect (Diptera: Cecydomyiidae). We have dealt with terpenoids, as the most important compounds of *M. mollis* belong to this group, and given previous records of increased terpenoid production following herbivory in various plant species (e.g., *5, 12, 13*).

MATERIALS AND METHODS

Mined and undamaged leaves, as well as leaves from stems with and without galls, were collected from a population of *Mintosthachys mollis* plants growing spontaneously in a locality within the Chaco

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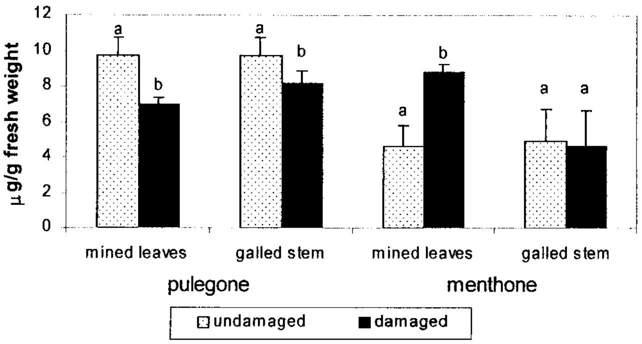

Figure 1. Concentration of pulegone and menthone found in *Minthosthachys mollis* oil from leaves either mined or growing near a gall, and the respective undamaged control leaves. Bars indicate standard deviation. Letters above the bars indicate significant differences within each pair of columns.

Mountain Woodlands of Central Argentina (Paso de la Tigra, Potrero de Garay, Córdoba Province). Seven samples (batches of 7−10 leaves each) from different plants were analyzed for each damage type and the respective undamaged control. Control leaves were from the same plants, and similar in size and age to those that were mined or gall-related. Mined leaves contained a new (inhabited) mine, which was removed for chemical analysis so that only undamaged tissue was analyzed.

The plant material was submitted to hydrodistillation in a micro Clevenger-like apparatus for 2 h and the volatile fraction was collected in hexane. Analyses were carried out with the use of a Perkin-Elmer Q-700 gas chromatograph equipped with a CBP-1 capillary column (30 m × 0.25 mm) and a mass selective detector. Analytical conditions were as follows: injector and detector temperatures, 250 °C and 270 °C, respectively; oven temperature programmed from 60 °C (3 min) to 240 °C at 4°/min; carrier gas, helium at a constant flow of 0.9 mL/min; source, 70 ev. The oil components were identified by a combination of mass spectral and retention time data, which were compared with those of authentic compounds and with those published in Zygadlo and Grosso (*14*). GC analyses were performed with a Shimadzu GC-RIA gas chromatograph fitted with a 30 m × 0.25 mm fused silica capillary column (phase polar) coated with Supelcowax 10. The GC operating conditions were the following: oven temperature programmed from 60 °C (3 min) to 240 °C at 4°/min; injector and detector temperatures, 250 °C; detector, FID; carrier gas, nitrogen at a constant flow of 0.9 mL/min. Identification of the components was performed by comparison of their retention times with those of pure authentic samples.

Comparisons between herbivore-damaged and undamaged leaves were performed using paired-sample *t*-tests.

Table 1. Chemical Composition of *Minthosthachys mollis* Leaf Oil Undamaged Leaves (Mean and Standard Error of 4 replicates)

component	percentage	SE
α-thujene	0.37	0.17
α-pinene	0.47	0.18
camphene	0.85	0.15
sabinene	0.65	0.36
β-pinene	0.34	0.16
β-myrcene	0.35	0.08
α -terpinene	0.73	0.12
δ-3carene	0.12	0.08
ρ-cymene	0.29	0.2
1,8 cineole	0.37	0.17
limonene	9.65	5.48
(z)β ocimenene	0.22	0.05
(e)β ocimenene	0.25	0.03
γ-terpinene	1.57	0.7
terpinolene	0.32	0.28
α-thujone	0.07	0.01
β-thujone	0.09	0.003
linalool	0.29	0.17
menthone	25.12	3.21
isomenthone	13.6	0.21
borneol	0.83	0.03
terpinen-4-ol	0.38	0.17
α-terpineol	1.4	0.49
menthol	0.38	0.22
pulegone	42.8	4.9
neral	0.63	0.06
carvone	0.71	0.33
geranial	0.76	0.14
α-cubebene	0.55	0.25
β-cubebene	0.33	0.13
piperitone	0.06	0.02
menthyl acetate	0.06	0.02
sabinyl acetate	0.06	0.02
piperitenone	0.47	0.43
β-bourbonene	0.06	0.02
β-elemene	0.52	0.16
β-caryophyllene	0.35	0.14
β-gurjunene	0.22	0.18
aromadendrene	0.22	0.18
α-cardinene	0.17	0.13
α-humulene	0.27	0.23
γ-cadinene	0.09	0.002
germacrene-d	0.5	0.26
β-selinene	0.56	0.12
α-muurolene	0.9	0.1
calamenene	0.06	0.02
spathulenol	0.57	0.53
caryophyllene oxide	0.47	0.43
globulol	0.37	0.33
α-santalol	0.17	0.13

RESULTS AND DISCUSSION

Although 50 different compounds were recorded in the essential oils of *M. mollis* (**Table 1**), two monoterpenes accounted for about 70% of their volume: pulegone = cyclohexanone, 5-methyl-2-(1-methylethylidene) and menthone = cyclohexanone, 5-methyl-2-(1-methylethyl), in coincidence with previous reports on this and other *Minthostachys* species (*15, 16*).

Leaves either mined or growing on a gall-bearing stem showed a significant decrease in pulegone concentration ($P = 0.031$ and $P = 0.016$, respectively) (**Figure 1**). Undamaged leaves from both categories had similar concentrations of pulegone, suggesting low variability in the spontaneously growing plants with regard to this compound. No other significant differences were found in relation to gall presence. Instead, mined leaves showed a remarkable increase in menthone concentration ($P = 0.016$), almost doubling the amount observed in nonmined (and either from galled or gall-free stems) leaves (**Figure 1**).

Biosynthetic paths of *M. mollis* could be similar to those observed in other Lamiaceae, where pulegone is a precursor of menthone (*15*, *17*, *18*). In that case, the present results would suggest an accelerated rate of pulegone transformation to menthone as a response to leaf miner activity. Instead, the low pulegone concentration observed in relation to gall presence could not be attributed to the same phenomenon, indicating differential responses from the plant according to the feeding habits of the insects, as proposed by Hartley and Lawton (*19*). Plants can respond in an herbivore-specific fashion even when different insect species with similar feeding habits are involved (*20*).

Minthostachys mollis is cultivated and gathered from the wild in Central Argentina to be used in medicinal infusions and in the preparation of commercial drinks. Like other species of *Minthostachys*, it is increasingly receiving attention as a potential commercial source of peppermint-like flavoring and as a natural biocide (*15*, *21*). All the oil components are found in herbs with the U.S. Food and Drug Administration status of "generally recognized as safe" (GRAS) (*21*). Menthone and pulegone have shown antimicrobial, fungistatic, allelopathic, insecticidal (*15*, *22*−*24*), and even psychoactive (*25*) properties. In many of these aspects, pulegone seems to have higher activity than menthone (*22*−*24*). The observed changes in amounts of these compounds seem likely to have economic implications, since the quality of the essential oil has been altered.

We believe, for all that has been discussed above, that inducible chemical changes in aromatic and medicinal plants deserve further study. These changes, which might be common and widespread, could have important economic consequences, particularly considering that the specific compounds on which commercial exploitation is based can be affected. The possibility of herbivore-induced chemical changes could be higher for aromatic or medicinal plants growing in cultivated systems, given the outbreaks of particular insect populations frequently associated with agroecosystem conditions.

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