

## Leaf volatile compounds and the distribution of ant patrolling in an ant-plant protection mutualism: Preliminary results on *Leonardoxa* (Fabaceae: Caesalpinioideae) and *Petalomyrmex* (Formicidae: Formicinae)

Carine Brouat<sup>a</sup>, Doyle McKey<sup>a</sup>, Jean-Marie Bessière<sup>b</sup>, Laurence Pascal<sup>a</sup>, Martine Hossaert-McKey<sup>a\*</sup>

<sup>a</sup> Centre d'écologie fonctionnelle et évolutive (CEFE), CNRS, 1919, route de Mende, 34293 Montpellier cedex 5, France

<sup>b</sup> Laboratoire de chimie macromoléculaire. UMR 5076, École nationale supérieure de chimie, 8, rue de l'École-Normale, 34296 Montpellier cedex 05, France

Received 24 October 2000; accepted 20 November 2000

**Abstract** — While observations suggest that plant chemicals could be important in maintaining the specificity and permitting the functioning of ant-plant symbioses, they have been little studied. We report here the strongest evidence yet for chemical signalling between ants and plants in a specific ant-plant protection symbiosis. In the mutualism between *Leonardoxa africana* subsp. *africana* and *Petalomyrmex phylax*, ants continuously patrol young leaves, which are vulnerable to attacks by phytophagous insects. We provide experimental evidence for chemical mediation of ant attraction to young leaves in this system. By a comparative analysis of the related non-myrmecophytic tree *L. africana* subsp. *gracilicaulis*, we identify likely candidates for attractant molecules, and suggest they may function not only as signals but also as resources. We also propose hypotheses on the evolutionary origin of these plant volatiles, and of the responses to them by mutualistic ants. © 2000 Éditions scientifiques et médicales Elsevier SAS

*Leonardoxa* sp. / ant-plant mutualism / ant-plant communication / leaf volatiles / ant attraction / methyl salicylate

### 1. INTRODUCTION

Species belonging to more than 100 genera of flowering plants and over forty genera of ants are involved in symbiotic ant-plant protection mutualisms [16]. Many of these symbioses involve only a restricted set of ant and plant partners, and some are species-specific. Ecological and evolutionary interactions in these symbioses are strong, and species involved show numerous specialisations. As in other intense interspecies interactions, many aspects of the functioning of these symbioses must be chemically mediated. However, the chemical ecology of ant-plant symbioses is virtually unexplored [49]. While nutritional aspects of

plant resources provided for ants have been studied in some cases (*Cecropia* [56]; *Acacia* [55]; *Macaranga* [33, 34]), the roles of plant chemicals as signals in these interactions have been very little investigated. Experiments have in some cases shown the role of (unidentified) plant chemicals in the orientation of ants to their host or to specific parts of their hosts [2, 20, 21, 24]. However, apart from some work on the role of plant chemicals in attracting specific ants that disperse and plant the seeds of specialised ant-epiphytes [17, 59], which can be compared with a larger body of work on seed-dispersal mutualisms involving non-symbiotic ants [36], compounds that may be responsible for the attraction of ants have not been identified.

One important aspect of the functioning of ant-plant protection mutualisms is the optimal allocation of ant patrolling activity to appropriate parts of the plant. The

\* Correspondence and reprints (fax: +33 4 67 41 21 38).

E-mail address: hossaert@cefe.cnrs-mop.fr (M. Hossaert-McKey).

plant's colony of symbiotic ants is usually maintained entirely by resources obtained, either directly or indirectly, from the host plant. The optimal level of investment by the plant in its ants, and thus ant colony size, is affected by the balance between costs and benefits to the plant of this investment [25]. From the plant's point of view, the patrolling worker force is thus a resource in short supply. As for plant chemical defences [45], selection should favour optimal allocation of biotic defences. In particular, selection should favour plant traits that cause ants to patrol those parts that are most likely to be attacked by herbivores, and/or those parts where herbivore attack would have the greatest impact on plant fitness, and to ignore or avoid parts less valuable and/or vulnerable. How might such 'allocation' of biotic defences by the plant be accomplished?

In some symbiotic protection mutualisms (e.g. *Cecropia* [22]), concentration of ant activity on young, vulnerable organs is achieved by the same simple mechanism as in facultative non-symbiotic mutualisms mediated by opportunistic ants attracted to extrafloral nectar: production of the resource is restricted to young leaves, floral buds, or other organs that are particularly vulnerable to herbivore attack and/or valuable to the plant (*Catalpa* [61]; *Prunus* [62]; see [39] for other examples).

In some other symbiotic protection mutualisms, however, patrolling activity of the ants appears to be decoupled from distribution of food resources offered by the plant. Some *Cladomyrma* ants, for example, protect leaves of the myrmecophytic tree *Crypteronia griffithii* Clarke ex Hook., even though this plant produces no nectar or food bodies [51]. The myrmecophytic tree *Leonardoxa africana* subsp. *africana* (Leguminosae) provides another example. Foliar nectar of this tree is the principal or sole food source of the plant's host-specific mutualist ant associate, *Petalomyrmex phylax* (Formicinae) [26, 46]. Nectaries are found only at the base of each leaflet of the pinnately compound leaf. They become active, and are visited by ants, only after the leaf matures. Nectar production is concentrated in a brief period around mid-day. In contrast, ant patrolling of leaf surfaces is completely restricted to tender young leaves, the entire surface of the leaf is patrolled, and patrolling is continuous over the 24-h cycle and throughout development of the young leaf [28]. The restriction of patrolling to young leaves is beneficial to the plant, because only un lignified young leaves are subject to herbivore attack; mature leaves possess mechanical and chemical defences and are virtually unattacked by phytophagous

insects. It is important to note that in this specialised symbiotic ant-plant mutualism, insects encountered on young leaves are unimportant as a food resource for the plant's specific associates. In fact, most specialist plant-ants do not eat the insects they kill while patrolling the host plant (see [27, 28] and references therein). Thus, in most systems, ants do not concentrate their activities on vulnerable organs because they obtain more insect food there.

How can this decoupling of ant activity on the plant from the apparent distribution of resources be explained? Two hypotheses appear plausible. First, in symbiotic mutualisms, in which an individual colony is associated with an individual plant for a long time, fitness of the ant colony depends not only on harvesting current resources but also on protecting plant parts that will provide resources in the future. Because fitness of members of the ant colony is tightly linked to survival and growth of the colony's host, rewards are not required to attract ants to vulnerable organs [3, 46]. All that is required is a proximate mechanism, a cue by which ants can recognise young leaves. Alternatively, young leaves may produce some as yet unidentified resource that attracts ants.

We present preliminary results on the role of plant chemicals in explaining the restriction of *Petalomyrmex* patrolling to young leaves of its host. We examine the following questions: (1) Are chemical signals responsible for the differential attraction of *Petalomyrmex* workers to young and mature leaves? (2) If so, what compounds could be important in ant attraction? (3) Do these compounds function solely as proximate signals, or might they be sought by ants as a resource? (4) Are the same compounds present in a closely related, non-myrmecophytic *Leonardoxa*?

## 2. MATERIALS AND METHODS

### 2.1. Study species

*Leonardoxa africana* subsp. *africana* is a myrmecophytic tree found in lowland forests of Cameroon [46–48]. This taxon is specifically associated with one mutualistic ant, *Petalomyrmex phylax*, which strongly protects the plant [29, 46]. The plant possesses swollen and hollow internodes, termed domatia, in which ants nest, and also provides extrafloral nectar to the ants. Extrafloral nectaries are functional only on mature leaves [26, 46]. *Petalomyrmex* workers actively patrol the young leaves of the host plant, continuously over the 24-h cycle and throughout the development of the leaf [28]. Young-leaf patrolling is a characteristic and apparently stereotyped behaviour. Patrolling workers

walk constantly over the leaf surfaces, stopping at intervals for brief periods, then continuing. They aggressively attack insects, such as small caterpillars, placed on the leaf [28]. Patrolling by *Petalomyrmex* results in a marked reduction of herbivory on young leaves [29]. Ants do not patrol the plant's mature leaves, which contain mechanical (lignin) and chemical (tannins) anti-herbivore defences and are virtually unattacked by phytophagous insects, even in the absence of ants [29, 46].

*L. a.* subsp. *gracilicaulis* McKey is a non-myrmecophytic sister taxon of *L. a. africana*. It occurs in southern Cameroon, Equatorial Guinea and Gabon, and is most common on hills (submontane forests and lowland forests transitional to them [26, 48]). *L. a. gracilicaulis* possesses no domatia, and is only involved in loose associations with opportunistic ants attracted to foliar nectaries active only on recently matured leaves [26].

## 2.2. Study sites

Behavioural tests and one set of leaf extractions were carried out in a population of *L. a. africana* near Ebodjé (2°34' N, 9°50' E), in the Campo Forest Reserve, southern province of Cameroon. The area is characterised by an equatorial climate with two rainy seasons and two dry seasons each year. The study in this population was conducted in March 1998. A second set of leaf extracts was prepared in March 1999 from *L. a. gracilicaulis* and *L. a. africana*, in a site near the village of Nkolo (3°15' N, 10°12' E), where the two plants occur sympatrically.

## 2.3. Solvent extracts of leaf compounds

Compounds from young and mature leaves of *L. a. africana*, and from young leaves of *L. a. gracilicaulis* were extracted by washing the leaves in hexane for one half hour. Such rapid extraction yields primarily volatile compounds [7]. About twenty young leaves, collected on twenty different trees, were soaked in 200 mL hexane during half an hour. For comparison with a roughly equivalent surface area of leaf, an extract of mature leaves was performed on ten leaves, collected on ten different trees. Following collection, concentration and transport, the extracts were stored at -4 °C for 3 months in France.

## 2.4. Field attraction tests for ants

In the Ebodjé site, we conducted tests to determine whether leaf extracts attracted ants, on eight adult trees occupied by mature colonies of *P. phylax*. Attraction tests were performed over a 10-d period in March 1998, always during the peak of ant activity on plant surfaces between 10.00 and 14.00 hours [28].

On each tree, we chose four mature leaves, at the same height in the tree to permit simultaneous observations of the four leaves. All leaves chosen had no ants on their surfaces before the beginning of the experiment. On each leaf, we delicately placed a triangle of filter paper. Each filter paper was chosen haphazardly from one of the four following treatments: 1) 50 µL young leaf extract of *L. a. africana*; 2) 50 µL mature leaf extract of *L. a. africana*; 3) 50 µL hexane, to test the effect of the solvent on ants; 4) only filter paper, to test the effect of the presence of the paper on ants.

During 5 min observation, we recorded ants that came into contact with each filter paper. Each ant that came and touched the border of the filter paper with its antennae or walked over the surface of the paper filter was scored as one. The total duration of the observation was 15 min, with fresh extract being added to filter papers every 5 min. On each of the eight experimental trees, we performed two to three attraction tests, each time using a different set of four leaves.

The effects of each treatment on the number of ants attracted were analysed using a General Linear Model procedure (PROC GLM [57], type III SS), experimental tree being considered as a random variable, and treatment as a fixed variable. Duncan's multiple range test was used to make comparisons among treatments.

## 2.5. Identification of compounds present in leaf extracts

The analysis was performed on leaf extracts of *L. a. gracilicaulis* and *L. a. africana* collected in March 1999, following the same protocol as for extracts used in 1998 for the attraction tests. The extracts were first concentrated in a rota-evaporator, at 50 °C water bath, to 5–10 mL. For the analysis of each extract, we used GC and combined GC-MS. The GC analyses were carried out using an Alltech® EC-1 column (length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm) on a Chromopack® CP 9003 (on-column injector, FID detector) with an oven temperature programmed from 50 to 200 °C at 5 °C·min<sup>-1</sup> held for a total run time of 40 min. The injection volume was 2 µL. Data analyses were carried out using Maestro II. Internal standards (20 µL of a solution at 200 ng·L<sup>-1</sup> nonane and dodecane) were added to the extract as a check to confirm calibration of retention times. Comparison with the known amounts of these compounds also allowed a rough estimate of the concentration of compounds found in the extracts. For each different compound that we found in our extracts, we estimated its relative quantity in percentage of the total amount of the compounds present in the extract.

To identify the compounds associated with peaks obtained with the GC analysis, gas-chromatographic-

**Table I.** Attraction of the ant *P. phylax* to four different treatments: hexane extract of young leaves of *L. a. africana*, hexane extract of mature leaves of *L. a. africana*, hexane only, or filter paper only. (range) Minimum and maximum numbers of attracted ants. Mean number of ants attracted for each treatment was compared by a Duncan test. Results for treatments with different letters are significantly different.

Treatment	Number of attracted ants, means $\pm$ SD (range)	Comparison of means ( $P = 0.05$ )
Young leaves	2.05 $\pm$ 2.59 (0–9)	A
Mature leaves	0.27 $\pm$ 0.63 (0–2)	B
Hexane	0.45 $\pm$ 0.81 (0–2)	B
Filter paper only	0.32 $\pm$ 0.94 (0–4)	B

mass spectrometric (GC-MS) analysis was conducted using a Hewlett-Packard 5890 chromatograph equipped with an Optima 5 (analogous to a DB5) capillary column (30 m  $\times$  0.2 mm interior diameter; film thickness 0.1  $\mu$ m). The column was temperature programmed as follows: 50 °C, 3 min isothermal, 3 °C  $\cdot$  min<sup>-1</sup> to 200 °C. The carrier gas was helium (0.6 mL  $\cdot$  min<sup>-1</sup>). Injector temperature was 200 °C. Detection was at 270 °C. The GC apparatus was coupled with a quadripole MS system (type 5971). The ion source was operated in the electron-impact mode (EI, 70 eV). Compounds were identified by their mass spectra and by their relative retention times. Some compounds remained unidentified because of their low concentration in our extracts.

### 3. RESULTS

#### 3.1. Ant attraction by plant compounds

The analysis of variance of numbers of ants attracted by each treatment showed a significant effect of the tree on which attraction tests were performed ( $F_{1,7} = 3.61$ ;  $P = 0.0104$ ). The effect of treatment on the number of attracted ants was also highly significant ( $F_{1,3} = 9.08$ ;  $P = 0.0003$ ). There was no significant treatment  $\times$  tree interaction ( $F_{1,21} = 1.15$ ;  $P = 0.3326$ ) on the number of attracted ants. The young leaf extract attracted more ants than did either the mature leaf extract or control filter papers (table I). This pattern was observed without exception in each of the replications of the tests. Plant compounds present in extracts of the young leaves of *L. a. africana*, either volatile substances or cuticular compounds that act by contact chemostimulation, thus seem to attract workers of the plant's mutualistic ant *P. phylax*.

#### 3.2. Compounds present in extracts of *Leonardoxa* leaves

Numerous compounds, mostly volatile constituents, were present in all three leaf extracts (table II). Sev-

enteen compounds, all common to both subspecies, were present in the two young-leaf extracts. Of these, fifteen were also present in *L. a. africana* mature-leaf extract, which contained no additional compounds not found in young leaves. Peaks 1–4 (table II) were present in the mature-leaf extract, but the amounts relative to those of other compounds could not be estimated. Compounds were much less concentrated in the mature-leaf extract, making separation of these peaks from the solvent peak more difficult than in the young-leaf extracts. The percentage contributions of different compounds in the mature-leaf extract are thus based on the totals of peaks 5–17, leading to an overestimation of their contributions compared to the same peaks in the young-leaf extracts.

Despite these imprecisions, the mature-leaf extract appeared to be dominated by heavier compounds (tri- and pentadecanes). The two young-leaf extracts contained less of these compounds. The young-leaf extracts were rich in 'green-leaf volatiles' [64, 65], corresponding to peaks 1–7. Together these compounds accounted for over 60 % of the compounds extracted from young leaves of the non-myrmecophyte, and almost 50 % of those from young leaves of the myrmecophyte.

Extract of young leaves of the myrmecophyte was very different from the other two extracts in containing methyl salicylate as the most abundant volatile compound (around 30 % of all compounds extracted). This compound could not be detected in extract of mature leaves of the myrmecophyte. In young-leaf extract of the non-myrmecophyte, methyl salicylate was a minor component, accounting for only 0.2 % of all compounds extracted.

### 4. DISCUSSION

Field attraction tests and chemical analyses clearly demonstrated that *P. phylax* was attracted by compounds present in extracts of young leaves of *L. a. africana*. The stereotyped and continuous patrolling of the mutualistic ant *P. phylax* on young and vulnerable parts of its host thus has a potential proximal explanation: amount of compounds present in leaves differs strongly between young and mature leaves, and *P. phylax* responds to these differences. Anatomical and histochemical studies are necessary to identify the source of attractant compounds in young leaves of *Leonardoxa*.

#### 4.1. Differences between extracts of young and mature leaves of the myrmecophyte

What cues could be at the base of the specific response of *P. phylax* workers to chemicals present in

**Table II.** Leaf compounds found in hexane extracts of *L. a. africana* (myrmecophyte) and *L. a. gracilicaulis* (non-myrmecophyte). Percentages are indicated in relation to the total composition of each extract.

Peak	Identification	Retention time	Young leaves <i>L. a. gracilicaulis</i>	Young leaves <i>L. a. africana</i>	Mature leaves <i>L. a. africana</i>
1	3-Hexanone	782	17.5	9.9	*
2	2-Hexanone	790	25.0	11.7	*
3	3-Hexanol	801	8.2	10.2	*
4	2-Hexanol	805	9.8	12.8	*
5	NI	830	2.2	1.0	6.0
6	3-Hexylacetate	855	0.3	1.2	0.5
7	2-Hexylacetate	860	0.2	0.8	0.4
8	Limonene	1 012	5.5	2.9	2.5
9	Linalool	1 087	2.8	0.6	0.3
10	Methyl salicylate	1 165	0.2	28.5	–
11	NI	1 176	0.5	1.8	–
12	NI	1 190	5.0	2.2	3.0
13	NI	1 210	5.1	2.0	3.0
14	Branched tridecane	1 268	4.2	4.3	8.0
15	Branched tetradecane	1 375	4.3	4.3	11.0
16	Branched pentadecane	1 460	2.2	2.0	12.0
17	Pentadecane	1 492	5.0	1.8	31.0

\* These compounds were present in *L. a. africana* mature-leaf extracts, but because these peaks were not well separated from the solvent peak, it was impossible to estimate relative concentrations. Relative concentrations of compounds in this extract are thus based on totals of peaks 5–17.

young leaves of their host? Three apparent differences exist between mature- and young-leaf extracts. First, 'green-leaf volatiles' appear to be more concentrated in young-leaf extract. In some plants, abundance and/or composition of green-leaf volatiles varies with leaf age, and phytophagous insects are capable of responding to such differences [40]. Second, tri-, tetra-, and pentadecanes appear more abundant in mature-leaf extract. Each of these types of compounds is known from floral odours of ten to fourteen genera of plants [38], and they commonly co-occur, as in *Leonardoxa*. Some of these compounds are also known to be constituents of cuticular wax [31], which might explain their greater abundance in mature-leaf extracts. As noted above, however, in the mature-leaf extract percentage of green-leaf volatiles is underestimated and that of tri- and pentadecanes is overestimated. We thus cannot quantify how different young- and mature-leaf extracts are in these respects.

The third difference between young- and mature-leaf extracts of the myrmecophyte is much more striking. While methyl salicylate could not be detected in mature-leaf extract, it was the most abundant compound in extracts of young leaves.

#### 4.2. Methyl salicylate: the most abundant compound in the young leaf extract of *L. a. africana*

Methyl salicylate is commonly emitted by plants, and appears to play diverse roles in interspecific interactions, depending on the plant part concerned. This compound functions in pollinator attraction in many plants [5, 37, 38, 52, 54], and a closely related compound, methyl-6-methyl salicylate, appears to play roles in seed dispersal by some ants [17, 59].

Methyl salicylate is also commonly encountered in leaf odours. A survey (not exhaustive) of recent literature produced records from leaf odours of at least eight genera (in seven families) for methyl salicylate, which usually occurs as part of a diverse mix of leaf volatiles [4, 11–13, 18, 19, 41–43, 53, 60]. Methyl salicylate is usually not dominant in the mixture of volatiles. However, this methyl ester is a major compound in leaf odour of at least one species, *Nicotiana tabacum* cv. Xanthi-nc [60]. The role of methyl salicylate in leaf odours appears defensive. It is sometimes a component of direct defences of the plant. It has a repellent effect on *Aphis fabae* Scop. [32]. Methyl salicylate has antiseptic activity (Duke's Phytochemi-

cal and Ethnobotanical Databases, ARS: <http://www.ars-grin.gov/duke/>), and some related compounds have fungistatic activity [59]. In other cases, its role in direct defences of plants is that of a messenger. Emission of methyl salicylate is involved, for example, in the local induction of resistance against pathogens, via transformation of this compound to salicylic acid [53, 60]. Methyl salicylate may also contribute to indirect plant defences, by attracting natural enemies of phytophagous insects. Methyl salicylate emitted by leaves of *Phaseolus lunatus* L. when the plant is attacked by the spider mite *Tetranychus urticae* Koch. attracts the natural enemy of this herbivore, the predatory mite *Amblyseius potentillae* Garman [18, 19]. Results of two studies (on *Acer* [43] and *Vitis* [42]) investigating effects of damage to leaves on production of volatiles also suggest a defensive role for methyl salicylate. In both studies, damage caused marked increases in the concentration of volatiles, including methyl salicylate in both cases.

#### 4.3. Green-leaf volatiles: other possible attractants for mutualistic ants of *L. a. africana*

Green-leaf volatiles appear to be more concentrated in the young-leaf extract of *L. a. africana* than in the extract from mature leaves. Are they potential attractants for *P. phylax*? Like methyl salicylate, green-leaf volatiles are reported as common components of numerous plant species belonging to a variety of plant families. They are also reported to be attractants for various phytophagous insects [40, 64], insect predators [63, 65] or even pollinating insects [54]. Their emission by the plant seems often associated with leaf damage. Hexanal, for example, released from wounded leaf surfaces, contributes to the increased response of *Azteca* ants to damaged leaves of *Cecropia obtusifolia* Bertol. [1]. In *L. a. africana*, however, ants regularly patrol intact young leaves, and preliminary experiments indicate that *P. phylax* does not respond to simulated herbivory on young leaves (C. Brouat, pers. obs.).

#### 4.4. Evolutionary origin of chemical signals emitted by the plant – adaptation or exaptation?

Did the chemical signals that attract *Petalomyrmex* ants to young leaves of their host plant originate as adaptations evolved by the plants in the context of symbiotic mutualism, or are they exaptations [30] – traits that were already present in the non-myrmecophytic ancestor and acquired a new function in symbiotic mutualism? One approach to this question is to determine whether compounds present in young leaves of the myrmecophyte *L. a. africana* are also present in the closely related but non-myrmecophytic *L. a. gracilicaulis*, which appears to

be basal in comparison with *L. a. africana* [47, 48]. We found that all compounds present in young leaves of *L. a. africana* were also present in young leaves of *L. a. gracilicaulis*. The differences between the two subspecies in chemical composition of extracts of young leaves are strictly quantitative. This suggests that compounds already present, and likely performing other roles, have acquired a new function in the myrmecophyte. If selection in the context of signalling in symbiotic mutualism has acted on leaf chemistry, its effect has been limited to changes in relative concentrations of pre-existing compounds. The major difference between young leaves of myrmecophyte and non-myrmecophyte apparent from this study is the dominance of methyl salicylate in the young-leaf extract of the myrmecophyte. The relative concentration of this compound as a proportion of all compounds extracted was over 100 times greater in the young leaf extract of the myrmecophytic *L. a. africana* than in that of *L. a. gracilicaulis*. The abundance of methyl salicylate in the young leaf extract of *L. a. africana* (about 30 % by mass of all volatiles in the young leaf extract) is highly unusual. In analyses of leaf extracts reported in the literature, relative percentages of methyl salicylate in the mix of volatiles are usually lower than 20 % [4, 11, 13, 18]. The dominance of methyl salicylate in the young leaf extract of *L. a. africana* in comparison with that of its non-myrmecophytic relative might represent a specialisation of plant odour driven by evolutionary interaction with its mutualistic ant.

#### 4.5. Evolutionary origin of response of the mutualistic ant to host-derived chemicals

Numerous plant volatiles elicit behavioural responses from ants and other insect predators or parasitoids. These chemical signals usually emanate from damaged leaves. Attraction to wounded leaves may be adaptive for predators because it increases the probability of encountering phytophagous-insect prey [1, 63]. *Petalomyrmex* workers are strongly attracted to ‘intact’ young leaves of the host. Wounding is neither necessary to elicit patrolling, nor does it increase patrolling intensity (C. Brouat, unpubl. data). However, intact young leaves of the myrmecophyte may mimic signals emitted by wounded leaves, and the response of their specific mutualist, adaptive in the context of plant protection, may be grounded in this response widespread in opportunistic predators.

Ants might also respond to plant chemicals because they are similar or identical to semiochemicals produced by the ants themselves. The major compound emitted by young leaves of *L. a. africana*, methyl salicylate, is similar to a compound produced by some ant species, methyl-6-methyl salicylate [35]. The latter

compound appears to be an alarm pheromone for some Ponerinae [23, 35]. For some *Camponotus* species, evidence suggests that this compound is a sex pheromone produced by mandibular glands of males [10, 59]. In the mutualism between *Camponotus femoratus* Fab. and neotropical epiphytes [59], this compound plays an important role in the recognition of epiphyte seeds by mutualistic ants. Seidel et al. [59] propose that seeds of ant-garden epiphytes attract *Camponotus* by chemically mimicking ant brood. Some 'green-leaf volatiles' are also produced by ants and play roles as pheromones [9, 14]. Were *Petalomyrmex* predisposed to recognise and respond to 'green-leaf volatiles' and methyl salicylate produced by their host, because these chemicals act as pheromones in this ant? Testing this hypothesis requires chemical studies of ants mutualistically associated with *Leonardoxa* species, and of their relatives.

#### 4.6. Why do ants patrol young leaves?

Field attraction tests and chemical analysis showed that response of ants to plant compounds provides a proximal explanation for the stereotyped patrolling of young leaves, and the absence of patrolling of mature leaves, by *P. phylax*. This ant possesses numerous specialised adaptations to its host-plant, with which it is obligately and exclusively associated [15, 46, 47]. Fitness of ants could thus be so tightly linked to plant growth and survival that direct rewards would not be necessary to induce ants to patrol young leaves [3, 46].

However, while *Petalomyrmex* workers apparently do not obtain food by patrolling young leaves, we cannot yet exclude the hypothesis that they obtain other resources, such as plant-derived antiseptic compounds. Some bees line their nest cavities with plant resin that protects against fungi associated with pollen [50]. Similarly, some epiphyte-specialist ants could use plant-derived compounds to protect their nests from microbial pathogens [59]. Methyl salicylate possesses antiseptic properties (Duke's Phytochemical and Ethnobotanical Databases, ARS: <http://www.ars-grin.gov/duke/>). Some 'green-leaf volatiles' are potent fungistatic compounds [8]. Do *Petalomyrmex* ants collect these compounds or their derivatives for their fungistatic properties? This hypothesis could explain some aspects of the behaviour of *P. phylax* (L. Gaume, pers. comm.; C. Brouat, pers. obs.). When patrolling young leaves of *L. a. africana*, workers of *P. phylax* often stop, appear to scrape the leaf with their first pair of legs and touch their antennae. This behaviour is also reported for the mutualistic ant of *Crypteronia griffithii*, *Cladomyrma maschwitzi* Agosti [51]. The significance of this behaviour is unknown.

In numerous ants, the metapleural gland often produces antiseptic secretions [6, 44, 58]. However, as Seidel et al. [59] suggest for another plant-ant, *P. phylax* could have lost the capacity to produce fungistatic compounds, and collect plant compounds that retard growth of microbial nest pathogens. Our study suggests the interest of examining products of the defensive glands of *P. phylax*.

## 5. CONCLUSIONS

Our preliminary results on field attraction tests and chemical analyses demonstrated that compounds present in young, but not mature, leaves of *L. a. africana* are attractive to workers of the plant's host-specific mutualist ant associate *Petalomyrmex phylax*. Attraction of ants to these substances may thus be the proximate mechanism explaining the restriction of ant patrolling young leaves. Allocation of the colony's limited force of patrolling workers solely to vulnerable parts of the plant is a behavioural trait beneficial to both the host plant and its ant colony. While the precise nature and evolutionary origin of plant-derived signals and of ant responses to them are not yet clear, exaptations – pre-existing traits that acquired new functions – of both plants and ants could explain the origin of this ant-plant signalling system. Investigation of the semiochemicals of *Leonardoxa*-associated ants and their relatives, and field attraction tests using odour components offered individually or mixed, are now necessary for deeper understanding. Our results also suggest avenues for approaching many other unexplored aspects of the chemical ecology of symbiotic ant-plant mutualisms, including mechanisms insuring the encounter of host plants and foundress queens.

## Acknowledgments

We thank the Ministry of Research and Higher Education of the Republic of Cameroon for permission to carry out the field component of this research. This study was funded by a grant from the CNRS (Programme 'Environnement, Vie et Sociétés'). We thank Alain Ngomi and Gabriel Debout for their assistance in the field and in collection of leaf extracts, Laure Grison for her technical assistance in the laboratory, Edmond Dounias for the loan of a field vehicle, and the APFT programme of the European Commission for its help in our stays in Yaoundé. Luc and Marie Moliko are thanked for their hospitality in Ebodjé, and Big John in Nkoloboundé. We thank the Institut Pasteur of Yaoundé for having kindly let us use their rota-evaporator. The manuscript was improved by discussions with Ambroise Dalecky, Laurence Gaume and Marc Gibernau.

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