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Comparison of the chemical composition of three species of smartweed (genus *Persicaria*) with a focus on drimane sesquiterpenoids

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A B S T R A C T

The genus *Persicaria* is known to include species accumulating drimane sesquiterpenoids, but a comparative analysis highlighting the compositional differences has not been done. In this study, the secondary metabolites of both flowers and leaves of *Persicaria hydropiper*, *Persicaria maculosa* and *Persicaria minor*, three species which occur in the same habitat, were compared. Using gas chromatography–mass spectrometry (GC–MS) analysis of extracts, overall 21/29 identified compounds in extracts were sesquiterpenoids and 5/29 were drimanes. Polygodial was detected in all species, though not in every sample of *P. maculosa*. On average, *P. hydropiper* flowers contained about 6.2 mg g FW^{-1} of polygodial, but *P. minor* flowers had 200-fold, and *P. maculosa* 100,000 fold lower concentrations. Comparatively, also other sesquiterpenes were much lower in those species, suggesting the fitness benefit to depend on either investing a lot or not at all in terpenoid-based secondary defences. For *P. hydropiper*, effects of flower and leaf development and headspace volatiles were analysed as well. The flower stage immediately after fertilisation was the one with the highest content of drimane sesquiterpenoids and leaves contained about 10-fold less of these compounds compared to flowers. The headspace of *P. hydropiper* contained 8 compounds: one monoterpene, one alkyl aldehyde and six sesquiterpenes, but none were drimanes. The potential ecological significance of the presence or absence of drimane sesquiterpenoids and other metabolites for these plant species are discussed.

1. Introduction

Terpenoids represent the largest class of plant secondary metabolites with over 50,000 known structures. They play important roles in the interaction of plants with their environment (Gershenzon and Dudareva, 2007). Nearly 100 sesquiterpene drimanes derived from drimenol are known; they possess a wide variety of biological activities including antibacterial, antifungal, anti-nematode, antifeedant, piscicidal, and molluscicidal properties (Jansen and de Groot, 2004). Polygodial, as best known proponent of this group of sesquiterpenoids, is present in a few plant genera, such as the Cannellaceae genera *Drymis* (Munoz-Concha et al., 2007) and *Tasmannia* (Read and Menary, 2000) as well as in some species of the *Persicaria* genus (formerly known as *Polygonum*) (Starkenmann et al., 2006); in fact the presence or absence of polygodial in different *Persicaria* species is being proposed as a chemotaxonomic discriminant between different sub-groups of that genus (Derita and Zacchino, 2011). This dialdehyde has been also found in bryophytes and pteridophytes (Asakawa et al., 2001) and it is even synthesized by Mediterranean nudibranchs of the genus *Dendrodoris*, where it plays a role as fish antifeedant (Avila et al., 1991). Polygodial has a pungent taste to mammals (Escalera et al., 2008; Kubo and Ganjian, 1981), and acts as an antifeedant for a number of herbivorous insects (Jansen and de Groot, 2004), including aphids (Asakawa et al., 1988; Powell et al., 1995), Colorado potato beetle (Caprioli et al., 1987; Gols et al., 1996), and moth larvae (Caprioli et al., 1987; Kubo and Ganjian, 1981). The neurological mode of action of polygodial is by opening TRPA1 ion channels on sensory neurons (Escalera et al., 2008).

Persicaria hydropiper L. (Polygonaceae), commonly known as water-pepper, is an annual plant, native to Eurasia, widely distributed as a plant of damp places and shallow waters, such as damp rides in woods, damp meadows, ditches and sides of streams, canals and ponds (Timson, 1966). A distinguishing characteristic

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of the species is that its leaves, and even more its inflorescences, have a pungent taste, due to polygodial. The compound is stored in epidermal cavities, also called valvate or irritant glands, present on leaves and tepals. The latter can contain up to 8.5% of polygodial in dry weight (Hagendoorn et al., 1994). When observed with a magnification lens or binocular these cavities appear like small translucent dots of approx. 0.5 mm in diameter (Lersten and Curtis, 1992).

Persicaria minor (Hudson) Opiz, also known as small water-pepper, is an annual plant, less common than *P. hydropiper*, but with similar morphological features and habitat preferences. One discriminating feature is the number of the epidermal cavities on the flower heads, which are significantly less on *P. minor* (Eggelte, 2007). Another trait that distinguishes the two species is the colour of the flowers, which is more intensely white or pink-purple in *P. minor* compared with a light green to white in *P. hydropiper*. When chewed upon, the flowers of *P. minor* present only a slightly pungent taste, compared with the strong taste of *P. hydropiper*.

Persicaria maculosa Gray (formerly *Polygonum persicaria* L., common name lady's-thumb) is also an annual weed of temperate regions, closely related to the other two and thriving in the same territory, although usually in drier habitats. The reason we included it in this study was the debated absence of cavities and pungency. According to Derita et al. (2008), specimens of this species in Argentina do have low numbers of cavities and produce small amounts of polygodial, while Hagendoorn et al. reported that the Dutch specimens analysed have no cavities, and, therefore, no polygodial at all (1994).

The aim of the present study was to analyse the distinctive differences and similarities in the chemical profiles of these three closely related species of the *Persicaria* genus occurring in geographical proximity in the Netherlands, and partly overlapping biotopes.

2. Results and discussion

2.1. Interspecific variation of the chemical composition of *Persicaria* spp.

Seeds of three species of the *Persicaria* genus, *P. hydropiper*, *P. minor* and *P. maculosa*, were collected in the woods in close range

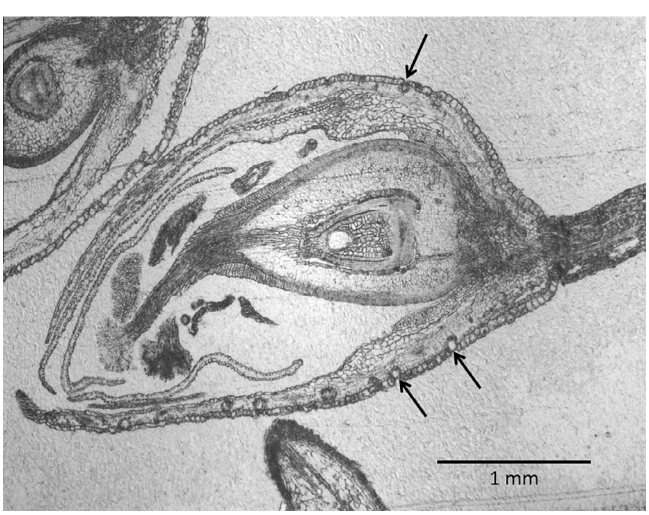

Fig. 1. Light micrograph of a longitudinal cross section of a paraffin embedded flower of *P. hydropiper*. Arrows indicate the valvate glands (cavities) where polygodial is stored.

of each other. The three species were identified by taxonomist Ronald van den Berg (Wageningen UR) (Supplementary Fig. S3 for images of the plants). Seven separate samples were taken from plants raised from the seeds: three developmental stages of flowers and four of leaves. In Fig. 1, a cross section of a *P. hydropiper* flower clearly shows the presence of the valvate glands in the tepals of the flowers. Samples were extracted in dichloromethane (DCM) and analysed by coupled gas chromatography–mass spectrometry (GC–MS). A total of 29 major metabolites could be identified across all three species, 15 of which were present in all. Nearly all (27/29) identified metabolites were terpenoids, most were sesquiterpenoids (21/29) and a quarter of those were drimanes (5/21) (Tables 1 and 2, structures in Fig 2, and mass spectra in Fig. S1). Table 1 shows the average relative abundance of the 29 metabolites in the flowers of the three species, while in Table 2 the chemical profiles of the leaves are listed. *P. hydropiper* was the species with the widest variety of metabolites detected in our analyses; the only compound present in the other two species, but not in water-pepper, was β-selinene. In general, flower samples contained more different and higher quantities of metabolites compared to leaves, with the exception of the three neophytadiene isomers, which were more abundant in the leaves, because they are degradation products of chlorophyll (Rowland, 1957).

The five sesquiterpene drimanes observed in the gas chromatogram were drimenol, polygodial, 9-*epi*-polygodial, drimenin and isodrimenin (Fig 2). It is known that 9-*epi*-polygodial is formed from polygodial by base-treatment (Cortés et al., 1998; Kubo and Ganjian, 1981), and there are other reports of this compound being an artifact of the GC–MS analysis (Asakawa et al., 2001). We confirmed that upon injection of a ^{1}H NMR verified reference standard of pure polygodial, also 9-*epi*-polygodial is observed, and that the ratio of the peaks versus 9-*epi*-polygodial varied from 1.6 to 0.8:1 depending on the injection temperature (Fig. S2). An earlier study, however, reports the ^{1}H NMR spectra for both epimers in *Drymis winterii* (Rodríguez et al., 2005). As our GC–MS method does not allow the reliable estimation of the ratios, we report the combined concentrations of both epimers under the label of polygodial.

Polygodial was the most abundant compound in *P. hydropiper* flowers, making up for 77% of the extract, equivalent to 6.2 mg g FW^{-1} (Tables 1 and 3). It was 200 times less abundant in *P. minor* flowers (0.032 mg g FW^{-1}), representing 25% of the total compounds, and 100,000 times less abundant in *P. maculosa* flowers (70 ng g FW^{-1}), representing 0.1% of total compounds (Tables 1 and 3). The drimane sesquiterpene lactones drimenin and isodrimenin were minor compounds when compared to polygodial, accounting for only 2% of the *P. hydropiper* flower extract. Relative to *P. hydropiper*, they were 10- and 100-fold less abundant in *P. minor* flowers, respectively, and not detected in *P. maculosa* flowers. Drimenol, the putative precursor of polygodial (Pickett, 1985) was only detected at low concentrations in *P. hydropiper* and not in the other species, presumably because it serves as an intermediate and it is promptly converted. Comparing leaves to flowers, polygodial was 10× more abundant in flowers of *P. hydropiper*, equally abundant in *P. minor* leaves and flowers and 10× less abundant in flowers than leaves of *P. maculosa*.

The low levels of polygodial in *P. maculosa* were earlier not found by Hagendoorn et al. (1994) for specimens collected in the Netherlands, but they are in line with the results of Derita et al. (2008) who detected low amounts of polygodial (0.54 mg g DW^{-1}) in leaves of *P. maculosa* from Argentina. Yet, by comparison, our specimen contains only 70 ng g FW^{-1}. Taking into account the FW/DW comparison, this is still 100 times less than accessions from Argentina.