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Alkaloid metabolism in thrips-Papaveraceae interaction: Recognition and mutual response

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ABSTRACT

Frankliniella occidentalis (Pergande), the Western Flower Thrips (WFT), is a polyphagous and highly adaptable insect of the order Thysanoptera. It has a broad host range but is rarely found on Papaveraceae, which might be due to deterrent effects of alkaloids present in most species of this family. In order to test the adaptive potential of WFT, we investigated its interaction with two Papaveraceae offered as sole feeding source. We found that WFT are able to live and feed on leaves of *Eschscholzia californica* and *Chelidonium majus*. Both plants respond to thrips feeding by the enhanced production of benzophenanthridine alkaloids. Furthermore, cell cultures of *E. californica* react to water insoluble compounds prepared from adult thrips with enhanced alkaloid production. During feeding, WFT take up benzophenanthridine alkaloids from either plant and from an artificial feeding medium and convert them to their less toxic dihydroderivatives. This was shown in detail with sanguinarine, the most cytotoxic benzophenanthridine. A similar conversion is used in plants to prevent self-intoxication by their own toxins. We conclude that WFT causes a phytoalexin-like response in Papaveraceae, but is able to adapt to such host plants by detoxification of toxic alkaloids.

Introduction

Western Flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae), is an insect native to the Western USA. It spread since the 1980s and became a pest worldwide within 20 years (Kirk and Terry, 2003) affecting crop and ornamental plants both in greenhouses and in the field (Yudin et al., 1986). Western Flower thrips (WFT) is the most effective vector of a number of Tospoviruses, of which Tomato Spotted Wilt Virus (TSWV) is the most prominent (Whitfield et al., 2005). WFT lives on plant leaves and fruits and inside inflorescences. More than 200 plant species from about 90 families can serve WFT as a feeding source (Brødsgaard, 1989) which indicates its high adaptive potential (Moritz, 2006). It is noteworthy that larval and adult WFT occupy the same ecological niches but individuals may change hosts during their development (Moritz, 2006). Like many other thrips, WFT feeds by piercing epidermal cells and sucking up their content including cytosol, chloroplasts, vacuoles and nuclei (Kirk, 1997). The damage resulting from thrips feeding thus differs from that caused by other herbivorous insects. It is characterized by induction of local lesions, silvering around the punctures and darkening of leaf due to desiccation (reviewed in Childers and Achor, 1995). Plant responses to thrips feeding have been reported in recent years but the actual knowledge remains fragmentary. *Arabidopsis thaliana* leaf discs and plants reacted to thrips feeding by increased biosynthesis of jasmonate, an ubiquitous mediator of plant defence (Abe et al., 2008), while infection by a tospovirus increased the concentration of salicylate, thus suppressing the plant's anti-herbivore response (Abe et al., 2012). The increase of jasmonate leads to a reduced rate of oviposition and population growth by WFT on *Arabidopsis thaliana*, *Brassica rapa* and *Triticum aestivum* (Abe et al., 2009; El Wakeil et al., 2010). It is unclear, however, which kind of thrips-deterring activity was triggered via the jasmonate system. The same is true for ethylene, another plant hormone. Elevated ethylene emission has been observed in several plant–thrips interactions (reviewed in: Childers and Achor, 1995; Moritz, 2006) but no defence activities downstream of the ethylene peak are known.

An effective and widely distributed reaction of plants to herbivory is the enhanced production of toxic secondary compounds, as various alkaloids, terpenes, phenolic compounds, etc. (cf. Dixon et al., 1995; Walling, 2000; Bruxelles and Roberts, 2001; Hammerschmidt and Kagan, 2001; Lambdon and Hassall, 2001). Similar responses to thrips feeding have not yet been reported although such molecules most probably can deter thrips from

Abbreviations: HPTLC, high performance thin layer chromatography; Rf, retardation factor; SD, standard deviation; WFT, Western Flower thrips.

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feeding: Maharijaya et al. (2012) have recently identified several secondary products that are likely involved in the resistance of *Capsicum* (Solanaceae) species to WFT feeding, i.e. tocopherols, a sesquiterpene, an unknown sterol and a number of alkanes. Plants producing cytotoxic pyrrolizidine alkaloids may benefit from their deterrent effects against WFT, as suggested from studies in two species of the genus *Jacobaea* (Asteraceae), showing higher feeding damage on those species that contain lower alkaloid levels than others, and vice versa (Macel et al., 2005; Cheng et al., 2011; Macel, 2011). Up to now, neither enhanced production of alkaloids in response to thrips invasion nor metabolic adaptation of the thrips to plant defence compounds are known.

Metabolic adaptations of other herbivorous insects to the phytochemicals of food plants are known from insect-host systems (Glendinning, 2002). The most detailed data are available from arctiids and similar insects that feed on *Senecio* (Asteraceae) species and cope with their toxic pyrrolizidine alkaloids (Hartmann et al., 2003, 2005; Langel and Ober, 2011). In this case, the metabolism is not only required to protect the insect but also to recruit the alkaloids for the insect's defence against animal predators (Narberhaus et al., 2005).

In the present study we investigate the interaction between WFT and two plant species that produce benzophenanthridine alkaloids, members of the benzylisoquinoline family with a high cytotoxic potential (Schmeller et al., 1997). The plants studied here are *Chelidonium majus* (L.) (Greater celandine) and *Eschscholzia californica* (Cham.) (California poppy), two species of the family Papaveraceae that are widespread both in the American hemisphere and Europe (Tutin et al., 1993) and thus may be considered as potential host plants of WFT. They contain a broad spectrum of benzophenanthridines and other benzylisoquinolines (cf. Preininger, 1986). Benzophenanthridine alkaloids are highly effective phytochemicals as they intercalate dsDNA, interact with cytoskeletal proteins and various SH-enzymes and disturb membrane potential-dependent enzymatic processes (Wolff and Knipling, 1993; Faddeeva and Beliaeva, 1997; Schmeller et al., 1997; Slaninova et al., 2001). Sanguinarine is regarded as the most toxic among the benzophenanthridine alkaloids, due to its planar, hydrophobic and cationic molecular structure, making it one of the strongest antimicrobials produced in plants (Wink et al., 1998; Slaninova et al., 2001). The biochemical, enzymatic and molecular aspects of benzophenanthridine biosynthesis have been studied in most detail in cell suspension cultures of *E. californica* and related plants (Zenk, 1994; cf. Ziegler and Facchini, 2008; Klein and Roos, 2009 for reviews).

Contact with microbial elicitors causes enhanced expression of biosynthetic and protective enzymes followed by the enhanced production of these benzophenanthridine alkaloids (Gundlach et al., 1992; Cline et al., 1993; Roos et al., 1998). Two signal pathways operate between pathogen detection and gene expression: low elicitor concentrations signal via activation of phospholipase A2 and subsequent shifts of cytoplasmic pH (Viehweger et al., 2002, 2006; Färber et al., 2003; Roos et al., 2006; Heinze et al., 2007; Angelova et al., 2010), high elicitor concentrations cause a peak of jasmonate, leading to enhanced expression of biosynthetic genes together with an oxidative burst (Gundlach et al., 1992; Färber et al., 2003). Benzophenanthridine producing cells are protected from self-intoxication by several mechanisms, among them the reduction to less-toxic derivatives by sanguinarine reductase, a cytoplasmic enzyme (Weiss et al., 2006; Vogel et al., 2010). With whole plants, the highest alkaloid response to microbial elicitors is found in young roots. Leaves originally contain low alkaloid levels, but are able to enhance alkaloid production upon contact with elicitor or wounding (Angelova et al., 2010).

The two plant species chosen for our study represent different modes of compartmentalization of benzophenanthridines: in *C. majus*, cells excrete alkaloids into laticifers which develop in leaves, stems and roots (Colombo and Bosisio, 1996), *E. californica* has no laticifers but mainly accumulates the alkaloids in idioblasts of the root cortex (Angelova et al., 2010).

With this background in mind, we explored the following questions:

1. Do WFT avoid feeding on benzophenanthridine producing Papaveraceae?
2. Can WFT adapt to food plants that produce benzophenanthridine alkaloids?
3. Can WFT detoxify these alkaloids?
4. Are WFT recognized by alkaloid-producing Papaveraceae and do the plants respond by enhanced alkaloid production?

Material and methods

Growth and maintenance of thrips population

The colony of *Frankliniella occidentalis* (WFT) was maintained on common bean plants (*Phaseolus vulgaris* (L.)) with fully developed leaves at a 16 h/8 h light-dark regime, 23 °C ± 2 °C and 75–80% relative humidity in a separate breeding room. Fresh bean plants (grown in greenhouse for about 10 days) were supplied every week.

For generation of first instar larvae, adult WFT were placed on bean leaf discs as described below and young larvae were collected from these plates 0–4 h after hatching.

Plant material and cell culture

P. vulgaris and *Eschscholzia californica* (Cham.) were grown in pots of garden soil in the greenhouse at 21 °C. *E. californica* cell cultures were grown in a modified Linsmeyer-Skoog medium on gyratory shakers at 24 °C as described previously (Viehweger et al., 2002). Leaves of *Chelidonium majus* (L.) plants were taken from the wild (yard of the Zoological Institute in Halle).

WFT on leaf discs of P. vulgaris and C. majus

Each well of 12-well plates (Greiner Bio-One, Essen, Germany) was filled with 1.5 mL 0.4% (w/v) agar (Carl Roth, Karlsruhe, Germany). Leaf discs (15 mm in diameter) were punched out from newly emerged green leaves of *P. vulgaris* or from leaves of *C. majus*. Leaf discs were placed on the agar after it was cooled to room temperature. WFT (adults or larvae depending on the experiment) were placed on the leaf discs. The 12-well plates were closed with glass lids, sealed with Parafilm (Pechiney Plastic Packaging, Chicago) and kept in a climate chamber at 23 °C ± 1 °C, 16 h/8 h light-dark cycles and relative humidity of 75%.

To study developmental time and survival rate, one freshly emerged first instar larva (0–4 h after hatching) was placed on each leaf disc and examined daily under a stereo microscope. To study alkaloid uptake and induction of alkaloid production in the plant, 15 first instar larvae were placed on each leaf disc. Larvae were collected and analysed for alkaloid uptake after a feeding period of 3 days.

WFT on E. californica

To study alkaloid uptake from *E. californica* and alkaloid production in the plant, leaflets of 1.5–2 cm length (corresponding to approximately 1.5–2.0 cm^2 leaf surface, estimated from a digital image) were cut from 20 to 25 d old *E. californica* plants from green house and put in agar (1% (w/v) in distilled water) in 20 mL plastic jars. 25 first instar WFT larvae were placed on the leaflets in each jar. The jars were sealed with Parafilm to avoid contaminations