SHORT COMMUNICATION

Phytochemical cues affect hunting-site choices of a nursery web spider (*Pisaura mirabilis*)
but not a crab spider (*Misumena vatia*)

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Abstract. Predaceous arthropods such as spiders are often adapted to hunting sites where their hunting success is greatest. We investigated the responses of two spiders to phytochemical cues that they potentially experience while hunting on leaves or flowers, and how these cues could influence their decisions where to forage. We compared the behavior of two sit-and-wait predators, *Pisaura mirabilis* and *Misumena vatia*, which hunt predominantly in the vegetation or on flowers, respectively. In choice tests, *P. mirabilis* frequently preferred leaves and leaf extracts to flowers and floral extracts and avoided substrates treated with the common floral scents β-caryophyllene and nerolidol (sesquiterpenes) in natural concentrations. In contrast, *M. vatia* did not show any preferences for any of the substrates and treatments offered. The lack of responses by *M. vatia* contrasts with earlier studies on another crab spider species (*Thomisus spectabilis*) that used phytochemical cues as a guide to rewarding flowers. The avoidance of many flowers, their extracts, and the floral scent compounds by *P. mirabilis* suggests that these cues may prevent the visitation by this and other generalised predators that potentially decrease the pollination success of a plant.

Keywords: Deterrence, optimal foraging, secondary metabolites

An underlying assumption of many optimal foraging models is that animals are behaviorally, morphologically and physiologically adapted to maximize their net rate of energy intake (Schoener 1971; Cowie 1977). A behavioral adaptation of predaceous animals is to choose foraging patches that are frequently visited by prey or to which the animals are best adapted (Krebs et al. 1974; Shafir & Roughgarden 1998). Some crab spiders, for example, show adaptations as sit-and-wait predators on flowers: they are able to change color for camouflage and enhance the attractiveness of flowers for pollinators due to their ultraviolet contrast against petals (Heiling et al. 2003). The high specialization on flowers by crab spiders is also reflected in a relatively narrow prey spectrum, which is limited to common flower visitor taxa (Nentwig 1986). Other non-web-building spiders hunt or ambush predominantly in the vegetation and thus capture a broader spectrum of prey taxa (Nentwig 1986). To benefit from their adaptations to different plant structures (vegetative or reproductive) or to specific visitors of these structures, spiders need to perceive and thus recognize those structures. Heiling et al. (2004) have shown that crab spiders (*Thomisus spectabilis*) use visual and olfactory flower cues for patch choice.

We experimentally tested for substrate choice behavior and a role of phytochemicals in two non-web-building spiders that utilize different plant parts as hunting sites: the crab spider *Misumena vatia* (Thomisidae), which typically sits and waits on flowers to catch flower visitors, and the nursery web spider *Pisaura mirabilis* (Pisauridae), which hunts in the vegetation. In concordance with their lifestyle, we expected *M. vatia* to be attracted to flower cues, while *P. mirabilis* may prefer leaves.

Between June and August 2008, we caught *M. vatia* and *P. mirabilis* spiders on fallow lands in Würzburg, Germany. We collected fifty-eight individuals of *M. vatia* on flowers of *Achillea millefolium*, *Aegopodium podagraria*, *Leucanthemum vulgare*, *Saponaria officinalis*, *Solidago canadensis*, *Trifolium pratense*, *Tripleurospermum maritimum*, while we collected all but one of 41 *P. mirabilis* from the vegetation (one individual was collected from an *Achillea millefolium* flower). We kept the spiders individually in small plastic containers in a climate chamber under long day conditions (day:night = 14:10 h, 26:19°C) and fed them with flies twice a week and continuously provided water as a small drop. We picked the plants used for the laboratory experiments in the same area.

In pair-wise choice tests, spiders were able to choose between different substrates including flowers vs. leaves of the same plant species (Experiment I), filter papers with extracts of flowers vs. extracts of leaves of the same plant species (Experiment II) and filter papers treated with synthetic floral scent compounds vs. unscented controls (Experiment III). The principal setup of these experiments (I–III) was the same: we placed individual spiders on pieces of cork (ca 30 cm²) in water-filled bowls, preventing spiders from escaping. On each of these islands, we attached two wooden sticks (height = 140 mm, diam. = 3 mm) in an upright position and attached the different substrates used in the tests to the tip of these sticks. The distance between the substrates (ca 1 cm) was chosen to be close enough that the spiders could freely change between the substrates without descending to the islands but large enough that spiders were forced to make a choice. Neon lamps from above illuminated the whole setup. After spiders were placed on the islands, we observed them for 1 h, recording their position on either substrate every 3 min. We used individual spiders for several tests but not repeatedly for the same treatment.

Experiment I: We placed freshly picked flowers and leaves from *Achillea millefolium*, *Centarea cyanus*, *Tanacetum vulgare* (all Asteraceae), *Medicago sativa* (Fabaceae) and *Saponaria officinalis* (Caryophyllaceae) in small water-filled vases. The vases were 1.5 ml standard microcaps, and we attached them on top of the wooden sticks. In each pair-wise test (flower vs. leaf of the same plant species), we adjusted the number of leaves and flowers or inflorescences so that both substrates represented approximately the same area, providing sufficient space for spiders to sit on.

Experiment II: We used the same five plant species to prepare leaf and flower extracts. We placed freshly chopped plant material into an extraction thimble and continuously extracted it with 50 ml n-hexane in a Soxhlet apparatus for three hours at a temperature of 85°C.
In order to determine those compounds in the extracts that frequently occur in flower and leaf scents (Knudsen et al. 2006), we analysed the extracts using a Varian 3800 gas chromatograph (GC) fitted with a 1079 injector and a ZB-5 column (5% phenyl polysiloxane; length, 60 m; inner diameter, 0.25 mm; film thickness, 0.25 μm; Phenomenex) and a Varian Saturn 2000 mass spectrometer. We filled 1 μl of the samples into a quartz vial in the injector port of the GC by means of the ChromatoProbe kit (Amirav & Dagan 1997). The injector split vent was opened, and the injector was heated at 40°C to flush any air from the system. After 2 min, the split vent was closed and the injector heated at 200°C until the end of the run. The split vent was again opened after 4.5 min. Electronic flow control was used to maintain a constant helium carrier gas flow rate (1.0 ml min⁻¹). The GC oven temperature was held for 4.5 min at 40°C, then increased by 6°C min⁻¹ to 300°C, and held for 15 min at this temperature. Mass spectra were taken at 70 eV with a scanning speed of one scan per second from m/z 30 to 650. We analyzed the data as described elsewhere (Dötterl et al. 2009), and used an internal standard (3-chloro-4-methoxytoluene) for quantification.

Experiment III: Since we expected that the phytochemical cues to which spiders respond are not specific to certain plant species, we used spider species, plant species and treatment (i.e., fresh plant material or extracts thereof) in one GLM, with the proportion of observations on flowers or flower extracts as response variable and spider species, plant species and treatment (i.e., fresh plant material or extracts thereof) as explanatory variables. In the GLM for tests with floral extracts (II) or scented filter papers (III) was obtained, disregarding observations during which the spider was not present on one of the substrates. Some spiders spent time on the islands, while others did not leave it during the entire period (P. mirabilis: 3.0% of all trials, M. vatia: 7.3%); these rare events were not included in the calculation of the proportion. We performed generalized linear models (GLM) with quasibinomial error distribution (accounting for the overdispersed data) in order to explore the parameters influencing the spiders’ choice. We analysed the tests with fresh plant material (Experiment I) and extracts (Experiment II) in one GLM, with the proportion of observations on flowers or flower extracts as response variable and spider species, plant species and treatment (i.e., fresh plant material or extracts thereof) as explanatory variables. In the GLM for tests with floral scent compounds (Experiment III), we used spider species, substance and concentration (mMol) as explanatory variables. Beginning with the full model, we reduced the models stepwise and compared them to the previous one with a $X^2$ test (Crawley 2005). Prior to the stepwise statistical analysis, we compared the full model to a null model (model with no explanatory variables) to validate the overall effect of the combined parameters. We tested individual parameters only if the full model had significantly more explanatory power than the null model (see Mundry & Nunn 2009). Additionally, we individually tested the proportions against the null hypothesis (assuming equal visitation of both treatment and control; i.e., proportion = 0.5) with a Wilcoxon test. All statistical analyses were performed using R 2.4.0 (R Development Core Team 2009).

In 93.3 and 97.5% of all trials with M. vatia and P. mirabilis, respectively, the spider chose one of the substrates within the first 10 min after the solvent had evaporated, a trial started.

Each trial (1-h period) yielded up to 20 observations from which the proportion of observations on flowers or flower extracts as response variable and spider species, plant species and treatment (i.e., fresh plant material or extracts thereof) as explanatory variables. In the GLM for tests with floral scent compounds (Experiment III), we used spider species, substance and concentration (mMol) as explanatory variables. Beginning with the full model, we reduced the models stepwise and compared them to the previous one with a $X^2$ test (Crawley 2005). Prior to the stepwise statistical analysis, we compared the full model to a null model (model with no explanatory variables) to validate the overall effect of the combined parameters. We tested individual parameters only if the full model had significantly more explanatory power than the null model (see Mundry & Nunn 2009). Additionally, we individually tested the proportions against the null hypothesis (assuming equal visitation of both treatment and control; i.e., proportion = 0.5) with a Wilcoxon test. All statistical analyses were performed using R 2.4.0 (R Development Core Team 2009).

In 93.3 and 97.5% of all trials with M. vatia and P. mirabilis, respectively, the spider chose one of the substrates within the first
Once a spider climbed up a wooden stick, it rarely descended to islands again. While _M. vatia_ often changed the substrates during the trial (3.0 ± 0.2 times, mean ± SE), _P. mirabilis_ was less likely to switch, with only 0.8 ± 0.2 changes of the substrate per trial. The responses to fresh plant material (Experiment I) were usually consistent with responses to extracts of the same plant species (Experiment II) for both species of spider, but the spiders’ choices between leaves and flowers differed strongly between plants (Table 1a).

_P. mirabilis_ strongly preferred leaves over flowers (and their extracts) in three out of five plant species, whereas _M. vatia_ did not show any preferences (Table 1a and Fig. 1a).

In trials where spiders were allowed to choose between filter paper treated with scent compounds and acetone-treated filter paper (Experiment III), the choices depended on the particular substance and spider species. Overall, the concentration of the compounds did not affect the spiders’ choices (Table 1b).

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In trials where spiders were allowed to choose between filter paper treated with scent compounds and acetone-treated filter paper (Experiment III), the choices depended on the particular substance and spider species. Overall, the concentration of the compounds did not affect the spiders’ choices (Table 1b). Similar to the previous tests, _M. vatia_ was less selective than _P. mirabilis_ (Table 1b and Fig. 1b). _M. vatia_ avoided filter paper treated with nerolidol, and _P. mirabilis_ avoided both nerolidol and β-caryophyllene (Fig. 1b). _P. mirabilis_ behavior was not affected by the green leaf volatiles _cis-3-hexen-1-ol_ and _cis-3-hexen-1-yl acetate_ (Φ ∼ 50.5, P ≥ 0.37, Wilcoxon test). Large amounts of nerolidol occurred in floral extracts of _S. officinalis_, and β-caryophyllene in _A. millefolium_. These substances may have triggered the preference of _P. mirabilis_ for leaves and leaf extracts in _S. officinalis_, and for leaf extracts of _A. millefolium_ over the respective flowers or floral extracts (Fig. 1). Living flowers of _A. millefolium_ were not avoided by _P. mirabilis_, suggesting that some substances were dissolved from the plant tissue and were thus present in the extracts that were not emitted by fresh plant material or were emitted in a lesser amount.

The results of our study imply that _P. mirabilis_ perceive phytochemical cues and use them to decide where to ambush for...
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