

“Ménage à trois”: The problem with three-way interactions involving predatory wasps

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Abstract

The wasp *Cotesia glomerata* is a parasitoid; eggs are laid within host caterpillars of the small cabbage white butterfly and immature stages complete development inside the hosts, eventually killing them when they emerge to pupate. To detect and locate hosts it is believed that *C. glomerata*, like many parasitoids, relies on odours released from plants following caterpillar feeding. The preferences of *C. glomerata* to different pairwise combinations of plants infested with host caterpillars, non-host caterpillars and both host and non-host caterpillars were measured using an olfactometer. *Cotesia glomerata* did not discriminate between any of the infested plants, suggesting this parasitoid cannot distinguish the odours released from plants in response to host and/or non-host caterpillar feeding. We suggest that odours are perhaps only general cues used by *C. glomerata* to locate caterpillar-infested plants, and a number of complementary cues (e.g. post-alighting visual and chemical cues) are then enlisted at closer range.

Keywords

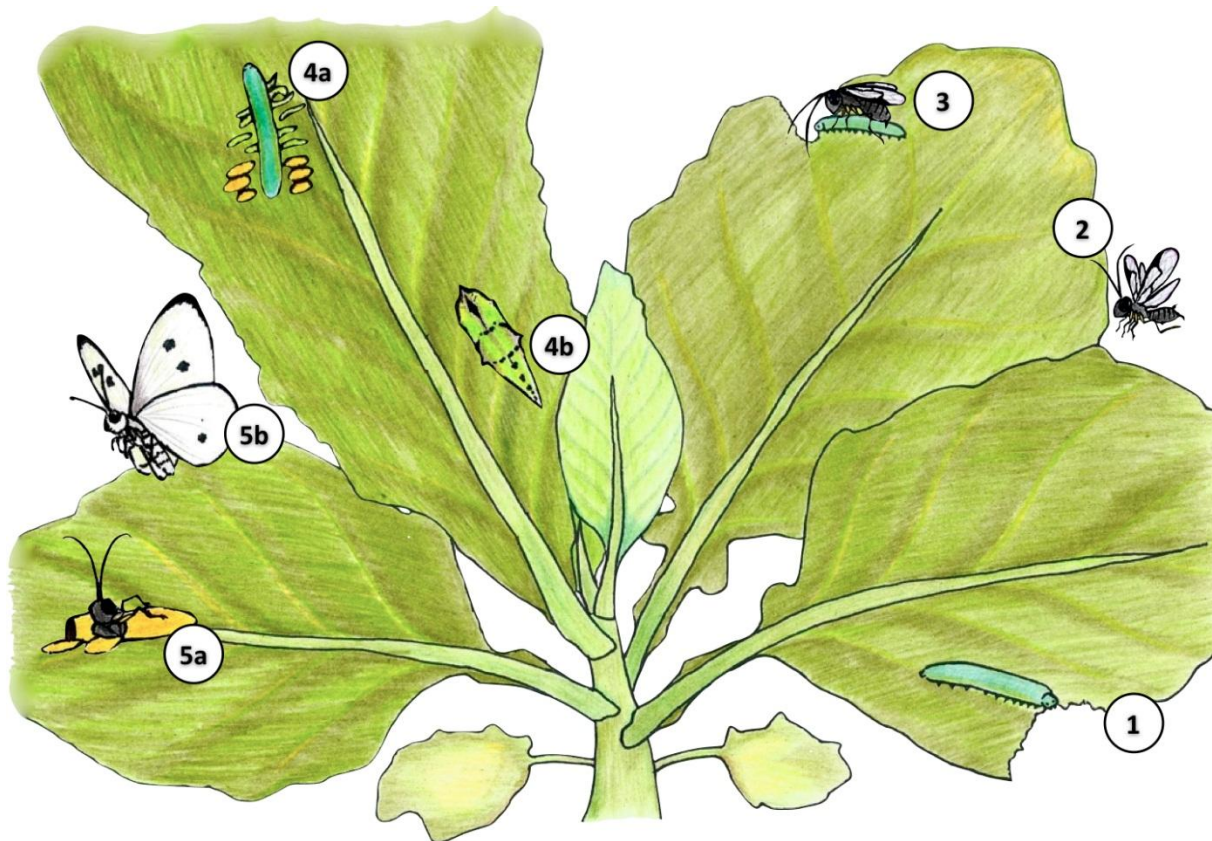
Cotesia glomerata, volatile organic compounds (VOCs), *Pieris rapae*, *Plutella xylostella*, olfactometer

Introduction

When herbivorous insects like the caterpillars of various butterflies and moths feed on plants, one response by the plant is to release odours in the form of volatile organic compounds (VOCs). There is much debate in the literature about the role(s) of VOCs ([Kessler & Heil, 2011](#)). We know that in some cases the blends of VOCs from plants are unique to the plant species and the particular herbivore or the combination of herbivores feeding on the plant ([Arimura et al., 2009](#)). Natural enemies of herbivores include parasitoids, which are a specialized group of insects (primarily wasps) which lay their eggs in or on other insects. Their young feed exclusively on the body of their host, eventually killing it. Many species of parasitoids are host-specific, i.e. they can use only a particular species or a limited range of herbivore hosts to complete their life cycle, but others attack a wide range of host species ([Godfray, 1993](#)). It is suggested that parasitoids use their ability to detect unique VOC signatures from herbivore-plant complexes to locate specific prey or host species ([Poelman et al., 2011](#)), hence a "three-way interaction" between plant, herbivore and natural enemy takes place.

The wasp parasitoid *Cotesia glomerata* is host-specific to pierid butterflies, which include the small cabbage white butterfly, *Pieris rapae*. Female *C. glomerata* use VOC cues to detect and locate sites where caterpillars are feeding ([van Dam et al., 2010](#)), and lay eggs into suitable hosts (Godfray, 1993). Subsequently, the leaf material that the caterpillars consume is diverted into nourishing the developing wasp larvae within the caterpillar. The wasp larvae eventually erupt through the body of their host, killing the caterpillar, before they spin silken cocoons on the leaf surface. Adult parasitoids later emerge to continue the next *C. glomerata* generation (see Figure 1 below).

Figure 1. Schematic diagram of the relationship between a Brassica plant, cabbage white butterflies *P. rapae*, and its parasitoid *C. glomerata*, featuring the following events: (1) feeding by caterpillar; (2) location of infestation site(s) by mated female *C. glomerata*; (3) parasitization of caterpillar by *C. glomerata*; (4a) emergence and pupation of wasp larvae from parasitized caterpillar; (4b) pupation of unparasitized caterpillar; (5a) emergence of adult *C. glomerata* from pupae; (5b) emergence of adult cabbage white butterfly. Not drawn to scale; illustrated by Gurion C.K. Ang.



The cabbage white butterfly is a cosmopolitan pest and its caterpillars have a voracious appetite for brassicaceous vegetable and oilseed crops including cabbage, broccoli, cauliflower and canola ([Troetschler et al., 1985](#)). Though no formal study has estimated the global economic losses due to cabbage whites, the caterpillars have the ability to bore into cabbage heads and defoliation rates can be considerable, resulting in significant loss in crop yield and quality ([Visser, 1986](#)). To improve natural control and reduce reliance on pesticides, much attention has been drawn to any natural enemy or biocontrol agent that can kill caterpillars, such as the parasitoid *C. glomerata*.

If, as has been commonly suggested, *C. glomerata* relies on VOCs to detect and locate prey (van Dam et al., 2010), and if VOC profiles are unique to the herbivore-plant complex, we would expect that *C. glomerata* can discriminate between VOCs produced by plants in response to feeding by host and non-host caterpillars to maximize its foraging efficiency. We address this question through a series of laboratory experiments, to determine behavioral preferences of *C. glomerata* to VOC blends actively produced from host (= *P. rapae*) or non-host (= the diamondback moth *Plutella xylostella*, another major agricultural pest which is not attacked by *C. glomerata*) infested plants. Additionally, we determine if the VOC blend from dually-infested plants (both host and non-host caterpillars) influences choice preference behaviour of the wasp in relation to singly-infested plants.

Methods

Plants and Insects

Commercially obtained common cabbage *Brassica oleracea* var. *capitata* cv. Sugarloaf seedlings were raised in a glasshouse to the 10-leaf stage and used for all experiments. In October 2011, *Cotesia glomerata*, *P. rapae* and a non-host species, *Pl. xylostella*, were field collected from a cabbage plot at Gatton, Australia (27°33'S, 152°18'E), and laboratory colonies were established.

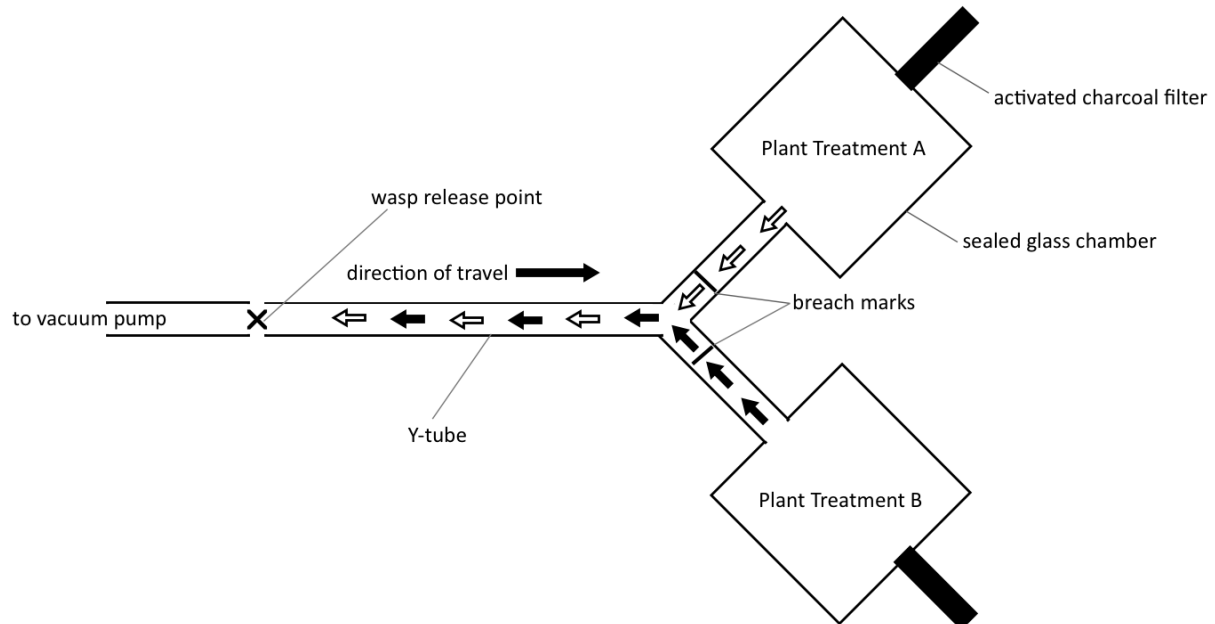
The *P. rapae* and *Pl. xylostella* caterpillar colonies were maintained on excised cabbage leaves and reared in ventilated culture boxes (30cm x 20cm x 10cm) at 20°C; pupae were regularly collected and left in ventilated cages (40cm x 40cm x 40cm) for emergence of adults. Adults were fed an aqueous honey solution (20% (w/v)) and laid eggs on potted cabbage plants that were replaced 2-3 times a week. Once eggs hatched and caterpillars began feeding on the leaf material, leaves were excised and placed into culture boxes.

Parasitised caterpillars were obtained by exposing up to three mated female *C. glomerata* to at least 40 *P. rapae* caterpillars, feeding on a plant, in a ventilated parasitisation cage (40cm x 40cm x 40cm). New batches of at least 40 caterpillars were provided each day over three consecutive days. During this time, female parasitoids were fed an aqueous honey solution (20% (w/v)). All caterpillars (parasitised or not) were handled and reared as described above for *P. rapae* until emergence and pupation of parasitoid larvae, which were then removed from the main culture and placed into a ventilated cage (40cm x 40cm x 40cm) for emergence of parasitoid adults.

Parasitoid Responses in Olfactometer

Behavioral responses of *C. glomerata* to different pairs of VOCs were measured using an olfactometer (Fig. 2) which comprised a glass Y-tube with a 0.8cm internal diameter, 7cm stem and 9.5cm arms at a 60° angle. Pairs of VOCs (emitted from various plant treatments) were presented in sealed glass chambers (15cm x 15cm x 15cm) at the end of either arm. To exclude potentially confounding ambient odours, air was drawn over activated charcoal filters when it entered the system. Air was drawn through the system at a rate of 1L/min with a vacuum pump (Figure 2).

Figure 2. Simplified diagram of the olfactometer, including the glass Y-tube where the parasitoid *C. glomerata* was released (Point X) and exposed to two VOC blends from different plant treatments (A and B) as indicated by the black and white arrows. The mark on each arm of the Y-tube indicates the point at which if breached, *C. glomerata* was scored to prefer a certain plant treatment. Not drawn to scale.



Naïve mated 2-5 day old female *C. glomerata* that had not previously experienced host caterpillars were isolated from the adult cage into individual gelatine pill capsules (1.5cm length; 0.8cm diameter) and then individually introduced to the end of the Y-tube stem 6cm from the Y-split. Each wasp was given up to 30 min in the olfactometer to respond. Once an individual moved beyond the 2.5cm breaching mark (Fig. 2) and into one of the Y-tube arms it was considered to have made a choice for the corresponding plant treatment in that arm. Non-responders that did not make a choice in 30 min were discarded. Wasps were used only once. The arrangement of the chambers and relative position of the Y-tube arms was swapped, and new plants were used every three replicates. Between every six replicates all glassware were washed in 95% ethanol and dried before use to ensure VOCs

adhering to the glass were removed. In this way, wasps were tested to each of the following pairs of plant treatments:

- (1) intact (i.e. un-infested) versus host (= *P. rapae*) infested plants;
- (2) intact versus non-host (= *Pl. xylostella*) infested plants;
- (3) host infested versus non-host infested plants;
- (4) host infested versus dually (both host and non-host) infested plants; and
- (5) non-host infested versus dually infested plants.

Plants were infested with a suitable number of host and/or non-host caterpillars to achieve approximately 30% leaf material consumption after 24h feeding, and caterpillars were removed from plants prior to olfactometer tests. Statistical significance between wasp responses to pairwise combinations of plant treatments was determined using Chi-square tests at the 5% level.

Results

Most females (88%) responded within 30 min after being introduced into the olfactometer.

Cotesia glomerata females were significantly attracted to infested plants over intact plants, regardless of whether the plants were infested with host or non-host caterpillars (Fig. 3):

intact plants versus host infested plants ($N=35$ females tested), $\chi^2 = 31.114$, d.f. = 1, $P <$

0.0001; intact plants versus non-host infested plants ($N=39$), $\chi^2 = 27.923$, d.f. = 1, $P <$ 0.0001.

In subsequent tests, no statistically significant difference was observed between any of the

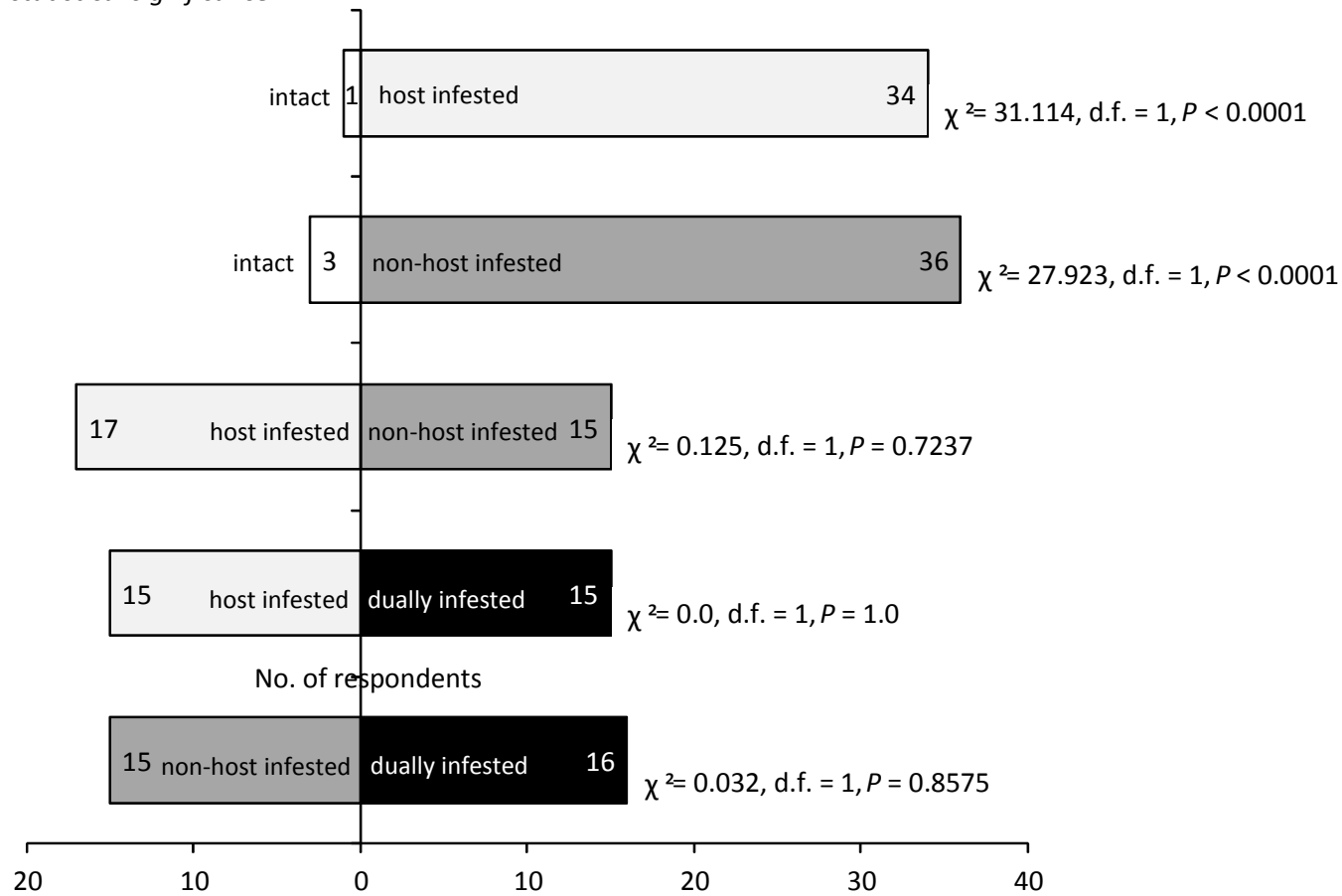
treatments in pairwise tests (Figure 3): host infested plants versus non-host infested plants

($N=32$), $\chi^2 = 0.125$, d.f. = 1, $P = 0.7237$; host infested plants versus dually (both host and non-

host) infested plants ($N=30$), $\chi^2 = 0.000$, d.f. = 1, $P = 1.0$; and non-host infested plants versus

dually-infested plant ($N=31$), $\chi^2 = 0.032$, d.f. = 1, $P = 0.8575$.

Figure 3. Olfactometer preferences of mated host-naïve female *C. glomerata* when presented with various pairwise combinations of plant treatments from intact plants (white), host (= *P. rapae*) infested plants (light grey), non-host (= *Pl. xylostella*) infested plants (dark grey), and/or dually (both host and non-host) infested plants (black). Numbers in bars reflect the number of respondents, and were used for the calculation of the χ^2 - and P- values where $P < 0.05$ indicates statistical significance.



Discussion

Parasitoids, and perhaps other natural enemies of herbivores, require reliable cues that indicate quarry identity, location and density to facilitate optimal foraging. As parasitoids are unable to evaluate caterpillar identity using visual assessment at a distance ([Wäckers, 2001](#)), many studies have established that VOCs produced from the plant-herbivore complex are used as detectable, reliable cues that parasitoids use to locate their caterpillar hosts ([Paré & Tumlinson, 1997](#); [Du et al., 1998](#)). The idea that VOCs mediate parasitoid attraction led to the assumption that “three-way interactions” involving plants, herbivore and parasitoid are species-specific, i.e. a particular species of plant under attack by a particular species of herbivore will release a specific blend of VOCs to attract a host-specific parasitoid ([Vet & Dicke, 1992](#)).

Our study provides some evidence that questions this VOC-mediated assumption of exclusiveness: naïve *C. glomerata* did not discriminate between host, non-host and dual infestation types suggesting this parasitoid cannot distinguish VOCs unique to the various plant-herbivore complexes. Hence for at least this parasitoid, it appears that unique VOCs do not mediate the interaction it has with its host *P. rapae* and the plant *B. oleracea* var. *capitata* cv. Sugarloaf, one of the many cultivars of brassicaceous vegetable that its host feeds on. A different plant cultivar may influence parasitoid behaviour in the olfactometer in unpredictable ways. It is also possible that VOC signatures from host infested and non-host infested plants are not unique to this particular plant-herbivore system. For example, [Kugimiya et al., \(2010\)](#) suggested that significant differences in the chemical composition of VOCs released from Chinese cabbage *Brassica rapa* (post *Pl. xylostella* infestation) made day-to-day VOC blends unique from each other. This, however, did not significantly influence the

attractiveness of an infested plant to *Cotesia vestalis*, a parasitoid of *Pl. xylostella*. This may imply that VOCs, though unique in chemical composition by our definition, are perhaps not unique to parasitoids like *C. glomerata* and *C. vestalis*. The most important point is that these parasitoids do not discriminate between apparently unique VOC blends, though why this is the case is still a matter for speculation.

Cotesia glomerata also showed a significant preference for infested plants over non-infested plants, regardless of whether that infestation was from its host or non-host. This implies that *C. glomerata* is attracted to feeding-damaged plants rather than the damage caused by caterpillars of its specific host, and suggests the role of VOCs as "broad-scale" attractive cues to this parasitoid. It is still possible, however, that *C. glomerata* relies on other volatile cues (e.g. host waste, different VOC signature from a different plant-herbivore complex), post alighting physical (vibrations) and short-range chemical cues released from the plant-herbivore complex to detect and locate suitable host caterpillars once in the vicinity or on the plant. Indeed, many other parameters apart from the conventional "VOC" school of thought may mediate the interaction between plant, herbivore and parasitoid.

Future studies which evaluate the post-alighting behavior of *C. glomerata* to host and non-host caterpillars will enable us to draw conclusions about the relationship between the VOCs from the plant-herbivore complex and its influence of the parasitoid's foraging behaviour. It will also be important to quantify and qualify differences in behaviour which occur with experienced females, which may become more efficient at caterpillar location once they "learn" or associate specific cues with successful foraging attempts.

The ability of a parasitoid to detect its host prey is paramount for the survival of subsequent generations, especially if the parasitoid and prey relationship is species-specific

([Weathersbee III et al., 2004](#)). However, there is much uncertainty in the evolution of “three-way interactions” involving agricultural pests and predators because crop plants including cabbages are highly selected cultivars which may perhaps be releasing unreliable signals (VOC blends) when under herbivore attack such that natural enemies respond less efficiently. It is also possible that natural selection has reduced the reliance of *C. glomerata* on a single type of cue (i.e. VOCs from the plant-herbivore complex) for caterpillar location to make it more dependent on a combination of cue types so as to improve its foraging efficiency – a complicated “ménage à trois” indeed!

If, as the results in this study suggests, a parasitoid’s ability for VOC distinction is limited to a certain extent, then parasitoids must rely on other cues or a combination of these to locate their prey in the natural situation. This ability for a parasitoid to be reliant on a range of complementary cue types to forage efficiently is a stable and effective strategy which has the potential to be pervasive among other parasitoids which exhibit similar life cycles.

This, too, may have implications for the manipulation of the environment to improve the efficacy of *C. glomerata* as a biocontrol agent of the pest butterfly *P. rapae*. If attractive cues to *C. glomerata* can be determined, they can be artificially (chemically) synthesized and applied onto crops to attract parasitoids and other predators earlier. This may limit the extent of damage done by the herbivores and provide growers with more savings.

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