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HOST SEARCHING OF PARASITIDS: DOES
INFOCHEMICALLY- COMPLEX HABITAT
AFFECT HOST SEARCHING EFFICIENCY OF
COTESIA WASPS AND COULD THIS AFFECT
INDIRECT DEFENSE OF PLANTS?

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ABSTRACT

Many bioassays on plant- parasitoid interactions have shown that some plant species and even cultivars attract parasitoids at different rates and this suggests that plants differ in their levels of indirect defense. However, such studies have largely focused on a single food chain involving plant-herbivore-parasitoid and ignored that plants growing in natural or agro-ecosystems are simultaneously infested by several species of insect herbivores and not just one. In a wind tunnel bioassay, we employed an ecologically relevant study of flight responses of mated females of a parasitoid, *Cotesia glomerata*, to volatile infochemical cues emitted by potted white cabbage plants. The plants were infested by *Pieris rapae*, *Mamestra brassicae*, or both *Pieris rapae* and *Mamestra brassicae*. The parasitoid preferred odors from *Pieris rapae* (host) - , *M. brassicae* (non-host)-, or *P. rapae* and *M. brassicae* (host and non-host) -infested plants to uninfested plants. When paired up with *M. brassicae*-infested plants, plants infested by both *P. rapae* and *M. brassicae* were more attractive. However, the parasitoid did not prefer odors from *P. rapae* -infested plants to *M. brassicae* -infested plants. We took a step further and compared indirect defense of two cultivars of white cabbage (*Brassica oleraceae* var.capitata) grown in a context of an agro-ecosystem. We compared parasitism frequencies of *Cotesia glomerata* and *C.rubecula* parasitizing their host caterpillars, *Pieris brassicae* and *P. rapae*, which had been feeding on the two cultivars (Badger shipper and Rivera). We hereby report that the cultivars significantly differed in their level of indirect defense. Rivera had a higher level of indirect defense than Badger shipper.

Key words: *Cotesia glomerata*; *C. rubecula*; *Pieris rapae*; *P. brassicae*; *Mamestra brassicae* ; *Brassica oleraceae*;infochemically-diversified habitat; host searching efficiency.

1.0 GENERAL INTRODUCTION

1.1 Self defense by plants

Plants are prone to damage by herbivorous insects (Van Loon et al., 2000; Shiojiri et al., 2001; Kessler & Baldwin, 2002; Dicke et al., 2003; Rostás et al., 2003; Mithöfer et al., 2005) and have developed various chemical defense mechanisms to offset this (Van Loon et al., 2000). The chemical defense mechanisms are broadly classified into two groups, namely, direct and indirect defense respectively (Dicke and Sabelis, 1988; Vet and Dicke, 1992; Turlings and Benrey, 1998; Van Loon et al., 2000; Dicke and Van Loon, 2000; Cortesero et al., 2000; Shiojiri, 2002; Kessler & Baldwin, 2002). Direct chemical defense occurs when secondary metabolites, constitutively or inductively produced by the plants, repel or prevent herbivorous insect from feeding on the plant; or become toxic to the insects following ingestion of a plant material (Van Loon et al., 2000; Lawrence & Novak, 2004). On the other hand, indirect chemical defense mechanism employs provision of foraging cues to natural enemies (predators or parasitoids) of the attacking insects (Dicke et al., 1990a; Takabayashi et al. 1991; Hoballah & Turlings, 2001; Dicke et al., 2003; Turlings & Wäckers, 2004; Schnee et al., 2006).

1.2 Indirect chemical defense

In defending themselves indirectly, plants employ mechanisms that initiate or improve provision of foraging cues to natural enemies of the attacking insects (Dicke et al., 1990a; Takabayashi et al. 1991; Cortesero et al., 2000; Hoballah & Turlings, 2001; Dicke et al., 2003; Turlings & Wäckers, 2004; Schnee et al., 2006). Upon damage by herbivorous insects, plants can produce extrafloral nectars for the attraction of natural enemies (Cortesero, 2000; Baldwin et al., 2001). Insect herbivory may also induce plants to synthesize and emit volatile infochemical cues (Dicke & Sabelis, 1988; Agelopoulos & Keller, 1994; Geervliet, 1997; Hoballah and Turlings, 2005), which direct natural enemies including parasitoids to the damaging herbivores (Dicke et al., 1990a; Vet & Dicke, 1992; Steinberg et al., 1993; Shiojiri et al., 2001; Kessler and Baldwin, 2002; Rostas et al., 2006). These infochemicals are also known as herbivore-induced plant volatiles (HIPVs) (D'Alessandro and Turlings, 2006). Through behavioral and chemical studies, parasitoids have been shown to be attracted to HIPVs that result from damage

caused by their herbivorous hosts (for example: Agelopoulos and Keller, 1994; Geervliet, 1997; Dicke et al., 1998; Shiojiri et al., 2001). Further studies have shown that HIPVs differ from other volatile infochemicals released during the intact state of a plant or when the plant is mechanically damaged (Whitman & Eller, 1990; Agelopoulos & Keller, 1994). Parasitoids can discriminate between these differences and opt to search plants that are damaged by their herbivorous hosts (Dicke et al., 1990b, 2003; Turlings et al., 1990; Steinberg et al., 1993). However, it is still not known whether parasitoids can distinguish volatile infochemical cues emitted by a plant damaged by both a host and a non-host caterpillar from the cues emitted by a plant infested by either a host or a non-host caterpillar. Under natural and even agro-ecosystem conditions, it is ordinary to find a given plant simultaneously being infested by a host and a non-host caterpillar for a certain parasitoid species. The same plant could also be neighbored by a plant of the same cultivar that is undergoing damage by an herbivore that is either a host or a non-host for the parasitoid. Natural and ordinary occurrences of such scenarios warrant a study into how volatile infochemicals emitted in such complex situations would influence host-searching behavior and efficiency of parasitoids.

The herbivore-induced plant volatiles (HIPVs) are a blend of various volatile organic chemical compounds (VOCs) and their compositions can be highly species- and interaction-specific (Von Dahl et al., 2006); the blends vary with the plant (species and cultivar) (Hoballah Fritzsche et al., 2002; Hoballah Fritzsche & Turlings, 2005) and the herbivore that feeds on it (Du et al., 1996) although some compounds are basic to all plants that suffer insect herbivory (see review by Dicke, 1999). Plants of the same species and even cultivar may emit volatile blends that are both qualitatively and quantitatively different when insect herbivores of different species infest the plants (see review by Dicke, 1999; Guerrieri et al., 1999; Hoballah & Turlings, 2005). Conversely, a single species of insect feeding on different plant species and cultivars may cause emission of different volatile chemical blends (Hoballah & Turlings, 2005). This plasticity in synthesis and emission of HIPVs as determined by interaction between plants and host caterpillars may influence host searching behavior, and hence efficiency of parasitoids. This could ultimately cause variation in the levels of indirect defense by various cultivars of a given plant species.

1.2.1 Plants likely differ in indirect defense

Several bioassays using different plants species such as cabbage (Geervliet, 1997; Kalule and Wright, 2004), maize (Hoballah Fritzsche et al., 2002) and cassava (Souissi, 1999) have demonstrated that various cultivars of such plants upon damage by insect herbivores attract parasitoids at different rates. This suggests that plants differ in their level of indirect defense both at species and cultivar levels.

1.3 Parasitoids in general

1.3.1 What are parasitoids?

Parasitoids are insects whose larval development takes place parasitically on or inside the bodies of other arthropods, mostly insects, (Doutt, 1959; Godfray, 1994; Geervliet, 1997; Turlings and Benrey, 1998, Siekmann et al., 2004; Fatouros, 2006). The larvae of parasitoid develop by feeding on the host (Doutt, 1959; Godfray, 1994). Following successful completion of their development, the parasitoids kill their hosts (Doutt, 1959; Turlings and Benrey, 1998; Beckage and Gelman, 2004; Fatouros, 2006). Most parasitoids are parasitic wasps or flies belonging to the order Hymenoptera or Diptera respectively (Fatouros, 2006), albeit hymenopterans are the majority (Doutt, 1959; Godfray, 1994). Several thousands (Doutt, 1959), up to 70, 000, (Fatouros, 2006) species of parasitoids have been described; though it is estimated that the total number of all existing parasitoid species is somewhere between one million to two million (Godfray, 1994).

Parasitoids are important insects; they can find practical use as biological control agents of pest insects (Doutt, 1959; Godfray, 1994). Moreover, parasitoids lend themselves to study by evolutionary ecologists (Godfray, 1994). This is due to their numerous variations on a comparatively simple life -cycle, the uncommonly direct link between observable behavior and quantifiable reproductive success; not to mention many other traits including ownership by many, the means of regulating sex ratio (Godfray, 1994). Not to be overstated is the ability of closely related species to differ not only in dietary specializations (Geervliet, 1997) but also the plasticity with which they learn and retain memory of infochemical cues (Bleeker, 2005).

1.3.2 Parasitoids for control of pest insect.

Many insects that serve as hosts for parasitoids are herbivorous (Turlings and Benrey, 1998). Some of these insect herbivores cause economically significant damage to important crops (Godfray, 1994). Parasitoids are, therefore, among the most promising natural enemies in attaining suppression of pest insects in agro-ecosystems (Murdoch and Stewart 1988; Pedersen and Mills 2004; Wanner et al., 2006). Through the process of endeavoring to reproduce itself, a parasitoid contributes to pest insect control. This occurs when the egg it deposits in/on a host develops into a larva, which grows by feeding in /on the body of the host (Godfray, 1994; Geervliet, 1997). The feeding and related activities of the parasitoid larvae cause developmental arrest of the host insect (Beckage and Gelman, 2004). The arrest occurs when the parasitoid activity inside or on its host impairs the feeding performance of the host insect on host plants (Dicke and Van Loon, 2000). This could confer some fitness on the host plant, for example, increased production of seeds (Dicke and Van Loon, 2000). Eventually the host insect dies when the parasitoid has developed and become independent (Doutt, 1959; Godfray, 1994; Beckage and Gelman, 2004). Through this, the parasitoids control pest insects in an environmentally safe manner (Dicke et al., 2003) as they specifically target their hosts and do not harm non-hosts.

1.3.3 Reproductive success of parasitoids.

Reproductive success of a parasitoid is contingent upon availability of a suitable host (Doutt, 1959; Godfray, 1994; Lewis et al., 1998; Siekmann et al., 2004; Bleeker, 2005; Wanner et al., 2006). Olfactory response of the parasitoid to such a host is the most important factor driving the reproductive success (Wang et al., 2004). Upon emergence from a cocoon, an adult female parasitoid may or may not find itself in a strange environment that is separated from its host populations (Vinson 1998; Wanner et al 2006). The implication here is that a mated female parasitoid may have to fly between various habitats in search of a suitable host on/in which it may oviposit her eggs (Fatouros et al., 2005). After landing on the host the wasp has to assess quality of the host. The gregarious species of parasitoid will have to judge on the number of its offspring that can be supported by the host and possible effect of the host on their individual sizes of the offspring. The parasitoid will also evaluate the number of hosts

that are on the plant, whether or not these hosts contain competitors and when to leave the plant (Poelman, pers.com.)

1.3.4 Host searching process.

Generally, searching behavior is an active movement through which insects hunt for resources (Bell, 1990). Until she successfully parasitizes her host, a parasitoid has to go through a series of different steps ranging from host habitat location, to host location, host acceptance, host suitability, and host regulation (Vinson 1976, 1998). However, the number and types of steps to be followed vary with the type of parasitoid-host relationship (Vinson 1998). The processes leading to successful colonization of a host by a parasitoid are mostly guided by chemical cues from both the herbivore-host and host plant/ feeding substrate harboring the herbivore (i.e., infochemical cues) (Vinson 1976; Lewis and Martin, 1990; Vet and Groenewold 1990; Turlings et al 1990, 1993; Vet and Dicke, 1992; Feener et al., 1996; Morehead 2001; Steidle and Van Loon, 2003; Fatouros et al., 2005a). Infochemical cues (i.e. HIPVs) emitted by the host plants undergoing damage by caterpillars, which play host to parasitoids are more detectable to the parasitoids over long range than infochemical cues coming from the host itself (Vet and Dicke 1992; Vinson 1998; Vet et al., 2002). However, these easily detectable, highly volatile infochemical cues that are emitted by host plants are not authentic indicators of host presence (Vet and Dicke, 1992). Impeccable infochemical cues that are host-specific and are, therefore, strong indicators of host presence usually come from hosts themselves (Morehead 2001; Fatouros et al., 2005b).

1.3.5 Parasitoid efficiency

In view of their small size, adult female parasitoids always have the formidable task of locating often small, scarce and well-hidden hosts in their complex habitats (Geervliet, 1997; Turlings and Benray, 1998). Accordingly, the parasitoids have to evolve high level of efficiency when searching for such hosts. They may make efficient and effective use of chemical signals that plants emit in response to herbivory by the hosts (HIPVs). Efficient host searching mechanisms are essential for a parasitoid's chances of survival and reproduction (Bell, 1990; Geervliet, 1997). A parasitoid is regarded as efficient in host searching when it employs minimum time and energy in locating the host.

Recent findings have shown that genetic diversity in parasitoids belonging to different populations and phenotypic plasticity of individuals, in addition to their physiological state; frequently result in considerable variations in the response to infochemical cues (Lewis and Martin, 1990).

1.3.6 Dietary specialization and infochemical use.

Level of dietary specialization of parasitoids could influence the plasticity of their host searching behaviors (Vet & Dicke, 1992; Steidle & Van Loon, 2003). The level of specialization determines the extent of specificity of information required during search for hosts. Explicit information on the identity of hosts available in a certain habitat should not be very valuable for parasitoids that attack a broad array of hosts, but will be enormously important for parasitoids that are more specialized at the herbivore level. What is more, information on the precise identity of the food plant/feeding substrate should be significant to parasitoids that attack one or more host species that occur on a single plant species/ feeding substrate (Vet & Dicke, 1992; Steidle & Van Loon, 2003).

1.4 The *Cotesia glomerata* and *C. rubecula* parasitoids.

Both *Cotesia glomerata* (L.) and *C. rubecula* (Marshall) are larval endoparasitoids of *Pieris* caterpillars. The parasitoids belong to the order: Hymenoptera and family: Braconidae. Both *C. glomerata* and *C. rubecula* are strongly attracted to plant odors resulting from host larval feeding damage on plants (Sato, 1979; Laing and Levin, 1982; Steinberg et al., 1993). Though they are phylogenetically related and share some traits, the parasitoids differ in some aspects (Geervliet, 1997). The differences are as given here below:

1.4.1 *Cotesia glomerata*

C. glomerata is a generalist in terms of caterpillar- and plant- host ranges; it can parasitize *Pieris rapae*, *P. napi*, *P. melete* and *P. brassicae* (Geervliet, 1997). Its major host in Europe is the gregariously feeding *P. brassicae* (Laing and Levin, 1982; Sato & Ohsaki, 2004). However, the Dutch population of *C. glomerata* can successfully parasitize other hosts like *Pieris rapae* and *P. napi* (Bleeker, 2005). The parasitoid can parasitize these caterpillars when they feed on brassicas or non-brassicas e.g. rosaceous plants. Thus *C. glomerata* is a generalist at both first and second trophic levels. Adult female is approximately 2.6 mm in length. Number of eggs laid by females per host

caterpillar is in the region of 25, hence the parasitoid is gregarious. A female prefers laying in younger instars (first or second instar) to older instars. She can lay a total of 1000-2200 eggs in her lifetime. The adult parasitoid emerges from 5th host instar.

1.4.2 *Cotesia rubecula*

On average, an adult female of this parasitoid measures 3 mm in length. Though it can parasitize *Pieris brassicae*, *P. napi* and *Plutella xylostella*, its preferred host is *Pieris rapae* (Geervliet, 1997). Though *C. rubecula* can be reared in the large cabbage white, *P. brassicae* (L.) (Wiskerke & Vet, 1994), there are fitness penalties that come with development in this host species (Geervliet 1994). The parasitoid prefers parasitizing a host that causes feeding damage on brassica plants; hence the parasitoid is a specialist at both first (caterpillar) and second (plant) trophic levels. The parasitoid is solitary as it lays a single egg per host caterpillar. In total it can lay up to 100 eggs during its entire lifetime. It mostly oviposits in first or second instar larvae, though it can also lay eggs in third instar (L3) caterpillars. The adult egresses from a 4th instar larva when the egg is laid in a first instar (L1) or second instar (L2) caterpillar, and from a 5th instar when oviposition was in an L3 caterpillar (Geervliet, 1997).

Besides the differences outlined above, some additional disparities between the two parasitoid species have lately come to the fore. Though they both find their hosts by relying on herbivore-induced synomones from plants, the two species differ in their use of associative learning (Geervliet, 1997). Under similar conditions, it has been shown that *Cotesia glomerata* can alter its preference in favor of the odors from host a plant after undergoing an oviposition experience on that plant while *C. rubecula* does not change its intrinsic preference (Geervliet, 1997). Recent work by Bleeker (2005) further demonstrates that the two parasitoid species differ in the length of time during which they retain information about the learned host-associated cues. *Cotesia glomerata* can retain such information for as long as five days while *C. rubecula* has such a short memory; it retains the information a day after learning it, but in subsequent days the information is considerably gone (Bleeker, 2005).

CHAPTER TWO.

2.0 RESEARCH QUESTIONS AND HYPOTHESES.

Plants are phenotypically plastic in regard to synthesis and emission of the volatile organic compounds (VOCs) (Dicke et al., 2003). Some plants have been shown to constitutively emit the volatile organic compounds even when not damaged by insect herbivores (D'Alessandro and Turlings, 2006). However, when the herbivores cause feeding damage, the plants increase the level of VOCs emission (D'Alessandro and Turlings, 2006). Such increase takes two dimensions, namely qualitative and quantitative. Furthermore, the phenotypic plasticity in plants can occur such that the same cultivar upon damage by different insect species can emit different profiles of VOCs (Takabayashi et al., 1991; Mattiacci and Dicke, 1995; Du et al., 1996; Dicke and Vet, 1999). This cultivar-herbivore interaction can cause production and emission of some novel HIPVs. Parasitoids can exploit this novelty in HIPVs emission to respond to volatiles indicative of their preferred hosts only (Sabelis & Van der Baan, 1983; Takabayashi et al. 1991; Cortesero et al., 1997; Du et al., 1996, 1998; Turlings and Benrey, 1998; Dicke and Vet, 1999; Ninkovic et al, 2001). In view of the foregoing, we posed the question: do closely related plant cultivars infested by the same herbivore species significantly differ in their level of indirect defense? Based on the very same observations that some novel HIPVs result from certain plant-herbivore interactions, we hypothesized that the related plant cultivars damaged by a similar species of herbivore would attract parasitoids at different rates. Thus the plants will vary in their level of indirect defense. The finding by Du et al. (1996) that HIPVs profile is a product of interaction between plant cultivar and the species of the attacking herbivore and that there is a differential response of parasitoids to these HIPVs begs the question: is there a difference in level of parasitism between caterpillars of closely related species feeding on a given plant cultivar? Based on the finding by Du et al. (1996), we hypothesized that even when feeding on the similar plant cultivars, two phylogenetically -related caterpillars would differ considerably in their level of parasitism.

HIPVs are known to be attractive to parasitoids in laboratory experiments on agricultural plants like Brussels sprouts, red cabbage (Geervliet, 1997; Dicke and van Loon, 2000; Fatouros, 2006) and maize (Turlings et al., 1990; Hoballah and Turlings, 2005). The HIPVs have also been revealed lately to function defensively under natural

conditions (Kessler and Baldwin, 2001; D'Alessandro and Turlings, 2006). Such findings have led to a significantly increasing level of interest in the HIPVs (Turlings et al., 1990; van Poecke & Dicke, 2004). This is with respect to the possibility of exploiting HIPVs for development of new strategies in crop protection (D'Alessandro and Turlings, 2006). So therefore, the first step towards testing this possibility is by attempting to answer the two ecological questions/ test the hypotheses already posed here above. In no doubt studies on indirect defense involving parasitoids have mostly been done under laboratory environmental conditions (Cortesero et al., 2000). Even though laboratory studies are helpful in dissecting the role of HIPVs in mediating indirect defense of plants by parasitoids, they may not be necessarily dependable forecasters of real parasitoid activity in the field. For sure in certain plant–herbivore systems it has been demonstrated that different insect species may elicit different odor blends, leading to preferential oriented flight of natural enemies to plants on which their specific hosts (Du et al., 1996; De Moraes et al., 1998; Guerrieri et al., 1999) were feeding. It remains to be authenticated, however, how this specificity in plant signals can be present amidst substantial variability in induced volatile blends caused by other factors. Main differences in the chemical composition can be found among different plant species (Hoballah Fritzsche et al., 2002), but within a species genotypic effects have also been found to be significant (Loughrin et al., 1995; Hoballah et al., 2002). What is more, abiotic factors such as humidity, temperature, light intensity, light cycle, and nutrient availability all can have an effect on the quantity and the quality of herbivore-induced plant odors (Cortesero et al., 2000; Gouinguéné and Turlings, 2002; D'Alessandro and Turlings, 2006). Hence study of indirect defense of plants as quantified by parasitism frequency of their herbivorous enemies, which may be influenced by the above factors would benefit if they were done in an agro-ecosystem situation. For that reason, we attempted to experimentally answer the two questions stated above by doing our studies in a context of agro-ecosystem.

The reality that HIPVs are largely produced as a consequence of herbivore-specific factors appears to make them relatively reliable as foraging cues for parasitoids (Vet & Dicke, 1992). However, significance of these volatile cues in the attraction of natural enemies under field conditions is still not very well understood (Ockroy et al., 2001). This is because until now, inducible indirect defense involving plant-herbivore-parasitoid interactions has largely been studied for simple linear food chains whereby plants are

infested by a single herbivore species (Shiojiri et al., 2001; Dicke et al., 2003). Nevertheless, in natural and even agroecosystems, it is common for plants to be simultaneously infested by numerous herbivore species at any given point in time (De Moraes et al, 2000; Shiojiri et al., 2001). Again, a plant infested by an insect could be neighbored by a plant of a similar cultivar that is undergoing damage by different species of insects. Some of these herbivores may play host to a particular species of parasitoids while others may not. What could result from the infestation by these numerous herbivores could be a spatial change in quality and quantity of infochemical information for the parasitoids (Lewis and Martin, 1990). Such a change may result in an infochemically-diversified habitat. This now makes it necessary for ecologists to focus their investigations on inducible indirect defense in which parasitoids are involved as key players against a back drop of an infochemically-complex habitat. Host-searching behavior is related directly to a parasitoid's capacity to locate and parasitize hosts under various conditions (Wang & Keller, 2002). Comparative studies of host searching efficiency of parasitoids employing ecologically relevant bioassays are an important instrument for evaluating adaptations for host location. This kind of comparison helps draw unequivocal conclusions that differences in host searching efficiency are certainly associated to the ecological factor under study (in this study infochemically- diversified habitat). In this regard, in the current study we focused on comparing host-searching efficiency of *Cotesia glomerata*, a generalist parasitoid under various levels of habitat heterogeneities/complexities. The question we attempted to answer was: Does level of habitat complexity affect host searching efficiency of a parasitoid? It is known that *C. glomerata* can easily detect and respond to HIPVs produced by its host in a simple habitat where its host is the only species of insect causing damage (Geervliet, 1997). However, it is not known yet how the complexity of HIPVs caused by infestation by two or more insect species could affect flight response and odor choice of the parasitoid. We hypothesized that the less complex the habitat was the higher would *C. glomerata* fly to the host -damaged plant and vice versa.

3.0 RESEARCH METHODOLOGY

3.1 Test for host searching efficiency of parasitoids.

3.1.1 Choice of current study system

The current study investigated host- searching behavior of *Cotesia glomerata* under an infochemically-heterogeneous/complex habitat caused by HIPVs resulting from infestation of a plant by two insect herbivores. This parasitoid can use *Pieris rapae* as a host. However, it cannot parasitize *Mamestra brassicae*. So, therefore, we employed *P. rapae* and *M. brassicae* as a host and a non-host respectively. The specific objective was to compare host searching efficiencies of *C. glomerata* as indicated by flight response to and odor choices of HIPVs in a context of infochemically- diversified habitats. We compared their flight responses to and odor choices of HIPVs emitted under various combinations of plant-host and/ or non-host caterpillar complexes.

3.2 Caterpillar rearing

Pieris rapae and *Mamestra brassicae* were reared on Brussels sprout plants in a climate room (20-22°C, 50-70% RH, 16L: 8D). The small white butterfly (*Pieris rapae*) and adult *Mamestra brassicae* moth, whose eggs develop into the two caterpillars respectively, were kept in gauze cages (40*30*30) holding whole Brussels sprout plants. The butterflies were maintained on water and honey held in a Petri dish placed inside the cage. Eggs laid by the butterflies developed into caterpillars.

3. 3. Parasitoid treatment

Parasitoids that were used in this experiment were 2-5days old mated female *C. glomerata*. The parasitoids were obtained from a laboratory culture. The laboratory culture originated from a starter culture, which had been established from the field cocoons collected within the vicinity of Wageningen. *C. glomerata* was reared on *P. brassicae* feeding on Cabbage cultivar Cyrus under the following conditions: 20-22°C, 50-70% RH, 16L: 8D. Cocoons of the parasitoids were collected from plants and stored in Petri dishes at temperature below 23°C to prevent egression of adults.

To get adult parasitoids, the cocoons in the Petri dishes were placed in glass/wire gauze cages measuring 30cm x 30cm x 30cm and incubated at 23°C under a photo period of 16L: 8D. The emerged parasitoids were maintained on water and honey. Water was

held in a 250- mm beaker. A clean white piece of paper was rolled to form a wick, and then dipped inside the beaker. It is the water that traveled up the paper through capillary action that was accessible to the parasitoids. This way it was not possible for the parasitoids to drown. The honey was applied on the outer wall of the cage. This was done so as to prevent the parasitoids from getting stuck on the honey. The parasitoids were kept under these conditions until they were used in a wind tunnel bioassay. Keeping all the emerged parasitoids together ensured that the females mated with males.

3.4 Odor sources.

Odor sources were based on six to eight weeks old individually potted plants. The plants belonged to one cultivar, white cabbage (*Brassicae oleracea var. capitata*, cv. Christmas drumhead). They were reared in green house under the following conditions: Temperature: 18°C; photoperiod: 16L: 8D; R.H: 70 %. Number of leaves per plant varied from six to ten.

Three different treatments were used as odor sources. These were:

1. Plant/Host Complex (PHC) comprising of the cabbage and host caterpillar (*Pieris rapae*). The plant was inoculated with ten first- instar caterpillars.
2. Plant/Non- host Complex (PNHC) comprising of the cabbage and a non-host caterpillar (*Mamestra brassicae*). The plant was induced with ten first- instar caterpillars.
3. A plant-Host/Non host-Complex (PH/NHC) in which the same plant was induced with both host and non-host caterpillars. The plant was induced with ten caterpillars of *P. rapae* and five *M. brassicae* caterpillars.

In all cases above first in-star larvae were used since Mattiacci and Dicke, (1995) found that the parasitoids prefer infochemical cues resulting from the damage by first in-star larvae to cues from later in-stars. Inoculation was done on intact potted plants and the caterpillars were left to feed for 24 hours immediately preceding the bioassay. The caterpillars were distributed fairly over top three leaves of the plant. This ensured a near-uniform damage on the experimental plants. Odor sources were also obtained from an undamaged plant (CC). This served as a control odor source.

3.5 Wind tunnel bioassay.

The intact potted plants with damaging caterpillars still feeding on them were used in the bioassay.

Two-choice tests were carried out in a wind tunnel suited to a daytime-active, flying insects. The parasitoids were released from a horizontal glass cylinder with both ends open, placed on a socket of 10 cm in height at a distance of 1 m from the odor sources. The cylinder is meant to prevent wasps from immediately flying towards the ceiling, and keep them in the airflow. With the aid of a mechanically damaged leaf of the same cultivar as the experimental plants, individual females were transferred from the emergence cages to the horizontal glass cylinders. They were placed on the bottom of the release point. The females were expected to initiate a flight and chose between the odor sources placed at the upwind end of the tunnel. Complete oriented flight that ended in landing in one of the two odor sources was recorded as a choice. Landings elsewhere in the set up or wasp that did not take off were recorded as not responding. Test for each parasitoid lasted a maximum of five minutes. Each parasitoid was offered only one flight opportunity.

3.6 Odor source combinations.

Flight responses of parasitoids were investigated in the following odor source combinations:

1. Undamaged plants (CC) were tested against Plant-Host caterpillar complex (PHC).
2. Undamaged plants (CC) were paired with Plant/Non- host caterpillar Complex (PNHC).
3. Undamaged plants (CC) were paired with plant-Host/Non- host complex (PH/NHC).
4. Plant-Host caterpillar Complex (PHC) was tested against Plant/Non- host caterpillar complex (PNHC).
5. Plant/Host caterpillar Complex (PHC) was tested against plant- Host/Non host- Complex (PH/NHC).
6. Plant/Non- host caterpillar Complex (PNHC) was tested against plant-Host/Non –host complex (PH/NHC).

Sixty-six parasitoids were tested for every combination of odor sources involving control plants (CC) against the induced plants (i.e., treatments 1-3). For the odor sources in

which the induced plants were paired against one another (i.e. treatments 4-6) one hundred and eleven parasitoids were tested.

Treatments 4-6 were thought to be progressively complex infochemically so we expected that the parasitoid would not clearly make out the volatiles induced by its host caterpillar. The end result would be a low flight response to plants harboring its host.

3.7 Field experiment.

Comparisons of indirect defense of two cultivars and parasitism frequency of their associated caterpillars under agro-ecosystem conditions.

3.7.1 Study site:

Field site for the study was located within the vicinity of Wageningen, The Netherlands.

3.7.2 Materials

Two cultivars of white cabbage (*Brassica oleracea* variety *capitata*) namely, Badger Shipper and Rivera were used. Each cultivar was planted in a plot of its own. Each plot measured 6M x 6M and had 49 plants. A spacing of 75 cm x 75 cm was adopted for the plants. The plots were spaced 6 M apart. In total there were 40 plots, twenty- two of which were grown with Rivera and the other eighteen with Badger Shipper. There were not enough seeds of Badger Shipper available and so the two cultivars could not be equally represented in the 40 plots. The 40 plots were divided over two sites, A and B, separated by 6 meters. We also induced experimental plants in half the plots for each cultivar with first instar larvae of *P. rapae* at the beginning of the experimental period to see if this could influence indirect defense of the two cultivars.

3.7.3 Rearing of caterpillars

Two caterpillars, *Pieris rapae* and *Pieris brassicae* were reared on Brussels sprout plant (*Brassica oleracea* gemminifera cv. Titurel) in a climatic room at 20-22°C, 50-70% RH and a 16L: 8D photo regime. The small white and large white butterflies whose eggs develop into the two caterpillars respectively were kept in gauze cages (40*30*30) holding whole Brussels sprout plants. The butterflies were maintained on water and honey provided on Petri dish placed inside the cage. Eggs laid by the butterflies developed into caterpillars.

3.7.4 Induction of plants with caterpillars

Cabbage plants grown in the field as explained above were inoculated with the caterpillars. First instar larvae were used. Leaves of Brussels sprout plants on which the caterpillars had been feeding in the laboratory were detached and placed in small plastic boxes for transportation to the field. The plants were alternately inoculated by the caterpillars, *Pieris rapae* and *P. brassicae*. If in one week *Pieris rapae* was used for the induction, then in the following week *P. brassicae* was used. Induction was done by placing ten caterpillars on the younger leaf in one plant per plot and leaving them to feed for four days. Use of a fine-tipped paintbrush aided this placement. This way it was expected that due to their feeding, the caterpillars would release into the host plant elicitors that would cause biosynthesis and emission of HIPVs. This would lead to attraction of natural populations of parasitoids (*Cotesia glomerata* and *C. rubecula*) available out in the field to the plant -source of HIPVs and end up parasitizing the caterpillars. On the fourth day following induction, the caterpillars were recollected for dissection to find out if and to what extent they were parasitized.

3.7.5 Method of recollection

The caterpillars were recollected by visiting the same plants that were inoculated and gently picking up the found caterpillars with a paint brush and placing them in vials labeled to make identity of each plot and cultivar of plant unique. It was noted the number of recollected caterpillars per plot over the entire experimental period.

3.7.6 Method of dissection

All the recollected caterpillars were dissected under a dissection microscope at a magnification of X100. All eggs and larvae of *Cotesia glomerata* (CG) and *Cotesia rubecula* (CR) were scored. Encountering more than one egg of *C. rubecula* in a caterpillar caused the caterpillar to be considered as superparasitised. For *C. glomerata* it was so if more than 35 eggs were encountered (Geervliet, 1997).

3.8 Statistical analyses.

3.8.1 Wind tunnel bioassay.

We expected most of *Cotesia glomerata* to fly to the infested plants regardless of whether damaged by host or non-host caterpillars when such plants were paired up with control (undamaged) plants. Additionally, we expected that the parasitoid could not differentiate, at a long distance (1m), the HIPVs from host and non-host damaged plants. Therefore we expected the percent responses towards all the odor source combinations of induced plants to be 50-50. We applied binomial probability function to test for any significant difference between odor choices of the paired odor sources.

3.8.2 Data from field experiment

Data from the field experiment were subjected to statistical analyses with the aid of statistical software, *GenStat 8th* Edition. Data on the recollected caterpillar as well as the fraction of their parasitism were binomially distributed and so we performed stepwise logistic regression. Three models resulted from such analyses: A Null model that indicates the overall deviance in the dependent variables (i.e. frequency of occurrence of recollected caterpillars and fractions of their parasitism) as determined by the variables cultivar, caterpillar, week, induction treatment and the interactions between these variables. We present a final model as a minimum adequate model containing only the independent variables that significantly influenced the outcome variables. The final model resulted from running the presented Full model, with all the independent variables and interactions between them followed by deletion of non-significant effects using the maximum-likelihood method. We used Post-hoc t-probability tests ($\alpha=0.05$) to analyze for statistical differences between groups within significant factors for all models.

Since the number of caterpillars recollected from the field for the two caterpillar species was binomially distributed, we modeled it as a dependent variable with fixed binomial totals of 10. This is because the number of caterpillars applied on each experimental plant was 10 per plot in every experimental week. We modeled the fraction of parasitized caterpillars as a dependent variable in a separate model relating the number of parasitized caterpillars to the binomial total of recollected caterpillars on that plant. We applied the same method when analyzing parasitism of *P. rapae* by the two parasitoids, *C. glomerata* and *C. rubecula*, running separate models per wasp species.

We ran a non-parametric test (*Mann-Whitney U*) to find out if there was any significant difference in the number of *C. glomerata* eggs between *Pieris brassicae* and *P. rapae* caterpillars. Chi-square test was used to analyze frequencies of super- and multi-parasitism by both *Cotesia* species. Superparasitism of *P. rapae* was regarded to occur if more than one egg of *C. rubecula* was found in the caterpillar. For *P. brassicae*, superparasitism occurred if more than 35 eggs of *C. glomerata* were found in a dissected caterpillar. Occurrence of eggs and/or larvae of both *C. glomerata* and *C. rubecula* in *P. rapae* caused the caterpillar to be considered as multiparasitised (Geervliet, 1997).

4.0 RESULTS

4.1 Field experiment

4.1.1 Frequency of recollection

Out of 2400 *Pieris rapae* caterpillars that were inoculated on the cabbage plants in the field over the 13-week experimental period, we managed to recollect 992; while for *P. brassicae* we recollect 1868 out of 2800. We detected a significant difference in the fraction of caterpillars recollect over the experimental period (indicated by weeks) (df=11, deviance=561.076, $P < 0.001$) (Fig.1). The fraction of recollect caterpillars also differed significantly for the caterpillar species (df=1, deviance =343.420, $P < 0.001$) (Stepwise Logistic Regression, Full model deviance=2537.998, df=519; Final model deviance=2537.998, df=519) (Fig.1) and cultivar (df =1, deviance = 8.730, $P = 0.003$). Only cultivar and week interacted to influence frequency of occurrence of recollect caterpillars (*cultivar * week*, df =12, deviance = 29.047, $P = 0.004$), while no interaction was detected between other factors.

Table 1: Measure of parasitism frequencies of *Pieris brassicae* and *P. rapae* caterpillars by *Cotesia glomerata* (C.g) and *C. rubecula* (C.r) on field grown Rivera and Badger Shipper cultivars of white cabbage (*Brassicae oleracea*).

	<u>Rivera</u>	<u>Badger Shipper</u>
Number of recollected caterpillars:		
<i>P. brassicae</i>	1046	821
<i>P. rapae</i>	577	411
Number of caterpillars parasitized:		
<i>P. brassicae</i>	645	380
<i>P. rapae</i>	297	139
Number of caterpillars parasitized by C. g:		
<i>P. brassicae</i>	617	372
<i>P. rapae</i>	146	54
Number of caterpillars parasitized by C. r:		
<i>P. brassicae</i>	28	8
<i>P. rapae</i>	151	85
Number of <i>C. glomerata</i> eggs:		
<i>P. brassicae</i>	2381	1570
<i>P. rapae</i>	591	240
Number of <i>C. rubecula</i> eggs:		
<i>P. brassicae</i>	20	8
<i>P. rapae</i>	77	53
Superparasitism by <i>C. rubecula</i> :		
<i>P. rapae</i> (<i>C. rubecula</i> + <i>C. rubecula</i> >1)	3	2
<i>P. brassicae</i> (<i>C. rubecula</i> + <i>C. rubecula</i> >1)	1	0
Superparasitism by <i>C. glomerata</i> :		
<i>P. brassicae</i> (<i>C. glomerata</i> + <i>C. glomerata</i> >35)	17	12
<i>P. rapae</i> (<i>C. glomerata</i> + <i>C. glomerata</i> >35)	0	0
Multiparasitism:		
<i>P. brassicae</i> (<i>C. glomerata</i> + <i>C. rubecula</i> >1)	98	63
<i>P. rapae</i> (<i>C. glomerata</i> + <i>C. rubecula</i> >1)	53	27

Fractions of recollected caterpillars over experimental weeks.

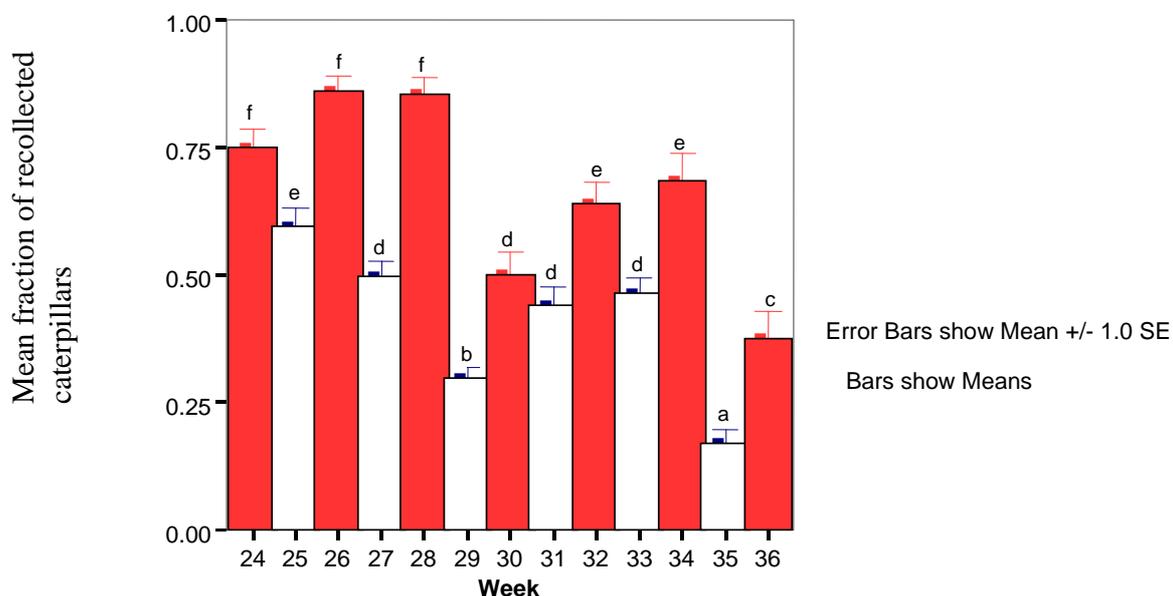


Fig. 1. Fractions of recollected caterpillars over experimental weeks. Means with different letters are significantly different according to Tukey-Kramer post hoc multiple mean comparison ($p < 0.05$); means with the same letters are not significantly different. Weeks with even numbers represent *P. brassicae*; odd numbers are for *P. rapae*.

4.1.2 Frequency of parasitism.

Frequencies of parasitism varied significantly with the study weeks (*week*: $df=11$ deviance=1188.673, $P<0.001$) (Fig. 2), cultivars (*cultivar*: $df=1$, deviance=51.962, $P<0.001$) (Table1) and caterpillar species (*caterpillars*: $df=1$, deviance=61.428, $P<0.001$) (Fig 3 and Table 1). *C. glomerata* parasitized *P. rapae* and *P. brassicae* at different frequencies between the two cultivars (*cultivar*: $df=1$, deviance=22.891, $P<0.001$). Parasitism frequencies of these caterpillars by *C. glomerata* were higher in Rivera than in Badger Shipper (Table1).

P. brassicae was parasitized by either *Cotesia glomerata* or *C. rubecula*. However, the low number of *C. rubecula* eggs recovered from *P. brassicae* did not warrant analysis.

Only cultivar and week interacted to determine frequency of parasitism (*cultivar*week*: $df=11$, deviance =73.554, $P<0.001$), while the other factors did not. Inducement treatment of the experimental plants with *P. rapae* at the beginning of the season did not affect frequency of parasitism (*treatment*: $df=1$, deviance =1.097, $p<0.295$).

Mean fractions of parasitism frequency of *Pieris brassicae* and *P. rapae* over the entire experimental period.

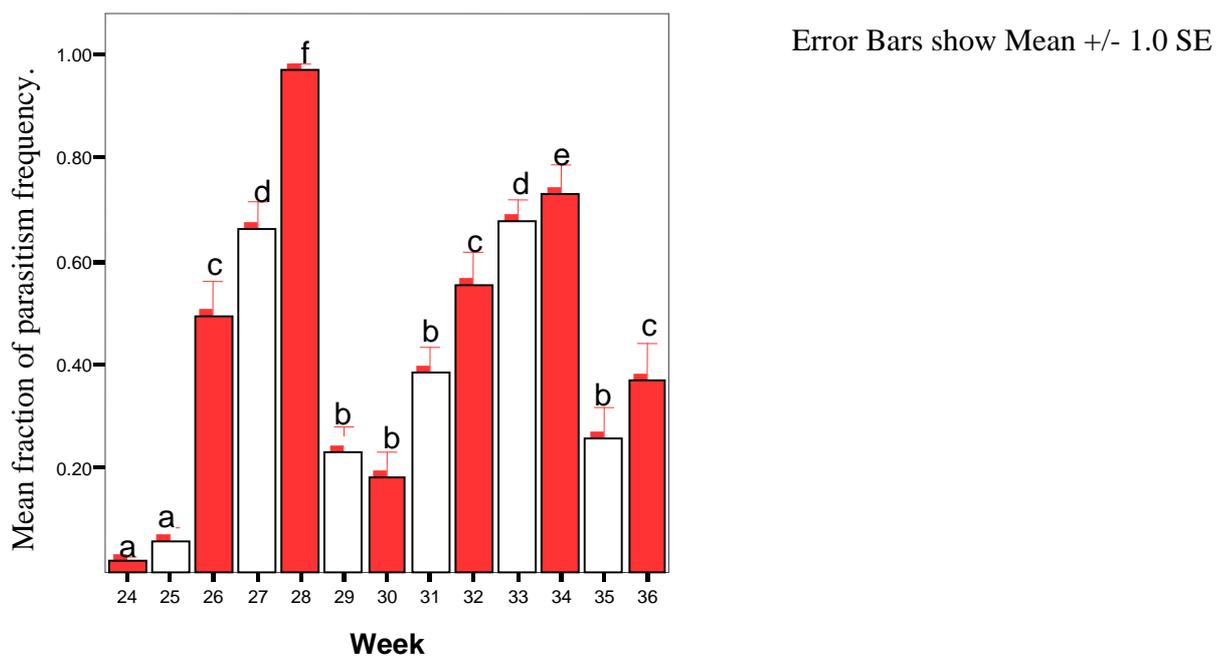


Figure 2 Mean fractions of parasitism frequency of *Pieris brassicae* and *P. rapae* over the entire experimental period indicated by weeks 24-36. Bars show means. Means with different letters are significantly different according to Tukey-Kramer post hoc multiple mean comparisons ($p < 0.05$); means with the same letters are not significantly different. Weeks with even numbers represent *P. brassicae*; odd numbers are for *P. rapae*.

Parasitism frequency per caterpillar per cultivar.

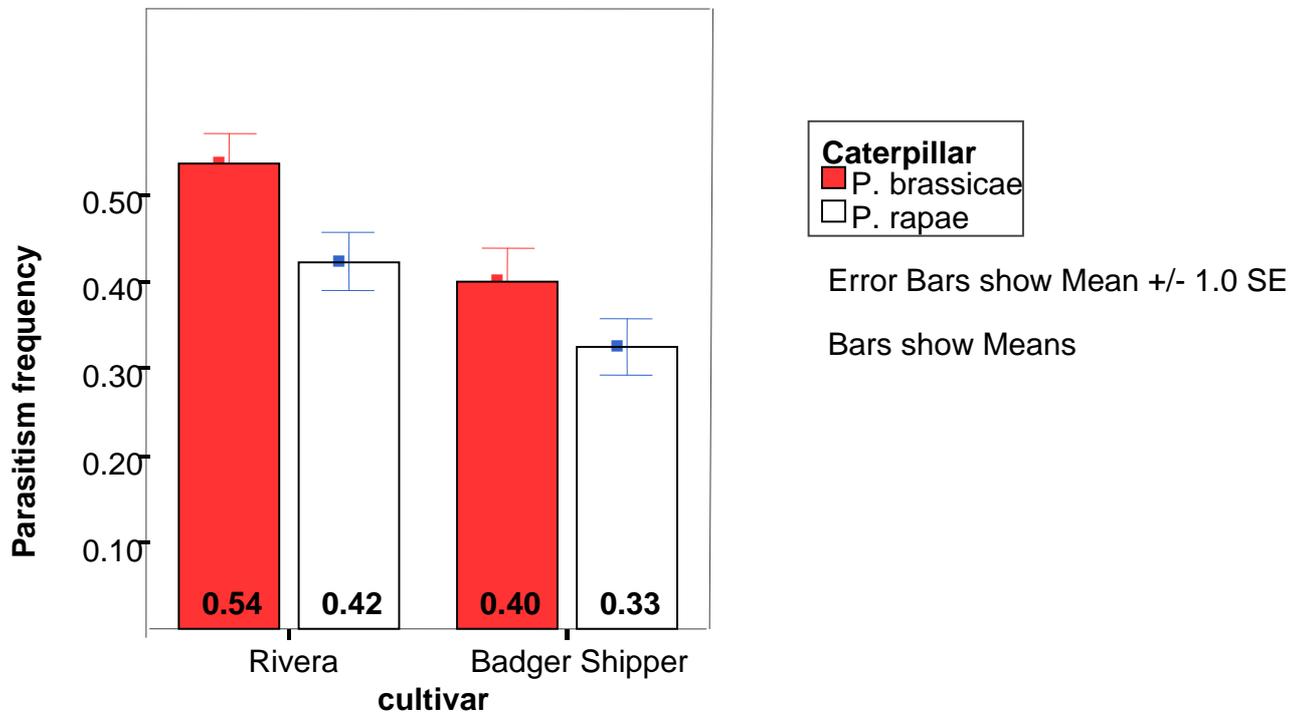


Fig. 3. Parasitism frequency of *Pieris brassicae* was higher than *P. rapae* (caterpillars: $df=1$, $P<0.001$). Parasitism frequency was higher on Rivera than Badger shipper (cultivar: $df=1$, $P<0.001$). Both analyses were based on Stepwise Logistics regressions.

P. rapae caterpillars were parasitized by *C. glomerata* or *C. rubecula*. Parasitisation frequencies of *P. rapae* by *C. rubecula* were slightly significantly different over cultivars (Rivera exhibited higher parasitism frequency than Badger Shipper) (cultivar: $df=1$, deviance = 4.020, $P=0.045$) (Stepwise Logistic Regression) but were largely different over the experimental weeks (week: $df=5$, deviance=98.848, $P<0.001$) (Stepwise Logistic Regression) (Fig.4).

Mean fraction of parasitism frequency of *Pieris rapae* parasitized by *Cotesia rubecula* over the experimental period.

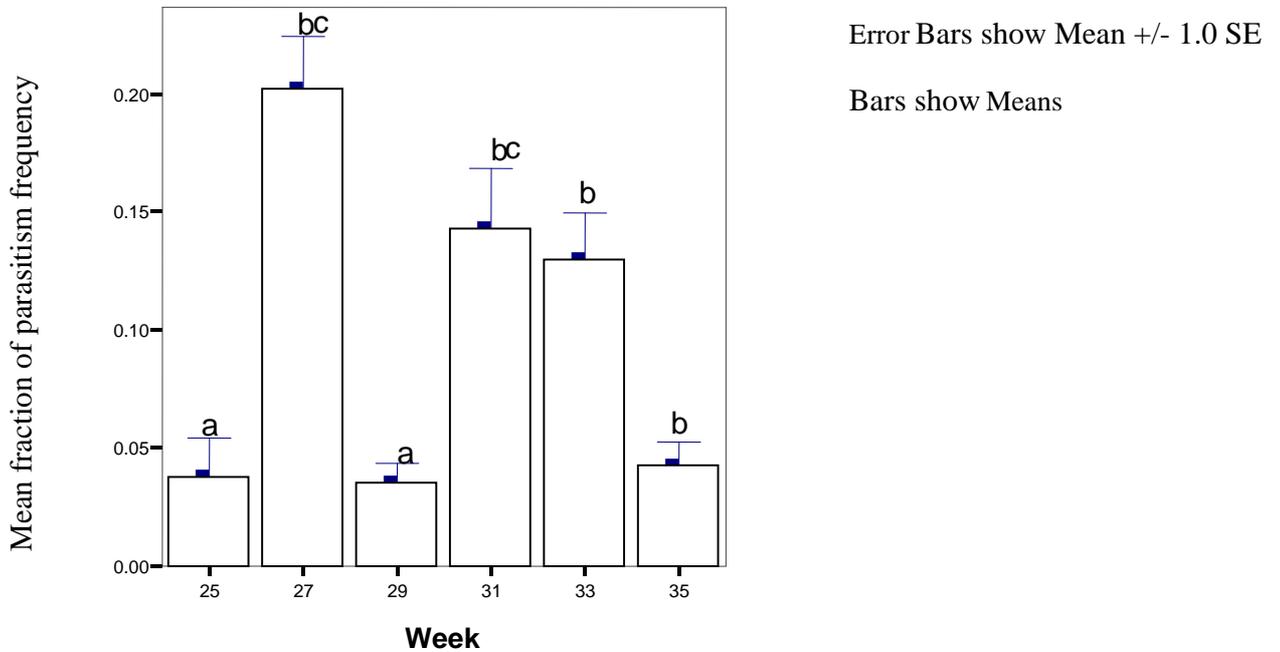


Figure 4. Mean fraction of parasitism frequency of *Pieris rapae* by *Cotesia rubecula* on Rivera and Badger Shipper over the growing season. Means with different letters are significantly different (Tukey-Kramer post hoc multiple mean comparison, $p < 0.05$); means with the same letters are not significantly different.

Time affected parasitization frequency of *P. rapae* by *C. glomerata* (*week*: $df=5$, deviance=265.757, $P<0.001$) (Fig. 5) and an interaction between cultivar and time (*cultivar and week*: $df=5$, deviance =33.047, $P<0.001$) significantly affected the parasitization as well.

Mean fraction of parasitism frequency of *Pieris rapae* parasitized by *Cotesia glomerata* over the experimental weeks.

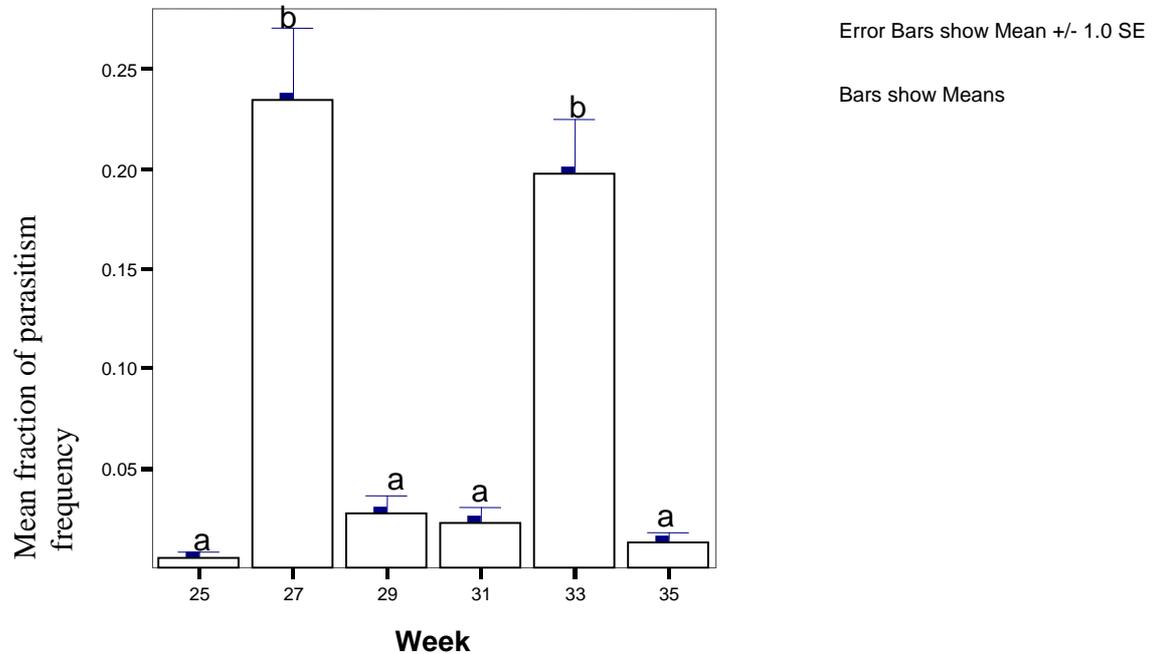


Figure 5. Mean fraction of parasitism frequency of *Pieris rapae* by *Cotesia glomerata* on Rivera and Badger Shipper over the growing season. Means with different letters are significantly different (Tukey-Kramer post hoc multiple mean comparison, $p < 0.05$); means with the same letters are not significantly different.

We found that *Pieris brassicae* had more eggs of *Cotesia glomerata* than *P. rapae* in either cultivars of white cabbage ($U = 2090.5$, $P < 0.001$) (Table 1 and Fig.6). Number of *C. rubecula* eggs in *P. rapae* was higher than in *P. brassicae* ($X^2 (1) = 64.935$, $P < 0.001$) (Table 1). More eggs of *C. rubecula* were found in *P. rapae* feeding on Rivera than on Badger Shipper ($X^2 (1) = 7.506$, $P = 0.006$) (Table 1). ANOVA could not be performed because data on number of eggs of *C. glomerata* and *C. rubecula* were not normally distributed, even after trying to normalize them.

Mean number of *C. glomerata* eggs per caterpillar per cultivar.

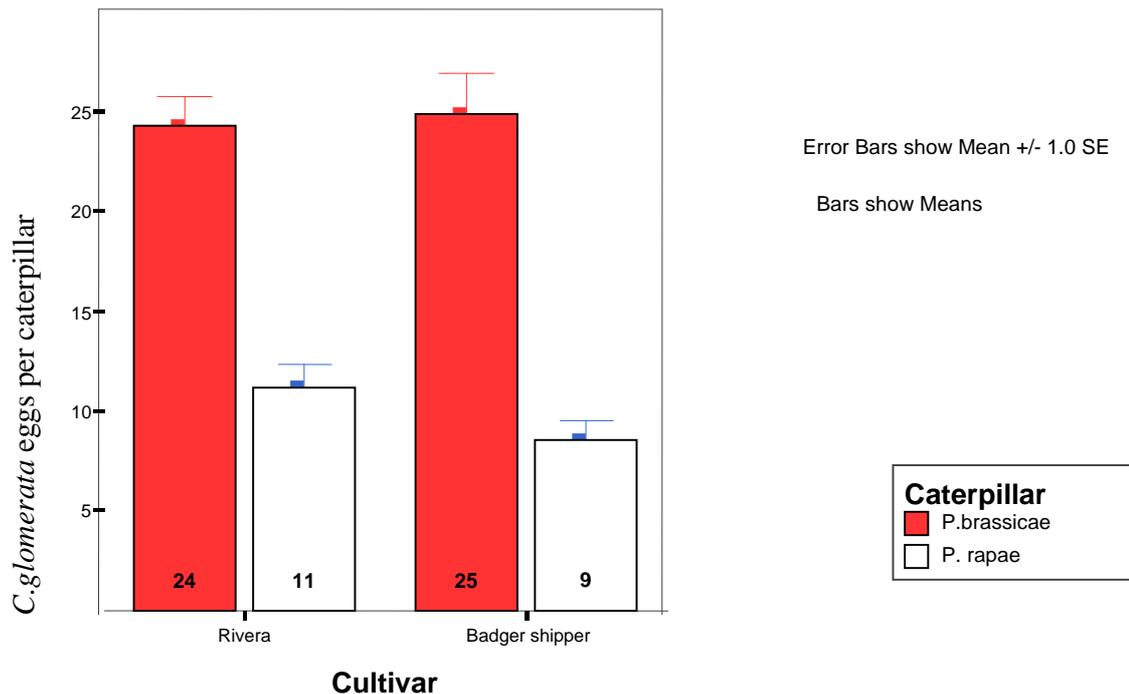


Fig. 6. Mean number of *Cotesia glomerata* eggs in *Pieris brassicae* and *P. rapae* over the 13-week experimental period. The mean is based on parasitized caterpillars only. *C. glomerata* eggs in *P. rapae* were more in Rivera than in Badger Shipper.

C. rubecula parasitized more *P. rapae* than *P. brassicae* ($X^2 (1) = 41.295, P < 0.001$) (Table 1). Parasitism frequency of the *P. rapae* by *C. rubecula* was higher in Rivera than in Badger shipper ($X^2 (1) = 9.328, P = 0.002$) (Table 1).

4.1.3 Frequency of Superparasitism and multiparasitism

Frequency of superparasitism of *P. brassicae* by *C. glomerata* did not differ between the two cultivars ($X^2 (1) = 0.862, P = 0.353$) (Table 1). No significant difference in frequency of superparasitism by *C. rubecula* was observed between *P. rapae* and *P. brassicae* ($X^2 (1) = 2.667, P = 0.102$). Superparasitism of *P. rapae* by *C. rubecula* did not differ between

Rivera and Badger Shipper ($X^2(1) = 0.667, P = 0.414$) (Table 1). Multiparasitism of *P. brassicae* by both *Cotesia glomerata* and *C. rubecula* was higher in Rivera cultivar than in Badger Shipper ($X^2(1) = 7.609, P = 0.006$) (Table 1).

The same trend was observed in *P. rapae* where multiparasitism by the two parasitoids was higher in Rivera ($X^2(1) = 8.450, P = 0.004$) (Table 1).

4.2 Wind Tunnel bioassay

When presented with a choice, female *Cotesia glomerata* exhibited a significant preferential oriented flight to a potted cabbage plant infested by both *Pieris rapae* and *Mamestra brassicae* when the other option was uninfested plant (Fig.7). When undamaged plant was paired up with *P. rapae*- or *M. brassicae*-infested plant, *C. glomerata* preferred odors emitted by the latter and flew towards it. However, the parasitoid did not exhibit a preference between *P. rapae*-infested plant and a plant infested by *M. brassicae* (Fig.7). The parasitoid preferred the odors from *M.brassicae/P.rapae*-infested plant to *M.brassicae*- or *P.rapae*-induced volatiles (Fig.7).

Flight responses of *Cotesia glomerata* females to different odor source combinations.

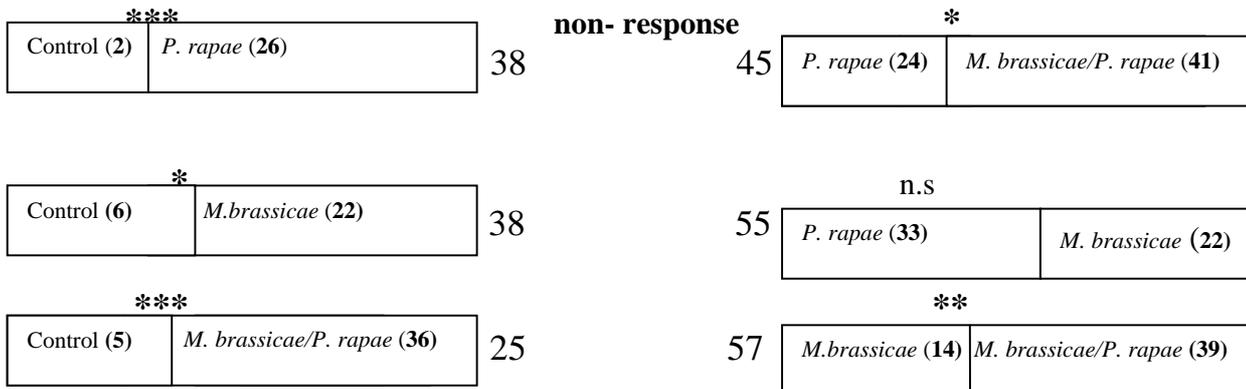


Figure 7. Flight responses of *Cotesia glomerata* females to different odor source combinations. Asterisks indicate significantly different odor preference within the binomial probability function (* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$ n.s. = nonsignificant odor preference). Numbers of responding wasps are in bracket; numbers close to the bars are those of wasps that did not respond to odor source combinations; control = uninfested plant. Bars not drawn to scale.

5.0 DISCUSSION

5.1 Wind tunnel bioassay

These results indicate that *C. glomerata* generally responds to volatiles emitted by cabbage plants infested by a host caterpillar (*P. rapae*), a non-host (*M. brassicae*) or both host and non-host caterpillars when the other option is an uninfested plant. Geervliet (1997) showed that *C. glomerata* preferred Brussels sprout infested by nonhost caterpillars (*Plutella xylostella* and *Mamestra brassicae*) to uninfested plants. Shiojiri et al. (2001) also reported that *C. glomerata* favored volatiles emitted by *P. rapae* (host)-infested cabbage to volatiles from uninfested cabbage, and that the parasitoid did not prefer *P. rapae* (host) - infested to *Plutella xylostella* (non-host)-infested cabbage. Our current data support these observations. We did not detect a statistical difference in odor choice of *C. glomerata* between *P. rapae*-infested plants and *M. brassicae*-infested plants (Fig.7).

It seems that when the cabbage plant is simultaneously infested by *P. rapae* and *M. brassicae* then the profile of volatile blends increases and that is why *C. glomerata* preferred *P. rapae* / *M. brassicae*-infested cabbage to *M. brassicae* or *P. rapae*-infested cabbage (Fig. 7). The current results suggest that to *C. glomerata* (a generalist parasitoid), long range (1m) flight response to HIPVs is not affected by whether the volatile synthesis and emission is induced by a host or non-host caterpillar. The observation that *C. glomerata* prefers *M. brassicae*/*P. rapae*-infested plants to *P. rapae* (host) -or *M. brassicae* (non-host)-infested plants indicate that at long range, what is important to a generalist parasitoid is the amount of HIPVs it can detect and not the specificity of such volatiles. This is despite the possibility that a plant that was infested by both *M. brassicae* and *P. rapae* could have produced larger quantity of HIPVs than the *M. brassicae*- or *P. rapae* – infested plants. Geervliet (1997) observed that naive *C. glomerata* discriminated over long range (1m), volatiles emitted by plants infested by host herbivores of various densities. Our current data seem to validate such observation. The data also support the hypothesis by Vet and Dicke (1992) regarding dietary specialization and infochemical use by parasitoids. They state that for a generalist parasitoid (at both first and second trophic levels), long-range detection and flight

response to HIPVs is not influenced by specificity of the herbivore inducing such volatiles. Based on our results, we hypothesize that the generalist parasitoid is not efficient when searching for a host in an infochemically-diversified/complex habitat. The parasitoid does not make out in-flight the plant infested purely by hosts. The parasitoid ends up losing energy and time by preferring plant odors induced by both host- and non-host caterpillars when the alternative plant odor is that purely induced by a host caterpillar. The probability of getting a host on the former plant will be lower than in the latter plant. Furthermore, a plant occurring in such a complex habitat will be less defended by the generalist parasitoid.

5.2 Field experiment

We detected a significant difference between the two caterpillars, *Pieris brassicae* and *P. rapae*, in the frequency at which they were recollected (Fig.1). More *P. brassicae* than *P. rapae* were recollected. This difference was affected in part by either the cultivars (Rivera and Badger Shipper) or time of the growing season (weeks). An interaction between these two factors further accounted for the difference in frequency of the recollected caterpillars. It is possible that the significant difference in frequency of recollected caterpillars as influenced by the cultivar was due to different architecture of the cultivars. Rivera had a bigger and thicker canopy than Badger Shipper. So it is likely that some caterpillars penetrated into the inner leaves of the Rivera and this probably shielded them from being spotted during recollection. Furthermore, with the passage of time the plants experienced different growth rates and hence differential rate of canopy formation. This could explain why time interacted with cultivar to partly influence the frequency of recollected caterpillars. Holding the other factors constant, it may well be the case that the different feeding habits of the two caterpillar species also impacted on the frequency of their recollection. *Pieris rapae* is a cryptic solitary feeder (Geervliet, 1997) while *P. brassicae* is an aposematic gregarious feeder (Laing and Levin, 1982; Harvey et al., 2003; Sato & Ohsaki, 2004). *Pieris rapae* is able to spread quickly within a plant (Fahring and Paloheimo, 1986). Possession of such a trait enables it to penetrate into inner leaves of a plant and so it may be difficult to relocate. The gregarious feeding and aposematic coloration resulting from sequestration of glucosinolates make *P. brassicae* easier to spot in a plant. The observation that more *P. brassicae* than *P. rapae*

was recollected in Rivera than in badger shipper (Table 1) could be due to difference in glucosinolate levels between the plant cultivars. This could be so because *P. brassicae* sequesters glucosinolates, which makes it very noticeable (i.e. aposematic).

Morphological traits of plants can influence host accessibility by the parasitoids (Cortesero et al., 2000). Such plant traits as shape, wax content and hardness of leaf can either aid or hamper movement of parasitoids after landing and hence influence the encounter rate with its host (Cortesero et al., 2000). Wax makes the leaf surface slippery and so the parasitoid can fall off the leaf when probing for the host. This fact cannot be left out when trying to account for difference in frequency of parasitism between the two cultivars. Likely Badger shipper cultivar had more wax (that negatively affected the parasitoids) than Rivera.

The biosynthesis of herbivore-induced synomones is *de novo* (Pare and Tumlinson, 1997; D'Alessandro and Turlings, 2006). Any compound that comes from herbivores and interacts with the plant on a cellular level is a potential elicitor of synomone production (Kessler and Baldwin, 2002). Thus far, herbivore-specific elicitors have been isolated from oral secretions of some lepidopteran species including *Pieris brassicae* (Kessler and Baldwin, 2002). The herbivore-induced synthesis of synomones may employ induction of enzyme activity (Mattiacci et al., 1995; Bouwmeester et al., 1999; Degenhardt and Gershenzon, 2000). An enzyme, β -glucosidase, which elicits HIPVs emission, has been identified in *Pieris brassicae* (Mattiacci et al., 1995). Variation in volatile profiles of plants infested by different herbivores suggests that each insect species may produce its own signature molecule(s) that allows plants to differentiate among herbivorous attackers (Takabayashi, et al., 1991; Paré et al., 1998). Though β -glucosidase has been identified in *P. brassicae*, it is unknown whether *P. rapae* also possesses β -glucosidase. If indeed it possesses the enzyme, it is not known if it is exactly the same as that possessed by *P. brassicae*. It is possible that the difference in parasitism frequency we noted between the caterpillars (Table 1 and Fig. 3) even when infesting similar cultivars was due to possession of different elicitor enzymes or different levels of the enzymes that ended up causing a differential emission of HIPVs between the two cultivars; thus varying degree of attractiveness to parasitoids.

HIPVs-blends are complex and comprise a range of different volatile organic compounds (VOCs), including alkanes, alkenes, aldehydes, alcohols, ketones, ethers,

esters and carboxylic acids (D'Alessandro and Turlings, 2006). Methanol (an alcohol) is one of the constituent compounds of VOCs (D'Alessandro and Turlings, 2006). It is one of the quantitatively most important VOCs (Von Dahl et al., 2006). Von Dahl et al. (2006), in their study of *Nicotiana attenuata*- *Manduca sexta* interaction found that methanol results from enzymatic demethylation of pectin by pectin methylesterase (PME). They demonstrated that wounding of *N. attenuata* by *M. sexta* increased methanol emissions, which are commonly at their highest levels during early stages of leaf development (Von Dahl et al., 2006). Oral secretions of *M. sexta* contained high PH and this activates PME. Pectin polymers account for 35% of the cell wall mass of dicotyledonous plants, and consist of α -D-galacturonic acid units with variable amounts of methyl esters. The esterification of pectin is thought to increase the fluidity of the cell wall. Demethylation of pectin occurs during leaf maturation. Since the cultivars we used are dicotyledonous, could be they possessed innately different levels of pectin, demethylation of which resulted in emission of methanol at varying levels.

Inducibility of indirect defenses requires information processing in the plant (Van Poecke and Dicke, 2002). Such information processing depends on two important signal transduction pathways: octadecanoid pathway that employs a plant hormone known as jasmonic acid (JA) as one of the most important compounds (Van Poecke and Dicke, 2002). Salicylic acid pathway in which a plant hormone, salicylic acid (SA) plays a crucial role is the other signal transduction pathway (Dempsey et al., 1999). Both the octadecanoid and the salicylic acid pathway play a significant role in the attraction of parasitoids (Dicke and van Poecke, 2002) as they activate defensive genes that initiate synthesis and emission of HIPVs (Tooker and De Moraes, 2005). The pathways may act antagonistically against each other (Van Poecke and Dicke, 2002); alternatively, they have been shown to act synergistically (Xu et al., 1994). The antagonistic or synergistic interactions of the two pathways depend on the plant-herbivore complex in question. Damage by herbivore insect causes an increase in the level of jasmonic acid, synthesis of which is regulated by an enzyme lipoxygenase (Reinbothe et al., 1994; Bell et al., 1995; Bleichert et al., 1995). Lipoxygenase catalyze the hydroperoxidation of certain C-18 unsaturated fatty acids, providing intermediates for formation of jasmonic acid (Bell et al., 1995). It could be the case that Rivera has a higher concentration of jasmonic acid than Badger Shipper and that could be the reason as to why the former cultivar exhibited

a higher indirect defense than the latter cultivar. Van Poecke and Dicke (2002) showed that herbivore-infested transgenic *Arabidopsis thaliana* plant that exhibits a highly reduced expression of *lox2*, a lipoxygenase isozyme and does not show wound-induced accumulation of JA was less attractive to *Cotesia rubecula* parasitoid than herbivore-infested wild-type plant of the same genotype.

Not only is lipoxygenase important in the production of JA but also green leaf volatiles (GLVs), like hexanol and (*Z*)-3-hexen-1-ol (Gardner, 1995). Chemoreceptors on the antennae of both *Cotesia rubecula* and *Cotesia glomerata* females respond to these green leaf volatiles (Smid, et al., 2002). Hence plant cultivars with low level of lipoxygenase will emit low amounts of the green leaf volatiles and it is likely that the parasitoids will respond to the (GLVs) differently. Such could be the case in our present study; the two cultivars we used exhibited different levels of indirect defense as shown by the difference in parasitism frequencies of the *Pieris* caterpillars between the cultivars (Table 1 & Fig.2).

Our current study detected a significant difference in parasitism frequency of the two *Pieris* caterpillars on the two cultivars of white cabbage (Fig.2). Discrete dissimilarities may occur between HIPVs from different plant species or cultivars (Geervliet, 1997; Hoballah and Turlings, 2005). Takabayashi et al., (1991) demonstrated that chemical differences between HIPVs coming from different apple cultivars infested by the same spider mite species are much more varied than differences between individuals of one apple cultivar infested by different spider-mite species. Therefore, genotype of a plant seems to play a significant role in affecting the composition of the chemical information emitted as a consequence of insect herbivory. In relation to indirect defense, it is expected that different cultivars of a given plant species may vary in their level of attractiveness to parasitoids. This could explain why differential parasitism as well as superparasitism frequencies were observed between the two cultivars (Rivera and badger Shipper) we used.

The indirect defense in plants is plastic as the induction and emission of HIPVs depends on the interaction between an array of biotic factors, such as plant hormones, herbivore-derived elicitors, associated microorganisms and abiotic factors, such as wounding, O₃ and CO₂ concentration, temperature and light, UV-radiation and many other factors (Gouinguéné and Turlings, 2002; D'Alessandro and Turlings, 2006; Rostas

et al., 2006). Interactions between these factors differentially activate various signaling pathways (Kessler and Baldwin, 2002). This will likely cause a spatio-temporal variation in HIPVs depending on what factors are in play where and at what time. Results of our current study can be discussed in light of the generality of the foregoing. This is because we found a statistically significant interaction between cultivar and time as a determinant of the frequency of parasitism of the two caterpillars on Rivera and badger Shipper. It is possible that HIPVs emitted by the two cultivars of white cabbage vary with time. This could be due to a time-dependent change in physiology of the plant cultivars, environmental (biotic and abiotic) conditions or both. Change in climatic conditions may not only influence physiology of a plant, it may also influence availability of microorganisms (pathogenic or not). Rostas et al., (2006) found that fungal infection reduces herbivore-induced plant volatiles other than green leaf volatiles in maize. This may not reduce parasitisation rate by naive parasitoids, which mainly depend on green leaf volatiles for host location. However, experienced parasitoids may be affected since they use specific volatiles other than green leaf volatiles (Bleeker et al., 2005). It may also be the case that microorganisms, availability of which may be influenced by such weather conditions as humidity and air temperature interacted with the two cultivars differently and this determines the production and emission of HIPVs.

Zhang et al., (1999) found that within a space of less than two months, expression of green leaf volatiles including (*Z*)-3-hexen-1-ol in a plant *Betula pendula* changed very significantly, mostly due to maturation of leaves of the plant. The same observation that maturation of plant leaves influences the level of green leaf emission can be invoked to explain our finding that the Rivera and Badger shipper cultivars showed difference in indirect defense. Indeed we observed that Badger shipper matured at a faster rate than Rivera. When we talk of maturation here we mean how soon a cultivar forms a crop (i.e. cabbage head) and the outer leaves become moribund. Changes in green leaf volatile production over the growing season could therefore have influenced parasitoid attraction, particularly the naive ones that respond mostly to green leaf volatiles.

In some instances, the process that leads to the alteration of plant volatile profiles seems to be dependent on the size of the herbivore infestation levels, the larval instar and the duration of feeding activity (Dicke et al., 1993; Tumlinson et al., 1993; Takabayashi et al. 1995; wind tunnel data on current study). It can further be suggested that the

observed temporal variation in parasitism frequency could as well be due to presence of natural populations of caterpillars resulting from deposition of eggs on the experimental plants by adult butterflies, availability and population density of which likely varied with the growing season. The possible implication here is that there was an overlap in the availability and density of natural populations of the feeding caterpillars of varying instars. Again, population dynamics of natural populations of parasitoids could have affected parasitism frequency. May be the there was a temporal variation in availability of such parasitoids.

The higher multiparasitism frequencies of *P. brassicae* and *P. rapae* when the caterpillars infested Rivera than when they infested Badger Shipper (Table 1) indicate that Rivera produced higher volatiles profile than badger Shipper. Therefore, most of both *Cotesia glomerata* and *C. rubecula* were attracted to volatiles from the Rivera.

Presence of a conspecific or heterospecific competitor influences a foraging decision made by a parasitoid (Visser et al., 1990). In a wind tunnel experiment, Geervliet (1997) demonstrated that *Cotesia glomerata* preferentially flew towards a cabbage plant infested with a *Pieris rapae* caterpillar when the other option was that of a similar plant on which *Cotesia rubecula* was parasitizing *P. rapae*. Presence of a natural enemy like a hyperparasitoid may also affect behavioral response of a parasitoid to volatile infochemical cues coming from an herbivore-damaged plant in a similar manner (Höller et al., 1994). Both these factors cannot be overlooked in trying to explain why we observed a temporal variation in parasitism in the two cultivars of cabbage we used. Could be the presence of natural enemies and competitors of the *Cotesia* parasitoids in the study site varied with time, and this influenced the parasitism frequency by *Cotesia* spp.

5.3 Conclusions

From the wind tunnel bioassay we conclude that naïve *C. glomerata* are less efficient when searching for hosts in an infochemically complex habitat. Results of the field experiment clearly indicate that closely related plant cultivars differ in their level of indirect defense. Again, *P. brassicae* was more parasitized than *P. rapae* even when they each infested similar cultivars.

5.4 Future prospects (field experiment)

In indirectly defending themselves, plants play an active role by mediating the interaction between entomophagous arthropods and herbivores. Chemical and morphological traits of plants can directly influence survival, fertility, and foraging success of parasitoids in or on hosts. These properties can also have indirect effects by affecting qualities of an herbivore that in turn affect the physiology, behavior, or development of parasitoids (Turlings and Benrey, 1998; Cortesero et al., 2000). Results from our field study clearly indicate that one cultivar (Rivera) had significantly higher level of indirect defense than the other (as measured by parasitism frequency of the caterpillars). However, it is still not known how the cultivar with a better indirect defense would affect life history traits of the parasitoid species. For example, physiology, flight response to hosts, sex ratio, and even development of the parasitoids are the traits that could be negatively affected. Hence it is important to initiate a study into these aspects by making use of Rivera cultivar. Long term success of biological control of pest insects using parasitoids would depend not only on having a plant that is good at attracting the natural enemy, but also one that does not negatively affect the life history traits of subsequent generations of parasitoids.

Our data also reveal that there was a seasonal variation in indirect defense. This suggests a time-dependent change in quantity and/or quality of phytochemicals including phytohormones. Accordingly, it would be interesting to study how this temporal variation of phytochemicals would affect quality and hence suitability of host caterpillars for the parasitoids.

Generally, not much is known about the influence of environmental factors on the synthesis and emission of HIPVs of plants including white cabbage. Water stress and soil nitrogen have been shown to have a strong bearing on the capacity of cotton plants to systemically release parasitoid-attracting volatiles (Cortesero, et al., 2000). Besides the water stress, season and light have also been shown to affect emission of volatile cues in lima bean (*Phaseolus lunatus*) plants (Takabayashi et al., 1994). These observations open an avenue for studying how agronomic conditions can be manipulated to find out the best practices that would result in optimal synthesis and emission of HIPVs by white cabbage (Rivera). Such knowledge is vital for shaping growing conditions that may enhance plant signaling and thereby improve the ability of parasitoids to effectively find hosts.

Physiology and biochemistry of the cultivar with a higher indirect defense (Rivera) needs to be studied. Such a study could help elucidate the key signaling pathways that principally account for HIPVs synthesis and emission. Ultimately, through breeding, cultivars with high indirect defense even at low level of infestation by herbivores can be generated. This can be done through over expressing a gene responsible for synthesis of HIPVs.

5.4.1 Future prospects (wind tunnel bioassay)

In the wind tunnel bioassay we studied odor choices of naïve females of *Cotesia glomerata*, however under natural and agro-ecosystem situations there exist both naïve and experienced parasitoids. In order to validate our current data it would be important if the same study system as we used on the wind tunnel bioassay was applied but using wasps that have experienced host related cues.

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