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# EFFECTS OF ALLELOCHEMICALS FROM FIRST (BRASSICACEAE) AND SECOND (*Myzus persicae and Brevicoryne brassicae*) TROPHIC LEVELS ON *Adalia bipunctata*

FRÉDÉRIC FRANCIS,[1,*] GEORGES LOGNAY,[2] JEAN-PAUL WATHELET,[2] and ERIC HAUBRUGE[1]

[1]*Unit of General and Applied Zoology*
[2]*Unit of General and Organic Chemistry*
*Gembloux Agricultural University*
*Passage des Déportés 2, B-5030 Gembloux, Belgium*

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**Abstract**—Three Brassicaceae species, *Brassica napus* (low glucosinolate content), *Brassica nigra* (including sinigrin), and *Sinapis alba* (including sinalbin) were used as host plants for two aphid species: the generalist *Myzus persicae* and the specialist *Brevicoryne brassicae*. Each combination of aphid species and prey host plant was used to feed the polyphagous ladybird beetle, *Adalia bipunctata*. Experiments with Brassicaceae species including different amounts and kinds of glucosinolates (GLS) showed increased ladybird larval mortality at higher GLS concentrations. When reared on plants with higher GLS concentrations, the specialist aphid, *B. brassicae*, was found to be more toxic than *M. persicae*. Identification of GLS and related degradation products, mainly isothiocyanates (ITC), was investigated in the first two trophic levels, plant and aphid species, by high-performance liquid chromatography and gas chromatography–mass spectrometry, respectively. While only GLS were detected in *M. persicae* on each Brassicaceae species, high amounts of ITC were identified in *B. brassicae* samples (allyl-ITC and benzyl-ITC from *B. nigra* and *S. alba*, respectively) from all host plants. Biological effects of allelochemicals from plants on predators through aphid prey are discussed in relation to aphid species to emphasize the role of the crop plant in integrated pest management in terms of biological control efficacy.

**Key Words**—Allelochemical, glucosinolates, isothiocyanates, ladybird, aphid, toxicity, tritrophic interactions.

*To whom correspondence should be addressed. e-mail: francis.f@fsagx.ac.be



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## INTRODUCTION

Many Brassicaceae species are cultivated as oilseed, vegetable, or fallow plants. In 1997, cabbages (*Brassica oleracea* L.) represented 10.7% of the world vegetable production. More than 11% of plant oil was produced from rape, *Brassica napus* L. (FAO, 1995, 1997). Brassicaceae crops are known to biosynthesize glucosinolates (GLS) as secondary metabolites. Myrosinase, a thioglucosidase enzyme (EC 3.2.3.1.), was found in plants containing GLS but was also detected in insects, such as the cabbage aphid, *Brevicoryne brassicae* L. (MacGibbon and Allison, 1968; Francis, 1999a). In the plant, myrosinase is located in the cytoplasm whereas GLS are stored in vacuoles (Grob and Matile, 1979). Glucosinolate degradation is initiated when cell integrity is destroyed (Birch et al., 1990), leading to the production of nitriles, thiocyanates, oxazolidinethiones, and isothiocyanates (ITC). The latter compounds may act as infochemicals in plant–animal interactions (Lamb, 1989) and can also display toxic effects and constitute part of the plant's defense against fungal and insect infestation (Heaney and Fenwick, 1995). ITC may be repellent or attractant, depending on the insect. While Brassicaceae specialists are stimulated to feed and oviposit by these substances, generalist herbivores are generally deterred.

Chemical cues from plants or herbivores are essential in the effective localization of the host/prey habitat and the searching behavior of entomophagous insects (Sauls et al., 1979). *Adalia bipunctata* L. is known for its polyphagy towards many aphid species (Hodek, 1959) even if some of them can present negative effects on the ladybird development (Hodek and Honek, 1996). The herbivore aphids we studied were *M. persicae* and *B. brassicae*. The latter is a Brassicaceae specialist whereas the first one is a generalist (more than 400 host plants; Quaglia et al., 1993). Due to its high polyphagy, *M. persicae* is found on Brassicaceae plants and tolerates the GLS and their degradation products (Nault and Stayer, 1972).

Host plant suitability for aphids does not necessarily meet the aphidophagous predator needs (Hodek and Honek, 1996). Determination of tritrophic interactions includes both practical and theorical interests. Plant–aphid–predator relations have been little studied in integrated pest management programs and raise some questions: how do the third trophic level interactions evolve in the presence of allelochemicals constituting plant defenses? Is the impact of specific plant secondary metabolites similar on the biology of herbivores and entomophagous insects? What are the active allelochemicals in aphids—native GLS or their degradation products? Indeed, GLS break down by enzymatic degradation to release volatile by-products, including mainly isothiocyanates. Brassicaceae species used in these studies were chosen for their glucosinolate profiles: sinigrin and sinalbin are mainly present (around sixfold more than total GLS in rape) in *Brassica nigra* L. and *Sinapis alba* L., respectively, while low GLS

content rape variety includes several GLS in low concentrations. This work was carried out to determine the toxicity of the Brassicaceae secondary substances on an aphid predator and to identify accurately the chemical compounds involved.

## METHODS AND MATERIALS

*Plant and Insect Rearing.* Broad beans (*Vicia faba* L.), black mustard (*Brassica nigra* L.), white mustard (*Sinapis alba* L.), and oilseed rape (*Brassica napus* L.) were grown in 10-cm-diameter plastic pots in three separate controlled environmental rooms at 20 ± 2°C temperature and 16L : 8D photoperiod. While beans were cultivated in pots containing a 1 : 1 mixture of perlite and vermiculite, Brassicaceae species were sown in 20-cm × 30-cm plastic trays containing ordinary compost and transplanted into plastic pots with the same compost when the plants had two true leaves.

Ladybirds, *Adalia bipunctata* L., *Acyrthosiphon pisum* (Harris), and *Myzus persicae* Sultzer had been reared in the laboratory for several years, whereas *Brevicoryne brassicae* L. was collected from a white mustard field in September 1998. While *A. pisum* was only reared on bean, the two other aphid species were mass reared on rape and white and black mustards. The Brassicaceae species were inoculated when they had five to six true leaves. Each plant species was cultivated in separate climate chambers.

For each combination of aphid species and host plant, 30 newly hatched larvae of *A. bipunctata* were isolated individually in 5-cm-diameter Petri dishes to avoid cannibalism and were kept at 20 ± 2° and 16L : 8D photoperiod. The larvae were collected from different egg clusters of the ladybird stock culture. Adults of the latter were reared in aerated plastic boxes (25 individuals per container) and fed every other day ad libitum with *A. pisum* reared on bean (optimal diet). Each experiment was repeated twice (a total of 60 individuals per beetle diet). Aphids stayed at least two weeks on their host plants before being removed from them and offered to the larvae ad libitum.

*Biological Observations.* Seven plant–prey combinations were tested in this work as ladybird diet: *M. persicae* reared either on *V. faba* (without GLS, considered as control), *B. napus*, *B. nigra*, or *S. alba* and *B. brassicae* reared either on *B. napus*, *B. nigra*, or *S. alba*. The Brassicaceae specialist aphid was not able to survive on bean.

After hatching, larvae were placed individually into 5-cm Petri dishes and observed every day to determine survival and appearance of nymphs until the pupal stage. Percent mortality at each development stage was analyzed by ANOVA after $\arcsin\sqrt{x}$ transformation (Dagnelie, 1973) and followed by mean comparisons by the Newman and Keuls method.

*Glucosinolate Analysis.* Glucosinolates were analyzed by high-perfor-

mance liquid chromatography (HPLC L-6200 and L-4000 UV detector, Merck-Hitachi) according to the ISO 9167-1 method, slightly modified. Plant, insect body, and honeydew samples were weighed and crushed in a hot methanol–water mixture (70 : 30 v/v). Glucosinolate enzymatic desulfatation was carried out on a DEAE column (Sephadex A-25) using commercial sulfatase. Desulfoglucosinolates were then analyzed by HPLC.

The *M. persicae* honeydew was collected on 20-cm × 20-cm glass plates placed just under the plants. After 48 hr of exposure, the plates were removed and rinsed with 10 ml of distilled water. To assess the occurrence of GLS in honeydew, samples were submitted to HPLC analysis as previously described. Sinigrin was used as the internal standard when *B. napus* and *S. alba* were involved whereas glucotropaeolin was added for *S. nigra* or aphid-related samples. Three replicates were considered for each analysis.

*Glucosinolate Hydrolysis Product Analysis.* White and black mustards contain high amounts of one major GLS (sinalbin and sinigrin, respectively), whereas the double zero variety of rape includes a mixture of low concentration GLS. We considered *B. nigra* and *S. alba* as two models, knowing that the low GLS content in rape would not allow easy detection of volatiles from aphids reared on it. That is why only aphids reared on both mustards were analyzed for the GLS hydrolysis products.

The volatile GLS metabolites from both aphid species (250 mg) reared on *B. nigra* were analyzed by use of a purge and trap unit (P&T Hewlett Packard) connected to a Hewlett Packard GC-MS system (HP5972 mass spectrometer coupled to a HP5890 series II gas chromatograph). Crushed samples were first maintained for 1 hr at 30 ± 0.2°C in thermostated glass tubes adapted to the P&T unit. They were purged with He at 4 ml/min for 11 min, the volatiles concentrated at room temperature on a Vocarb 3000, and finally desorbed by flash-heating to 250°C. The molecules of interest were automatically transferred to the GC-MS through a split-splitless injector at 250°C (splitless mode). They were separated on a HP5-MS (5% phenyl-dimethylpolysiloxane) column (30 m × 0.25 mm, *df* = 1 μm). The temperature program was from 40°C (1 min hold) to 180°C at 6°C/min then to 280°C at 15°C/min. MS spectra were obtained in the EI mode at 70 eV (scanned mass range from 30 to 300 amu). Analytes were identified on the basis of their retention times and by interpretation of MS fragmentation patterns. The recorded spectra were finally compared to those of the Wiley 238.L spectral library.

Nonvolatile GLS degradation products from both aphid species (samples each of ~250 mg) reared on *S. alba* were extracted with 500 μl pure diethyl ether for 12 hr. Solutions were injected as such in the splitless injector using the aforementioned GC-MS conditions. Quantification was performed by GLC with exactly the same chromatographic parameters on a Hewlett Packard (HP6890) apparatus equipped with a flame ionization detector maintained at

250°C. Two hundred micrograms of phenethyl-ITC was used as internal standard. The response factor was fixed to 1. The measurement of allyl-ITC was not performed due to its high volatility and the potential losses which could occur during crushing and transfer of the samples to the P&T system.

## RESULTS

*Ladybird Mortality.* When total mortality was considered (Figure 1), significant differences were observed according to the aphid species ($F = 60.46$, $P < 0.001$). There was no difference in ladybird mortality when fed with *M. persicae* reared on the three Brassicaceae species and broad bean used as control ($F = 0.87$, $P = 0.494$). When *B. brassicae* was used as ladybird prey, highly significant differences in total mortality were observed depending on the host plant ($F = 447.97$, $P < 0.001$). Mortality of ladybirds fed *B. brassicae* reared on *B. napus* was lower than when they were fed aphids from *B. nigra* or *S. alba* ($q = 0.141$; $P < 0.01$). However, mortality rates on both mustard species reared aphids were not different ($q = 0.097$; $P > 0.05$).

Mortality of each larval stage of *A. bipunctata* fed a combination of aphid species and host plant was assessed (Figures 2a and b). Change of *M. persicae*

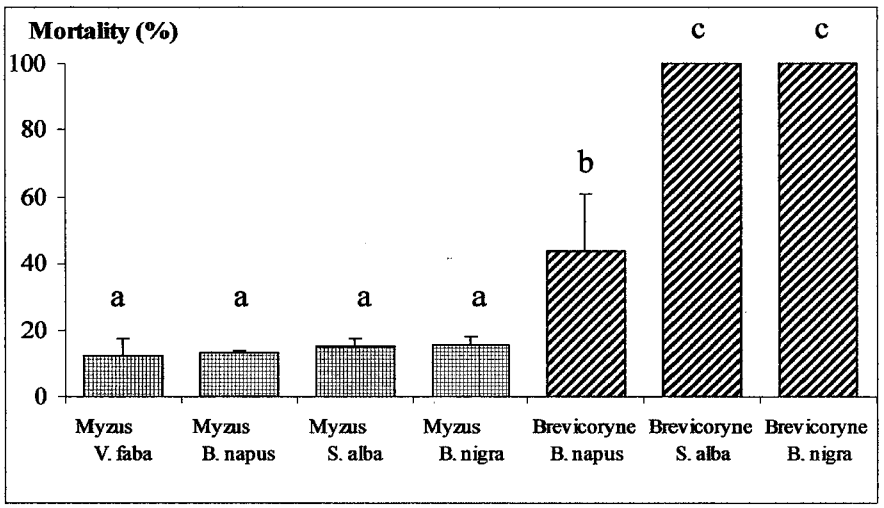


FIG. 1. Total mortality rates of *Adalia bipunctata* fed with different combinations of host plant and aphid species ($N = 60$). Error bars represent standard deviation from the mean and letters indicate significant differences at $P = 0.05$.

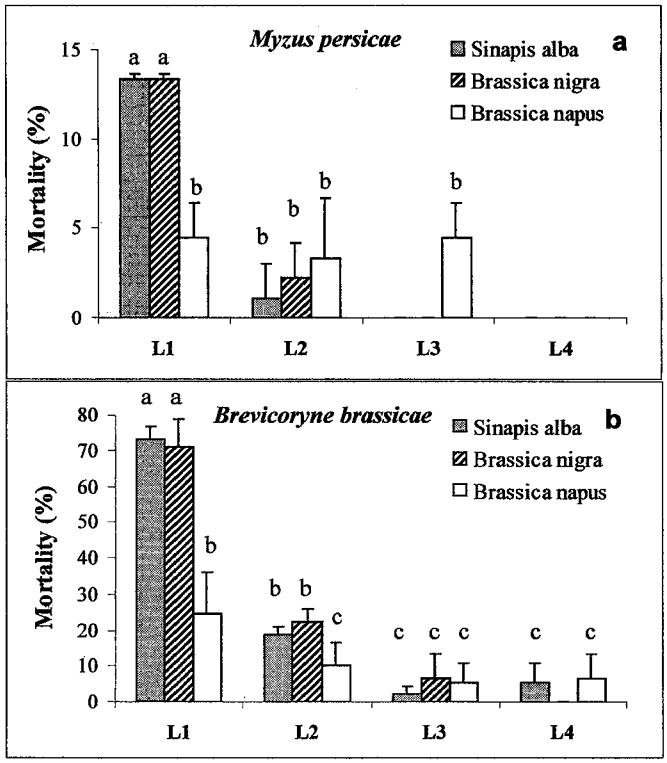


FIG. 2. Effect of aphid prey and host plant on larval mortality rates of *Adalia bipunctata* fed with *Myzus persicae* (a) and *Brevicoryne brassicae* (b) reared on *Brassica napus*, *Brassica nigra*, or *Sinapis alba* ($N = 60$ for each one). Error bars represent standard deviation from the mean and letters indicate significant differences at $P = 0.05$.

host plant displayed difference of mortality rates for first and third larval stages ($F = 40.41$ and $P < 0.001$; $F = 65.65$ and $P < 0.001$, respectively). Whether *B. napus* host plant induced lower mortality than the two mustard species ($q = 0.094$ and $P < 0.01$ for both mean tests), no difference was observed between the two latter plants ($q = 0.064$ and $P > 0.05$). Ladybird mortality rates observed for the three *M. persicae* host plants at the second larval stage were not different ($F = 0.60$ and $P = 0.579$).

A change of *B. brassicae* host plant induced a significant difference in mortality rates at the first and second larval stages ($F = 27.42$ and $P = 0.001$; $F = 5.16$ and $P = 0.048$, respectively). Mortality rates of *A. bipunctata* fed *B. brassicae* reared on both mustard species were higher than when fed aphids reared on rape ($q = 0.354$ and $P < 0.01$; $q = 0.132$ and $P < 0.05$ for first and second

TABLE 1. GLUCOSINOLATE CONTENTS IN PLANT AND APHID SPECIES[a]

| Glucosinolate | Host plant leaf | *Myzus persicae* | | *Brevicoryne brassicae* total aphid |
|---|---|---|---|---|
| | | Total aphid | Honeydew | |
| *Brassica napus* | | | | |
| Glucobrassicin | 0.16 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.00 | 0.10 ± 0.00 |
| Gluconasturtin | 0.49 ± 0.04 | 0.11 ± 0.01 | 0.06 ± 0.01 | 2.80 ± 0.77 |
| 4-OH-Glucobrassicin | <0.01 | <0.01 | ND | ND |
| Progoitrin | 0.57 ± 0.00 | <0.01 | ND | <0.01 |
| Glucoraphanin | 0.37 ± 0.01 | 0.33 ± 0.06 | 0.26 ± 0.12 | 2.91 ± 1.07 |
| Total | 1.59 ± 0.04 | 0.47 ± 0.05 | 0.34 ± 0.09 | 5.81 ± 1.13 |
| *Sinapis alba* | | | | |
| Sinalbin | 8.83 ± 0.15 | 0.72 ± 0.10 | 1.12 ± 0.29 | 180.96 ± 15.83 |
| Gluconasturtin | 1.82 ± 0.01 | 0.28 ± 0.02 | 0.35 ± 0.04 | ND |
| 4-OH-Glucobrassicin | <0.01 | ND | ND | 1.92 ± 0.37 |
| Brassicanapin | <0.01 | ND | ND | <0.01 |
| Progoitrin | 0.28 ± 0.06 | 0.65 ± 0.11 | 0.03 ± 0.00 | 3.28 ± 0.16 |
| Total | 10.93 ± 0.13 | 1.65 ± 0.09 | 1.50 ± 0.43 | 185.16 ± 14.36 |
| *Brassica nigra* | | | | |
| Sinigrin | 8.45 ± 0.54 | 1.75 ± 0.39 | 1.92 ± 0.17 | 132.88 ± 17.12 |
| Sinalbin | 0.23 ± 0.04 | <0.01 | ND | 4.89 ± 0.37 |
| 4-OH-Glucobrassicin | 0.79 ± 0.03 | <0.01 | <0.01 | 8.32 ± 1.72 |
| Progoitrin | 0.07 ± 0.01 | 1.58 ± 0.48 | <0.01 | 2.21 ± 0.41 |
| Total | 9.54 ± 0.42 | 2.33 ± 0.46 | 1.92 ± 0.17 | 148.20 ± 15.03 |

[a] Values are in μmol. $g^{-1}$FW. GLS amounts of *M. persicae* honeydew (in μmol) were determined from the glass plate rinsing solution. ND = glucosinolates were not detected.

larval stages, respectively). No difference was observed between aphids reared on *B. nigra* or *S. alba* ($q = 0.243$ and $P > 0.05$). *B. brassicae* host plant did not induce differences in mortality rates of third and fourth larval stage ladybirds ($F = 0.26$ and $P = 0.779$; $F = 1.88$ and $P = 0.232$, respectively).

*Glucosinolate Analysis.* The glucosinolates found in aphid samples and in host plant leaves are presented in Table 1 and 2. Small quantities of glucobrassicin, brassicanapin, and glucoraphanin were detected in both aphid species reared on the low-GLS-content rape. Larger quantities of sinalbin and sinigrin were found in aphid samples collected on *S. alba* and *B. nigra*, respectively. Larger differences in GLS content were observed between the aphid species. Higher GLS amounts were found in *B. brassicae* than in *M. persicae* samples. Complementary analysis of honeydew from the latter reared on each Brassicaceae species revealed traces of sinalbin and sinigrin in *S. alba* and *B. nigra*, respectively (Table 1). Honeydew production by *B. brassicae* was so small that analysis was not possible.

*Glucosinolate Hydrolysis Product Analysis.* Regardless of the host plant

TABLE 2. GLUCOSINOLATES FOUND IN HOST PLANT AND APHID SPECIES ANALYZED

| Common name | Glucosinolate side chain |
|---|---|
| Sinigrin | allyl |
| Sinalbin | *p*-hydroxybenzyl |
| 4-OH-glucobrassicin | 4-OH-3-indolylmethyl |
| Progoitrin | 2-OH-3-butenyl |
| Brassicanapin | 4-pentenyl |
| Gluconasturtin | 2-phenylethyl |
| Glucoraphanin | 4-methylsulfinylbutyl |
| Glucobrassicin | 3-indolylmethyl |

(*B. napus*, *B. nigra*, and *S. alba*), the GC-MS chromatograms of volatiles from *M. persicae* only displayed β-farnesene, a well known aphid alarm pheromone (Pickett et al., 1992). No glucosinolate degradation product was detected for *M. persicae* (Table 3). *B. brassicae* samples displayed substantial amounts of ITC(s) and nitrile(s) according to the GLS present in the aphid host plant (Table 3). Allyl-ITC and allylnitrile were found in aphids reared on *B. nigra*, which biosynthesizes sinigrin. Analyses of aphids collected on *S. alba*, which contains sinalbin (4-hydroxybenzyl-GLS) by the purge-and-trap method, did not reveal the occurrence of ITC. As no peak was detected by this method, the unsuccessful recovery of 4-hydroxybenzyl-ITC was attributed to its low volatility. Diethyl ether extraction was then undertaken. Diethyl ether extracts of *B. brassicae* reared on *S. alba* had two peaks: benzyl-ITC and benzyl-nitrile. The occurrence of benzyl-ITC was surprising as this compound is characteristic of glucotropaeolin (benzyl-glucosinolate) degradation. Further analysis was carried out to be sure that this was not an analytical artifact. Commercial thioglucosidase was added to an aqueous

TABLE 3. VOLATILE CONSTITUENT ANALYSIS OF *Brevicoryne brassicae* AND *Myzus persicae* EXTRACTS BY GC-MS (TOTAL ION CURRENT)[a]

| Aphid and host plant species | GLS hydrolysis products | Others |
|---|---|---|
| *Brevicoryne brassicae* | | |
| On *Sinapis alba* | Benzyl-ITC ($R_t$ = 22.87) | |
| | Benzylnitrile ($R_t$ = 17.27) | |
| On *Brassica nigra* | Allyl-ITC ($R_t$ = 10.53) | |
| | Allylnitrile ($R_t$ = 4.70) | |
| *Myzus persicae* | | |
| On *Sinapis alba* | | β-farnesene ($R_t$ = 24.60) |
| On *Brassica nigra* | | β-farnesene ($R_t$ = 24.63) |

[a] Names of identified compounds are followed by their retention time ($R_t$).

solution of sinalbin (purified from *S. alba*; 2 mg/ml); 4-hydroxy benzyl-ITC was identified by GC-MS. This result indicates that sinalbin metabolism in *B. brassicae* is more complex than suspected. We hypothesize that the loss of an hydroxy group is linked to the insect extract and not to the analytical method. This observation needs further investigation.

Quantitative evaluation of the benzyl-ITC production from specialist aphids was assessed and revealed a constant ITC/associated nitrile ratio (5.59 ± 0.37; benzyl-ITC/benzonitrile) for all replicates. The benzyl-ITC that was produced from the *B. brassicae* samples was 3.0 ± 0.2 μmol/g fresh material ($N = 4$ replicates).

## DISCUSSION

In association with visual cues, chemical composition plays an important role in host plant location by herbivorous insects (Prokopy and Owens, 1983). Fraenkel (1959) suggested that secondary plant metabolites were directly involved in food searching by insects. The impact of allelochemicals is not limited to the second trophic level, interactions are observed when entomophagous insects use synomones emitted by plants as cues for prey location. Volatile chemical cues employed by natural enemies may originate from the plant upon which the host is feeding, the host itself, other organisms associated with the host, or chemicals released as a result of interactions between the host and its food plant (Lewis and Martin, 1990). Release of herbivore-induced plant volatiles that act as reliable and detectable host location cues for parasitoids and predators has previously been demonstrated for several tritrophic systems (Dicke et al., 1990; Turlings et al., 1990).

The chemical traces of aphids, even in the absence of insects themselves, induce changes in ladybird oviposition behavior (Carter and Dixon, 1984). In this work, GLS were found in the honeydew of *M. persicae* reared on the three Brassicaceae host plants Weber et al. (1986) detected 3.6–28.7 μmol/g (dry weight) of GLS in the peach-potato aphid depending on the rape variety (low or high GLS content). Isothiocyanates and nitriles were detected by GC-MS in *B. brassicae* infesting either *B. nigra* or *S. alba*. The presence of benzyl-ITC was quite surprising; 4-hydroxy benzyl-ITC, the normal product of sinalbin degradation was not found. Loss of the hydroxy group seems to be due to the *B. brassicae* enzymatic extract but needs to be more extensively studied from a biochemical point of view. Benzyl-ITC, a typical glucotropaeolin degradation product, is toxic to some insects (Bartlet and Mikolajczak, 1989). These molecules emitted by the Brassicaceae specialist aphid could be used as infochemicals by entomophagous insects. Assays on the *B. brassicae* parasitoid *Diaeretiella rapae* demonstrated the role of volatiles from aphids as kairomones. Indeed,

they responded to odors of infested cabbage leaves or aphids alone but not plants uninfested by aphids, suggesting an innate odor preference for crucifer-feeding aphids (Reed et al., 1995). If Brassicaceae pest natural enemies use these infochemicals for their own benefit, generalist predators could use the ITC produced by *B. brassicae* as allomones to avoid this aphid species. When two aphid species were offered to *A. bipunctata* (*M. persicae* and *B. brassicae* or the latter and *A. pisum*), the generalist predator first consumed the other, then the Brassicaceae specialist species (Francis, personal communication). This rejection is directly linked to the food suitability represented by the aphid species (Hodek and Honek, 1996). No ladybird was able to reach the adult stage when fed with the specialist aphid reared on both mustard species. When prey was collected from rape, twofold lighter adults emerged (compared to control food, *M. persicae* on bean) and no reproduction was observed (Francis, 1999b).

Isothiocyanates, GLS degradation compounds, stimulate olfactory receptors of generalist herbivores such as *Aphis fabae*. Both pentenyl- and butyl-ITC are repellent to this aphid species (Isaacs et al., 1993). Alkenyl-ITCs are characteristic defensive chemicals of Brassicaceae. Chemical precursors that break down to release ITC have shown biological activity against pathogens and insect pests of Brassicaceae (Lamb, 1989; Dawson et al., 1993). The higher toxicity of *B. brassicae* prey to generalist predators can be objectively explained by the presence of these ITC compounds. Indeed, allyl-ITC and methyl-ITC are toxic to *A. bipunctata* (0.189 ppm $< LD_{50} <$ 0.308 ppm and 0.218 ppm $< LD_{50} <$ 0.551 ppm, respectively) after a 24-hr exposure (Francis et al., 1999). Moreover, myrosinase, the enzyme that catalyzes GLS degradation, was detected in *B. brassicae* but not in *M. persicae* (Francis, 1999a). ITC or nitriles were not found in the latter species reared on Brassicaceae species. Only intact GLS were observed in this aphid species reared on mustards or rape (0.47–2.33 $\mu$mol/g fresh aphid weight). On the other hand, high amounts of GLS were found in *B. brassicae* reared on both mustard species while lower allelochemical levels were observed on rape (25–30 times less). Weber et al. (1986) detected more than 60 $\mu$mol/g (when expressed on a fresh weight basis) of GLS in *B. brassicae* reared on high GLS content rapes.

Glucosinolates are known to display biological properties towards insects. While they are oviposition stimulants or phagostimulants (Hicks, 1974; Reed et al., 1989; Traynier and Truscott, 1991) for many Brassicaceae herbivore specialists, they also serve as repellents for most generalist crop pests (Blau et al., 1978; Erickson and Feeny, 1974). Foliar concentrations of GLS found in both mustard species were much higher than in rape (around sixfold more) and were in accordance with the results of Hopkins et al. (1998). Total GLS content in white mustard leaves were 50–70 $\mu$mol/g of dry weight, which corresponded to a concentration of 10–15 $\mu$mol/g GLS when expressed in fresh weight. Whereas 12.5 $\mu$mol/g (in dry weight) of total GLS was found in 00 rape leaves, more than

60 μmol/g was detected in a high-GLS variety of rape (Weber et al., 1986). Similar GLS concentrations (expressed in fresh weight), were reported in this study. Variations in GLS contents of cultivated species were tested to obtain resistant varieties of plant against herbivores. *Sinapis alba*, containing high amounts of sinalbin, was shown to be less susceptible to damage by insect pests than *B. napus* with low GLS content. The specialist aphid, *B. brassicae*, was found mainly on young tissues containing the highest total GLS levels, while the generalist aphid, *M. persicae*, was found predominantly on older plant parts (Hopkins et al., 1998). According to these authors, aphid distribution is directly linked to GLS concentration in plant.

Other secondary plant compounds are toxic to nonspecialist herbivores and to natural enemies of herbivores. Toxicity of *Aphis sambuci* seemed to be assumed by the passage of the glycoside sambunigrin from the plant *Sambuscus nigra* into the aphid. Several other species of aphids also seem to be poisonous to coccinellids, including *A. bipunctata*, and are rejected by ladybirds due to presence of allelochemicals, including *Macrosiphum aconitum* feeding on *Aconitum* (including aconitin), *Aphis nerii* infesting oleanders (including oleandrin and neriin), and *Aphis jacobaeae* feeding on *Senecio* sp. (including pyrrolizidine alkaloids) (Hodek and Honek, 1996).

Allelochemicals from plants are partly responsible for host specificity of herbivorous insects (Schoonhoven, 1981). As specialized organisms, insects developed many defense strategies. Their responses towards secondary metabolites vary considerably. Some insects have evolved adaptations to resist these allelochemicals, including stocking them as defense substances against natural enemies (Harborne, 1993). Others evolved biochemical systems to metabolize and assimilate secondary plant substances. Detoxification mechanisms can contribute to neutralizing the toxic potential of some compounds (Yu, 1984; Yu and Hsu, 1993). These adaptations range from chemical modification of toxin, its transformation into a nontoxic conjugated compound, or its sequestration. Allyl- and benzyl-ITC were metabolized by glutathione *S*-transferase (GST) from two lepidopterous generalist species (*Spodoptera frugiperda* and *Trichoplusa ni*), but no activity was detected with the specialist *Anticarsia gemmatalis* (Wadleigh and Yu, 1988). While the generalist aphid *M. persicae* displayed induction of the GST system when reared on Brassicaceae species (*S. alba*, *B. nigra*, *B. napus*), induction was not observed in the *B. brassicae* specialist herbivore whatever the host plant (Francis, 1999a).

Adaptation of the aphid predator, *A. bipunctata*, to crucifer allelochemicals is facilitated by induction of GST (Francis, 1999a). Indeed, the increase of GST activity was linked to the plant GLS levels predominantly with *B. brassicae* aphid. We hypothesize that the latter accumulates the plant secondary substances, constituting an aphid defense against their natural predators. More than the volatile emission as chemical cues, this phenomenon of bioaccumulation of

high GLS amounts in prey would imply an aphid limited consumption by entomophagous insects.

In conclusion, studies on integrated pest management must include both herbivore and plant trophic levels to determine the plant allelochemical impact on the third trophic level, the beneficial entomophagous insects. To obtain effective biological control, determination of the involved aphid species is not sufficient. Semiochemicals from plants, directly or through herbivore prey, must be taken into account as a potential toxin or reliable infochemical in relation to the efficacy of pest control by natural enemies. Effectiveness of biological control by coccinellids is influenced by many factors, among them the host plant species of the pest. If natural enemies attack a wide variety of herbivores that occur on the same host species, information from the plant may become very important (Vet and Dicke, 1992). The use of generalist predators is not always a general solution to control aphid infestations. This work confirms the need for a case-by-case consideration when setting an integrated pest management program, including chemical ecology approaches.

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