Intraspecific variation of host plant and locality influence the lepidopteran-parasitoid system of *Brassica oleracea* crops

S. Santolamazza-Carbone¹, P. Velasco¹, J. Selfa², P. Soengas¹, M. E. Cartea¹

¹Misión Biológica de Galicia, Consejo Superior de Investigaciones Científicas (MBG-CSIC), P.O. Box 28, 36080, Pontevedra, Spain

²Universitat de València, Departament de Zoologia, C/ Dr. Moliner 50, 46100 Burjassot (València), Spain

Corresponding author: S. Santolamazza-Carbone

Misión Biológica de Galicia (CSIC), P.O. Box 28, 36080, Pontevedra, Spain

E-mail address: anaphes@gmail.com; ssantolamazza@mbg.csic.es

Short title: Variations of lepidopteran-parasitoid system in *Brassica oleracea* crops

Abstract

The aim of the study was to investigate the attractiveness to herbivores and parasitoids of two cultivars of *Brassica oleracea*, namely *B. oleracea* var. *acephala* (kale) and *B. oleracea* var. *capitata* (cabbage), which exhibit differences of morphological and biochemical traits. To this end, field samplings were replicated at seven localities during one season in Galicia (NW Spain). Three specialist and three generalist lepidopteran species were sampled. A total of 7,050 parasitoids were obtained, belonging to eighteen genera and twenty-two species. The results showed that: 1) parasitism rate and parasitoid species richness changed with locality and was higher in cabbages, although

this crop had lower herbivore abundance than kales, 2) the proportion of specialist herbivores was higher in cabbages, whereas generalists dominated in kale crops, 3) the abundance of the egg parasitoid *Telenomus* sp., and the larval parasitoids *Cotesia glomerata* and *Diadegma fenestrale* was higher in kale crops, 4) parasitism rate of *P. rapae* larvae and pupae and *M. brassicae* eggs were higher in kales. In contrast with the notion that plant structural complexity provides physical refuge to the hosts and can interfere with parasitoid foraging, parasitism rate was higher on cabbage plants, which form heads of overlapped leaves. Possibly different chemical profiles of cultivars also influenced host-parasitoid relationship. These results suggest that top-down and bottom-up forces may enhance cabbage crops to better control herbivore pressure during the studied season. In Spain, information on natural occurring parasitoid guilds of kale and cabbage crops is still scarce. The data provided here about parasitoid species diversity, richness and parasitism rate, contribute to fill this gap and also represent a critical first step for conservation biological control plans of *Brassica* crops.

Keywords: biological control, cabbage, kale, insect-plant interaction, parasitoid community

Introduction

Cole crops such as kale, cabbage, kohlrabi, broccoli, cauliflower and Brussels sprouts belong to the same species, *Brassica oleracea* L., which is native to Europe and China, and cultivated throughout the world (Gupta 2009). In Spain, kale (*Brassica oleracea* var. *acephala*) and cabbage (*Brassica oleracea* var. *capitata*) crops are mostly grown in the north-western regions, such as Galicia, which dedicated up to 6,534 ha to

cultivation of both crops, representing the 46% of the Spanish field used for production of these vegetables (MARM 2009). Kales and cabbages are often greatly negatively affected by several lepidopterous species, whose larvae feed on leaves, causing a significant reduction of their economic value (Ordás and Cartea 2008). In NW Spain, the most common lepidopteran herbivores are the generalists *Mamestra brassicae* L. (Noctuidae), *Autographa gamma* L. (Noctuidae) and *Evergestis forficalis* L. (Pyralidae), and the specialists *Plutella xylostella* L. (Yponomeutidae), *Pieris rapae* L. (Pieridae) and *P. brassicae* L. (Pieridae) (Cartea et al. 2009). In this country, insect herbivores are generally controlled with chemical insecticides, although growing public concerns about the hazard for humans and environment and the economic cost makes this practice undesirable. The use of parasitoids as biological control agents of these crops has received little attention in Spain, and currently no biological control plans using natural enemies have been developed.

The quality of host plant, including morphology, size, nutrients and defence compounds, is a critical component of insect-plant interaction (Bernays and Chapman 1994). Secondary defence metabolites produced by *Brassica* plants, such as glucosinolates, upon damages by chewing herbivores become exposed to the plant enzyme myrosinase that hydrolized it, resulting in toxic compounds such as isothiocyanates and nitriles, that generally decrease performance of herbivores (Hopkins et al. 2009). These metabolites, are responsible for different patterns of insect attack (bottom-up defence) and their impact may reach other trophic levels (i.e. predators and parasitoids) through the food web (Ode 2006; Hopkins et al. 2009; Gols and Harvey 2009). Hence, the ability of parasitoids to control a pest population would depend not only on the interactions with its host, but also on the morphology and biochemistry of the host plant (Turlings and Benrey 1998; Ode 2006; Hopkins et al. 2009). In addition,

formation of specific volatile glucosinolate breakdown products increase attraction of several specialist parasitoids (top-down defence), and some species can even discriminate among volatiles emitted by different cultivars of the same plant (Gols and Harvey 2009). The cultivation of *B. oleracea* as an important human food crop plant has resulted in a reduction of total glucosinolate levels compared to wild type plants (Gols and Harvey 2009). However, despite *B. oleracea* cultivations had a relatively similar glucosinolate pattern, different concentration of aliphatic and indolic glucosinolates permit to carry on ecological studies with these plants (Poelman et al. 2008). The concentration of main glucosinolates, such as the aliphatics sinigrin and glucoiberin and the indolic glucobrassicin, may differ in kale and cabbage plants (Charron et al. 2005; Nilsson et al. 2006; Velasco et al. 2007; Cartea et al. 2008). In particular, it has been shown that sinigrin makes the major contribution of glucosinolates to kales, whereas glucobrassicin and glucoiberin may be higher in cabbages (Velasco et al. 2007; Cartea et al. 2008). Although sinigrin has a negative impact on generalist insect herbivores, which are more sensitive to the breakdown product of this defence metabolite, this metabolite also acts as feeding and oviposition stimulant for a wide range of specialist crucifer-feeding insects (Shields and Mitchell 1995; Sarfraz et al. 2006; Hopkins et al. 2009). On the other hand, glucoiberin was found to be negatively correlated with herbivore abundance, species richness and biodiversity (Poelman et al. 2009; Kos et al. 2011).

Kales and cabbages also differ in term of plant size and architecture (Ordás and Cartea 2008). Kale plant produces a strong-growing rosette of long-petioled, detached, elongated leaves with wavy margins, whereas cabbage architecture shows a higher complexity, with short stem and inwards, overlapped, curled leaves surrounding the terminal bud, which form the typical head (Pimentel 1961). Because parasitoid's host

finding capacity (i.e. the number of hosts found per unit of time) is negatively correlated with the increase of plant complexity (Gingras et al. 2002), we expected higher parasitism rate of herbivores harboured by kale cultivar. Of particular interest also is the role of leaf epicuticular waxes on plant-insect interactions, because glossiness may have an antixenotic (non-preference) effect, as showed for several phenotypes of kale with glossy leaves, which were positively correlated to plant resistance to lepidopterans (Picoaga et al. 2003).

The main objective of this study was to address whether plant cultivar and locality affected lepidopteran abundance and influenced parasitoid species diversity, richness and performance. Special attention has been paid on whether plant cultivar exerts different attraction of generalist and specialist herbivores. We expected higher herbivore abundance in kale crops because of the higher content of sinigrin. In particular, more specialists than in cabbages are expected, because there is evidence that this category of herbivores may be more responsive to sinigrin (see Hopkins et al. 2009 and references therein). Furthermore, because in Spain information on natural occurring parasitoid guilds of kale and cabbage crops is still limited, the data provided here about parasitoid communities, contribute to fill this gap and represent a critical first step for conservation biological control plans of *Brassica* crops.

Materials and methods

Experimental design

The study was replicated at seven localities across Galicia (NW Spain) (Fig.1), from June to November 2010. According to Martínez Cortizas and Pérez Alberti (2000), the climate of the region is classified as Atlantic humid, without long frost periods and with mild temperatures throughout the year. The average temperature ranges between 8-

10 °C in winter and 20-25°C in summer. Four fields were located at Pontevedra province (Pontevedra, 42° 24'N, 8° 38'W; Parada, 42° 23'N, 8° 32'W; Lalín, 42° 38'N, 8°10'W and Soutelo ,42° 33'N, 8°18'O), and the other three at Ourense province (Leiro, 42°22'N, 8° 9'O), Lugo province (Guitiriz, 43° 12'N, 7°53'W) and A Coruña province (Oroso, 43°1'N, 8° 26'W), respectively (see Supplementary materials S1 for climatic details of the localities).

The experimental plots were small agricultural fields embedded in an agricultural landscape, composed primarily of other cole crops, maize crops, vineyards and pastures. Later-successional plant communities such as old fields, hedgerows (i.e. an unmanaged row of trees or shrubs separating fields) and woodlots were also present. Seedlings of a local variety of kale (MBG-BRS0103) and cabbage (MBG-BRS0408), kept at the *Brassica* germplasm bank at the Misión Biológica de Galicia (MBG-CSIC), were germinated in greenhouse on February 2010 and then transplanted at each location on April-May 2010. At each site, an approximately 400 m² experimental plot, split in two subplots, was established. Subplots were planted with cabbages (N = 150) and kales (N = 150), arranged in six rows of 25 plants of each cultivar (80 cm between rows and 50 cm between plants). No chemical control was carried on in the plots to avoid interference with pest and parasitoid development. Samplings for parasitoid detection were performed monthly, covering the vegetative growth period of the crops and the flight periods of the pests (Cartea et al. 2009). On each sampling date, all the plants of the plots were carefully inspected for the presence of lepidopteran eggs, larvae and pupae. The whole plant was checked and all the insect stages on leaves were collected. However, *P. xylostella* eggs and the mining first instars larvae were not sampled due to their small size. Also, *M. brassicae* pupae were not sampled as they are buried in the soil. Eggs of *A. gamma* and *E. forficalis* were not sampled due to the difficulty to

recognize them. In Spain, all the lepidopteran species studied are bi- or multivoltine, thus we expected that samplings did not significantly reduce their abundance in the plots. Field collected material was transported to the laboratory, identified and placed in plastic 10cm-diameter-Petri-dishes, labelled with the collection date, locality, host identity and host plant. The larvae were reared individually in Petri-dishes and fed with fresh kale leaves. Lepidopteran larvae and pupae were reared until adulthood or until emergence of parasitoids. Egg hosts were maintained until larvae or parasitoids emerged. All rearing was carried out at room temperature (20 ± 2 °C) and under natural photoperiod.

Ecological parameters

The impact of parasitoids on lepidopteran host populations was calculated as the mean parasitism rate (i.e. the ratio between the overall number of parasitized hosts and the number of sampled hosts) per locality and sampling date. Unhatched host eggs and host pupae or unhatched parasitoid cocoons were dissected under the microscope to account for unemerged adult parasitoids.

After taxonomic identity and host-range was ascertained, the abundance of parasitoid species per cultivar, locality and sampling date was calculated. Also, the existence of different parasitoid guilds (i.e. egg, egg-larval, larval, larval-pupal and pupal parasitoids) and their distribution between the crops was studied.

Species diversity per locality, cultivar and sampling date was assessed by using the Shannon-Wiener diversity index (H'), following the equation:

$$H' = \sum_{i=1}^S p_i \ln p_i$$

Where p_i is the ratio between the numbers of emerged parasitoids from the species i and the total number of emerged parasitoids from the n species. The Shannon-Wiener index increases when the number of taxa increases or the distribution of the species becomes more even.

Species richness was calculated as the number of different taxa recorded per locality, cultivar and sampling date.

Taxonomic identity

We thank Dr. Santiago Bordera at University of Alicante (Spain) for the aid in the identification of part of the ichneumonid parasitoids; Antoni Ribes (Spain) for the identification of Chalcidoidea wasps; Dr. Bernard Pintureau at INRA of Lyon (France) for the identification of Trichogrammatidae; Dr. José V. Falcó at University of Valencia (Spain) for the identification of the braconid parasitoids, and Dr. Andrew Polaszek at Natural History Museum (UK) for the determination of Scelionidae. The dipteran parasitoids were identified by Dr. Hans Peter Tschorsnig at Naturkundemuseum of Stuttgart (Germany). The new host-parasitoid relationships and the new species for Spain were identified by Dr. Jesús Selfa at University of Valencia (Spain). The voucher specimens are conserved at the Misión Biológica de Galicia.

Statistical analyses

The effect of plant cultivar, locality (fixed factors) and sampling date (random factor), and the interaction between them, on overall herbivore abundance and on the abundance of the most prominent lepidopteran and parasitoid taxa was investigated by using two-way analysis of variance (ANOVA). Insect count data were $\log_{10}(x+1)$ transformed prior the analyses.

Differences of the proportion of generalist (i.e. *M. brassicae*, *A. gamma*, *E. forficalis*) and specialist (i.e. *P. xylostella*, *P. rapae*, *P. brassicae*) herbivores between cultivars were assessed by using a logistic regression with binomial proportion and logit link function.

The influence of plant cultivar, locality (fixed factors) and sampling date (random factor) on parasitoid species richness and diversity was analyzed by using a Generalized Linear Mixed Model (GLMM) with Poisson distribution and logarithm link function.

Variation of parasitism rate estimated in kale and cabbage crops across localities was assessed by Generalized Linear Model with binomial proportion (logistic regression) and logit link function. The binomial proportion (i.e. number of parasitized hosts/ number of sampled hosts) was treated as the response variable, whereas plant cultivar, locality and interactions were the independent variables. Sample date was adopted as random factor. Parasitism rate of the most abundant lepidopterans, such as *M. brassicae*, *P. xylostella* and *P. rapae*, were investigated separately, sorted by developmental stage, as parasitism rate of eggs, larvae and pupae reflects different parasitoid guilds. Because the main parasitoid of *P. xylostella* is a larval-pupal species (i.e. attacks larvae but emerge from the pupae), parasitism rate of this moth was calculated by pooling the number of sampled larvae and pupae. Significance was declared at $P < 0.05$. Statistical tests were carried out using the GenStat12.1 software package (VSN International Ltd, Hemel Hempstead, UK).

Results

Hosts and parasitoid biodiversity

A total of 3,870 eggs, 1,979 larvae and 556 pupae of lepidopterans were collected in the kale plots, whereas in the cabbage plots, 1,479 eggs, 922 larvae and 399 pupae were found. Three specialist (*P. rapae*, *P. brassicae*, *P. xylostella*) and three generalist (*M. brassicae*, *A. gamma*, *E. forficalis*) lepidopteran species were found. Across localities, crops and samplings, *M. brassicae* (larvae, N = 2,209), *P. xylostella* (larvae and pupae, N = 1,048), and *P. rapae* (larvae and pupae, N = 489) were the most abundant pests. *M. brassicae* represented the 76% of the collected larvae (N = 2,901) whereas, *P. xylostella* and *P. rapae* represented the 27% and 13% of the overall collected larvae and pupae (N = 3,856), respectively. An amount of 3,206 parasitoids (2,159 solitary and 1,047 gregarious) emerged from hosts sampled in kale crops, whereas from cabbage crops, 3,844 parasitoids (825 solitary and 2,949 gregarious) were obtained. However, the parasitism rate achieved was 28% and 36% in kale and cabbage crops, respectively.

The identity of 16 genera (12 Hymenoptera and 4 Diptera) and 19 species of primary parasitoids, and 3 genera of hymenopteran hyperparasitoids, was ascertained. Hymenopterans of the families Braconidae, Ichneumonidae, Encyrtidae, Pteromalidae, Eulophidae, Scelionidae and Trichogrammatidae were found. The dipteran parasitoids belonged exclusively to the family Tachinidae. The parasitoid complex, their relative abundance, host range and parasitism rate are shown in Table 1. The quantitative and qualitative assemblage of parasitoids per cultivar and locality, including species richness and diversity, are shown in Tables 2 and 3. The parasitoid species collected fall into 5 guilds, based on host stage attacked: egg (2 genera), egg-larval (2 species), larval (12 species), larval-pupal (5 species) and pupal parasitoids (3 species). Among them, 15 koinobionts (i.e. permit continued host development) and 8 idiobionts (i.e. prevent

further host development after the attack) species (Askew and Shaw, 1986) were represented.

Impact of cultivar and locality on lepidopterans and parasitoids

Overall herbivore abundance, taking into account all stages of the six Lepidoptera species, was significantly higher in kale than in cabbage crops ($F_{1,4030} = 10.77$, $P = 0.037$), and also changed depending on locality ($F_{6,4030} = 4.75$, $P < 0.001$) (Table 4). Plant cultivar ($F_{1,600} = 12.32$, $P < 0.001$) and locality ($F_{6,600} = 5.42$, $P < 0.001$) influenced the abundance of the gregarious parasitoid *Cotesia glomerata*, of the larval parasitoid *Diadegma fenestrale* (plant cultivar: $F_{1,1047} = 4.72$, $P = 0.003$; locality: $F_{6,1047} = 5.33$, $P < 0.001$), and of the egg parasitoid *Telenomus* sp. (plant cultivar: $F_{1,114} = 136.48$, $P < 0.001$; locality: $F_{6,114} = 6.77$, $P < 0.001$), which were significantly more abundant on kales. However, the abundance of *Trichogramma* sp. was affected only by locality ($F_{6,1043} = 6.77$, $P < 0.001$). The abundance of the sampled developmental stages of *M. brassicae*, *P. xylostella* and *P. rapae* were not affected by plant cultivar or locality (data not shown).

Furthermore, plant cultivar had an impact on the proportion of generalist and specialist herbivores found across the season (Wald statistic = 5.10, d.f. = 1, $P = 0.024$). In kale crops, the proportion of generalist lepidopterans was higher (85%) in comparison with cabbage crops (68%), whereas specialist herbivores were more abundant in cabbages (32%) than in kales (15%).

Plant cultivar had a significant effect on parasitism rate (Wald test = 5.9, d.f. = 1, $P = 0.015$), being higher in cabbage than in kale crops. Also, locality (Wald test = 1001.2, d.f. = 6, $P < 0.001$) and the interaction locality $\times$ plant cultivar (Wald test = 90.60, d.f. = 6, $P < 0.001$) were found significant. In fact, within locality, parasitism rate

was significantly different between crops in five out of seven localities, and it was higher in cabbage crops in four out of seven localities (Fig. 2). Parasitoid species diversity did not change between cultivars ($F_{1,7042} = 0.11$, $P = 0.746$) or localities ($F_{1,7042} = 0.27$, $P = 0.586$), whereas species richness was significantly higher in cabbage than in kale crops ($F_{1,7042} = 7.03$, $P = 0.010$) and also depended on location ($F_{6,7042} = 12.3$, $P = 0.028$) (Tables 2 and 3).

When considering species-specific effect of plant cultivar and locality on parasitism rate, we found that plant cultivar (kales) had a significant effect on parasitism rate of *P. rapae* larvae and pupae (plant cultivar: Wald test = 5,70 d.f. = 1, $P = 0,029$), although locality did not (Wald test = 12,24, d.f. = 6, $P = 0,057$). The interaction locality $\times$ cultivar was found significant (Wald test = 14,16, d.f. = 6, $P = 0,028$). However, parasitism rate of *P. rapae* and *P. brassicae* eggs was not affected by any variable. Parasitism rate suffered by *M. brassicae* larvae was not influenced by plant cultivar (Wald test = 0,42, d.f. = 1, $P = 0,519$), whereas locality (Wald test = 20,72, d.f. = 6, $P = 0,002$) and the interaction locality $\times$ cultivar were significant (Wald test = 13,82, d.f. = 6, $P = 0,032$). In addition, parasitism rate of the egg masses of this moth changed significantly depending on plant cultivar (Wald test = 4,3, d.f. = 1, $P = 0,039$), being more parasitized on kales, and on locality (Wald test = 904,5, d.f. = 5, $P < 0,001$). The magnitude of parasitism rate of *P. xylostella* larvae and pupae was not affected by plant cultivar (Wald test = 3,07, d.f. = 1, $P = 0,080$), whereas locality was significant (Wald test = 22,75, d.f. = 6, $P < 0,001$).

Table 4 shows the mean percent parasitism rate sorted by plant cultivar, host taxa, host developmental stage and locality. *P. xylostella* larvae suffered the highest attack in both kales and cabbages crops (77.6% and 81.9%, respectively), followed by *A. gamma* larvae (54.8% and 59.9%, respectively), *P. rapae* larvae and pupae (45.2%

and 37.1% respectively), *P. brassicae* larvae (38% and 38.3%, respectively), and *M. brassicae* eggs (35.5% and 23.1%, respectively), with significant differences between cultivars. The fluctuation of parasitism rate, host density and parasitoid abundance during the season in both crops is resumed in Figs. 3 A-B.

Parasitoid community and host range

P. rapae presented the greatest assemblage of parasitoid species (8 primary parasitoids and 2 secondary parasitoids), followed by *A. gamma* (7 primary parasitoid and 1 secondary parasitoid) and *M. brassicae* (6 primary parasitoids and 1 secondary parasitoid). *P. xylostella* was attacked by 4 primary parasitoids and 2 secondary parasitoids, among which the most abundant, in both cultivars and across the localities, was the ichneumonid *Diadegma fenestrale* (Holmgren) (Tables 2 and 3). *E. forficalis* was rarely found, and it was only attacked at Oroso, in kale crops, by the larval, gregarious parasitoid *Cotesia glomerata* L. (Hymenoptera: Braconidae), which parasitized the 3.3% of the hosts.

The egg-larval parasitoid *Copidosoma floridanum* (Ashmead) (Hymenoptera: Encyrtidae) which exclusively attacked *A. gamma*, accounted for 39.8% of the parasitoids emerged. Indeed, this value means that this parasitoid was the most represented in the study, although it does not imply that *C. floridanum* was the main responsible for the biological control of *A. gamma* in the field. In fact, only 8.8% of *A. gamma* pupae were attacked by this polyembryonic wasp, which may produce many hundreds of clonal broods from a single egg. The 16.2% of *A. gamma* larvae were controlled by both the tachinids *Voria ruralis* (Fallen) and *Pales pavidus* (Meigen), and by the ichneumonids *Camponotus annulatus* (Gravenhorst), *C. latrator* (Gravenhorst) (16.2%) and *Microplitis spinolae* (Nees) (1.5%). This last finding is very relevant, since

this is the first record of *M. spinolae* for Spain, and the first time that *C. latrator* is reported in association with *A. gamma*.

C. glomerata parasitized up to 3.3% of the *P. rapae* larvae population. Larvae of *P. rapae* were also attacked by both the tachinids *Epicampocera succincta* (Meigen) and *Phryxe vulgaris* (Fallen) (10%), and the braconid *Cotesia rubecula* (Marshall) (27.6%). The pupal parasitoid *Pteromalus puparum* L. (Hymenoptera: Pteromalidae) was found frequently, and emerged from 14.3% of the hosts. The egg parasitoid *Trichogramma* sp. emerged from the 10.3% of *P. rapae* eggs. The gregarious hyperparasitoid *Baryscapus galactopus* (Ratzeburg) (Hymenoptera, Eulophidae) emerged from just one *P. rapae* pupa.

The main parasitoid complex which attacked *P. brassicae* included *C. glomerata* and the tachinid *P. vulgaris*, which attacked the 32.3% and 16.5% of the larvae, respectively. *C. rubecula* and *Hyposoter ebeninus* (Gravenhorst) (Hymenoptera: Ichneumonidae) were sporadic.

The 5% of *M. brassicae* larval population was controlled by the braconid *Microplitis mediator* (Haliday) and just occasionally by *C. rubecula*, *Chelonus inanitus* L. (Hymenoptera: Braconidae) and *Hyposoter didymator* (Thunberg,) (Hymenoptera: Ichneumonidae). *H. didymator* is reported here for the first time in association with *M. brassicae*. The 20% of the *M. brassicae* eggs were parasitized by *Trichogramma* sp. (possibly *T. cacoeciae*) and the 16% by the scelionid *Telenomus* sp.

The highest parasitism rate was caused by *D. fenestrata*, which controlled up to 78.2% of the population of *P. xylostella*. This parasitoid peaked in both cultivars during July-August (80% of parasitism rate) (data not shown). The secondary parasitoid *Mesochorus pectoralis* (Ratzeburg) (Hymenoptera: Ichneumonidae) emerged from 7.4% of the pupae. This species is reported here for the first time in Spain. This

hyperparasitoid also sporadically parasitized *M. mediator* (primary parasitoid of *M. brassicae*), *M. spinolae* (primary parasitoid of *A. gamma*), and *C. rubecula* (primary parasitoid of *P. rapae*). The hyperparasitoid *Pteromalus semotus* (Walker) (Hymenoptera, Pteromalidae) and the larval parasitoids *C. plutellae* and *Diadromus subtilicornis* (Gravenhorst) (Hymenoptera: Ichneumonidae) were rarely found (Table 1).

Discussion

In this study, we tested the impact of intraspecific variation of host plant on the attractiveness to herbivores and natural enemies. As expected, Lepidopteran abundance was significantly higher in kales than in cabbages, although the overall parasitism rate and parasitoid species richness showed the opposite trend. This last finding does not agree with the prediction that the host plants with higher structural complexity could provide physical refuge to the hosts and negatively affect the host finding ability of natural enemies (Andow and Prokrym 1990; Gingras et al. 2002). Although top-down and bottom-up forces interact in complex ways in brassicaceous plants (Poelman et al. 2008; Gols and Harvey 2009), our results suggested that in the study systems both forces helped cabbage crop on limiting herbivore pressure.

Previous studies conducted on kale and cabbage varieties grown in NW Spain, including those used in the present investigation, have shown that sinigrin content was higher in kales, whereas glucobrassicin and glucoiberin may be dominant in cabbage cultivars (Velasco et al. 2007; Cartea et al. 2008). Our finding on insect distribution on different plant cultivars agree with the prevision that kale plants should be most visited by lepidopterans because sinigrin is known to act as a potent feeding and oviposition

stimulant for a wide range of crucifer-feeding insects (Shields and Mitchell 1995; Sarfraz et al. 2006; Hopkins et al. 2009), whereas glucoiberin may have a repellent/deterrent effect (Poelman et al. 2009; Kos et al. 2011). A synergistic interaction between the reduction of herbivore density caused by natural enemies and host-plant resistance level is expected (Hare 1992). More resistant host-plant should produce allelochemicals which impose a high metabolic stress to herbivores, sufficient to also increase their susceptibility to natural enemies. For instance, the slow-growth-high-mortality hypothesis tested with *P. rapae* and the larval parasitoid *C. glomerata*, predicts that prolonged development in herbivorous insects results in greater exposure to natural enemies and a subsequent increase in mortality (Benrey and Denno 1997). In our crop systems, the parasitism rate suffered by *P. rapae* larvae and pupae, mainly due to *C. glomerata*, *C. rubecula* and to the dipteran parasitoids, was significantly higher on kale plants (45.2%) than on cabbages (37.1%), although their abundance on plant cultivars did not differ. Thus, variation of foliar nutritional content of these crops possibly affects insect herbivore development and the performance of the natural enemies. However, our finding also agree with the study reported by Pimentel (1961), who showed that *P. rapae* larvae experienced greater parasitism rate on flat, opened leaf *B. oleracea* varieties, such as collard and kale, than on heading or curly-leafed plants, such as cabbage, Brussels sprouts and broccoli. Furthermore, resistant host-plant may release higher concentration of herbivore-induced volatile blends to attract parasitoids. In fact, parasitoids of herbivores specialized on Brassicaceae use specific breakdown products of glucosinolates, such as isothiocyanates and nitriles, as attractant (synomones) to find their hosts (Hopkins et al. 2009; Gols et al. 2012).

The proportion of specialists over the total amount of sampled herbivores was two-fold higher in cabbage (32%) than in kale (15%) crops. Defence compounds

produced by brassicaceous plants typically stimulate higher acceptance by specialist herbivores (Hopkins 2009), although it has been also shown that insect herbivory occurring early in the season affects the performance of subsequent colonizers by inducing significant changes in plant chemistry (Poelman et al. 2009). Specialist herbivores may be able to neutralize plant defences by way of detoxification, excretion and sequestration of noxious compounds (Ode 2006). *Pieris spp.* for example, can redirect the course of the normal hydrolysis reaction catalized by the enzyme myrosinase upon insect feeding, producing nitriles instead of toxic isothiocyanates, which the larvae excrete with their frass (Hopkins et al. 2009). Similar counter-adaptations have been developed by *P. xylostella*, which larvae possess in the gut a glucosinolate sulfatase enzyme that lead to the conversion of glucosinolates to desulfoglucosinolates, rather than nitriles and isothiocyanates (Ratzka et al. 2002). Actually, because of the ability of specialist lepidopterans to cope with plant defence metabolites, possibly other foliar chemical constituents, such as nitrogen, may be more important for larval performance than glucosinolate concentration (Awmack and Leather 2002). On the other hand, although the generalist *M. brassicae* can detect glucosinolates by receptor cells on the sensilla and its oviposition is induced by damaged plants (Hopkins et al. 2009), detoxification of plant defence compounds is less adopted due to the high metabolic cost involved (Schramm et al. 2012). This situation may determine a negative impact of host-plant chemistry on immature stages of larval parasitoids of *M. brassicae*. In fact, parasitism rate achieved by larval parasitoids was lower (6.7% and 5.7% in kale and cabbage, respectively) in comparison with the performance of the egg parasitoid complex (35.5% and 23.1% in kale and cabbage, respectively). Interestingly, although the abundance of *M. brassicae* eggs did not differ between cultivars, the relative abundance of the egg parasitoid species and consequently the parasitism rate achieved

was higher on kales. This finding suggests that kale bottom-up direct defences are poorly effective against *M. brassicae*, although it finally provided a higher herbivore control through the parasitoids (plant mediated indirect defence).

This study also provides a representative portrait of the Lepidoptera-*B. oleracea*-parasitoids tri-trophic system in NW Spain. In contrast to the greater attention paid to the lepidopteran herbivores, the diversity of their parasitoids complex and the breadth of the host range is still poorly known (Shaw et al. 2011). In this investigation, reliable data on the host-range for each parasitoid species was provided, and includes two new species for Spain (*Campoletis latrator* and *Mesochorus pectoralis*) and two new host-parasitoid relationships (*Autographa gamma*-*Campoletis latrator* and *Mamestra brassicae*-*Hyposoter didymator*).

The 86% of the identified primary parasitoids have the host range limited to one genus inside *Brassica* crops (Table1). This finding highlights the importance of the presence of undisturbed, late successional non-crop habitats, which could harbour alternate hosts, promoting the natural enemy recruitment (Marino and Landis 1996). In contrast, the generalist, obligatory, hyperparasitoid *M. pectoralis* attacked four different host families. This hyperparasitoid belongs to the small hymenopteran subfamily Mesochorinae (Ichneumonidae), which at present has 15 known species in the Iberian Peninsula (<http://ponent.atspace.org>). Hyperparasitism has traditionally been viewed as injurious to biological control efforts as the antagonistic interaction of hyperparasitoids may limit populations of the beneficial primary parasitoid (McDonald and Kok 1991; Frago et al. 2012), although Rosenheim (1998) pointed out that actually obligate hyperparasitoids may improve top-down control by stabilizing host-parasitoid fluctuations. In the present investigation, *M. pectoralis* emerged from 7.4% of the parasitized *P. xylostella* pupae, which could produce a negative impact on the efficiency

of the primary host, the larval-pupal parasitoid *D. fenestrata*. In fact, larval parasitism suffered by *P. xylostella* was higher in cabbages (81%) than in kales (77.6%), likely because the abundance of the hyperparasitoid was 3-fold higher in kales (Table 2 and 3). Another interesting trait of the studied parasitoid web is the accumulation of koinobiont species in both crops. According to Askew and Shaw (1986), koinobionts are generally specialists because of their need to interact with a living host, while idiobiont species are often considered generalist. If competition among parasitoid species contributes in determining the species richness, then idiobionts by virtue of their broader host range would be expected to increase under conditions of low competition and *vice versa* (Mills 1993).

The present study indicated that the efficiency of natural biological control of *M. brassicae* varied depending on host stage and cultivar. *M. brassicae* larvae supported six species of primary parasitoids, whereas only the egg parasitoids *Telenomus* sp. and *Trichogramma* sp. appeared to be promising candidates for biological control, with a mean parasitism rate of 37.1%. This parasitism rate is higher than those reported by Bianchi et al. (2005), who estimated that *Trichogramma* spp. and just marginally *Telenomus* spp., achieved an overall parasitism rate of 12.4% of *M. brassicae* egg batches. The existence of a high parasitism rate on the egg stage of *M. brassicae* is optimal from the perspective of crop protection, because plant injury can be completely prevented. The micro-hymenopterans such as *Trichogramma* and *Telenomus* have high dispersal ability thanks to their small size, hence, the absence of *Telenomus* in all but one locality of cabbage crops is difficult to explain (Tables 2 and 3). Considering that both cultivars grew close (< 1 m) in the subplots, this finding cannot be explained by environmental factors.

P. xylostella is one of the most destructive insect pests of *Brassica* crops (Talekar 1993). It is reported to be resistant to almost every insecticide applied in the field, including those of new chemistries, which led to increase the efforts worldwide to develop IPM programs based on the use of natural enemies (Sarfraz and Keddie 2005). Although over 135 parasitoids species are reported to attack various life stages, the most effective control is achieved by relatively few hymenopteran species belonging to the ichneumonid genera *Diadegma* and *Diadromus*, the braconid genera *Microplitis* and *Cotesia*, and the eulophid genus *Oomyzus* (Sarfraz et al. 2005). In the present study, *D. fenestrata* strongly controlled *P. xylostella*, with a parasitism rate ranging between 50-100%. It was regularly observed in both crops, including those where the host was less abundant. However, in contrast with the findings by Hambäck et al. (2010), we found a significant relationship between the presence of *D. fenestrata* and plant genotypes. Considering its efficiency, *D. fenestrata* may provide an effective biological control of *P. xylostella* on crops undisturbed by insecticides. Possibly, the augmentation releases of this parasitoid early in May-June could reduce the peak reached in July, as showed by Noda et al. (2000) in Japan.

In summary, this study suggested that intraspecific variation of host plant should play an important role in structuring the abundance and diversity of the associated insect herbivores and natural enemies. Indeed, this one-season investigation represents a first glimpse on the insect-plant interactions in *Brassica* agricultural systems and a long-term study, that addresses more accurately the relative contribution of top-down and bottom-up defences on regulating pest communities, is required. This study also highlights the potential of native parasitoid guilds for biological control of lepidopterans feeding on *B. oleracea* crops, and showed as the magnitude of the parasitism rate and the right species to use in biological control plans may vary depending on plant cultivar.

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Figures and tables captions

Fig.1 Location of the seven experimental plots of kale and cabbage in Galicia (NW Spain).

Fig. 2 Mean parasitism rate of all the stages of the sampled host taxa, sorted by plant cultivar and locality. Kale corresponds to white bars and cabbage to the grey bars. Black bars show the mean total parasitism rate per locality. Error bar represents $\pm$ SE. Bars sharing different letter are significantly different ($P < 0.05$). Comparisons of parasitism rate between plant cultivars in each locality and then across the localities were done by using logistic regression.

Fig. 3 A, B Overall parasitoid and herbivore number and percent parasitism rate estimated for each sampling date, in kale (A) and cabbage (B) crops. Herbivore number includes all the sampled stages. Error bars of the mean parasitism rate are too small to be seen.

Table 1. Parasitoid species status, habits, host-parasitoid relationships, total number and relative abundance (i.e. percentage of the total amount of individuals collected).

Table 2. Parasitoids emerged from hosts collected in kale crops, sorted by locality. Species diversity (H') was calculated by using the Shannon-Wiener diversity index. Richness is expressed as the number of different taxa per locality and sampling date.

Table 3. Parasitoids emerged from hosts collected in cabbage crops, sorted by locality. Species diversity (H') was calculated by using the Shannon-Wiener diversity index. Richness is expressed as the number of different taxa per locality and sampling date.

Table 4. Mean percent parasitism (mean $\pm$ SE) rate suffered by each host depending on its developmental stage, sorted by plant cultivar and locality (data are not transformed). N = total host sample size. Differences of total parasitism rates between cultivars have been obtained by using logistic regression. Groups labelled with (*) are significantly different ($P < 0.05$).

Supplementary Materials

S1. Temperature and relative humidity from June to November 2010. The number of days with minimum temperature (min) above 10°C, below 0 °C and 10 °C and the number of days with maximum temperature (max) above 10 °C, 20 °C and 30 °C are also reported. Environmental data of Leiro, Lalín and Soutelo were provided by official climate stations of the regional government (Xunta de Galicia, www.meteogalicia.es), located close to the study areas. Data from Pontevedra, Guitiriz, Oroso and Parada were obtained from climate stations of the Misión Biológica de Galicia, located near to the plots.

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