Combining flowering and banker plants to improve efficiency of parasitoids in the control of cabbage pests

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Dedication

This thesis is dedicated to God, my mother Ms. C. Carrillo Gutierrez, my father Mr. H. J. Zamora Carrillo, my brother Mr. L. I. Zamora Carrillo, and my nephew Master A. F. Zamora Carrillo.

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Abstract

Failures of biological control programs have been associated with factors, such as rearing systems of pest antagonists being of low quality, lack of food for pest antagonists and low efficiency of release methods among others. To contribute to the solution of these problems, techniques for improving the quality of rearing systems and strategies of habitat management were evaluated. In this context, the main goals of this study were, to evaluate different techniques for improving the quality of *Diaeretiella rapae* and *Encarsia tricolor* rearing systems (Chapter 2), to study the effect of flowering plants on the fitness and performance of the target parasitoids (Chapter 3), to study whether in the first five days after beginning of the experiment, banker plants could reduce the hot spots of pests infestation significantly and whether flowering plants improve the efficiency of banker plants (Chapter 4). In the second chapter, the approaches, type of host plant (broccoli var. Marathon F1, Brussels sprouts var. Hilds Ideal and cauliflower var. Freemont), architecture of the plant (with and without pruning) as well as cage size (0.21 m^3 and 0.02 m^3) were evaluated for improving the quality of the target rearing systems. The pruning involved cutting the roots, old and large leaves (> 15 cm) in the 4^{th} and 8^{th} week after sowing. Regarding the host plant experiments, the broccoli treatment had the best effect on most measured variables. For *D. rapae* experiment, the broccoli treatment had 182 mummies per plant more than cauliflower. For *E. tricolor* experiment, broccoli treatment had 88 mummies per plant more than cauliflower. Additionally, for both parasitoids, the incidence of powdery mildew was less in broccoli treatment compared to the other treatments. Considering the architecture experiments, pruning treatment had better performance than the control. For *D. rapae* experiment, pruning treatment had 106 mummies per plant more than the control, plants had no powdery mildew and parasitoids lived 2.57 days longer. For *E. tricolor* experiment, pruning treatment had 114 mummies per plant more than the control, plants had no powdery mildew and parasitoids lived 12 days longer. In the cage size experiments, the large cage had better effects than the small cage in most measured variables. For *D. rapae* experiment, the large cage had 69 mummies per plant more than the control. For *E. tricolor* experiment, the large cage had 121 mummies per plant more than the control and parasitoids from this treatment lived 7.5 days more than the control. In conclusion, the use of pruned broccoli plants in large cages substantially improved the quality of both rearing systems. In the third chapter, the attractiveness between different food resources (host-plant complex and flowering plant species) was compared. Thereafter, the effect of different food resources on the parasitoid longevity in climate chamber experiments, the impact of flowering plants on the parasitoid fecundity in greenhouse experiments as well as the influence of flowering plants on the performance of the target parasitoid in the field were measured. The parasitoid performance in the field was measured in terms of number of mummies and pest individuals, as well as the percentages of female/male parasitoids, parasitoid emergence and hyperparasitism by other species. The outcomes of the attractiveness experiments were: i) both adult parasitoids had access to the floral nectar of alyssum and buckwheat, but not to the faba beans; ii) alyssum had higher attractiveness to *D. rapae* compared to all other flowering plants; iii) the parasitoid *D. rapae* showed a higher preference for the host plant complex compared to flowering plants, except in the case of alyssum; iv) alyssum was more attractive to *E. tricolor* compared to buckwheat and v) the parasitoid *E. tricolor* showed a similar response for the host plant complex and flowering plants. The climate chamber and greenhouse experiments showed that the fitness of both parasitoid species was substantially increased given that: i) the *D. rapae* longevity on sources of high quality (honey and nectar) was 4.37 times as long as on sources without sugar (control and water) and 8.91 times for *E. tricolor*; ii) the *D. rapae* longevity was 2.07 times on buckwheat treatment as long as on alyssum and 1.53 times for *E. tricolor*; iii.) For *D. rapae*, the treatment with flowering plants (mix of alyssum and buckwheat) had 29.14 mummies more than the control, and iv) the number of mummies produced by *E. tricolor* did not differ significantly between the flowering plant treatment and the control. Finally, field experiments determined that the number of *D. rapae* and *E. tricolor* mummies with the flowering plant treatment were 2.1 and 1.4 times as much as the control, respectively. In conclusion, flowering plants can to attract parasitoids and play an important role in optimising the fitness of parasitoids. In the last chapter, it was first measured whether banker plants can give an opportune control at the beginning of the hot spots of the target pests and second whether the flowering plants can improve the efficiency of banker plants. The results showed that the parasitism rate in banker plant treatment was 55.5% for *D. rapae* and 39.9% for *E. tricolor*, respectively. The parasitism rate was estimated as the number of mummies related to the events of oviposition during the five days of the experiment divided by the initial number of hosts. It was also observed that flowering plants did not improve the efficiency of banker plants. Finally, it was concluded that the evaluated techniques in this study improve the fitness and performance of the parasitoid *D. rapae* in the field, but the efficiency of this techniques is not enough. Therefore, the optimal techniques to produce parasitoids in rearing systems as well as the role of banker and flowering plants are discussed as strategies to optimise the efficiency of parasitoids in terms of fitness and agronomical efficiency (percentage of mummies) and to contribute to the integrated pest management.

Keywords: *Brevycorine brassicae; Aleyrodes proletella; Diaeretiella rapae; Encarsia tricolor;* Banker plants; Flowering plants; Improved quality of rearing systems

Zusammenfassung

Das Scheitern der biologischen Bekämpfung von Schadarthropoden hängt oft mit Faktoren wie der geringen Qualität von Züchtungssystemen, einer zu geringen Menge an Nahrung und Wirten für Nützlinge oder einer geringen Effizienz der Freilassungsmethoden zusammen. Als Beitrag zur Lösung dieser Probleme befasst sich diese Arbeit mit der Bewertung und Verbesserung von Aufzuchtsystemen und Habitatmanagement, um die Qualität und Leistungsfähigkeit von Parasitoiden zu verbessern.

In diesem Zusammenhang hatte diese Studie die Hauptziele, verschiedene Techniken zur Verbesserung der Qualität von *Diaeretiella rapae*- und *Encarsia tricolor*-Zuchtsystemen (Kapitel 2) zu bewerten, den Einfluss von Blühpflanzen auf die Fitness der Parasitoide unter kontrollierten Bedingungen sowie die Leistung im Feldversuch zu messen (Kapitel 3), zu untersuchen, ob Banker Plants den Schädlingsbefall in den ersten fünf Tagen nach dem Start des Experiments reduzieren können und ob Blühpflanzen die Wirkung von Banker Plants verbessern (Kapitel 4).

Die Wirtspflanzen (Broccoli var. Marathon F1, Rosenkohl var. Hilds Ideal und Blumenkohl var. Freemont), Behandlung der Pflanzen (mit und ohne Beschneidung) sowie zwei Größen von Käfigen (0,21 m^2 und 0.02 m^3) waren die Faktoren, die im zweiten Kapitel untersucht wurden, um die Qualität der Züchtungssysteme zu verbessern. Beschnitten wurden die Wurzeln, sowie alte und große Blätter (> 15 cm) in der 4. und 8. Woche nach Aussaat. Bei der Untersuchung der Wirtspflanzen hat die Brokkolivariante hinsichtlich der meisten Parameter am besten abgeschnitten. Somit waren auf den Brokkolipflanzen 182 *D. rapae*-Mumien und 88 *E. tricolor*-Mumien mehr als auf Blumenkohl. Unabhängig von der Parasitoidenart trat in den Varianten mit Brokkoli weniger Echter Mehltau auf als in den anderen Varianten. In den Experimenten zur Pflanzenarchitektur schnitten die Varianten mit Wurzel- und Blattschnitt besser ab als die ohne. Hinsichtlich *D. rapae* wurden 106 mehr Mumien pro Pflanze beobachtet als in der Kontrolle. Diese Pflanzen hatten keinen Echten Mehltau und die Parasitoide lebten 2,57 Tage länger. Bei den Varianten mit *E. tricolor* wurden 114 mehr Mumien pro Pflanze auf beschnitten Pflanzen gefunden als auf Kontrollpflanzen, die Pflanzen hatten keinen Echten Mehltau und die Parasitoide lebten 12 Tage länger. In den Versuchen zur Käfiggröße hatte ein großer Käfig einen besseren Effekt auf die meisten gemessenen Parameter als ein kleiner Käfig. Pflanzen im großen Käfig hatten 69 *D. rapae*-Mumien mehr pro Pflanze als die Kontrolle. Zudem wurden in großen Käfigen 121 *E. tricolor*-Mumien pro Pflanze mehr gefunden und die adulten Parasitoide dieser Art lebten 7,5 Tage länger als in der

Kontrolle. Abschließend betrachtet zeigte die Verwendung von beschnittenen Brokkoli-Pflanzen in großen Käfigen eine Verbesserung der Züchtungssysteme beider Parasitoide.

Im dritten Kapitel wurde die Attraktivität verschiedener Nahrungsressourcen vergleichend betrachtet (Wirt-Pflanze Komplex und Blühpflanzenart). Nachdem der Effekt von verschiedenen Nahrungsressourcen auf die Lebensdauer der Parasitoide unter kontrollierten Bedingungen in der Klimakammer erfasst wurde, wurden Untersuchungen zum Einfluss von Blühpflanzen auf die Fertilität und Leistungsfähigkeit, bzw. der Parasitierung im Gewächshaus durchgeführt. Zur Bewertung der Leistung der Parasitoide wurden die Anzahl an Mumien und Schädlingen, sowie der Anteile an weiblichen/ männlichen Parasitoide, geschlüpften Parasitoiden und Hyperparasitismus durch andere Arten erfasst. In den Experimenten zur Attraktivität wurde Folgendes beobachtet: 1. Beide Parasitoide haben Zugang zum Blütennektar von Steinkraut und Buchweizen, jedoch nicht zu dem der Ackerbohne; 2. Steinkraut wies gegenüber *D. rapae* die höchste Attraktivität auf; 3. *D. rapae* zeigte eine hohe Präferenz für den Wirt-Pflanze Komplex verglichen mit Blühpflanzen; 4. Steinkraut hatte im Vergleich mit Buchweizen eine höhere Attraktivität auf *E. tricolor*; 5. *E. tricolor* zeigte eine ähnliche Präferenz für den Wirt-Pflanze Komplex und Blühpflanzen.

Die Klimakammer- und Gewächshausexperimente zeigten im Wesentlichen, dass die Qualität beider Parasitoide erhöht wurde: 1. Die Lebensdauer von *D. rapae* und *E. tricolor* war 3,37- bzw. 7,91-mal länger bei Nahrung hoher Qualität (Honig und Nektar) im Vergleich zu Nahrungsquellen ohne Zucker; 2. Die Lebensdauer von *D. rapae* und *E. tricolor* war 1,07- bzw. 0,53-mal so lang mit Buchweizen gegenüber der Variante mit Steinkraut; 3. Die Anzahl der gefundenen *D. rapae*-Mumien war in der Behandlung mit blühenden Pflanzen (Mischung aus Buchweizen und Steinkraut) war um 29,14 Mumien höher als in der Kontrolle; 4. Die Anzahl der gefundenen *E. tricolor* Mumien war in allen Behandlungen ähnlich. Im Feldversuch zeigte sich schließlich, dass die ausgesuchten Blühpflanzen die Anzahl der *D. rapae*- und *E. tricolor*-Mumien um das 2,1- bzw. 1,4-fache erhöhten. Zusammengefasst zeigte sich, dass Blühpflanzen in der Lage sind die Parasitoide anzulocken und eine wichtige Rolle bei der Optimierung ihrer Fitness spielen.

Im letzten Kapitel wurde zunächst untersucht, ob Banker Plants eine rechtzeitige Bekämpfung zu Beginn der Besiedlung des Zielschädlings bieten können und zudem, ob Blühpflanzen die Wirkung der Banker Plants steigern können. Die Ergebnisse zeigten, dass die Parasitierungsrate durch *D. rapae* und *E. tricolor* in Varianten mit Banker Plants 55,5%, bzw. 39,9% betrugen. Die Parasitierungsrate wurde als die Anzahl an Mumien (parasitierte Wirte) während der ersten fünf Tage des Experiments geteilt durch die Anzahl der Wirte zu Beginn

definiert. Darüber hinaus wurde beobachtet, dass blühende Pflanzen die Effizienz der Banker Plants nicht verbessert haben. Schließlich verbesserten die untersuchten Techniken die Fitness und Leistung der Parasitoide in den Feldversuchen, jedoch ist die Effizienz dieser Techniken nicht ausreichend. Deshalb werden die optimalen Techniken zur Produktion von Parasitoiden in Zuchtsystemen, sowie die Rollen von Banker Plants und blühenden Pflanzen als Strategien diskutiert, um die Effizienz der Parasitoide hinsichtlich der Fitness, der agronomischen Effizienz oder dem Anteil an Mumien zu verbessern.

Schlüsselwörter: ***Diaeretiella rapae; Encarsia tricolor; Brevycorine brassicae; Aleyrodes proletella;*** **Banker Plants; Blütenpflanzen; Verbesserte Qualität von Zuchtsystemen**

Abbreviations

ANOVA	Analysis of Variance
BCA	Biological Control Agent
CBC	Conservation biological control
°C	Degree Celcius
Fig.	Fig.
GLM	General linear model
HPC	Host Plant Complex
H	Hour
HSD	Honestly Significant Difference
IPM	Integrated Pest Management
L: D	Light: Darkness Photoperiod
RH	Relative humidity
%	Percentage
SE	Standard Error

Note: For all measurements the SI units and their derivatives were used

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1. General introduction

Mass rearing systems of pest antagonists being of low quality (Van Lenteren, 1991, 1993, 2003), deficiency of food for pest antagonists in the field (Landis *et al*., 2000) and unsuitable releasing methods (Collier and Van Steenwyk, 2004) are causes associated with the low efficiency of biological control programs.

The present work was focused on the following pest antagonists: *Diaeretiella rapae* and *Encarsia tricolor*. These species are parasitoids of *Brevicoryne brassicae* (cabbage aphid) and *Aleyrodes proletella* (cabbage whitefly), respectively. The cabbage aphid and whitefly are important pests of many cultivar groups of the *Brassica* genus, including broccoli, cauliflower, napus, and turnip among others. These pests lead to serious direct damage when they feed on the plant and indirect damage when they produce honeydew that favours the growth of sooty mould and hence reduce the photosynthetically active area of the plant. Moreover, the cabbage aphid can also transmit viruses (DeBarro and Carver, 1997). These pests are mainly managed with agrochemicals, bearing an absolute risk for residues in the produce which is a matter of health concern for consumers, are harmful to the environment and force development of pest resistance if intensively used. Hence, the trend of consumer demand for products free of pesticides and the necessity to evaluate alternative strategies for eco-friendly integrated pest management is arising. These pests are only associated with cabbage crops (brussels sprouts, broccoli, cauliflower among others) which have high importance in Germany according to the Federal Ministry of Food and Agriculture of Germany. In 2013, a total of 5836 ha were planted with white cabbage, 4241 ha with cauliflower, 2172 ha with broccoli, 1915 ha with red cabbage and 1873 ha with kohlrabi, meaning that cabbage covered around 18% of the areas under cultivation outdoors.

The koinobiont, solitary, endoparasitoids treated in this study were *D. rapae* and *E. tricolor*. They are the only parasitoids of *B. brassicae* and *A. proletella*. These parasitoids have a higher preference for these pests than others (Williams, 1995; Freuler *et al*., 2003; Kant, 2012). The parasitoid *D. rapae* (McIntosh) (Hymenoptera: Aphidiinae) is of Western Palearctic origin and has spread to other parts of the world (Carver and Stary, 1974). The parasitoid *E. tricolor* (Hymenoptera: Aphelinidae) is native to Europe and this is more frequent in Mediterranean and all Palearctic regions (Evans, 2002; Hernandez *et al*., 2003). These parasitoids survive through seasons and can live in diverse ecozones of the earth, showing a high plasticity. However, the agronomic efficiency in terms of parasitism percentage by these parasitoids is not enough to avoid strong yield losses.

Consequently, this study focused on the evaluation of techniques for enhancing the quality of rearing systems as well as habitat management strategies for improving the fitness and performance in the field of the selected parasitoids. The tested approaches for enhancing the quality of rearing systems were host plant, plant architecture and cage size. The evaluated habitat management techniques were flowering and banker plants. These techniques minimize the negative effect of many practices of the modern agriculture, such us domestication of plant species, monoculture, use of pesticides among others. During the domestication process of crop plants, often genes responsible for the production of secondary metabolites for pest resistance have been discarded. The monoculture offers a high quantity of food, increasing the permanence and performance of the pest individuals. Additionally, the richness and abundance of plant species that gives refuge, provides food and alternative hosts to pest antagonists has been reduced. The use of pesticides kills pest antagonists, increases the production costs, and gradually generates resistance in the pest (Altieri, 1999; Balmer *et al*., 2014).

In second chapter, techniques to optimise the quality of mass rearing systems were studied because they can be used to obtaine parasitoids for releasing by hand for augmentation programs or to provide banker plants for conservation biological control (Van Lenteren, 2003; Pickett *et al.*, 2004). Hence, the poor quality of rearing systems can negatively affect the efficiency of biological control agents for the management of pest in farmer´s crops as well as in research programs despite the possible high potential of these organisms (Van Lenteren, 1991, 1993, 2003; Frank, 2010). Nonetheless, the study about strategies or optimising the rearing systems has received little attention and should be a fundamental matter of concern in biological control.

Techniques to optimising the rearing systems were studied because at the beginning of this research, parasitoids lived two or three days, despite they were provided with food of high quality. Additionally, banker transplants from rearing systems had a high incidence of powdery mildew, low number of herbivores per plant, low number of mummies per plant, small size of mummies, in the cuticle of the herbivores grew fungi, and hence these rearing plants could not be used neither as provision of parasitoids for the fitness experiments nor as banker plants for transplanting in the field. Therefore, in second chapter, techniques for improving the quality of rearing systems were evaluated in order to produce parasitoids and banker plant to transplant in field of high quality. When natural enemies are reared for a long time, their fitness is reduced because of endogamy (Geden *et al*., 1992; Hoekstra, 2003) and conditions of high nursing (Lewis *et al*., 2003). Endogamy refers to mating between individuals of a small group of a species. With respect to the conditions of high nursing, such as optimal microclimate conditions and high availability of food, host, and mate, the insects reduce the searching intensity as well as the ability to adapt, reproduce and survive in agroecosystems. The solution for this problem is to reintroduce new populations from field to the rearing system (Nunney, 2003). Nevertheless, the quality of a rearing system is also determined by other management aspects (Freuler *et al*., 2003; Frank, 2010; Jandricic *et al*., 2014), such as the host plant (variety, subspecies, cultivar group among others), plant architecture and cage size. Hence, in this chapter, the hypothesis was that the management of rearing systems is a factor influencing the satisfactory development of the plant and hence the herbivore and parasitoid fitness, causing a cascade effect.

The genetic characteristics of the host plant and the optimal management of a rearing plant are factors determining the size and the reproductive rate of the host (Price *et al*., 1980; Ellis *et al*., 1996). The size of the herbivore is important because large herbivores result in parasitoids that live longer (Hardy *et al*., 1992; Silva *et al*., 2011). It is due to the fact that the parasitoid larvae have a higher availability of nutrients (Jervis, 1998). The effect of the host size on the *D. rapae* and *E. tricolor* longevity has been demonstrated by Kant *et al*., (2012) and Williams, (1995), respectively. Rearing plants with a high number of hosts and parasitoids that live a long time contribute to obtaining a high production of mummies (Kant, 2012). The quantity of mummies per plant is a relevant factor determining the number of needed banker plants. A high number of mummies per plant reduces the required number of banker transplants per hectare (Frank, 2010). Plants of high quality should also have a high tolerance to the target herbivore and low incidence of non-target organisms. These factors allow obtaining plants that live longer, the leaves are not quickly wilted, the stems are straight, the number of herbivores and number of mummies per plant is high, the frequency of renovation as well as work and costs linked with these activities are low (Frank, 2010).

The effect of host plants, plant architecture and cage size were evaluated to get rearing systems of high quality. Host plants (cultivar groups) have an important role on the life history parameters of the herbivore and parasitoid fitness (Freuler *et al.*, 2003; Bayhan *et al.*, 2007; Frank, 2010; Augustin, 2012; Jandricic *et al*., 2014) because pests generate different degrees of selection pressure on plants and as a result of this, cultivar groups or varieties of the same species have various levels of resistance to herbivores. In this sense, to get rearing plants with a high production of pest antagonists, this is relevant the use of host plants with a high tolerance to the herbivore and low resistance by antibiosis and antixenosis to the target herbivore as well as a low incidence to non-target organisms. Non-target organisms are a source of contamination that compete for photoassimilates with the target herbivores and reduce the quality of the rearing plant (Frank *et al*.,2010). Tolerance is the capacity of the plant of remaining vigorous with a high population of the pest (Strauss and Agrawal, 1999; Juenger and Lennartsson, 2000). Antibiosis is defined as different mechanisms of the plant, interfering negatively in the biology and development of the pest and antixenosis refers to properties of plants, avoiding the infestation of the pest (Teetes, 2007).

The plant architecture is an aspect influencing the physiology of the plant as well as the tolerance towards abiotic and biotic stress and the lifespan of the rearing plants. The plant architecture can be modified by pruning. The pruning treatment of this work focused on cutting old leaves, roots and leaves higher than 15 cm. Due to the lack of knowledge in this topic, the pruned treatment was chosen based in preliminary experiments. Old leaves pruning avoids the growing populations of *Myzus persicae* (personal observation). Plants with root pruning require less water, the transpiration is less (Reich, 1997) and hence the relative humidity is low. Low relative humidity avoided the presence of powdery mildew (Agrios, 1997). Pruning of larger leaves (higher than 15 cm) prevents the overlapping of leaves. The overlapping of leaves is another factor that should be avoided in rearing systems because favours high relative humidity and the development of fungi (Personal observation).

The last studied factor was the cage size which influences the quantity of light. The quantity of light is important because plants as cabbage that normally develop outdoors when they grow in cages, the little quantity of light can produce elongation and weakness (Ballaré, 1994; Lambers *et al*., 2008).

The indicators to measure the quality of the target rearing systems in this chapter were size of the herbivore, longevity of the parasitoid, number of mummies per plant as indicator of virulence, contamination of the non-target organism powdery mildew as well as adaptation after the transplanting when rearing plants are used as banker plants (Hardy *et al*., 1992; Williams, 1995; Frank, 2010; Kant *et al*., 2012). Additionally, the plant physiological variables, relative humidity and quantity of light in some experiments were measured to explain the results of the mentioned variables.

The objective of the third chapter was to evaluate the effect of different flowering plant species on the performance of the target parasitoids. For this purpose, the following topics were studied: the access to the nectar of selected flowering plants by parasitoids; the attractiveness of different food resources to parasitoids in laboratory experiments; the effect of various food resources on the longevity of the target parasitoids in climate chamber experiments, the influence of flowering plants on the parasitoid fecundity under greenhouse conditions and the effect of flowering plants on the performance of the target parasitoids under field conditions in terms of the number of mummies, percentage of parasitoid emergence, percentage of female parasitoid, percentage of male parasitoid and hyperparasitoid percentage from other species.

Flowering plants produce volatiles, attracting naturally occurring parasitoids and minimising migration of release parasitoids (Orre Gordon *et al.*, 2003; Bianchi and Wäckers, 2008; Pineda and Marcos García, 2008). Moreover, these plants represent an important source of food to parasitoids. Food is important for feeding, mating, oviposition, searching behaviours and metabolism of parasitoids (Leatemia *et al*., 1995; Rivero and Casas, 1999; Begum *et al*., 2006; Jervis *et al*., 2008; Varennes *et al*., 2015).

Parasitoids are omnivorous hence they can consume floral resources as well as the food provided by the host (Thompson, 1999; Fiedler and Landis, 2007; Jervis *et al*., 2008; Wäckers *et al*., 2008). Nectar is a solution composed principally of sucrose, glucose, fructose and water (Chalcoff *et al*., 2006), playing a significant role in the survival and fecundity of many parasitoids. Pollen is a source of protein, but no all parasitoids have mouthpart specialisations to consume this food (Jervis, 1998). Besides, vegetative features of selected plants may improve the fitness of biological control agents (Landis *et al*., 2000; Rebek *et al*., 2006), given that they reduce the adverse effect of extreme conditions in terms of humidity and/or temperature and provide favourable microclimatic conditions for the parasitoids.

As studies on flowering plants have reported positive benefits of this technique as well as negative and neutral too, a critical selection of flowering plants was conducted in this chapter because, i) crop pests could also exploit floral resources (Begum *et al*., 2006), ii) the accessibility to floral resources by parasitoids can be limited (Patt *et al*., 1997), iii) every flowering plant species can offer different kinds of benefits; for example, in *D. tasmanica*, alyssum had a better effect on the longevity, but buckwheat had a better effect on fecundity, iv) the comparative effect of a determined flowering plant species can be different in every natural enemy species; for example, buckwheat had a better effect than alyssum on the longevity of *Aphidius ervi* (Araj *et al*., 2006) and *Gonatocerous* spp. (Irvin *et al*., 2007) but in a study of Irvin *et al,* 2006 with *Dolichogeneidea tasmanica* was shown that alyssum-fed parasitoids lived longer than those fed on buckwheat and v) organisms of the fourth trophic level can benefit from flowering plants, minimizing significantly the efficiency of parasitoids on the management of the target pests (Araj *et al*., 2009).

The flowering plants alyssum *(Lobularia maritima)*, buckwheat *(Fagopyrum sculentum)* and faba bean *(Vicia faba)* in this study were chosen based on their reported benefits for programs of conservation biological control, including the availability of the seeds in the market, they are neither invasive nor offer benefits for cabbage pests, buckwheat begins the blooming before than alyssum (personal observations) but alyssum has longer blooming period which guarantees availability of floral resources throughout all growing season, (Hogg *et al*., 2011), buckwheat has high relation sucrose/hexose (Vattala *et al*., 2006), alyssum and buckwheat flowers are neither deep nor tubular which avoid the attraction of butterflies (Barret *et al*., 1996), they have a short time passage from sowing to blooming compared with other plant species (only six-eight weeks), they are known to be attractive and improve the fitness of several pest antagonists (Begum *et al*., 2006; Irvin *et al*., 2006; Irvin *et al*., 2007; Webb, 2010, Hogg *et al*., 2011, Jamont *et al*., 2013) and they have other uses (Alyssum and buckwheat are used for management of weeds according to Platt *et al*, 1999 and nectar for bees as well as faba beans buckwheat and faba beans are food for people or animals).

The objectives in the fourth chapter were to evaluate whether banker plants can give an opportune control and whether flowering plants can improve the efficiency of banker plant produced under controlled conditions. Banker plant is a system integrated by a plant and pest individuals with its respective natural enemy species that the farmer uses in the field in order to introduce and/or to spread antagonists that attack crop pests (Frank, 2010; Huang *et al*., 2012). On the majority of the cases banker plants only introduce the parasitoids and improve the management of the pest during the season of cropping but in some cases can also to help to parasitoids to overcome the seasons (Frank, 2010).

These systems combine aspects of augmentation and conservational biological control and can be used for improving the efficiency of both predators (Ramakers and Voet, 1996) and parasitoids (Jacobson and Craft, 1998; Goolsby and Ciomperlik, 1999; Van Driesche *et al*., 2008). Released parasitoids with banker transplants in the field is more efficient and cheap than multiple releases by hand with paper bags or cards (Stacey, 1977; Goolsby and Ciomperlik, 1999; Conte *et al*., 2000; Pickett *et al*., 2004; Frank, 2010). It is due to the fact that mummies released by hand could be killed during the manipulation of these in harvesting (Bigler, 1993) and/or transport (Fernández and Nentwig, 1997) and parasitoids that born from the plant have a better adaptation that those from releasing by hand (Goolsby and Ciomperlik, 1999; Pickett *et al*., 2004). Additionally, mummies from banker plants are protected from wind, high temperature or other factors in the underside of the leaf. Banker plants could have attractiveness to naturally occurring parasitoids because in some cases the plant elicits allelochemicals when the pests attack their tissues (De Moraes *et al*., 1998). For instance, *D. rapae* is able to recognize volatiles that produce plants when they are attacked by pests (Reed *et al*., 1995; Bradburne and Mithen, 2000; Hopkins *et al*., 2009) and similar results were observed in *A. ervi* (Wickremasinghe and van Emden, 1992; Guerrieri *et al*., 1993; Du *et al*., 1998).

Banker plant produced in rearing systems under control conditions were utilised because with the appropriate technique, these have a better quality than those produced in open rearing systems (Goolsby and Ciomperlik, 1999) for the following reasons: i) production of banker plants in open rearing systems are affected by biotic, abiotic and management stress. For example, production of parasitoid banker plants is not possible in places with high incidence of hyperparasitoids; ii) during the production of banker plants in open rearing systems, these plants need time for growing, the establishment of the host and then for the reproduction of the parasitoid. Therefore, if the environmental conditions favour the development of the pest, the increasing the pest populations during this time could be very high and then very difficult to manage even with agrochemicals (Stacey, 1977; Conte *et al*., 2004; Pickett *et al*., 2004).

The banker plants of this study were produced with non-alternative host because, despite Pike *et al*., (1999) reported 60 hosts (herbivore species) for *D. rapae* recognized worldwide, in preliminary experiments of this study, it was observed that these wasps did not lay eggs on *Aphis fabae*, *Sitobion avenae*, *Macrosiphum euphorbiae* and *Rophalosiphum maidis* and only lay eggs on cabbage aphids like *B. brassicae*, and *Myzus persicae*, confirming the results of Freuler *et al*., (2003), who found that this parasitoid only lay eggs in cabbage aphids in Central Europe. It can be explained taking into account that the host range of *D. rapae* and other parasitoids can depend on the geographical place (Baer *et al*., 2004; Antolin *et al*., 2006 and Le Relac *et al*., 2011). It means that, the genetic variability and flow of genes may influence the performance of parasitoids to utilise the herbivores species (Baker *et al*., 2003).

1.1. Objectives

1.1.1. Main objective

The main objective of this study was to improve the efficiency of *D. rapae* and *E. tricolor* by use of techniques to obtain rearing systems of high quality, and habitat management techniques, i.e. flowering plants and banker plants.

1.1.2. Specific objectives

1) To evaluate the effect of different host plants (cultivar groups), plant architectures and cage sizes on the quality of *D. rapae* and *E. tricolor* rearing plants (Chapter 2).
2) To study the effect of different food resources on the fitness and field performance of the parasitoids *D. rapae* and *E. tricolor* (Chapter 3).
3) To observe if banker transplants can reduce the hot spots or initial points of pest infestation (Chapter 4).
4) To examine whether flowering plants can improve the efficiency of *D. rapae* and *E. tricolor* banker transplants for the management of *B. brassicae* and *A. proletella,* respectively (Chapter 4).

1.2. Hypotheses

The following hypotheses were evaluated in this study:

1) The host plant and the management have an effect on the quality of the rearing systems (Chapter 2).
2) Nectar provided by flowering plants improves the fitness and performance of the parasitoids *D. rapae* and *E. tricolor* (Chapter 3).
3) Banker plants can achieve an opportune control of the hot spots of the target pests (Chapter 4).
4) Flowering plants can improve the efficiency of banker plants (Chapter 4).

2. Improving quality of *Diaeretiella rapae* and *Encarsia tricolor* rearing systems

Abstract

Improved quality of rearing systems can enhance the efficiency of pest antagonists released by hand or by banker plants. The parasitoids *Diaeretiella rapae* M´Intosh (Hymenoptera: Aphidiidae) and *Encarsia tricolor* Förster (Hymenoptera: Aphelinidae) are present on crops under open field conditions but their efficiency is insufficient to maintain pests below the economic injury level. To improve the quality of the rearing systems of both parasitoids, the effect of three host plants: Broccoli var. Marathon F1, Brussels sprouts var. Hilds Ideal and Cauliflower var. Freemont; two architectures of the plant, namely with and without pruning; as well as two sizes of cages, at 0.21 m^3 and 0.023 m^3 were evaluated. Indicators of quality or measured variables were the size of the herbivore, number of mummies, longevity of parasitoids, and incidence of powdery mildew among other variables. With respect to the host plant experiments, broccoli treatment had the best performance in almost all measured variables. For *D. rapae* experiment, broccoli treatment had 182 mummies per plant more than cauliflower, and 88 for *E. tricolor* experiment. Additionally, for both parasitoids, the incidence of powdery mildew was less in broccoli treatment compared to the other treatments. Regarding the plant architecture experiments, pruning treatment had better performance than control. For *D. rapae* experiment, pruning treatment had 106 mummies per plant more than control, plants had no powdery mildew and parasitoids lived 2.57 days longer. For *E. tricolor* experiment, pruning treatment had 114 mummies per plant more than the control, plants had no powdery mildew and parasitoids lived 12 days longer. In the experiments of cage size, the large cage treatment had better performance that small cage in most of the measured variables. For *D. rapae* experiment, the large cage had 69 mummies per plant more than the control. For *E. tricolor* experiment, the large cage had 121 mummies per plant more than the control and parasitoids from this treatment lived 7.5 days longer than the control. In conclusion, the use of pruned broccoli plants in large cages substantially improved the quality of both rearing systems.

Key words: Rearing system quality; *Diaeretiella rapae; Encarsia tricolor; Brevicoryne brassicae; Aleyrodes proletella*

2.1. Introduction

The poor quality of rearing systems can negatively affect the efficiency of pest antagonist for the management of pests in crops as well as in research programs, despite the possible high potential of these organisms (Van Lenteren, 1991, 1993, 2003; Frank, 2010). The rearing systems have particular importance because they are used to rear pest´s antagonists under artificial conditions as mass produce for releasing by hand for augmentation programs or provide banker transplants for conservation biological control (Van Lenteren, 2003; Pickett *et al.*, 2004). Nevertheless, the study about strategies or optimising the rearing systems has received little attention and should be a primary matter of concern in biological control.

In this study, the parasitoid-pest systems: i) *Diaeretiella rapae* - *Brevicoryne brassicae* (cabbage aphid) and ii) *Encarsia tricolor* - *Aleyrodes proletella* (cabbage whitefly) were chosen. The cabbage aphid and whitefly are two important pests for many cultivar groups of the *Brassica* genus. The parasitoids *D. rapae* and *E. tricolor* are the respective natural enemies of these pests.

At preliminary experiments of this research, there were no differences on the longevity of parasitoids fed with food of high or low quality, parasitoids lived a few days as well as plants from rearing systems had a high incidence of powdery mildew, low number of herbivores, low number of mummies, small size of mummies, in the cuticle of the herbivores grew fungi and hence these plants could not be used as banker plants. Hence, in this chapter, techniques for improving the quality of rearing systems were evaluated to produce parasitoids, and banker transplants of high quality.

When natural enemies are reared for a long time, the fitness of them is reduced because of endogamy (Hoekstra, 2003) and conditions of high nursing (Lewis *et al.*, 2003). Endogamy refers to mating among individuals of a small group of a species, having negative consequences in the fitness of organisms. With respect to the conditions of high nursing such as optimal microclimate conditions and high availability of food, host, and mate, the insects reduce the searching capacity and ability to adapt, reproduce and survive in agroecosystems. The solution of this problem is to reintroduce new populations from field to the system of rearing (Nunney, 2003). Nonetheless, the quality of a rearing system is also determined by other management aspects (Freuler *et al.*, 2003; Frank, 2010; Jandricic *et al.*, 2014) such as the host plant (variety, subspecies, cultivar group among others), plant architecture and cage size. In this chapter, the hypothesis was: the host plant and the management of rearing systems is a factor influencing the quality of the plant and hence the herbivore and parasitoid fitness, causing a cascade effect.

The genetic characteristics and the optimal state of a rearing plant are factors determining the size and the reproductive rate of the host (Price *et al*., 1980; Ellis *et al*., 1996). The size of the herbivore is important because large herbivores result in parasitoids that live longer (Hardy *et al*., 1992; Silva *et al*., 2011). It is due to the fact that the parasitoid larvae have a higher availability of nutrients (Jervis, 1998). The effect of the host size on the *D. rapae* and *E. tricolor* longevity has been demonstrated by Kant *et al*., (2012), and Williams, (1995), respectively. The reproductive rate of the host is proportional to the produced number of mummies per plant. Rearing plants with a high number of hosts and parasitoids that live a long time contribute to obtaining a high production of mummies. The quantity of mummies per plant is a relevant factor determining the number of required banker plants. A high number of mummies per plant reduces the needed number of banker transplants per hectare (Frank, 2010). Additionally, plants with a high tolerance to the target herbivore and a low incidence of non-target organisms are important factors in the quality control of the rearing plant. These factors allow obtaining plants with long life, the leaves are not quickly wilted, stems are straight, the number of herbivores and number of mummies per plant is high, the frequency of renovation as well as work and costs linked with these activities are low (Frank, 2010).

The effect of host plants, plant architecture and cage size were evaluated because the genetic of the plant as well as the management have influence in the development of the plant, target organisms and non-target organisms in the rearing systems (Bayhan *et al.*, 2007; Frank, 2010; Jandricic *et al*., 2014; Jahan *et al*., 2014). Host plants (cultivar groups) have an important role in the life history parameters of the herbivore and parasitoid fitness (Freuler *et al.*, 2003; Bayhan *et al.*, 2007; Frank, 2010; Augustin, 2012; Jandricic *et al*., 2014) because pests generate different degrees of selection pressure on plants and as a result of this, cultivar groups or varieties of the same species have various levels of resistance to herbivores. In this sense, in order to get rearing plants of high production of pest antagonists, this is relevant the use of host plants with a high tolerance to the herbivore and low resistance by antibiosis and antixenosis to the target herbivore as well as a low incidence to non-target organisms. Non-target organisms are sources of contamination that compete for photoassimilates with the target herbivores and reduce the quality of the rearing plant (Frank *et al*., 2010). Tolerance is the capacity of the plant of remaining vigorous with a high population of herbivores (Strauss and Agrawal, 1999; Juenger and Lennartsson, 2000). Antibiosis is defined as different mechanisms of the plant, interfering negatively in the biology and development of the pest and antixenosis refers to properties of plants, avoiding the infestation of the pest (Teetes, 2007).

The plant architecture is an aspect influencing the plant physiology and hence the tolerance towards abiotic and biotic stress as well as the lifespan of the rearing plants. The plant architecture can be modified by pruning. Due to the lack of information in this topic, this treatment was chosen based on preliminary experiments. The pruning treatment of this work focused on cutting old leaves, roots and leaves higher than 15 cm. Old leaves pruning avoids the growing populations of *Myzus persicae* (personal observation). Plants with root pruning have a lower requirement of water and hence the transpiration is less (Reich, 1997) as well as the relative humidity inside of the rearing cage, avoiding the presence of powdery mildew (Agrios, 1997). Pruning of leaves higher than 15 cm of length avoids the overlapping of leaves. The overlapping of leaves is another factor that should be considered because favours the development of fungi (Personal observation). The cage size was the last considered factor which influences the quantity of light. The cabbage crops grow outdoors and when they develop in cages, the low quantity of light can produce elongation and weakness (Lambers *et al*., 2008).

The indicators to measure the quality of the target rearing systems were chosen based on the related literature. These indicators were size of the herbivore, parasitoid survival, number of mummies per plant as indicator of virulence, contamination of the non-target organism powdery mildew and adaptation after the transplanting when rearing plants are used as banker plants (Hardy *et al*., 1992; Williams, 1995; Frank, 2010; Kant *et al*., 2012). Additionally, variables of the physiology of the plant, relative humidity and quantity of light in some experiments were measured to explain the results of some measured variables. Hence, the main objective of this chapter was to evaluate different host plants (cultivar groups), plant architectures and cage sizes.

2.2. Materials and Methods

2.2.1. Plants and insects

The host plants broccoli (variety Marathon F1, Hild, Germany), brussels sprouts (Hilds Ideal, Hild, Germany) and cauliflower (Hybrid Fremont, Germany) were chosen, taking into account information reported in literature (Freuler *et al.,* 2003 and Bayhan *et al.,* 2007) and personal observations. Populations of herbivores and parasitoids used in the experiments were collected from brussels sprout plants from the adjacent fields to the institute and after reared under controlled conditions. To raise cabbage plants, seeds were sown in plastic pots (12 cm diameter) filled with black peat substrate (Fruhstorfer Erde, type Nullerde). The seedlings were maintained in nursery for four to six weeks. Brussels sprouts infested with nymphs of all instars of *B. brassicae* and larvae of 3rd and 4th instar of *A. proletella* were used for rearing *D. rapae* and *E. tricolor*, respectively. The rearing of the parasitoids was undertaken in mesh cages measuring (20 × 20 ×32) cm and the environmental conditions in the climate chambers involved a temperature range of 20 - 25 °C, 40 – 60% R.H., and a photoperiod of L 16h: D 8h.

2.2.2. Effect of the host plant on the quality of the target rearing systems

A completely randomised design with three treatments and seven replicates was used for the experiments with *D. rapae* and *E. tricolor* in order to study the effect of the host plant on the quality of the target rearing systems under greenhouses conditions. The treatments were the host plants: i) broccoli, ii) brussels sprouts, and iii) cauliflower. The experimental unit or plot was a cage (0.37 x 0.25 x 0.25) m, wooden bottom, frames of wire, and mesh on the sides. Every cage had a two months old cabbage plant of the respective cultivar group depending on the treatment. In the *D. rapae* experiment, first, every plant was infested with approximately fifty adults of *B. brassicae* and after two weeks, ten female parasitoids were released per cage. For *E. tricolor* experiment, first, every plant was infested with approximately fifty adults of *A. proletella* and after four weeks, ten female parasitoids were released per cage. At the end of the experiment, for every experimental unit, the longevity of 14 female parasitoids, the size of 10 herbivores and the production of mummies were measured. The incidence of powdery mildew was measured every week during the two months of the experiment by counting the number of plants that had 25% of the leaves with the presence of the sickness. Longevity was defined as the number of days for which an adult parasitoid lived. To measure the longevity, an individual female parasitoid between 1-24 hours old, without deformities, without experience, unmated and unfed was released in a 9 cm Petri-dish which had two holes covered with fine mesh netting for ventilation and two holes for introducing the parasitoids and food. A flower of buckwheat was provided per day as a source of food. To get wasps between 1-24 hours old, mummies from the experimental plants were collected at random in gel capsules and marked with the name of the respective treatment. The measurements of wasp longevity were taken every 24 hours. The size of herbivores was measured with a Digital Microscope VHX-500F Keyence. The production of mummies was evaluated counting the number of mummies per plant. The hyphotesis of these experiments was that the host plant (cultivar groups) has effect on the quality indicator of the target rearing systems.

2.2.3. Effect of broccoli plant architectures on the quality of the target rearing systems

Having observed that the cultivar group or host plant broccoli (variety Marathon F1, Hild, Germany) was the most successful host plant for *D. rapae* and *E. tricolor* rearing in the last experiments, the next aim was to evaluate the effect of plant architectures in order to improve the quality of the target rearing systems. A completely randomised design with two treatments and seven replicates was conducted independently for each parasitoid. The treatments were: i) broccoli plants with root, old and large leaves (>15 cm) pruning and ii) broccoli plants without pruning. The experimental unit or plot consisted of a 2 months old broccoli plant inside a cage. The pruning was done thirty and sixty days after sowing. Two months after the introduction of the parasitoids in the cages, the variables longevity of parasitoids, herbivore size and production of mummies were measured. The incidence of powdery mildew was measured every week. The management of every experimental unit and the measurement of the variables were conducted same as the last experiments. Due to the lack of knowledge about the effect of plant architecture on the quality of rearing systems, the pruning treatment evaluated in this experiment was chosen based on preliminary trials with different types of pruning, taking into account the factors, namely: i) high tolerance to hydric stress; ii) the no elongation of the plants inside cages because elongated plants generally die very fast; iii) low falling of leaves, preventing loss of herbivore individuals and mummies on formation. When leaves fall, the mummies can be affected by the soil temperature, water, and predators, among others and second, the herbivore dies as well as the koinobiont parasitoids, needing a living herbivore to reach the mummy stage; iv) leaves with less of 15 cm of length, which have strong and thick tissues and v) the no overlapping of leaves, avoiding the development of powdery mildew and entomopathogenic fungi that attack herbivores or parasitoid hosts. The pruning of old leaves was chosen in order to prevent the development of populations of *Myzus persicae*. The pruning of leaves higher than 15 cm was also selected due to the fact that larger leaves are more susceptible to pathogens. The root pruning was chosen because this permits to obtain smaller plants, with short leaves, without elongation and high tolerance to hydric stress. Additionally, these plants have no overlapping of leaves and the falling of leaves is low. So, the hypothesis was that plants with the pruning allow obtaining high number of mummies, high size of host and parasitoids that live longer.

2.2.4. Effect of the pruning on some indicators of the vigour of broccoli plants

A completely randomised design, with two treatments and three replicates, was utilised under greenhouse conditions in order to justify, why plants from pruning treatment were stronger. The treatments were: i) plants with root, old and large leaves (>15 cm) pruning and ii) plants without pruning (control). The pruning of the plants was done one and two months after the sowing. Broccoli plants were sown in a 12 cm diameter plastic pots. The experimental unit consisted of a pruned broccoli plant inside a cage (0.37 x 0.25 x 0.25) m. The plants were not exposed to the strain of the insects. The vigour of the plant was measured in terms of the fresh matter of leaves, fresh matter of the stems, length of the main stem, number of leaves per plant, length of the leaves per plant, size of the leaf petiole, percentage of dry matter and relative humidity inside cages. The variables were measured after two months of the last pruning. The leaves and stems were placed inside paper bags and introduced during 48 hours in an oven to 75 °C to measure dry matter. Thereafter, the percentage of dry matter was estimated with the formula:

$\left(\left[{}^{\text{dry matter}}/_{\text{fresh matter}}\right] * 100\right)$. The relative humidity was recorded every fifteen minutes during seven days using miniature data loggers (Tiny Tag) and subsequently, the average per day was calculated.

The hypothesis was root pruning allows obtaining small-sized plants, without elongation, a low percentage of fresh matter and shorter leaves (less of 15 cm).

2.2.5. Effect of size of the cages on the quality of the target rearing systems

Having observed that pruned broccoli plants is a suitable technique to rear *D. rapae* and *E. tricolor* parasitoids in the last experiments, the next goal was to determine whether the size of the cage had a significant impact to optimise the quality of the target rearing systems. A completely randomised design with two treatments and seven replicates was conducted independently with each parasitoid to evaluate the effect of the size of the cages on the quality of the rearing systems under greenhouse conditions. The treatments were: i) large cages (0.51 x 0.76 x 0.56) m and ii) small cages (0.37 x 0.25 x 0.25) m. The experimental unit or plot consisted of a pruned broccoli plant for the small cage and 6 plants for the large cage. Two months later, the variables herbivore size, production of mummies, parasitoid longevity, and adaptation of the rearing systems under field conditions after transplanting and the intensity of photosynthetic active radiation were measured. The intensity of photosynthetic active radiation (400 nm-700 nm) was measured on the base and the top of the plant inside of the cages with a Licor lightmeter LI-250-A (Lincoln, Nebraska USA). The adaptation of the rearing plant after transplanting to outdoor crop fields was measured to ascertain whether these materials can be used as banker plants. A satisfactory adaptation in the field was defined as plants surviving at least two weeks without a change of colour (green to red) and wilted leaves after transplanting in the field. To measure this variable, the plants from cages were sowed in the field. The management of every experimental unit or plot and the methodology to measure the other variables was the same like last experiments. The hyphotesis was that the cage size influences the quality indicators of the target rearing systems.

2.2.6. Statistical analysis

A Log Rank Test (Mantel-Cox) was used to compare survival curves. For the other variables, a one-way ANOVA (General Linear Model) followed by a Tukey's test was used. Kruskal-Wallis test was used when the data showed no normal distribution and the test of Tamhne-T2 when the data had no homogeneity of variances. The level of significance was $p<0.05$ for all tests. All analyses were performed with SPSS 23 IBM.

2.3. Results

2.3.1. Effect of the host plant on the quality of the target rearing systems

For *D. rapae* experiment, the factor host plant had a significant effect on all measured parameters except for longevity (Fig. 2.1, 2.2, 2.3 and 2.4). In Table 2.1, the means, standard error and the differences between treatments of the experiments are shown. The largest mean size of cabbage aphid adults was obtained from broccoli and the lowest size from brussels sprouts and cauliflower. The size of cabbage aphid adults from broccoli treatment was 1.8 times as larger as cauliflower treatment. The number of mummies was highest on broccoli as the host plant followed by brussels sprouts and cauliflower. Broccoli treatment had 182 mummies per plant more than cauliflower. After two months, the powdery mildew incidences were 57, 100 and 100% in broccoli, brussels sprouts and cauliflower, respectively.

Table 2.1. Mean and standard error of the measured variables in the experiment titled effect of different host plants on the quality of *D. rapae* rearing plants. Different letters indicate significant differences at 5% level.

Variable	Herbivore or parasitoid	Treatment	Mean ± Standard error
Survival (days)	*D. rapae*	Broccoli	4.21 ± 0.32 a
	X^2=2.261, *df*=2, p=0.323 n=14	Brussels sprouts	3.92 ± 0.53 a
	Log Rank (Mantel-Cox)	Cauliflower	3.42 ± 0.31 a
Herbivore size (mm)	Adults of *B. brassicae*	Broccoli	1.33 ± 0.023 a
	F=12.112, *df*=2, p=0.005 n=7	Brussels sprouts	1.24 ± 0.016 b
	Tukey's Test	Cauliflower	1.23 ± 0.025 b
Number of mummies/plant	*D. rapae*	Broccoli	252.14 ± 21.58 a
	F=42.63, *df*=2, p<0.001 n=7	Brussels sprouts	124.71 ± 6.34 b
	Tukey's Test	Cauliflower	69.28 ± 10.60 c

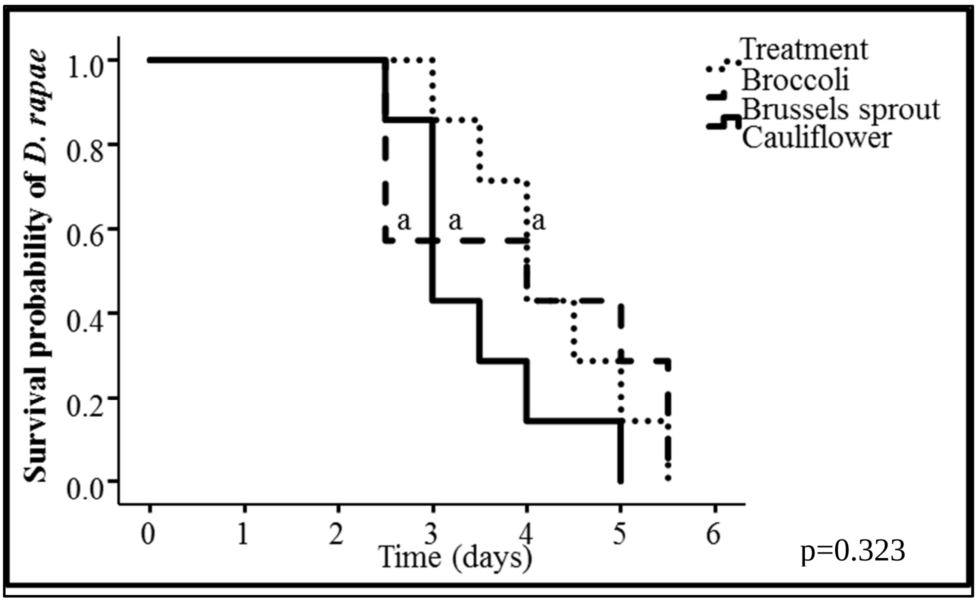

Fig. 2.1. Effect of different host plants on the survival of *D. rapae* females. Different letters indicate significant differences at 5% level according to the Log Rank (Mantel-Cox) Test n=14.

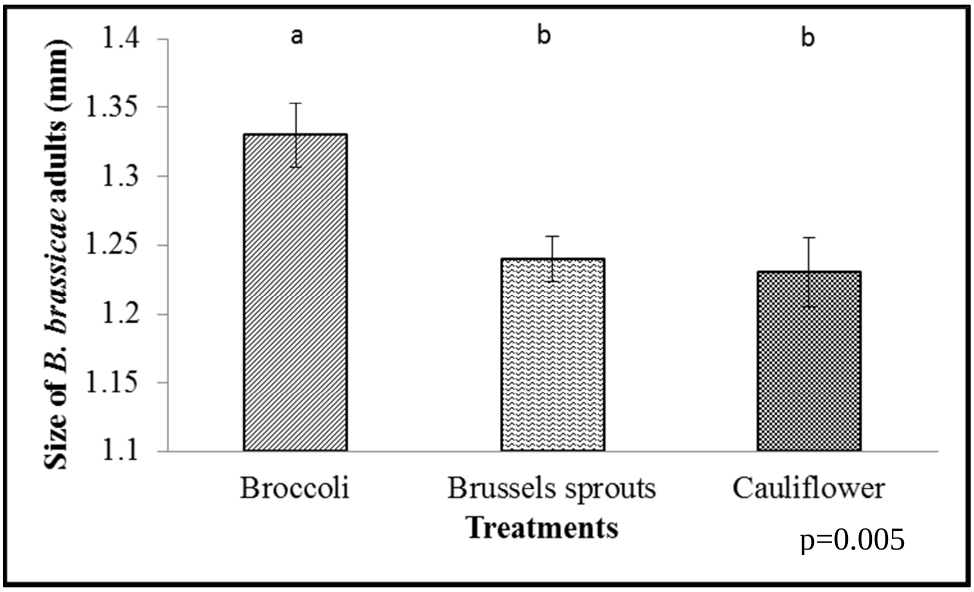

Fig. 2.2. Effect of different host plants on the size of *B. brassicae* adults. Different letters indicate significant differences at 5% level according to Tukey's Test n=7.

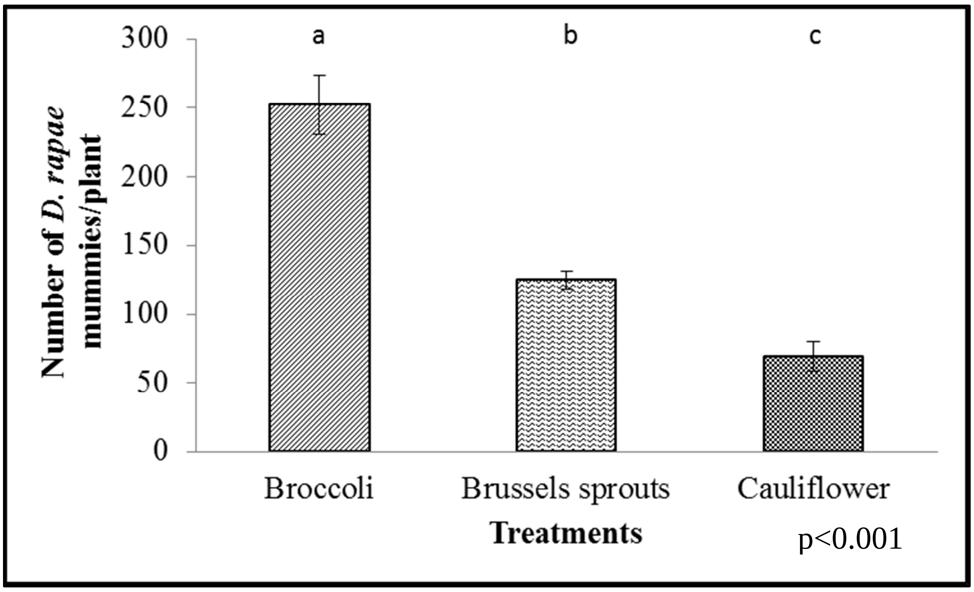

Fig. 2.3. Effect of different host plants on the number of *D. rapae* mummies per plant. Different letters indicate significant differences at 5% level according to Tukey's Test n=7.

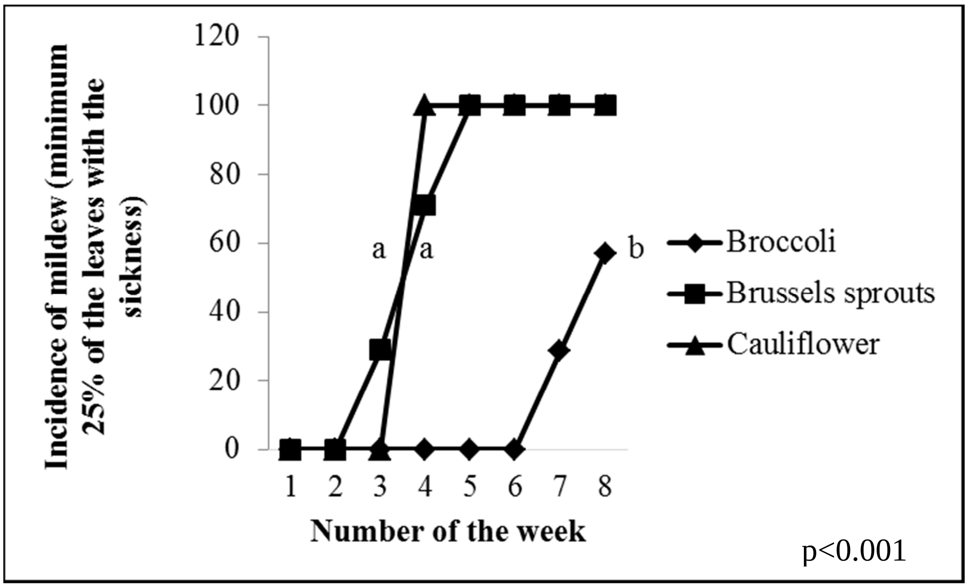

Fig. 2.4. Effect of different host plants on the incidence of powdery mildew in the *D. rapae* rearing. Different letters indicate significant differences at 5% level.

For *E. tricolor* experiment, the factor host plant had a significant effect on all measured parameters except survival (Fig. 2.5, 2.6, 2.7 and 2.8). In Table 2.2, the mean, standard error and the differences between treatments of the experiments are shown. The largest value of 4th instar larva of cabbage whiteflies was found on broccoli and brussels sprouts as host plants while cauliflower showed the smallest size. The herbivore size from broccoli treatment was larger by 12% compared to the cauliflower treatment. In this study, the number of mummies was highest on broccoli as the host plant followed by brussels sprouts and cauliflower. Broccoli treatment had 88 mummies per plant more than cauliflower. After two months, the powdery mildew incidences were 57, 100, and 100% in broccoli, brussels sprouts and cauliflower, respectively.

Table 2.2. Mean and standard error of the measured variables in the experiment titled effect of different host plants on the quality of *E. tricolor* rearing systems. Different letters indicate significant differences at 5% level.

Variable	Herbivore or parasitoid	Treatment	Mean ± Standard error
Survival (days)	*E. tricolor* X^2=0.329, *df*=2, p=0.849 n=14 Log Rank (Mantel-Cox)	Broccoli Brussels sprouts Cauliflower	4.5 ± 0.81 a 5.1 ± 0.68 a 4.71 ± 0.83 a
Herbivore size (mm)	Larvae of 4th instar of *A. proletella* F=23.475, *df*=2, p<0.001 n=7 Tukey's Test	Broccoli Brussels sprouts Cauliflower	1.02 ± 0.008 a 0.99 ± 0.013 a 0.91 ± 0.010 b
Number of mummies/plant	*E. tricolor* F=36.066, *df*=2, p<0.001 n=7 Tukey's Test	Broccoli Brussels sprouts Cauliflower	120.42 ± 9.55 a 71.71 ± 6.73 b 32.14 ± 5.09 c

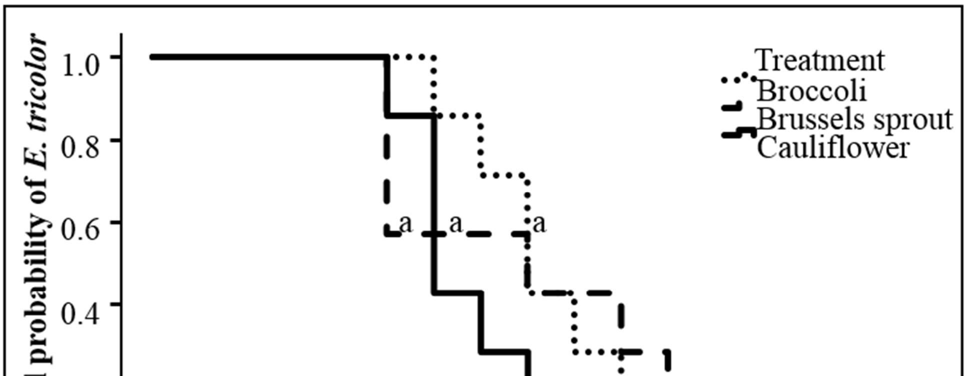

Fig. 2.5. Effect of different host plants on the survival of *E. tricolor* females. Different letters indicate significant differences at 5% level according to the Log Rank (Mantel-Cox) Test n=14.

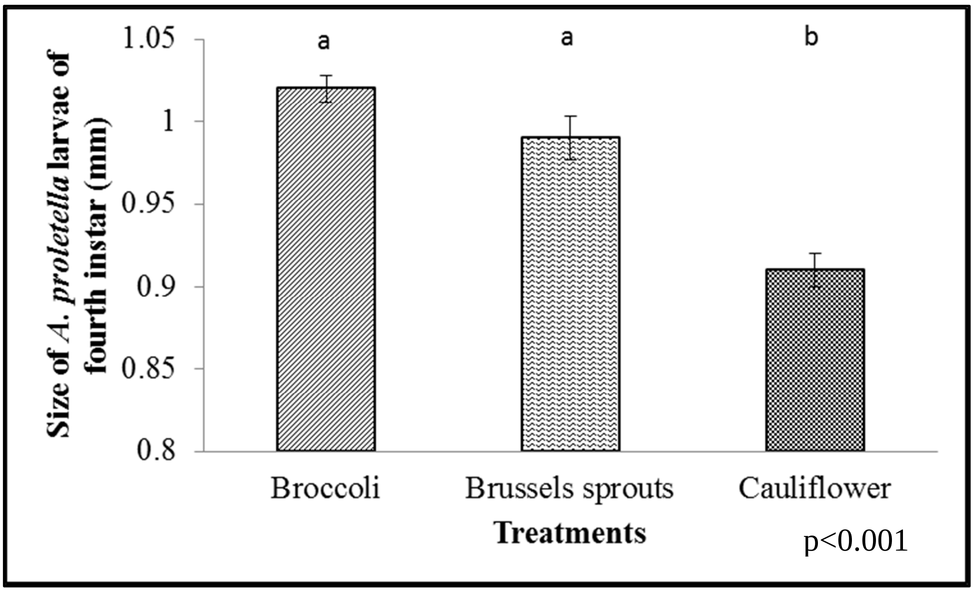

Fig. 2.6. Effect of different host plants on the size of *A. proletella* larvae of 4th instar. Different letters indicate significant differences at 5% level according to Tukey's Test n=7.

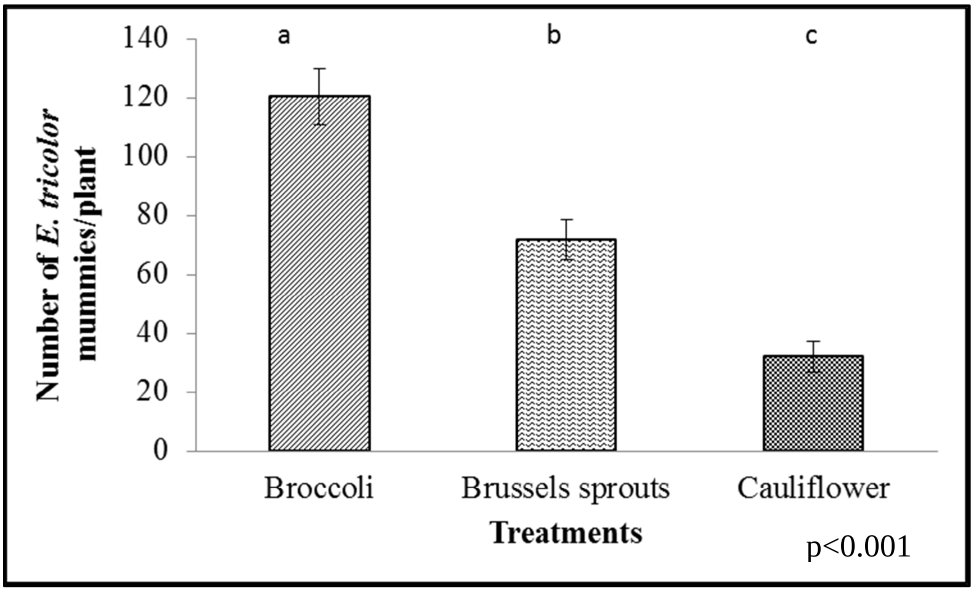

Fig. 2.7. Effect of different host plants on the number of *E. tricolor* mummies per plant. Different letters indicate significant differences at 5% level according to Tukey's Test n=7.

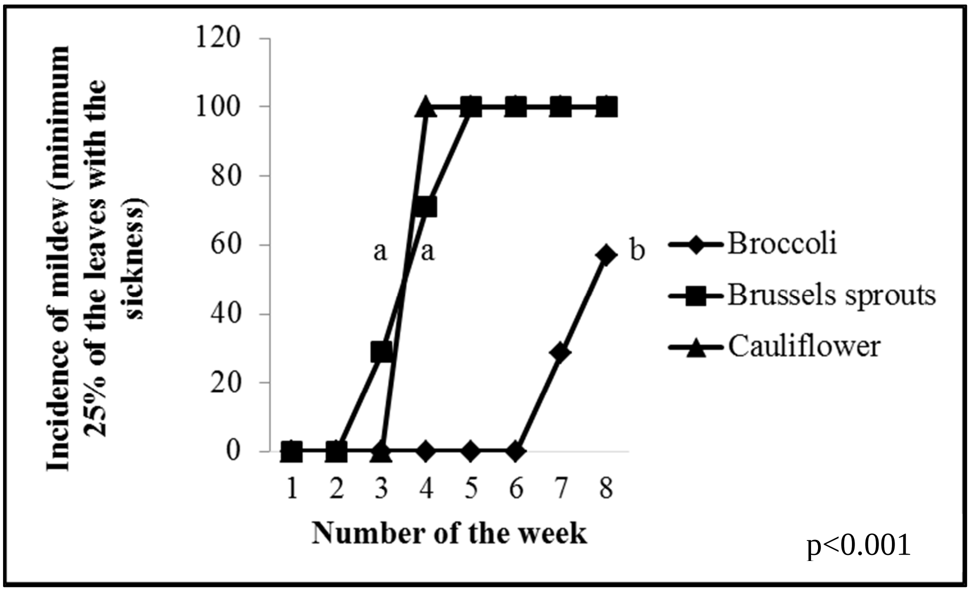

Fig. 2.8. Effect of different host plants on the incidence of powdery mildew in the *E. tricolor* rearing. Different letters indicate significant differences at 5% level according to Tukey's Test.

2.3.2. Effect of broccoli plant architecture on the quality of the target rearing systems

For *D. rapae* experiment, the pruning treatment showed better values in all measured variables compared to control (Fig. 2.9, 2.10, 2.11 and 2.12). The mean, standard error and the differences between treatments are shown in Table 2.3. Females of *D. rapae* lived 2.5 days longer on pruned plants. Pruning also increased the herbivore size by 12.9% and the number of mummies by 47.69%. Moreover, two months after releasing the parasitoids, pruned plants were still strong while the 50% of the control plants were already dead due to the high severity of powdery mildew.

Table 2.3. Mean and standard error of the measured variables in the experiment titled effect of plant architecture in the *D. rapae* performance in the field.

Variable	Herbivore or parasitoid	Treatment	Mean ± Standard error
Survival (days)	*D. rapae* X^2=5.4, *df*=1, p=0.02 n=14 Log Rank (Mantel-Cox)	Pruning Control	7 ± 0.852 a 4.43 ± 0.429 b
Herbivore size (mm)	Adults of *B. brassicae* F=44.953, *df*=1, p<0.001 n=7 Tukey's Test	Pruning Control	1.4 ± 0.022 a 1.24 ± 0.011 b
Number of mummies/plant	*D. rapae* F=16.096, *df*=1, P=0.002 n=7 Tukey's Test	Pruning Control	330 ± 19.95 a 223.43 ± 17.53 b

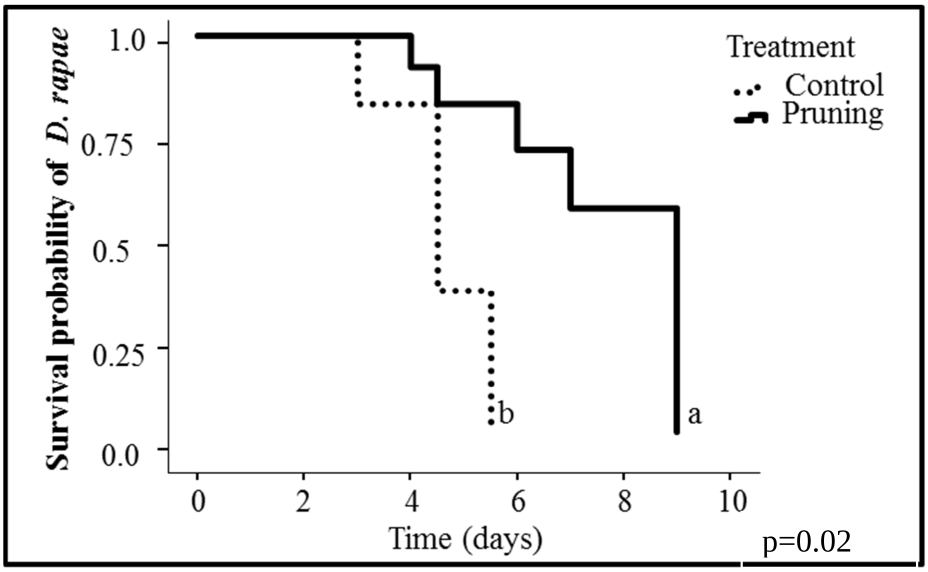

Fig. 2.9. Effect of different plant architectures on the survival of *D. rapae*. Different letters indicate significant differences at 5% level according to the Log Rank (Mantel-Cox) Test (n=14).

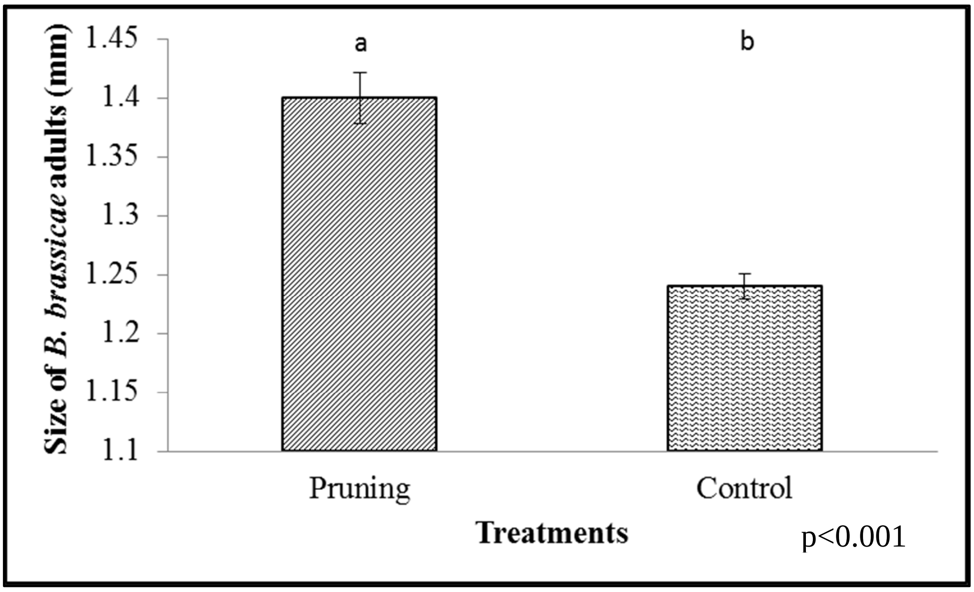

Fig. 2.10. Effect of different plant architectures on the herbivore size of *B. brassicae* adults. Different letters indicate significant differences at 5% level according to Tukey's Test (n=7).

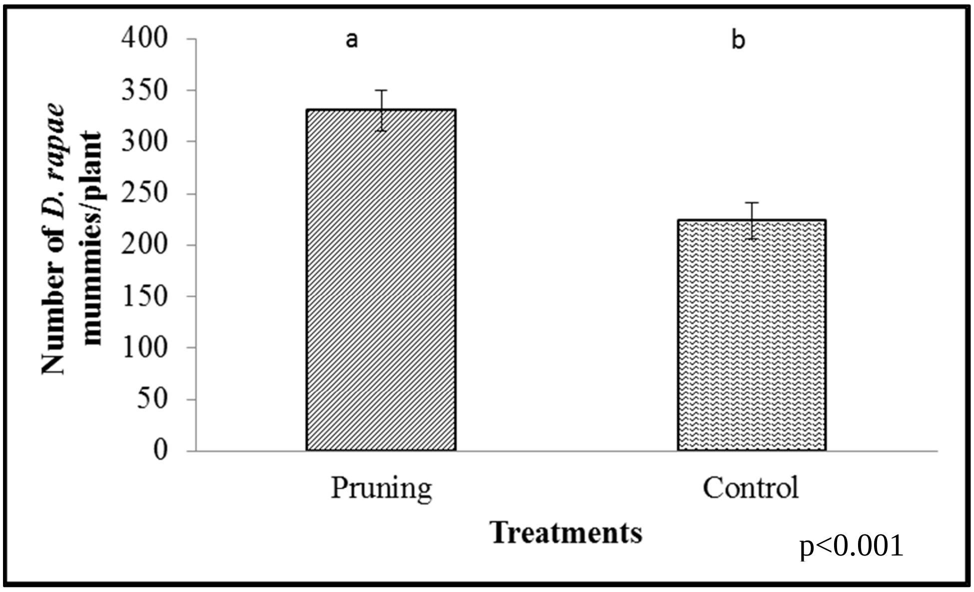

Fig. 2.11. Effect of different plant architectures on the number of *D. rapae* mummies per plant. Different letters indicate significant differences at 5% level according to Tukey's Test (n=7).

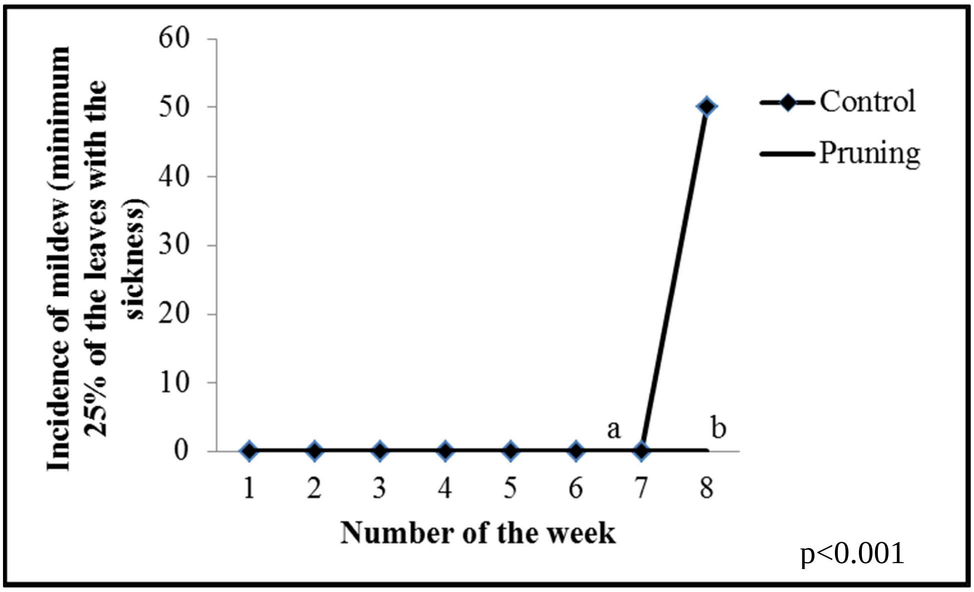

Fig. 2.12. Effect of different plant architectures on the incidence of powdery mildew in *E. tricolor* rearing plants. Different letters indicate significant differences at 5% level according to Tukey's Test.

For *E. tricolor* experiment, the pruning treatment showed better values in all measured variables compared to the control (Fig. 2.13, 2.14, 2.15 and 2.16). The mean, standard error and the differences between treatments are shown in Table 2.4. Females of *E. tricolor* from pruning treatment lived 12 days longer than in the control. Pruning treatment increased the herbivore size by 8.8%, and had 114 mummies per plant more than control. Moreover, two months after releasing the parasitoids, pruned plants were still strong while the 25% of control plants were already dead due to the high severity of powdery mildew. In fig. 2.17, 2.18, 2.19 and 2.20, plants without and with pruning from *D. rapae* and *E. tricolor* experiments are shown.

Table 2.4. Mean and standard error of the measured variables in the experiment titled effect of plant architecture in the *E. tricolor* performance in the field.

Variable	Herbivore or parasitoid	Treatment	Mean ± Standard error
Survival (days)	*E. tricolor* X^2=14.11, *df*=1, p<0.001 n=14 Log Rank (Mantel-Cox)	Pruning Control	17.93 ± 0.862 a 5.93 ± 0.82 b
Herbivore size (mm)	Larvae of 4th instar of *A. proletella* F=22.4, *df*=1, p<0.001 n=7 Tukey's Test	Pruning Control	0.98 ± 0.013 a 0.9 ± 0.011 b
Number of mummies	*E. tricolor* F=122.54, *df*=1, P<0.001 n=7 Tukey's Test	Pruning Control	243.57 ± 9.55 a 129.43 ± 3.878 b

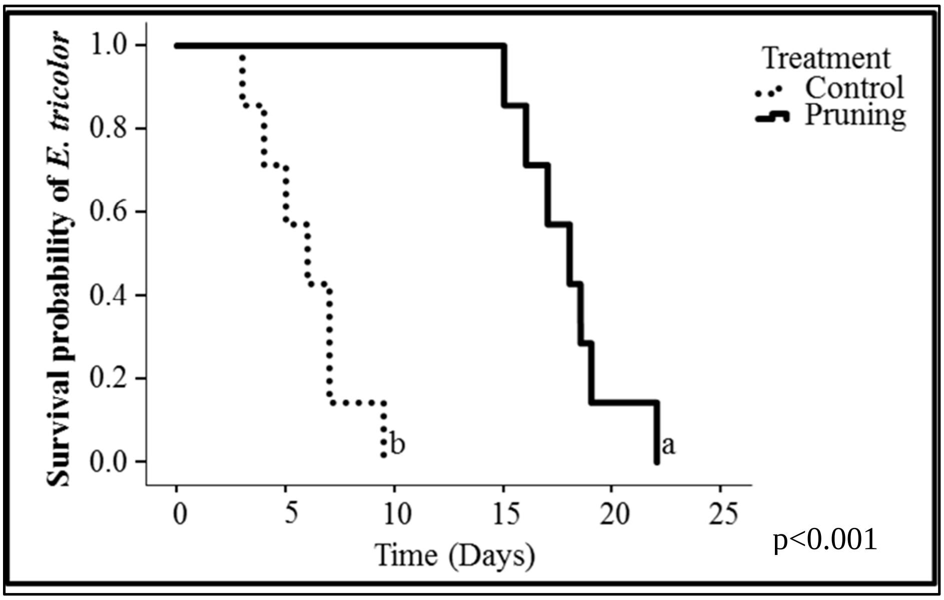

Fig. 2.13. Effect of different plant architectures on the survival of *E. tricolor* females. Different letters indicate significant differences at 5% level according to the Log Rank (Mantel-Cox) Test (n=14).

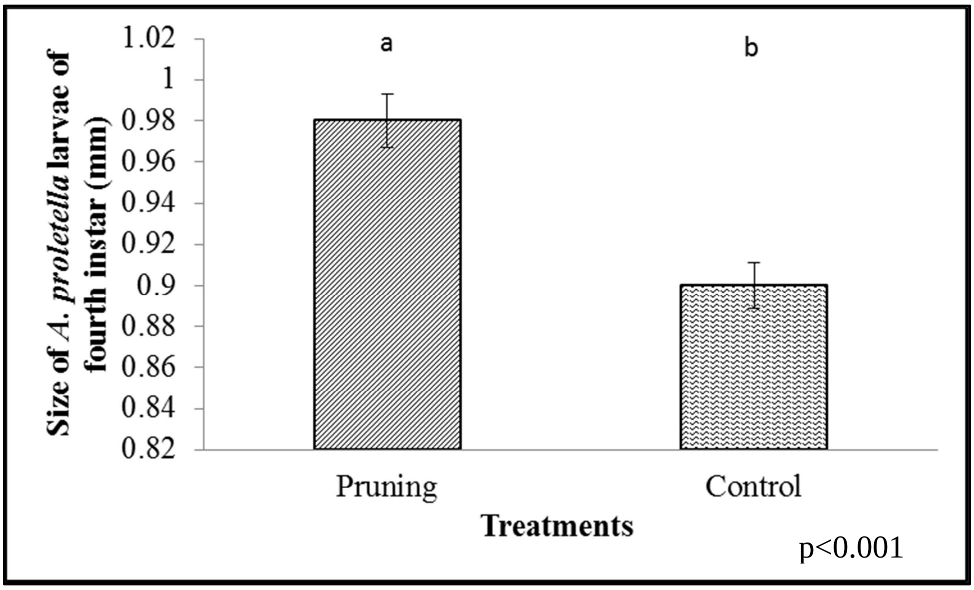

Fig. 2.14. Effect of different plant architectures on the size of *A. proletella* larvae of fourth instar. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey's Test (n=7).

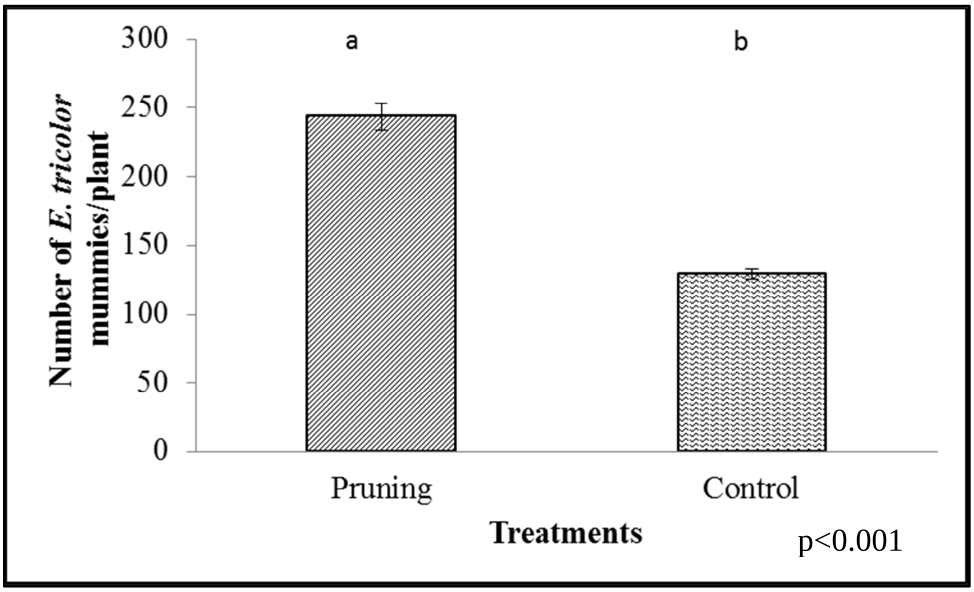

Fig. 2.15. Effect of different plant architectures on the number of mummies of *E. tricolor*/plant. Mean±SE are presented.Different letters indicate significant differences at 5% level according to Tukey's Test (n=7).

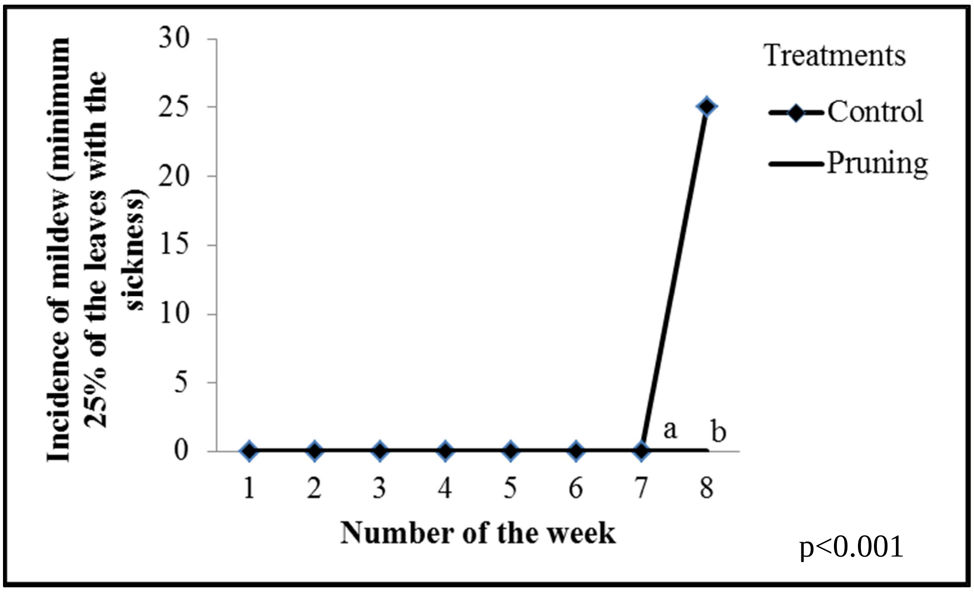

Fig. 2.16. Effect of different plant architectures on the incidence of powdery mildew in *E. tricolor* rearing plants. Different letters indicate significant differences at 5% level according to Tukey's Test (n=7).

Fig. 2.17. Four-months-old rearing plants of *D. rapae* without pruning.

Fig. 2.18. Four-months-old rearing plants of *D. rapae* with pruning.

Fig. 2.19. Four-months-old rearing plants of *E. tricolor* without pruning.

Fig. 2.20. Four-months-old rearing plants of *E. tricolor* with pruning.

2.3.3. Effect of the pruning on some indicators of the vigour of broccoli plants

The indicators of plant vigour, the fresh matter of the stems (Fig. 2.22), length of the main stem (Fig. 2.23), length of the leaf (Fig. 2.25) and relative humidity inside the cages (Fig. 2.28) differed statistically among treatments. Results for fresh matter of the leaves (Fig. 2.21), number of leaves per plant (Fig. 2.24), the length of the leaf petiole (Fig. 2.26) and percentage of dry matter (Fig. 2.27) in the pruned plant treatment were statistically equal to those obtain in the control. Mean and standard error of the measured variables are shown in table 2.5.

Pruning plants were smaller but stronger compared to the control. It was supported with the variables used as indicators of the plant's vigour. Pruned plants had a low fresh matter of the stems, shorter length of the main stem and shorter length of the leaves. Pruned plants had 44.26% less fresh matter of the stems, the length of the main stem was 39.56% shorter, the length of the leaf was smaller by 21% and the relative humidity inside of the cages was less by 18.6% compared to the control (Fig. 2.28).

Table 2.5. Mean and standard error of the measured variables in the experiment titled effect of different plant architectures on some variables of plant physiology. Different letters indicate significant differences at 5% level.

Variable	Statistical difference of the variables	Treatment	Mean ± Standard error
Fresh matter of the leaves (g)	F=6.1, *df*=1, p=0.069 n=3 Tukey's Test	Pruning	14.07 ± 0.95 a
		Control	19.50 ± 1.97 a
Fresh matter of the stems (g)	F=8.152, *df*=1, p=0.046 n=3 Tukey's Test	Pruning	10.03 ± 0.44 b
		Control	14.47 ± 1.49 a
Length of the main stem (cm)	F=7.811, *df*=1 p=0.049 n=3 Tukey's Test	Pruning	14.33 ± 1.33 b
		Control	20.00 ± 1.52 a
Number of leaves per plant	X=1.6 , *df*=1, p=0.197 n=3 Tukey's Test	Pruning	9.66 ± 0.33 a
		Control	10.33 ± 0.33 a
Length of the leaf (cm)	F=10.631, *df*=1, p=0.031 n=3 Tukey's Test	Pruning	8.20 ± 0.17 b
		Control	10.06 ± 0.54 a
Length of the leaf petiole (cm)	F=0.007, *df*=1, p=0.939 n=3 Tukey's Test	Pruning	4.40 ± 0.11 a
		Control	4.43 ±0.39 a
Percentage of dry matter per plant (%)	F=4.637, *df*=1, p=0.098 n=3 Tukey's Test	Pruning	18.77 ± 0.01 a
		Control	15.01 ± 0.00 a
Relative humidity inside the cages Data took during eight days every fifty minutes	F=9.027, *df*=1, P=0.04 n=3 Tukey's Test	Pruning	42.84 ± 2.57 b
		Control	50.81 ± 0.62 a

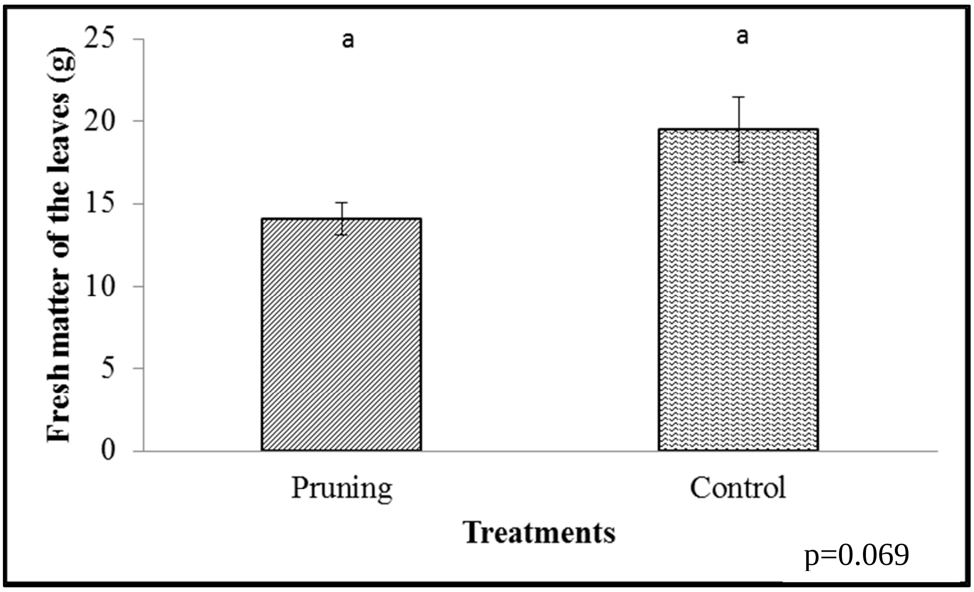

Fig. 2.21. Effect of different architectures on the fresh matter of the leaves of broccoli plants (variety Marathon F1, Hild, Germany). Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey's Test (n=3).

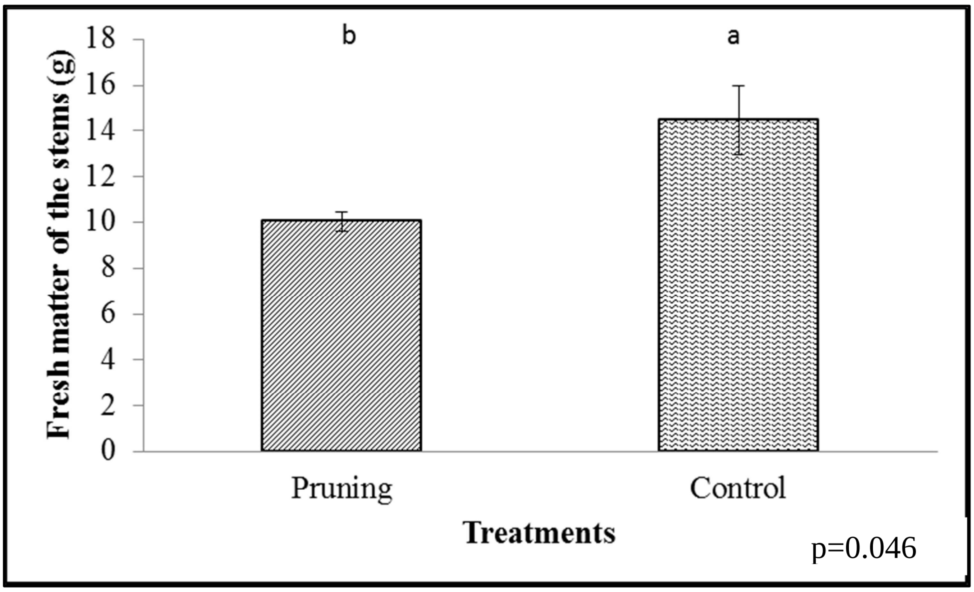

Fig. 2.22. Effect of different architectures on the fresh matter of stems of broccoli plants (variety Marathon F1, Hild, Germany). Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey's Test (n=3).

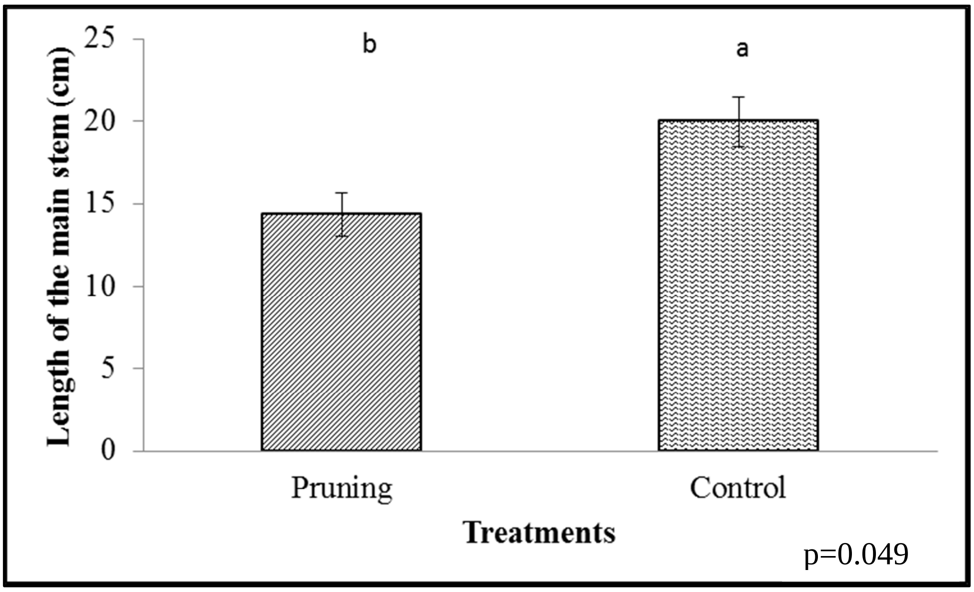

Fig. 2.23. Effect of different architectures on the length of the main stem of broccoli plants (variety Marathon F1, Hild, Germany). Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey's Test (n=3).

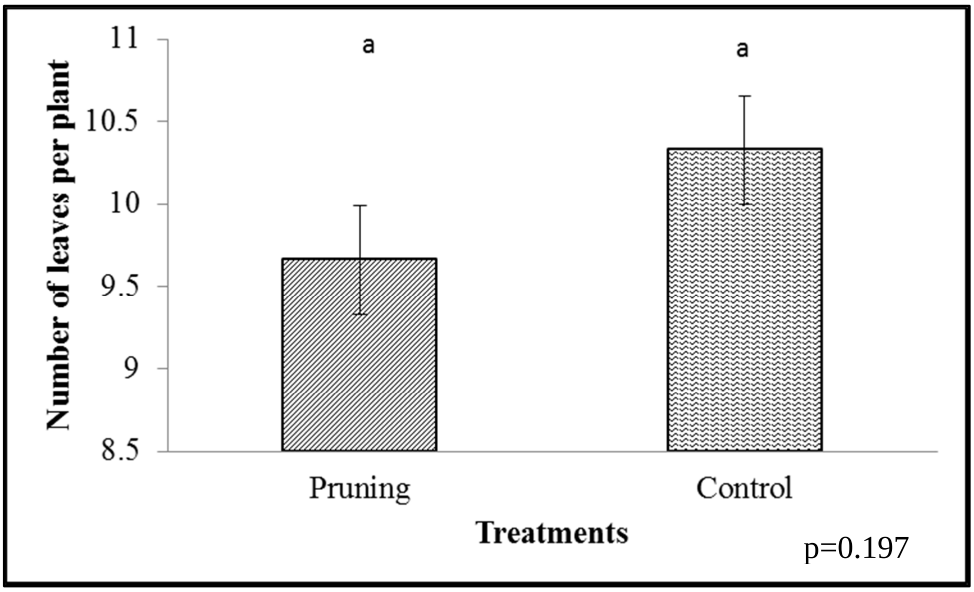

Fig. 2.24. Effect of different architectures on the number of leaves per plant of broccoli plants (variety Marathon F1, Hild, Germany). Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey's Test (n=3).

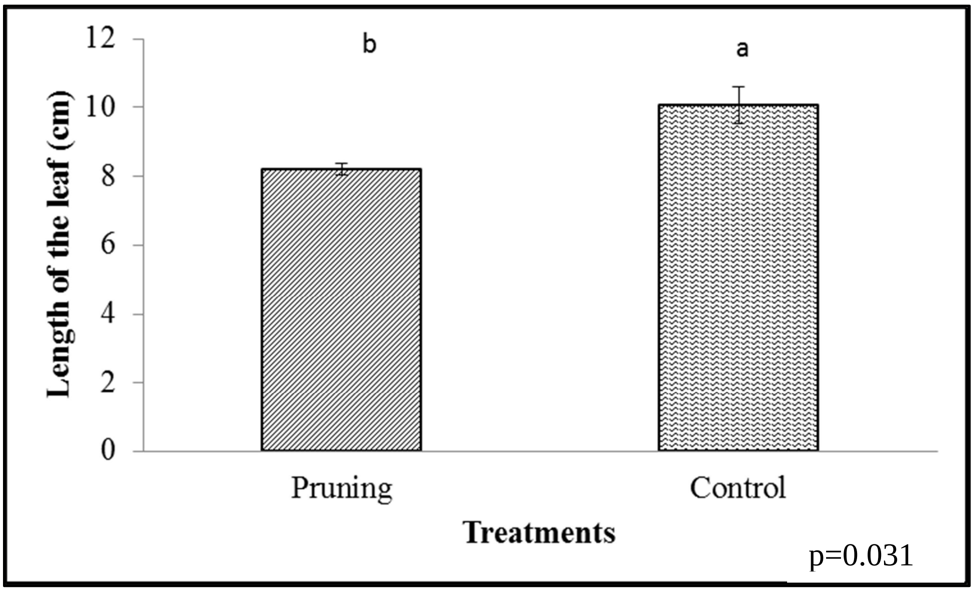

Fig. 2.25. Effect of different architectures on the length of the leaf (cm) of broccoli plants (variety Marathon F1, Hild, Germany). Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey's Test (n=3).

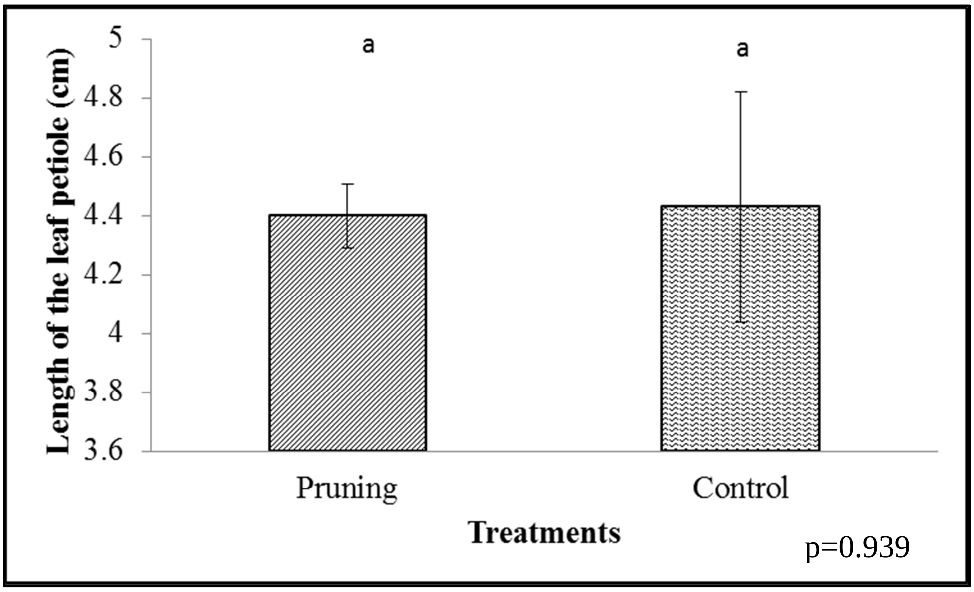

Fig. 2.26. Effect of different architectures on the length of the leaf petiole of broccoli plants (variety Marathon F1, Hild, Germany). Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey's Test (n=3).

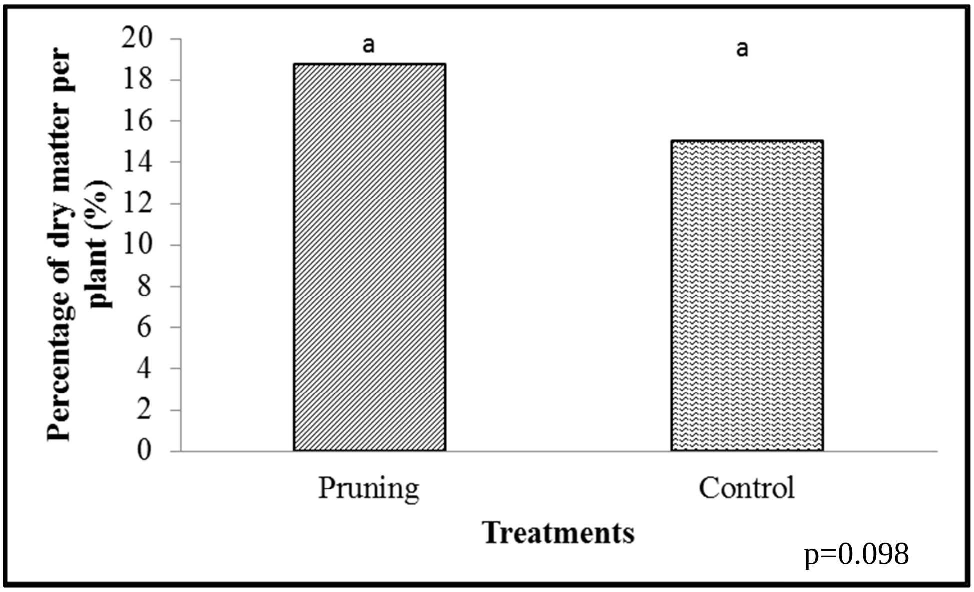

Fig. 2.27. Effect of different architectures on the percentage of dry matter of broccoli plants (variety Marathon F1, Hild, Germany). Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey's Test (n=3).

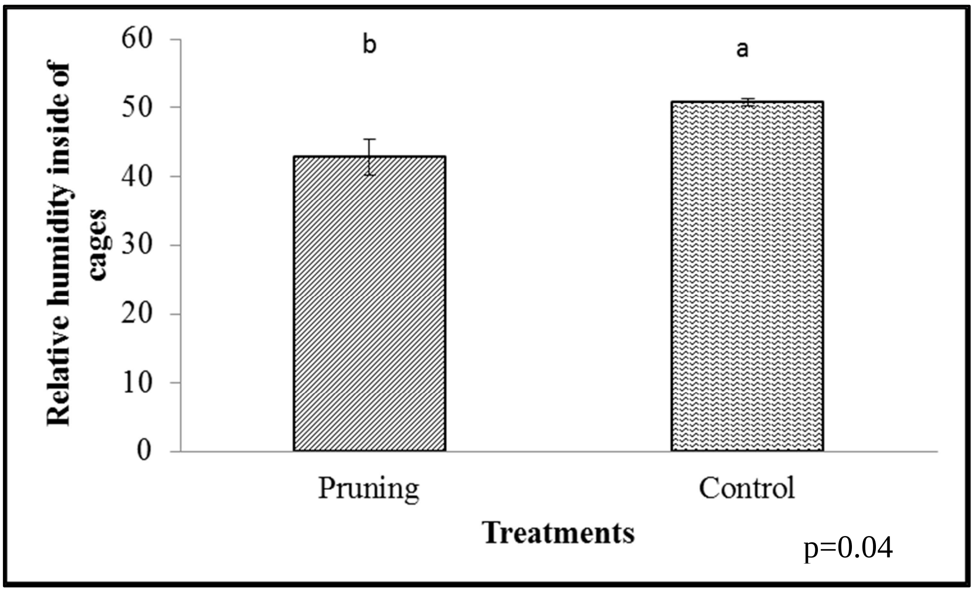

Fig. 2.28. Effect of different architectures on the relative humidity inside of the cages of broccoli plants (variety Marathon F1, Hild, Germany). Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test n=3.

2.3.4. Effect of cage size on the quality of the target rearing systems

For *D. rapae* experiment, the treatments showed significant differences in all measured parameters except survival, and adaptation after transplanting (Fig. 2.29, 2.30, 2.31). The mean, standard error and the differences between treatments are showed in table 2.6. Large cages increased the herbivore size by 6.2% and this treatment had 69.72 mummies per plant more than the control.

Table 2.6. Mean and standard error of the measured variables in the experiment titled effect of different cage sizes on the quality of *D. rapae* rearing plants. Different letters indicate significant differences at 5% level.

Variable	Herbivore or parasitoid	Treatment	Mean ± Standard error
Survival (days)	*D. rapae* X^2=0.013, *df*=1, p=0.91 n=14 Log Rank (Mantel-Cox)	Large cage Small cage	7.21 ± 0.406 a 6.93 ± 0.848 a
Size of herbivore (mm)	Adults of *B. brassicae* F=11.36, *df*=1, p=0.006 n=7 Tukey's Test	Large cage Small cage	1.41 ± 0.014 a 1.31 ± 0.026 b
Number of mummies/plant	*D. rapae* F=16.62, *df*=1, p=0.002 n=7 Tukey's Test	Large cage Small cage	417.29 ± 8.95 a 347.57 ± 14.54 b

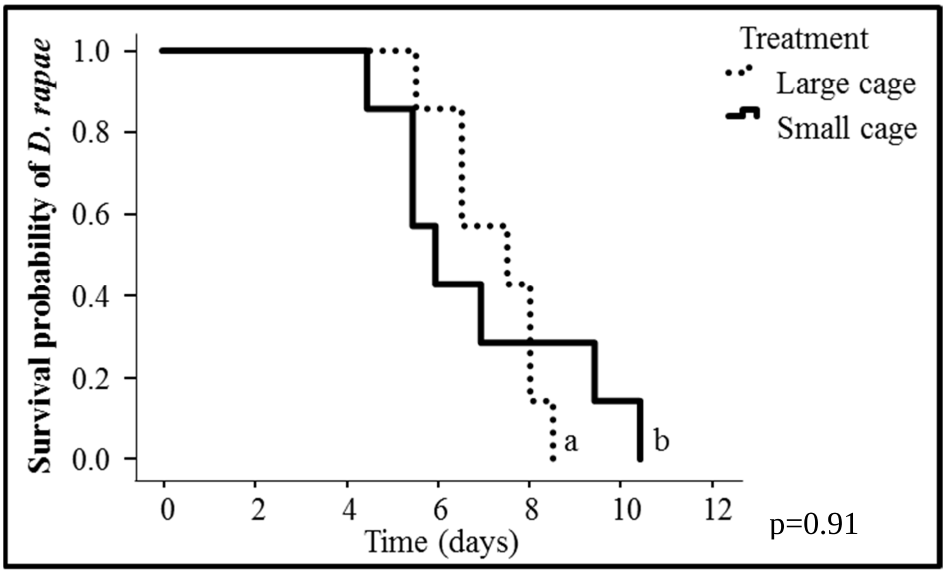

Fig. 2.29. Effect of different cage sizes on the survival of *D. rapae* females. Different letters indicate significant differeces at 5% level according to the Log Rank (Mantel-Cox) Test (n=14).

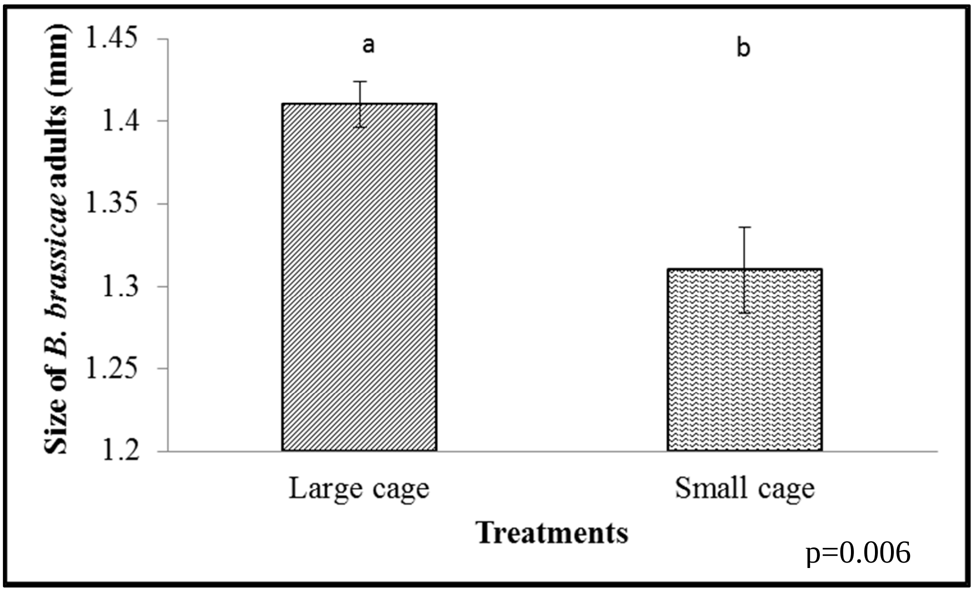

Fig. 2.30. Effect of different cage sizes on the herbivore size of *B. brassicae.* Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey's Test (n=7).

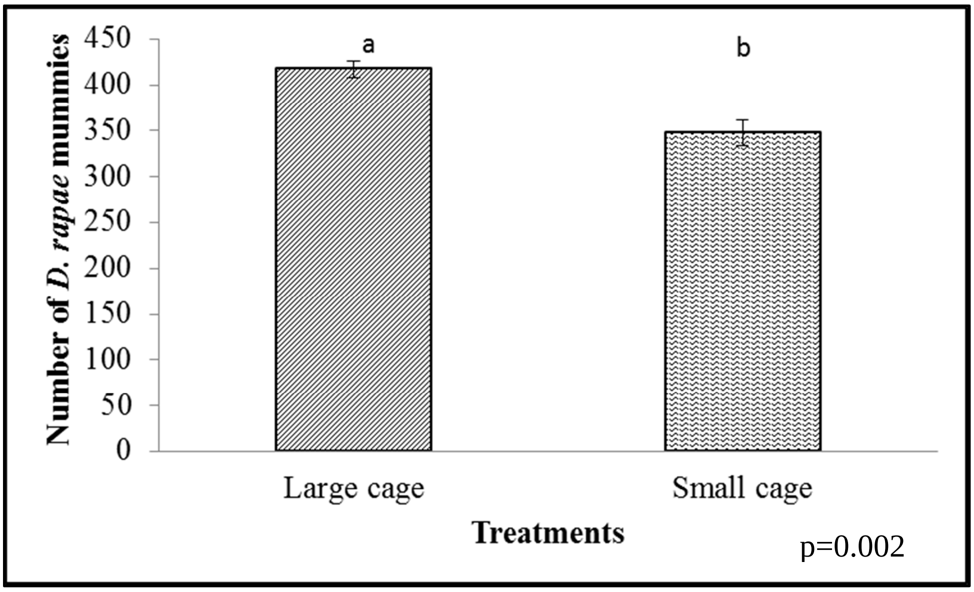

Fig. 2.31. Effect of different cage sizes on the number of *D. rapae* mummies. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey's Test (n=7).

For *E. tricolor* experiment, the factor cage size showed significant differences in all measured parameters except in adaptation after transplanting (Fig. 2.32, 2.33, 2.34 and 2.35). The mean, standard error and the differences between treatments are showed in table 2.7. The *E. tricolor* females lived eight days longer in the large cage treatment than those in small cages. Larvae of 4th instar of *A. proletella* reared on large cages were 9.09% larger than those in the small cages. Rearing plants in the large cage had 121 mummies per plant more than those in the control. Both treatments revealed plants with satisfactory adaptation in the field when rearing plants were transplanting in the field as banker plants. Additionally, inside large cages, the intensity of photosynthetic active radiation µmol m^{-2} s^{-1} (400 nm-700 nm) was 22.89% higher, as compared to that inside small cages. Inside large cages, the mean of the intensity of photosynthetic active radiation was 45.62 ± 2.60 and inside small cages was 37.12 ± 2.57.

Table 2.7. Mean and standard error of the measured variables in the experiment titled effect of different cage sizes on the quality of *E. tricolor* rearing systems. Different letters indicate significant differences at 5% level.

Variable	Herbivore or parasitoid	Treatment	Mean ± Standard error
Survival (days)	*E. tricolor* X^2=6.5, *df*=12, p=0.011 n=14 Log Rank (Mantel-Cox)	Large cage Small cage	23.57 ± 2.50 a 16.07 ± 0.948 b
Size of herbivore (mm)	Larvae of fourth instar of *A. proletella* F=20.0129, *df*=1, p<0.001 n=7 Tukey's Test	Large cage Small cage	1.08 ± 0.017 a 0.99 ± 0.011 b
Number of mummies	*E. tricolor* F=29.8125, *df*=1, p<0.001 n=7 Tukey's Test	Large cage Small cage	386.86 ± 20.48 a 265.57 ± 8.53 b

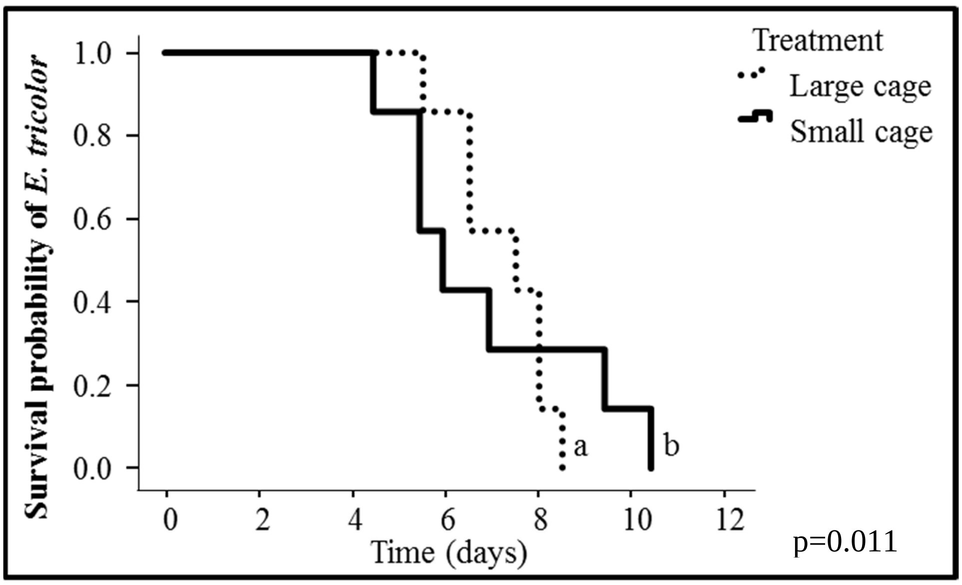

Fig. 2.32. Effect of different cage sizes on the survival of *E. tricolor* females. Different letters indicate significant differences at 5% level according to Log Rank (Mantel-Cox) Test (n=14).

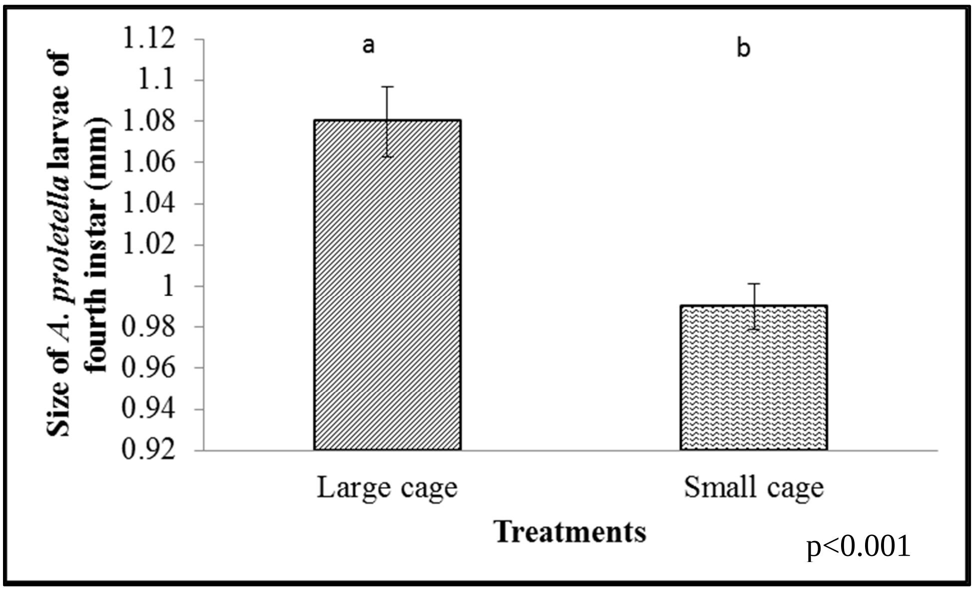

Fig. 2.33. Effect of different cage sizes on the size of *A. proletella* larvae of 4th instar. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey's Test (n=7).

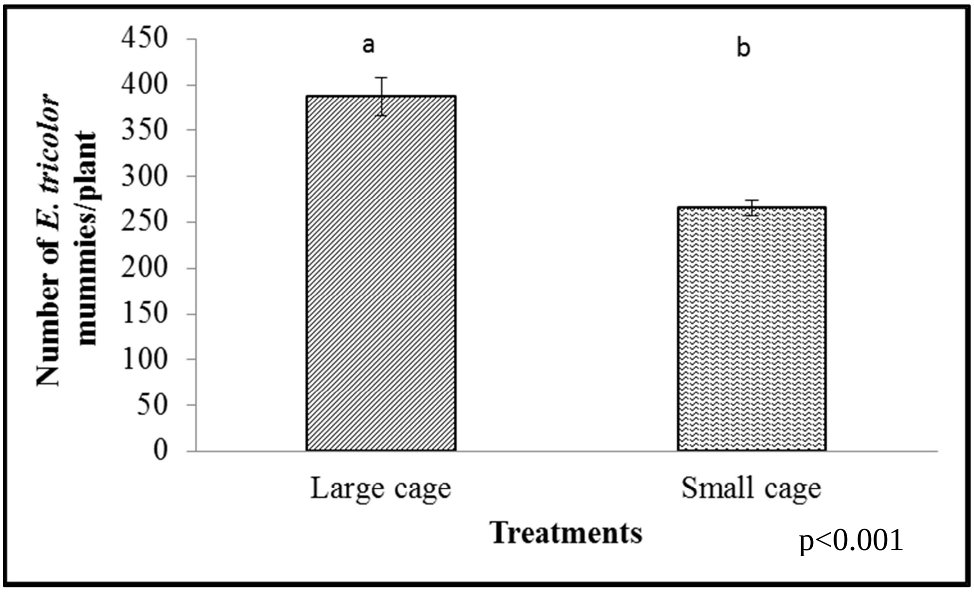

Fig. 2.34. Effect of different cage sizes on the number of *E. tricolor* mummies/plant. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey's Test (n=7).

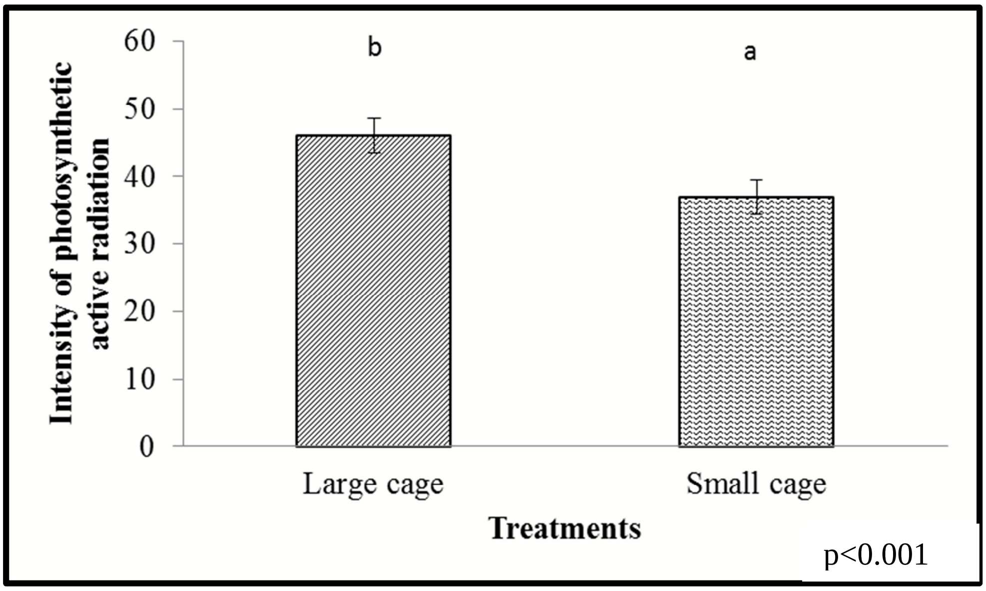

Fig. 2.35. Effect of different cage sizes on the intensity of photosynthetic active radiation µmol m^{-2} s^{-1} (400 nm-700 nm). Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test (n=3).

2.4. Discussion

The techniques of mass production of pest antagonists plays a significant role in programs of biological control because the management of rearing systems mediates a relevant effect on the fitness of pest antagonists and hence influences the efficiency of these organisms to control pest in the crop fields (Van Lenteren, 1991, 1993, 2003; Frank, 2010). Hence, the purpose of this chapter was to explore different approaches to providing a set of techniques in order to improve the quality of the rearing plants. The evaluated approaches were host plant, plant architecture and cage size. In this research, the variables evaluated as indicators of quality were chosen based on literature (Van Lenteren, 2003; Frank, 2010). They were herbivore size, parasitoid longevity, number of mummies, incidence of powdery mildew and adaptation of the rearing plants after transplanting in the field.

Host plants have been reported to possess an effect on the development of the target and non-target organisms (Freuler *et al.*, 2003; Bayhan *et al.*, 2007; Frank, 2010; Augustin, 2012). It is consistent with this study because the host plant (cultivar group) had an effect on the herbivore size, and incidence of powdery mildew as well as parasitoid fitness.

The herbivores size is a relevant indicator of quality because this variable is positively related to the longevity of the adult parasitoids (Kant *et al*., 2012, and Williams, 1995). The relation between host plant and herbivore is justified due to the host plant resistance by antibiosis (Ellis *et al*., 1996, Frank, 2010). Resistance by antibiosis refers to mechanisms of the plant that affect the development of the herbivores in terms such as reproductive rate, survival, size among others. In our case, broccoli had the less resistance by antibiosis to the target herbivores (i.e. cabbage aphid and cabbage whiteflies).

The number of mummies per plant is other relevant indicator of the quality of rearing plants used as banker plant for transplanting, given that a high quantity of mummies per plant reduce the number of banker plants used per ha/cycle in the field, frequency of renovation, as well as work and costs linked with these activities (Frank, 2010). In this study, the factor host plant influenced the number of mummies and this was also observed in the findings of Freuler *et al.,* (2003), who found that savoy cabbage *(Brassica oleracea* L. convar. *capitata* var. *sabauda* L.*)* had a higher production of *D. rapae* mummies than turnip *(Brassica rapa* L. var. *rapifera* subvar. *Majalis).* Jandricic *et al.*, (2014) also find an effect of different host plant crops on the production of *Aphidius colemani* parasitoids. This author observed that on barley and wheat the production of *A. colemani* wasps was higher than this on oats or rye.

The number of mummies depends on the longevity of parasitoids –parasitoids that live more time lay more eggs- and also in the number of herbivores. In this study, the high number of mummies did not depend on the longevity, given that in this variable, there were no significant differences among treatments. The fact that the host plant influenced the number of mummies can be explained because of this variable is positively correlated with the number of herbivores as it was mentioned by kant, (2000). Indeed, in this study, plants with a high number of mummies also had a high number of herbivores (personal observation) and satisfactory development of the pest reflected in the size of the herbivores. According to the literature, the host plant has a strong effect on the development of herbivore population levels (Amjad and Peters, 1992; Yue and Liu, 2000; Frank, 2010). These differences have been justified as host plant resistance by antibiosis, antixenosis, as well as tolerance levels to populations of the pest (Ellis *et al*., 1996) due to the biochemical composition and morphological structure of the host plant. Plants with a high tolerance and low resistance by antibiosis or antixenosis to the target herbivore lead to the obtaining of a high production of herbivores and hence their antagonists during an extended period (Frank, 2010; Pickett *et al*., 2004).

The incidence of the non-target organism is a factor that disrupts the lifetime of the rearing plants and hence it merits a careful study (Frank, 2010). In this research, the host plant had an influence on the incidence of powdery mildew, and broccoli treatment had the lowest incidence of this sickness. After two months, the 100% of brussels sprouts and cauliflower plants died while only the 60% of the broccoli plants died due to powdery mildew.

Considering the architecture of the plant, this study was the first to examine the influence of this factor on rearing systems quality. Pruned plant treatment improved the indicators of the vigorous of the plant and hence the size of the herbivore, the longevity of parasitoids, the number of mummies and the lifespan of the plants, as compared to the control (without pruning). Plants with pruning were smaller and stronger. The vigour of the plant is important because plants can live a long time due to the fact that they can tolerate better: i) biotic stress by target herbivores which may affect the plant health, generating early senescence of leaves and death of the plant and ii) abiotic stress by wind, rain and extreme temperature which could break the banker plants and dislodge the mummies of the leaves (Frank, 2010). These findings are corroborated with the reported by Bender, (1996) and Reich, (1997), who observed that the proper root and leaves pruning lead to vigorous plants, leaves with strong tissues and thus a high tolerance to abiotic and biotic stress.

According to Price *et al*., (1980), in vigorous plants, the development of the pest is appropriate maybe due to the suitable nutritional content of the plant. In this study, pruned plants had higher size of the herbivore.

In this research, two months after releasing the parasitoids, pruned plants were still strong while the control plants were already dead, indicating that pruning elongates the lifespan of the plants. Pruned plants were resistant to powdery mildew, given that the architecture of the plant could have an effect on the transpiration of the plant and hence on the relative humidity within the cages. Indeed, the relative humidity inside cages from pruning treatment was lower than in the control by 18.6%.

The results of cage size experiments showed that size of the herbivores, the number of *D. rapae* mummies, the number of *E. tricolor* mummies and the *E. tricolor* longevity was higher inside large rather than small cages. This is possible given that the quality of light is a factor linked to vigorous plants and the strength of the plant has an important direct effect on the herbivore development and indirect effect on the parastoid fitness. When the quality of the light was measured, the plants inside large cages had 22.89% more light compared to small cages, meaning that this variable could have an effect on the quality of the rearing systems.

In summary, the findings of this research clearly demonstrate that for both parasitoids, the use of pruned broccoli plants inside large cages can lead to herbivores of large size, a low incidence of powdery mildew, parasitoids with increased longevity, high mummy production, strong plants with satisfactory field adaptation and a high lifespan. This Chapter confirm that the suitable management of rearing systems for the mass production of pest antagonists can have a substantial impact on the lifespan of banker plants produced under controlled conditions as well as on the fitness of pest antagonists.

3. The role of flowering plants in improving the fitness and performance of *Diaeretiella rapae* and *Encarsia tricolor* parasitoids

Abstract

In agroecosystems, the lack of food resources is a factor influencing the fitness and efficiency of parasitoids. Given that parasitoids are omnivorous, if hosts are absent, they have to rely on alternative food sources, i.e. nectar of flowers. However, the screening of these flowering plant species that offer nectar under particular conditions of study is paramount before their introduction into the crop field because studies have shown that the effect of flowering plants could be positive, neutral and/or negative in Integrated Pest Management (IPM). The systems of this study were *Diaeretiella rapae* - *Brevicoryne brassicae* (cabbage aphid) and *Encarsia tricolor* - *Aleyrodes proletella* (cabbage whitefly). The cabbage aphid and whitefly are important pests of many cultivar groups of the *Brassica* genus. These pests are mainly managed with agrochemicals that affect human health and are harmful to the environment. In this context, the parasitoids *D. rapae* and *E. tricolor* are natural enemies of these pests, respectively. Hence, the aims of this research were to explore the access of the parasitoids to the nectar of the selected flowering plant species and the effect of flowering plants on the attraction, survival, fecundity and performance of the microhymenopterans *Diaeretiella rapae* M´Intosh 1855 (Hymenoptera: Aphidiidae) and *Encarsia tricolor* 1878 Förster (Hymenoptera: Aphelinidae). The parasitoid performance was measured in terms of the number of mummies, the number of pest individuals, percentage of female parasitoids, percentage of male parasitoids, percentage of parasitoid emergence and hyperparasitoid percentage of non-target species. Dual choice bio tests were designed to compare the attractiveness of different food resources offered by a flowering plant or herbivore plant complex. Overall, six choice experiments were performed with each parasitoid species, whereby each experiment was replicated 50 times. The six choice conducted experiments were alyssum vs. buckwheat, alyssum vs. faba bean, buckwheat vs. faba bean, herbivore-plant complex HPC (plant infested with the herbivore) vs. alyssum, HPC vs. buckwheat and HPC vs. faba bean. Subsequently, to evaluate the effect of different food resources on the survival probability of *D. rapae* and *E. tricolor,* a completely randomised design with seven treatments and twenty replicates per treatment was conducted. The treatments were control, water, honey, non-host honeydew, host-honeydew, nectar of buckwheat and nectar of alyssum. Thereafter, a randomised complete design with two treatments (flowering plants and control) and seven replicates was carried out to estimate the effect of flowering plants on the fecundity of the target parasitoids.

The flowering plants were buckwheat and alyssum. Finally, a randomised complete design was done with two treatments - i.e. with and without flowering plants - and six repetitions. The results of food attractiveness experiments were: i) both adult parasitoids had access to the floral nectar of alyssum and buckwheat, but not to the faba beans; ii) alyssum had higher attractiveness for *D. rapae* compared to all other flowering plants; iii) in the experiments with the parasitoid *D. rapae*, it was observed that the flowering plants never had higher attractiveness that the host plant complex (HPC), and v) the parasitoid *E. tricolor* showed the same response for HPC and flowering plants. The climate chamber experiments showed that the fitness of both parasitoid species was substantially increased given that: i) the *D. rapae* longevity on sources of high quality (honey and nectar) was 4.37 times as long as on sources without sugar (control and water) and 8.91 times for *E. tricolor*; ii) the *D. rapae* longevity was 2.07 times on buckwheat treatment as long as on alyssum and 1.53 times for *E. tricolor*; iii) the longevity of *D. rapae* was 3.89 times longer in the treatment of buckwheat compared to host honeydew and 1.19 times longer for *E. tricolor* and iv) For *D. rapae*, the treatment with flowering plants (mix of alyssum and buckwheat) had 29.14 mummies per plant more than the control and there were no differences for *E. tricolor*. Finally, in the field experiments, it was observed that the flowering plants treatment had 47 *D. rapae* mummies per plant more than the control and 15 for *E. tricolor*. In conclusion, the selected flowering plants can to attract parasitoids and play a major role in optimising the fitness of the target parasitoids but these techniques are not enough for a satisfactory management of the target pests.

Keywords: *Diaeretiella rapae; Encarsia tricolor; Brevicoryne brassicae; Aleyrodes proletella;* Flowering plants; Survival; Fecundity; Parasitism percentage

3.1. Introduction

A lack of food sources in agroecosystems and the low capacity to find the host by the parasitoid are factors constraining the fitness and efficiency of parasitoids. Food is necessary for searching behaviours and metabolism (Leatemia *et al*., 1995; Rivero and Casas, 1999; Begum *et al*., 2006; Jervis *et al*., 2008, Varennes *et al*., 2015). Parasitoids are omnivorous and hence they can live longer with an absence or a low number of hosts if there are alternative food resources as nectar (Thompson, 1999; Jervis *et al*., 2008; Wäckers *et al*., 2008).

Agroecosystems are usually monocultures with few plants that offer food to pest antagonists (Altieri, 1999). A technique to provide alternative food is the use of flowering plants (Landis *et al*., 2000 and Jonsson *et al*., 2008), providing nectar and/or pollen to pest antagonists (Zamora-Carrillo *et al*., 2011). Nectar plays a significant role in the effectiveness of parasitoids. Siekmann *et al*., (2001) observed that nectar reduces the probability of starvation and increases the longevity of the parasitoid *Cotesia rubecula* through providing essential nutrients. Pollen is a major source of protein for the formation of eggs. However, some parasitoid species cannot take pollen because they have not mouthpart specialisations to consume this food (Jervis, 1998).

In this research, the systems of study were *Diaeretiella rapae* - *Brevicoryne brassicae* (cabbage aphid) and *Encarsia tricolor* - *Aleyrodes proletella* (cabbage whitefly). The cabbage aphid and whitefly are important pests of many cultivar groups of the *Brassica* genus. These pests are mainly managed with agrochemicals that can damage human health if residues remain on the produce and can be harmful to the environment. The parasitoids *D. rapae* and *E. tricolor* are solitary, koinobiont endoparasitoids of *B. brassicae* and *A. proletella*, respectively (Williams, 1995 and Kant, 2012) and their use could help to reduce or avoid the use of chemicals.

These parasitoids are present in the cabbage fields but the efficiency of these parasitoids is insufficient to maintain the pest populations in levels below economic thresholds. The parasitoid *D. rapae* feeds on honeydew and nectar (Kant, 2012) and *E. tricolor* on honeydew, hemolymph and nectar (Augustin, 2012). Therefore, flowering plants can offer nectar as alternative food for improving the parasitoid survival during host searching and/or enhancing the fecundity. Fitness in this study refers to survival and fecundity. In addition, flowering plants may produce volatiles that attract parasitoids (Bianchi and Wäckers, 2008; Fujinuma *et al*., 2010; Kopta *et al*., 2012; Orre Gordon *et al*., 2013). Additionally, the vegetative features of these plants may offer a kind of shelter for the parasitoids (Landis *et al*., 2000; Rebek *et al*., 2006) reducing the adverse effect of extreme conditions of humidity and/or temperature. The screening of flowering plant species is paramount before their introduction into the crop field (Araj *et al*., 2006). Studies have shown that the effect of flowering plants could be positive as well as neutral or negative. It is due to the fact that, first, floral resources have also been pointed out as food for crop pests (Begum *et al*., 2006). For example, Baggen and Gurr (1998) found that many pests of the order Lepidoptera feed on floral nectar. Second, some flowering plant species have a better effect than others on the fitness of parasitoids (Hogg et al., 2011). Third, parasitoid accessibility to nectar is not always possible due to the relation between structure sizes of flowers and insects (Patt *et al*., 1997). Fourth, every plant can offer different kinds of benefits. For example, in *Dolichogenidea tasmanica*, alyssum had a better effect on the longevity, but buckwheat had a better effect on fecundity. Fifth, the effect of a determined flowering plant species can be different in every natural enemy species. For example, buckwheat had a better effect than alyssum on the longevity of *Aphidius ervi* (Araj *et al*., 2006) and *Gonatocerous* spp. (Irvin *et al*., 2007). But in a study of Irvin et al, (2006) with *D. tasmanica* was observed that alyssum had a better effect on the longevity of this parasitoid, as compared to buckwheat. Sixth, some flowering plants can benefit the fourth trophic level, reducing significantly the effect of parasitoids on the management of the target pests (Araj *et al*., 2009).

Hence, the aims of this research were: i) to explore the access to the nectar of selected flowering plants by parasitoids, ii) to study the attractiveness of different food resources to parasitoids in laboratory experiments, iii) to evaluate the effect of different food resources on the longevity of the target parasitoids in climate chamber experiments, iv) to measure the influence of flowering plants on the parasitoid fecundity under greenhouse conditions, and v) to observe the effect of flowering plants on the number of mummies, percentage of parasitoid emergence, percentage of parasitoid females and males, hyperparasitoid percentage of non-target species under field conditions.

The hypotheses of this chapter were that: i) the target parasitoids have access to the floral resources, ii) the chosen flowering plants have high attractiveness to the target parasitoids, iii) flowering plant nectar can improve the longevity and fecundity of the target parasitoids, iv) flowering plants have effect on the number of mummies, percentage of parasitoid emergence, percentage of parasitoid females, percentage of parasitoid males and hyperparasitoid percentage of non-target species under field conditions.

3.2. Materials and methods

3.2.1. Plants, herbivores and parasitoids

The insects *B. brassicae*, *D. rapae*, *A. proletella* and *E. tricolor* were collected from brussels sprouts grown in the fields of the Institute of Horticultural Production Systems (Hannover, Germany) and mass reared under controlled conditions. The mass rearing of the insects was undertaken in mesh cages of (51 x 76 x 56) cm, and the host plant was broccoli (Marathon F1, Hild, Germany). The mesh cages were in climate chambers at 20 - 25 °C, with relative humidity between 40 – 60 % and a photoperiod of 16:8 h L:D. 1-24 hours old, without experience, unmated and unfed parasitoids were used for all experiments. To determine the sex of individuals of *D. rapae*, it was observed the presence or absence of ovipositor in the last part of the abdomen. For *E. tricolor* experiment, it was counted the number of segments in the flagellum of antennas which have seven in females and six in males. To get 1-24 hours old parasitoid adults, mummies of both parasitoid species were kept in gelatine capsules until hatching (Fig. 3.1). To raise broccoli plants, seeds were sowed in plastic pots (12 cm diameter) filled with black peat substrate (Fruhstorfer Erde, type Nullerde) and the pots were maintained in the nursery for a period of 4-6 weeks. The plants were fertilised once a week with Wuxal Top N (Wilhelm Haug GmbH, Germany), a foliar fertilizer with macro and micronutrients. To obtain flowering plants, seeds were sown directly in 12 cm diameter plastic pots.

Fig. 3.1. Parasitoids between 1-24 hours old in gel capsules

The flowering plants used in this study were:

i) Alyssum *(Lobularia maritima)* (brassicacea) (Begum *et al*., 2006; Irvin *et al*., 2006; Irvin *et al*., 2007; Webb, 2010, Hogg *et al*., 2011, Gontijo *et al*., 2013) variety snows crystals (Fig. 3.2 a).

ii) Buckwheat *(Fagopyrum esculentum)* (polygonacea) (Kopta *et al*., 1996; Stephens *et al*., 1998; Tylianakis *et al*., 2004; Begum *et al*., 2006; Irvin *et al*., 2006 and Irvin *et al*., 2007) variety Bamby (Fig. 3.2 b).

iii) Faba bean *(Vicia faba)* (fabaceae) (Du *et al*., 1998 and Jamont *et al*., 2013) variety hangdown grünkerning (Fig. 3.2 c).

These flowering plant species were chosen based on the following benefits found in the literature, the seeds are available in the market, they are neither invasive nor attract cabbage pests, buckwheat begins the blooming before than alyssum (personal observations), but alyssum has longer blooming period, they promote availability of nectar for parasitoids during all crop season (Hogg *et al*., 2011), buckwheat has a high relation sucrose/hexose (Vatala *et al*., 2006), alyssum and buckwheat flowers are neither deep nor tubular which avoid the attraction of butterflies, they have a short time passage from sowing to blooming compared with other plant species (only six-eight weeks), they are known to be attractive and improve the fitness of several pest antagonists (Begum *et al*., 2006; Irvin *et al*., 2006; Irvin *et al*., 2007; Webb, 2010, Hogg *et al*., 2011, Jamont *et al*., 2013) and they have other uses (Alyssum and buckwheat are used for management of weeds according to Platt *et al*., 1999 and nectar for bees, buckwheat is food for people or animals).

Fig. 3.2. Flowering plants a) alyssum *(Lobularia maritima)* variety snows crystals, b) buckwheat *(Fagopyrum sculentum)* variety Bamby, and c) Faba bean *(Vicia faba)* variety hangdown grünkerning.

3.2.2. Attractiveness and access to the food resources

Dual choice bio-tests were designed to compare the attractiveness of different food resources. The food resources of the bio-tests are explained in Table 3.1 and the dual choice bio-test in Table 3.2. The set-up for every experiment was a Petri dish which had a hole of 1 cm diameter in the centre to introduce the insects and the two options (Fig. 3.3). The size of Petri-dishes for *D. rapae* was 14 cm and for *E. tricolor* was 6 cm. The *D. rapae* parasitoids were observed for a maximum of 30 minutes, while *E. tricolor* parasitoids for 120 minutes. The observational time and size of the cages were determined, taking into consideration results of preliminary experiments. The test arena was less than *D. rapae* because preliminary experiments in 14 cm Petri dishes showed that behavioural response of 200 individuals of *E. tricolor* to resources within a 2 h period was very low (only 10% gave a response) and the maximum time that *D. rapae* and *E. tricolor* needed to choose an option were 5 and 120 minutes, respectively. The possible response events were: i) option 1, ii) option 2 and iii) no response. If the test did not give a response, it was repeated with another setup to achieve 50 observations per experiment. A preference for one of the resources was classified when the parasitoid visited the resource for a minimum of 10 seconds. Direct access to nectar was documented by pictures. The attractiveness was defined in this study as the capacity of a flowering plant or the pest-infested cabbage leaf to generate visual and/or olfactory signals that induce the movement of the target parasitoid to the respective plant tissue.

Table 3.1: Description of the different choices offered to *Diaeretiella rapae* and *Encarsia tricolor* parasitoids in the experiments concerning the attractiveness of food resources.

Food source	***D. rapae*** **(14 cm bio-test arena)**	***E. tricolor*** **(6 cm bio-test arena)**
Alyssum *Lobularia maritima*	4 flowers 4 cm² leaf	4 flowers 1 cm² leaf
Buckwheat *Fagopyrum esculentum*	4 flowers 4 cm² leaf	4 flowers 1 cm² leaf
Faba bean *Vicia faba*	1 flower 3 stipules with extra-floral nectaries 4 cm² leaf	1 flower 1 stipules with extra-floral nectaries 1 cm² leaf
Plant – Herbivore – Complex (PHC)	Honeydew: 4 cm² of one leaf with more than 40 aphids, removed before the start of the experiment.	Honeydew and herbivore:1 cm² with at least 10 larvae of third or fourth stage (pupa).

Table 3.2: Dual choice biotest conducted to compare the attractiveness of different food resources. All tests were carried out for each parasitoid independently. HPC:Host-Plant complex

	Dual choice bio-test
1	Alyssum vs buckwheat
2	Alyssum vs faba bean
3	Buckwheat vs faba bean
4	HPC vs alyssum
5	HPC vs buckwheat
6	HPC vs faba bean

Before beginning the experiments, five parasitoids were released to monitor their responses to potential light gradients in the room. All resources were placed perpendicular to the light gradient on opposite sides of the Petri dish.

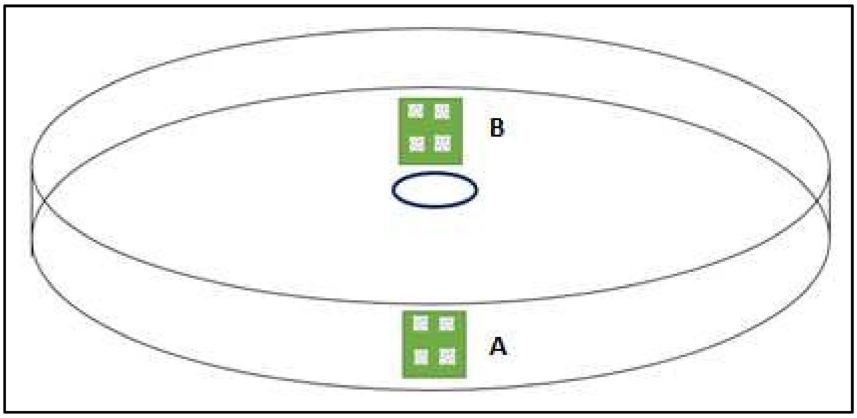

Fig. 3.3. Set-up for the experiments about the attractiveness of food resources to *D. rapae* and *E. tricolor.* Green squares: a piece of leaf, white squares: flowers or hosts, blue circle: hole to introduce parasitoids, A: option 1 and B: option 2.

3.2.3. Effect of food resources on the survival of the target parasitoids

An experiment of seven treatments and twenty replicates over time was used to evaluate the effect of different food resources on the survival probability of *D. rapae and E. tricolor.*

Treatments included:

(1) Control (no food)

(2) Water only

(3) An undiluted honey solution for *E. tricolor* and 50 % honey solution for *D. rapae*

(4) Non-host honeydew (produced by *Aphis fabae*)

(5) Host honeydew (produced by *B. brassicae* or *A. proletella*)

(6) Floral nectar from alyssum (provided as one inflorescence with 2 or 3 flowers during anthesis per day)

(7) Floral nectar from buckwheat (provided as one inflorescence with 2 or 3 flowers during anthesis per day)

To compare survival curves, the longevity of the parasitoids was measured. Longevity here refers to the number of days for which the adult parasitoid lived. The longevity of both parasitoid species was tested separately for males and females by exposing them to various food resources. Based on preliminary experiments, the best setup for every experimental unit was a 9 cm Petri dish lid placed on a 14 cm petri dish lid. The smaller lid had two holes of 2 cm covered with mesh for ventilation and two holes of 1 cm diameter to introduce the parasitoids or replace food resources (Fig. 3.4). For the water treatment, a filled Eppendorf tube containing a filter paper was used to provide this resource. The same design was used for the supply of 80% honey solution to *D. rapae*, while in the setup of *E. tricolor* a droplet of undiluted honey less than 1 mm in diameter was placed. The inflorescences of buckwheat and alyssum from 8-week-old potted plants were introduced directly without cutting the plant (Fig. 3.5). The presence of nectar in nectaries was confirmed visually in buckwheat directly in flowers (small drops of nectar) and through the colour of the pollen grains for alyssum. When the pollen shows yellow colour, it is guaranteed that nectar is present. To prevent parasitoids from escaping or getting stuck in the Eppendorf tubes, all holes were sealed with plasticine. To provide honeydew, four large aphids or whiteflies in a small piece of leaf were replaced in the setup each day. The availability of all resources was checked every day and the survival of parasitoids was recorded daily. The environmental conditions in the climate chambers involved a temperature range of 19.85 ± 1.01 °C, 65.1% ± 11.62 R.H. and a photoperiod of L 16h : D 8h.

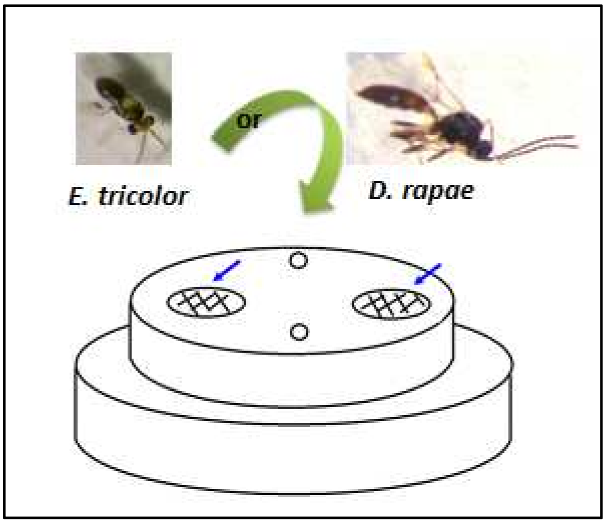

Fig. 3.4. Basic set-up to measure the longevity of the parasitoids in the water, control, honey, non-host honeydew and host honeydew treatment.

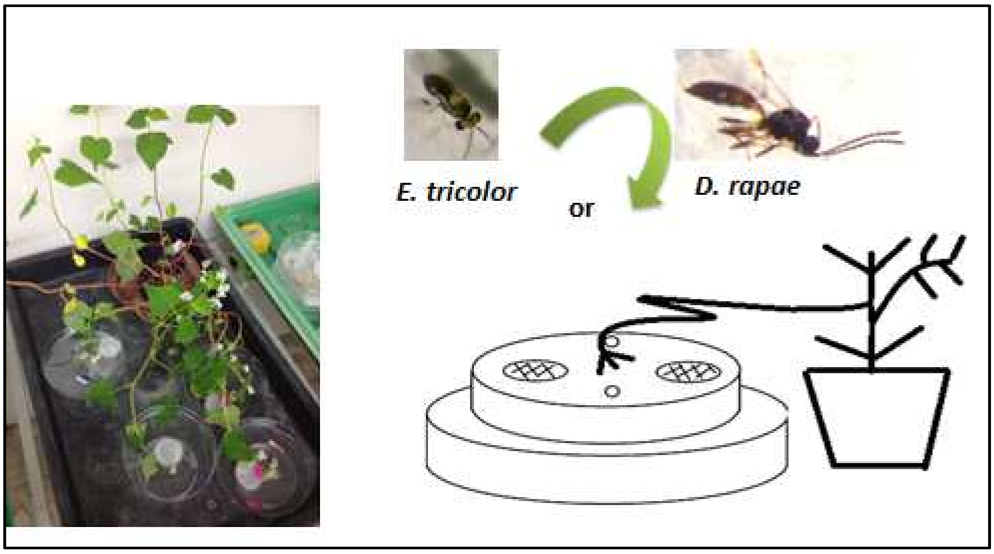

Fig. 3.5. Set-up of the flowering plant treatment on the longevity of the parasitoids.

3.2.4. Effect of the food resources on the realised fecundity of the target parasitoids

A greenhouse experiment with two treatments and seven repetitions over time was conducted in order to study the impact of flowering plants on the realised fecundity of each parasitoid species. The experiments were carried out in the period from September to November 2014. The treatments were: i) flowering plants, and ii) control. The experimental unit was a cage experiments (46 x 25 x 25) cm with a broccoli infested plant and one female parasitoid (Fig. 3.6.). For *D. rapae* experiment, every plant had 100-120 adult aphids and for *E. tricolor* 80-100 whitefly larvae. Since in previous experiments, alyssum had the best value for attractiveness and buckwheat for longevity of parasitoids, both were simultaneously offered in the flowering plant treatment. At the beginning of the experiment, each flowering plant had between 10-20 flowers at anthesis. Based on the expected life parameter of the parasitoids, the experiments were terminated after 22 and 26 days for *D. rapae* and *E. tricolor*, respectively and the number of mummies was counted. The environmental conditions inside the greenhouse involved a temperature range of 18.4 ± 0.21 °C, 51.54 % ± 3.66 R.H. and a photoperiod of L 16h : D 8h.

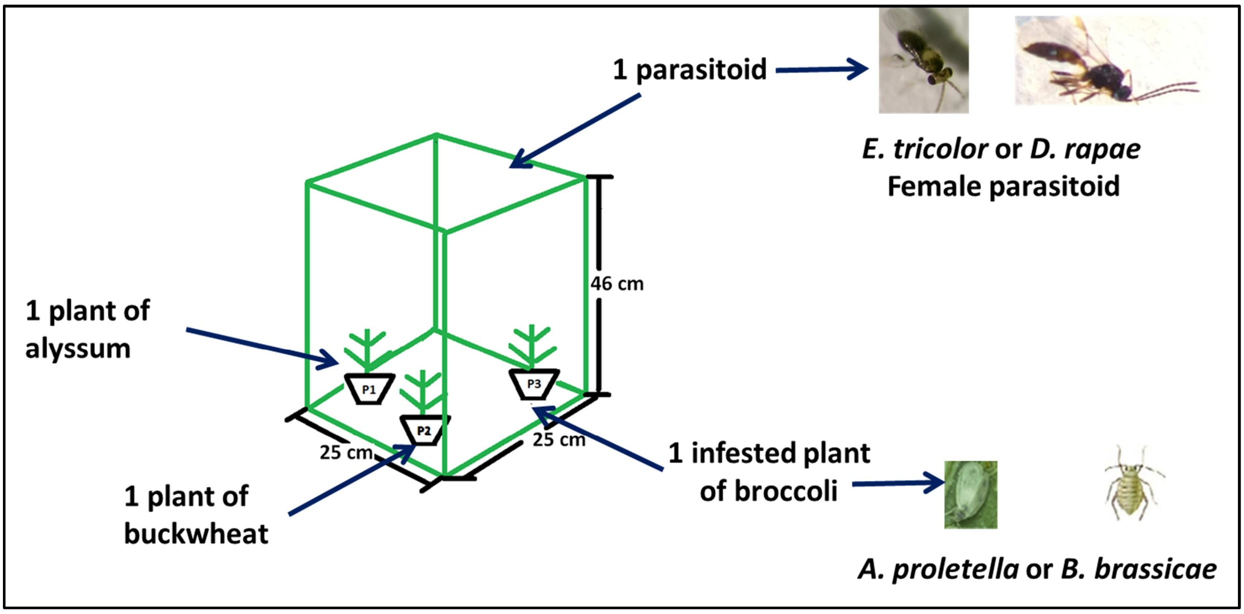

Fig. 3.6. Set-up of the flowering plant treatment in the experiment, effect of flowering plants on the realised fecundity of the target parasitoids. This experiment was done independently for each parasitoid.

3.2.5. Effect of flowering plants on the performance of the target parasitoids under open field conditions

To evaluate the effect of flowering plants on the performance of parasitoids during the first generation under open field conditions, a completely randomised design with two treatments was conducted in the period from June to September 2014. The experiment was repeated twice to get six replicates per treatment. The experimental unit or plot was an area of 4 x 3 m in size with twelve uninfested broccoli plants placed along the plot margins, three infested plants with 100-120 *B. brassicae* adults and three infested plants with 100 – 120 *A. proletella* larvae between 3rd and 4th instar (Fig. 3.7). The infested plants were placed in the middle of the plot on a 2 m circle. The centre of the circle was either free from flowering plants (control treatment) or contained two alyssum (50-70 flowers) and two buckwheat (10-20 flowers) flowering plants (flowering plant treatment). At the beginning, twenty unmated, unfed female parasitoids of each species were released in the centre. The distance between plots was 6 m distance. Taking into account the period from the beginning of the experiment until the formation of mummies of second generation, the experiments were terminated after 22 and 26 days for *D. rapae* and *E. tricolor*, respectively. The performance of parasitoids was measured in terms of the number of aphids, whitefly larvae, number of *D. rapae* mummies, number of *E. tricolor* mummies, emerged parasitoid adults, the number of parasitoid females and males and hyperparasitoid of non-target species. A random sample of 100 *D. rapae* and 50 *E. tricolor* mummies per experimental unit or plot were collected for breeding in gelatine capsules to count the emerged adults, the number of female and male parasitoids and hyperparasitoid of non-target species.

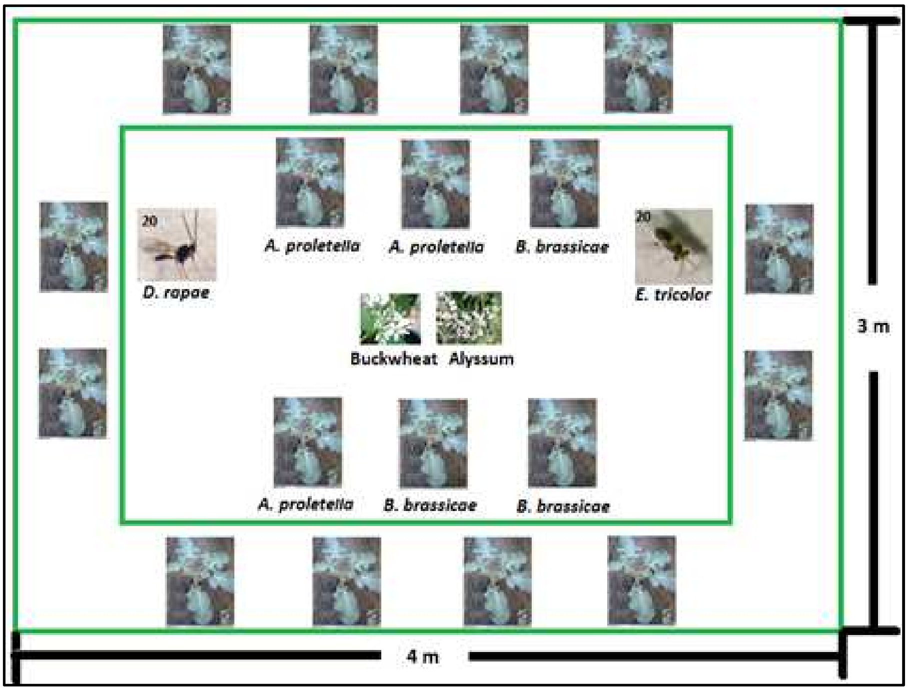

Fig. 3.7. Set-up of the flowering plant treatment in the field experiment to observe the effect of flowering plants on the performance of *D. rapae* and *E. tricolor* experiments. The control had the same set-up but without flowering plants.

3.2.6. Statistical analysis

The attractiveness of food sources to the parasitoids was compared with Fischer tests. The Kaplan–Meier function was used to generate the graphics of survival probability with the data of longevity. Survival curves were compared with a Log Rank Test (Mantel-Cox). For the other variables, the Generalized Linear Model GLM was used if the data were normally distributed, whereas the Kruskal-Wallis test was used when the data had no normality. The treatment means were compared using a Tukey's test (HSD). When the data had no homogeneity of variances, Tamhane's T2 was used. The level of significance was $p<0.05$ for all tests. All analyses were carried with SPSS 23 IBM.

3.3. Results

3.3.1. Access to the floral resources and attractiveness of food resources

Direct observations indicated that adults of both parasitoid species had access to both the floral nectar of alyssum and buckwheat but not to the faba bean floral nectar (Fig. 3.8, 3.9, 3.10 and 3.11). The parasitoids also fed on nectar, but they did not consume pollen from the studied flowering plants.

Fig. 3.8. Access of *Diaeretiella rapae* to the nectar of alyssum flowers

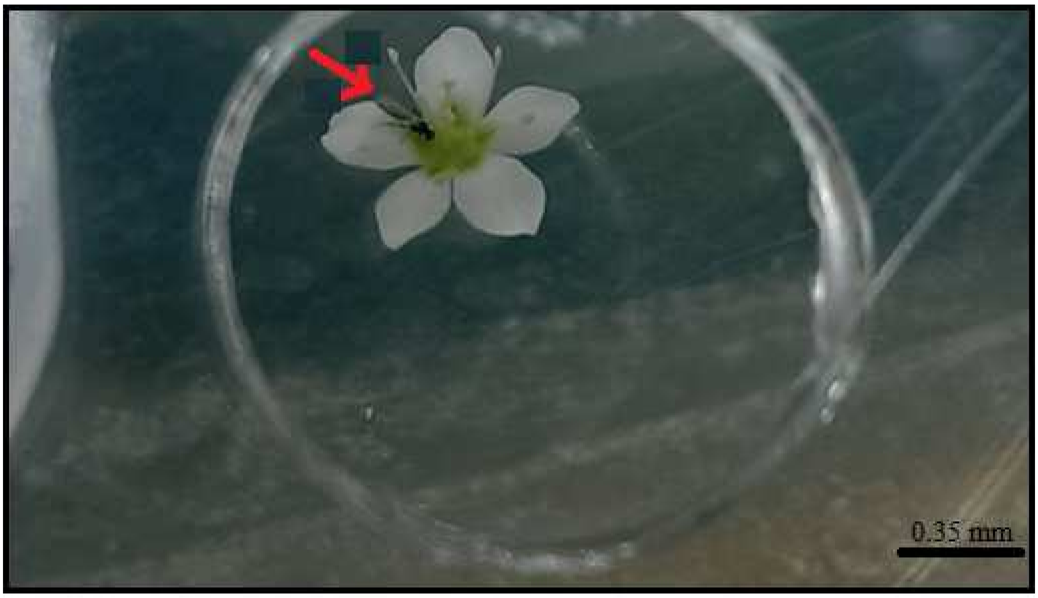

Fig. 3.9. Access of *Diaeretiella rapae* to the nectar of buckwheat flowers

Fig. 3.10. Access of *Encarsia tricolor* to the nectar of alyssum flowers

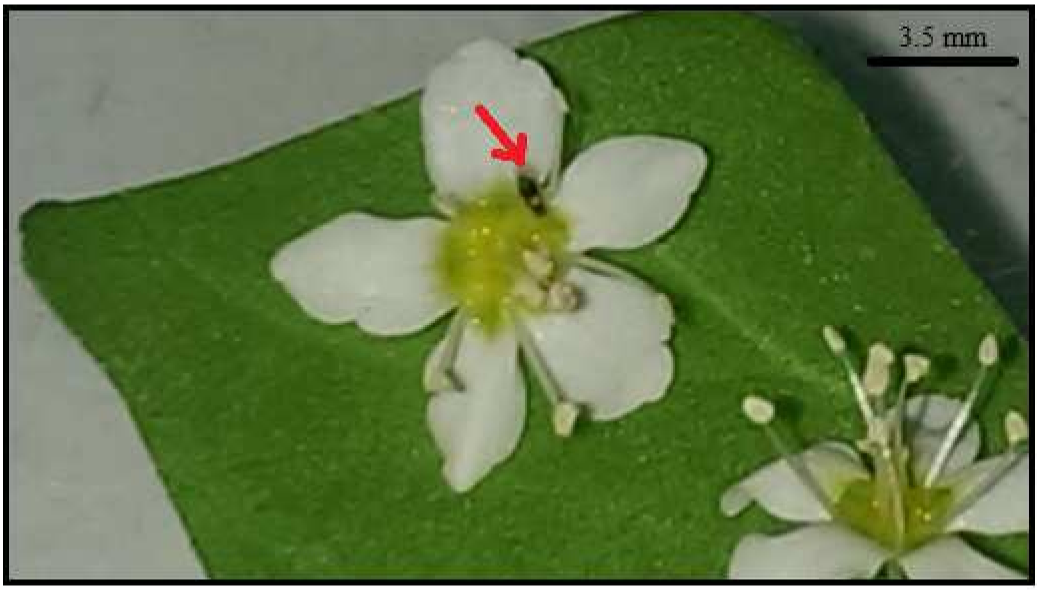

Fig. 3.11. Access of *Encarsia tricolor* to the nectar of buckwheat

Considering attractiveness of food resources, most of the tested *D. rapae* and *E. tricolor* parasitoids responded positively to the food sources within 30 and 120 minutes after introduction into the arena, respectively. Within the experimental period, the 25.37 % of *D. rapae* and 44.44 % of *E. tricolor* did not respond at all. The *D. rapae* and *E. triolor* parasitoids exhibited clear differences in their behavioural response (Fig. 3.12 and 3.13). Among the flowering plants, if alyssum was offered in combination with buckwheat or faba bean, *D. rapae* strongly preferred alyssum. For *E. tricolor*, alyssum had higher attractiveness than buckwheat, and there was not difference between alyssum and faba bean. Finally, buckwheat only had higher attractiveness, as compared with faba bean for *E. tricolor* but not for *D. rapae*. If one of the floral resources - i.e. alyssum, buckwheat or faba bean - was offered in combination with the herbivore plant complex, *E. tricolor* showed the same preference for any of the resources. By contrast, *D. rapae* indicated a clear preference for its herbivore plant complex if offered in combination with either buckwheat or faba bean. Though, in combination with alyssum, the herbivore plant complex lost attractiveness for *D. rapae*.

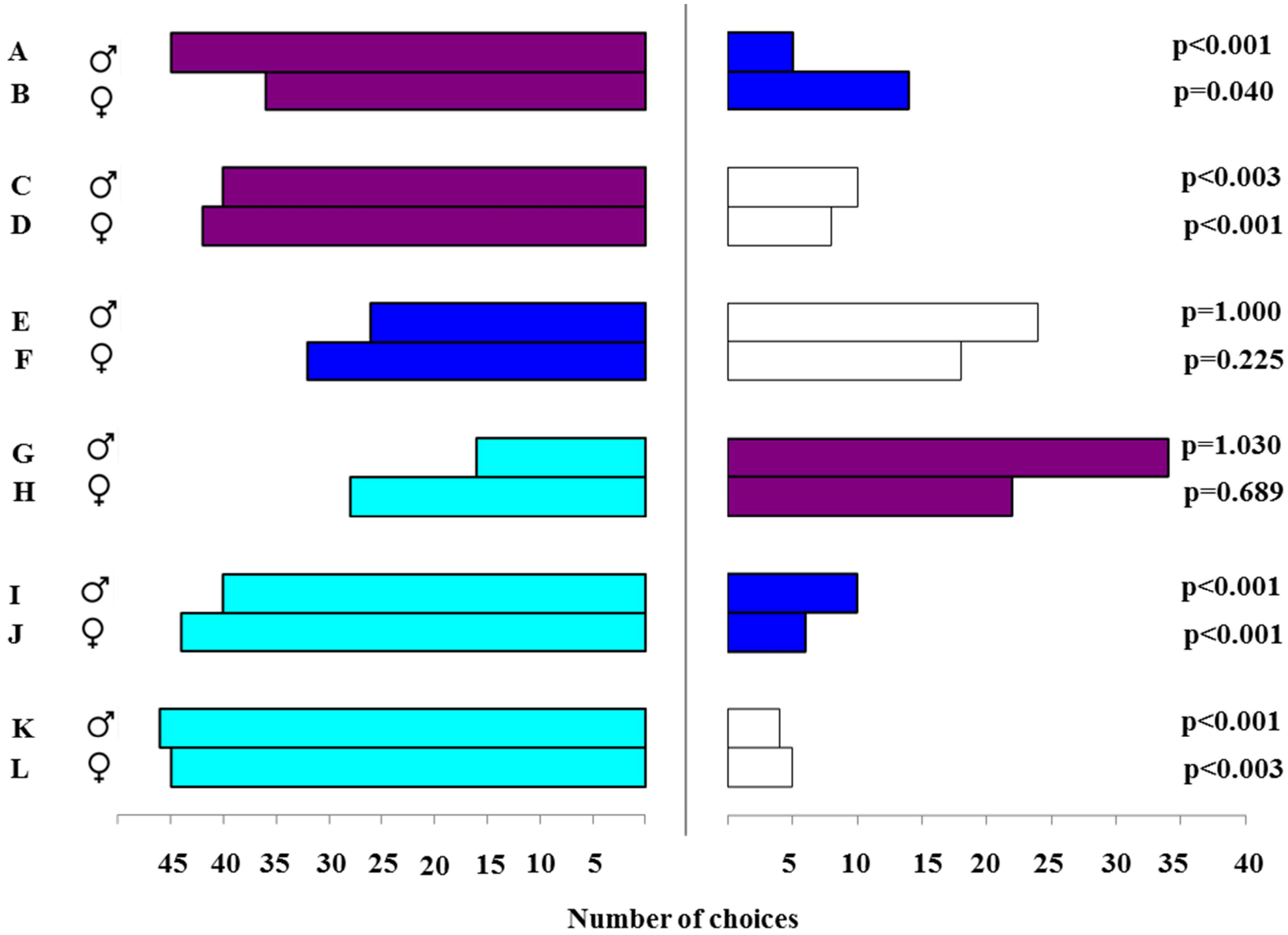

Fig. 3.12. Response of female and male *D. rapae* in two-choice experiments. Bars indicate the frequency of parasitoids choosing between two food resources. A-L refers to different experiments (n=50). Purple: alyssum, strong blue: buckwheat, white: faba bean and aquamarine: herbivore-plant complex. Null hypothesis is rejected when *p- value* < 0.05.

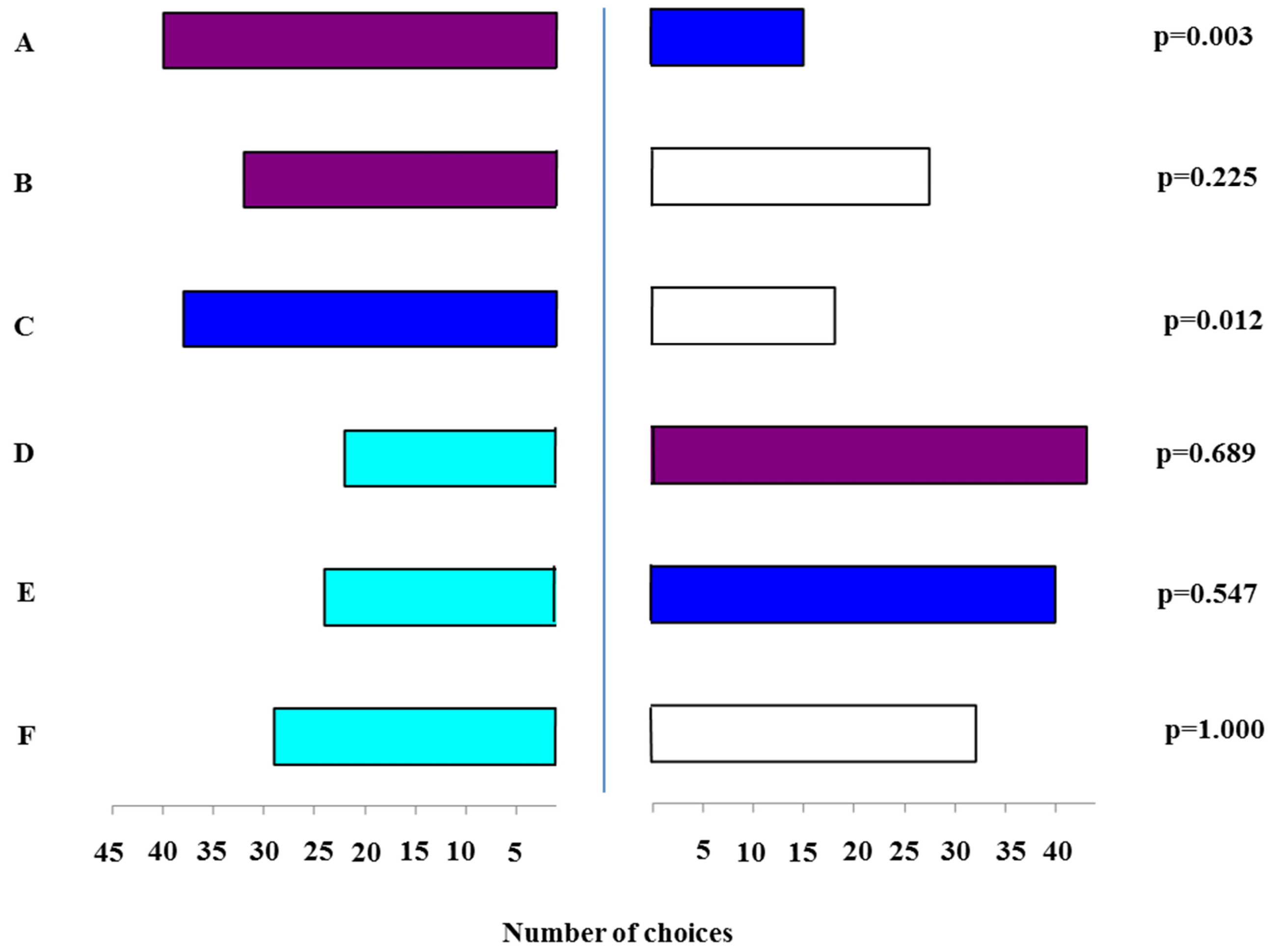

Fig. 3.13. Response of female *E. tricolor* in two-choice experiments. Bars indicate the frequency of parasitoids choosing between two food resources. A-F refers to different experiments (n=50). Purple: alyssum, strong blue: buckwheat, white: faba bean and aquamarine: herbivore-plant complex. Null hypothesis is rejected when *p- value* < 0.05.

Flowering plants should require a low number of agronomical practices. Although, it was not the objective of this experiment, it is important mentioning that faba bean had a high incidence of pests, meaning that this species is not suitable as flowering plant. This plant species was attacked by gray mold (Fig. 3.14 a), powdery mildew (Fig. 3.14 b), rust (Fig. 3.14 c), and thrips (Fig. 3.14 d).

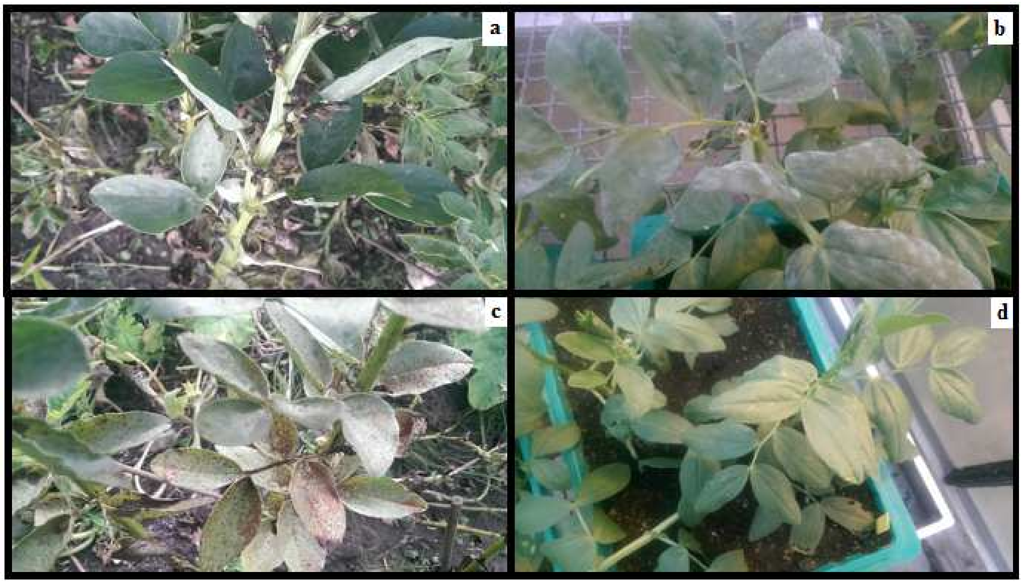

Fig. 3.14. Phytosanitary problems of *Vicia faba* during its growing a) gray mold, b) powdery mildew, c) rust, and d) thrips.

3.3.2. Effect of food resources on the survival of the target parasitoids

The survival curves of the parasitoid wasps were significantly different among treatments of the experiments with *D. rapae* females (X^2=209.9, *df*=6, p<0.001) (Fig. 3.15), *D. rapae* males (X^2=168.3, *df*=6, p<0.001) (Fig. 3.16), *E. tricolor* females (X^2=230.6, *df*=6, p<0.001) (Fig. 3.17), and *E. tricolor* males (X^2=189.61, *df*=6, p<0.001) (Fig. 3.18). The differences between the treatments are shown in Table 3.3.

Table 3.3: Mean and standard error of the measured variables in the experiment titled effect of different food resources on the *D. rapae* and *E. tricolor* survival. Different letters indicate significant differences according to Log Rank Test (Mantel-Cox).

Treatment	*D. rapae* females	*D. rapae* males	*E. tricolor* females	*E. tricolor* *males*
Honey	7.4 ± 0.2 b	6.15 ± 0.2 b	70.65 ± 1.1 a	31.85 ± 1 a
Buckwheat	15.2 ± 0.4 a	8.5 ± 0.15 a	12.8 ± 0.4 b	11.65 ± 0.3 b
Alyssum	6.2 ± 0.2 c	5.5 ± 0.3 b	8.65 ± 0.2 d	7.25 ± 0.3 c
Host Honeydew	2.8 ± 0.2 e	2.05 ± 0.1 c	7.2 ± 0.2 e	3.95 ± 0.2 d
Non-Host Honeydew	2.35 ± 0.2 e	2 ± 0.1 c	8.8 ± 0.7 c	5.95 ± 0.5 c
Control	1.7 ± 0.1f	1.6 ± 0.1 d	3.45 ± 0.15 g	3.05 ± 0.15 e
Water	3.8 ± 0.2 d	1.45 ± 0.1 d	4.35 ± 0.3 f	3.42 ± 0.2 de

When *D. rapae* and *E. tricolor* parasitoids were fed on honey and nectar, they lived 6 and 20.12 days longer than those fed without sugar sources (control or water), respectively. When the effect of flowering plants on the survival of parasitoids was compared, it was observed that *D. rapae* and *E. tricolor* parasitoids lived 6 and 4.27 days longer on buckwheat compared to alyssum, respectively. The parasitoid *D. rapae* showed no significant differences between non-host honeydew and honeydew treatments, while *E. tricolor* wasps fed on non-host honeydew lived 1.8 days longer than those fed on host honeydew treatment. The higher effect of nectar treatments over honeydew treatments was confirmed because this food increased the survival of the target parasitoids 6.55 days in *D. rapae* and 3.6 days in *E. tricolor.* All *D. rapae* females and males died at 8th and 9th day, respectively except in the treatment of buckwheat. The *D. rapae* parasitoids with the treatment of buckwheat had a life between 9-17 days for females and 7-10 days for males (Fig. 3.15 and 3.16). All females and males of *E. tricolor* died at 13th and 9th day respectively except in the treatments of buckwheat and honey. The *E. tricolor* parasitoids with the treatment of buckwheat showed a life span between 9-16 days for females and 9-15 days for males (Fig. 3.17 and 3.18). The maximum longevity of *D. rapae* females, *D. rapae* males, *E. tricolor* females and *E. tricolor* males in alyssum was 7, 7, 11 and 9 days respectively while in buckwheat longevity amounted to 17, 10, 16 and 15 days accordingly.

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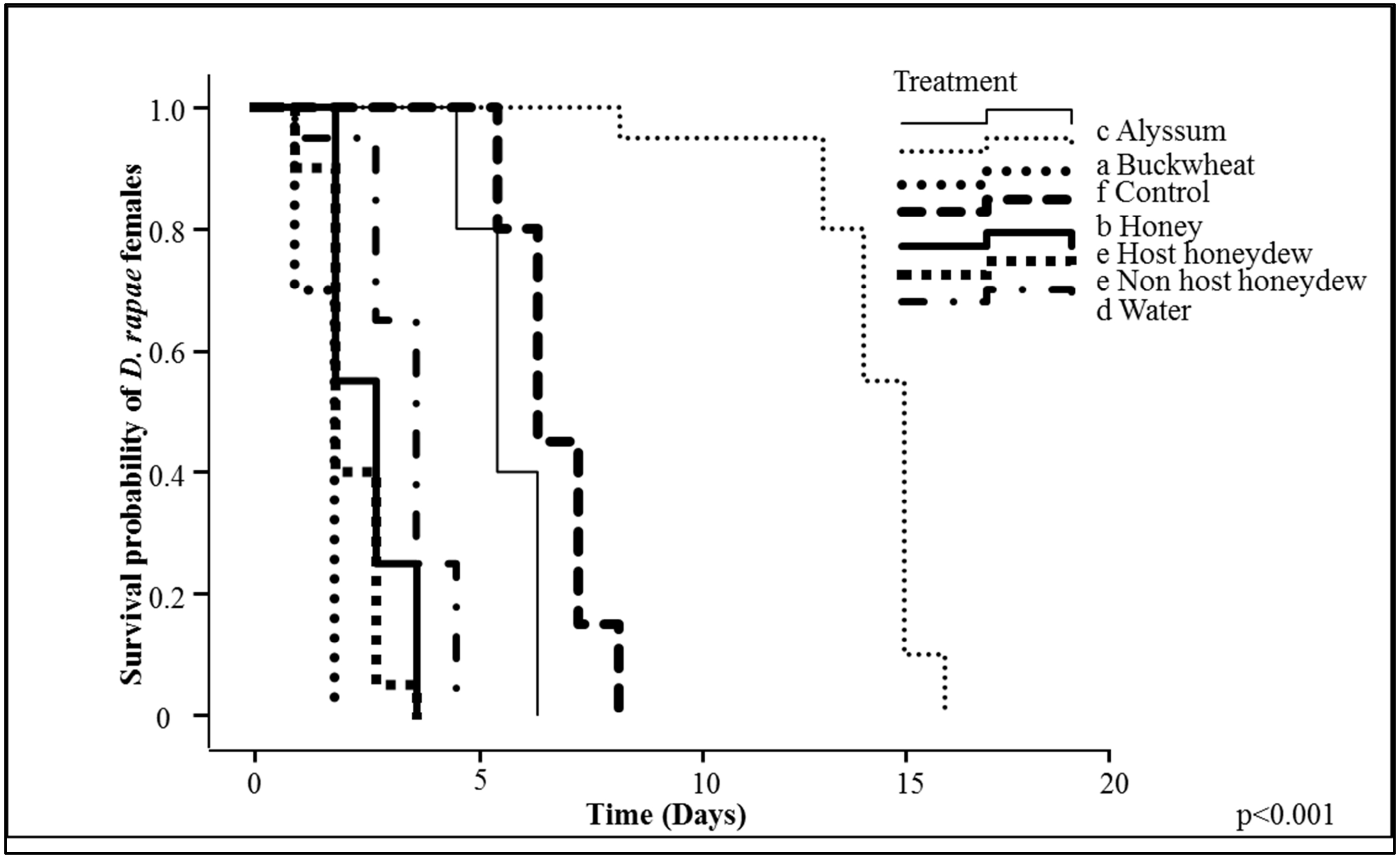

Fig. 3.15. Effect of several food sources on the survival of *D. rapae* females. Different letters indicate significant differences according to Log Rank Test (Mantel-Cox) n=20.

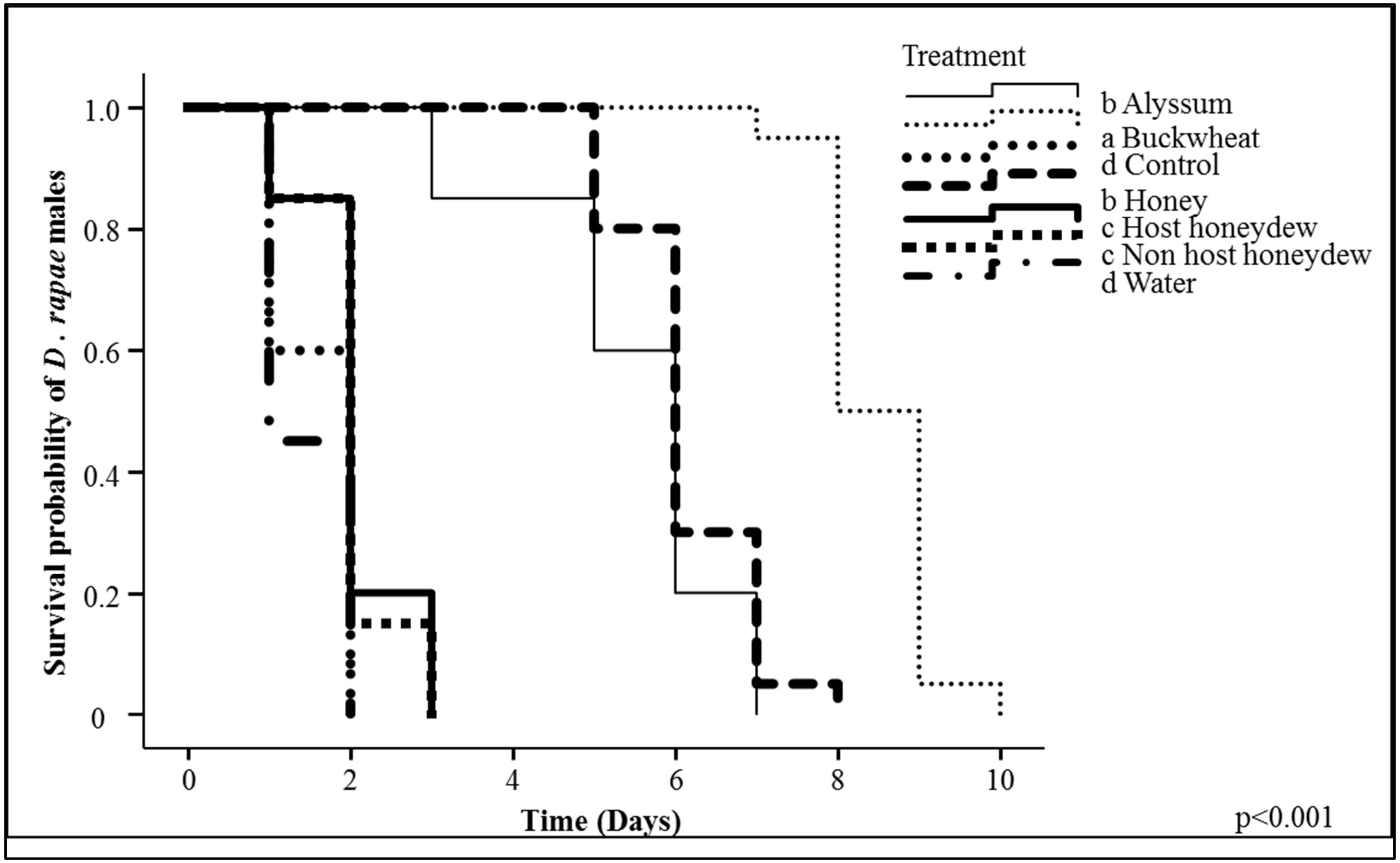

Fig. 3.16. Effect of several food sources on the survival of *D. rapae* males. Different letters indicate significant differences according to Log Rank Test (Mantel-Cox) n=20.

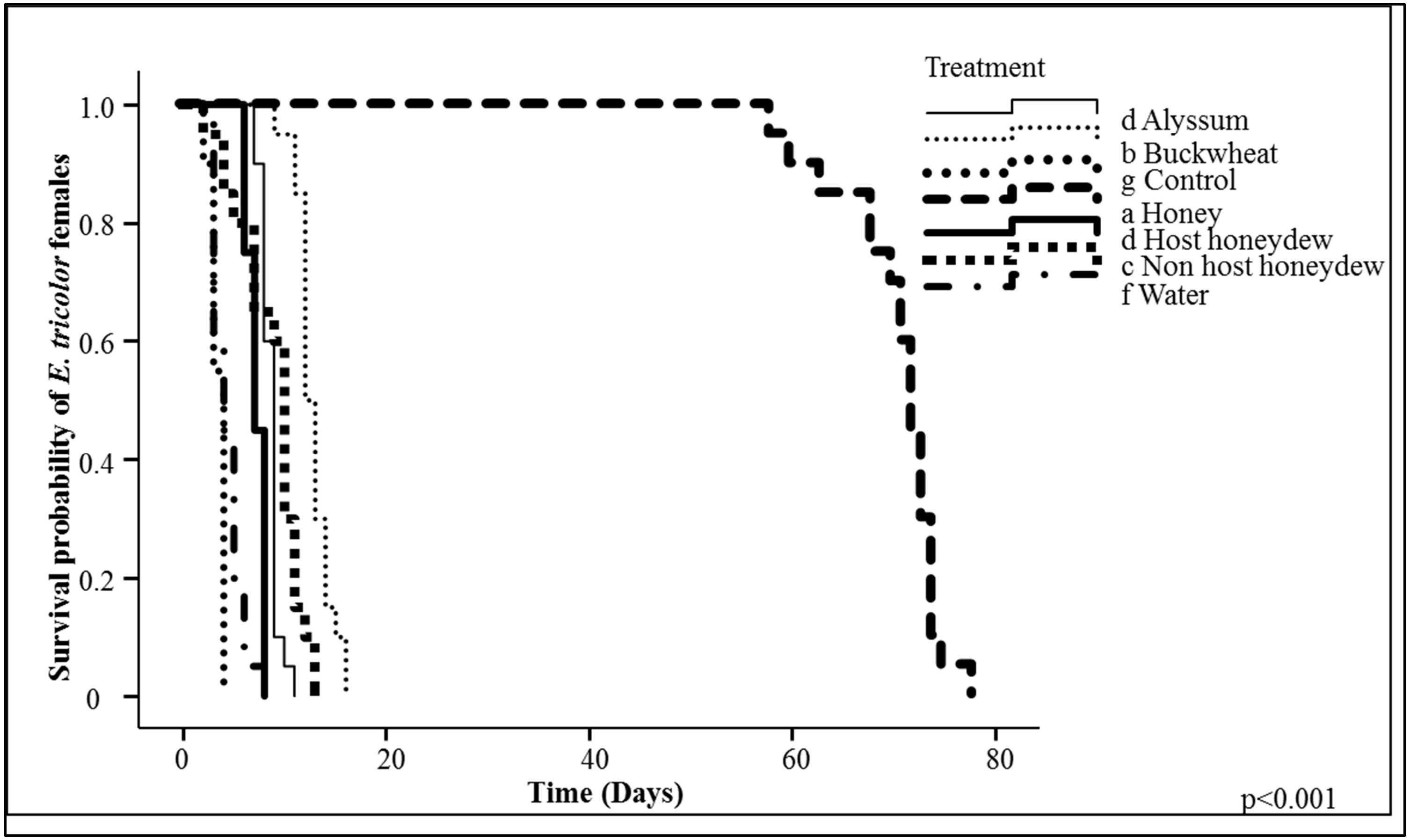

Fig. 3.17. Effect of several food sources on the survival of *E. tricolor* females. Different letters indicate significant differences according to Log Rank Test (Mantel-Cox) n=20.

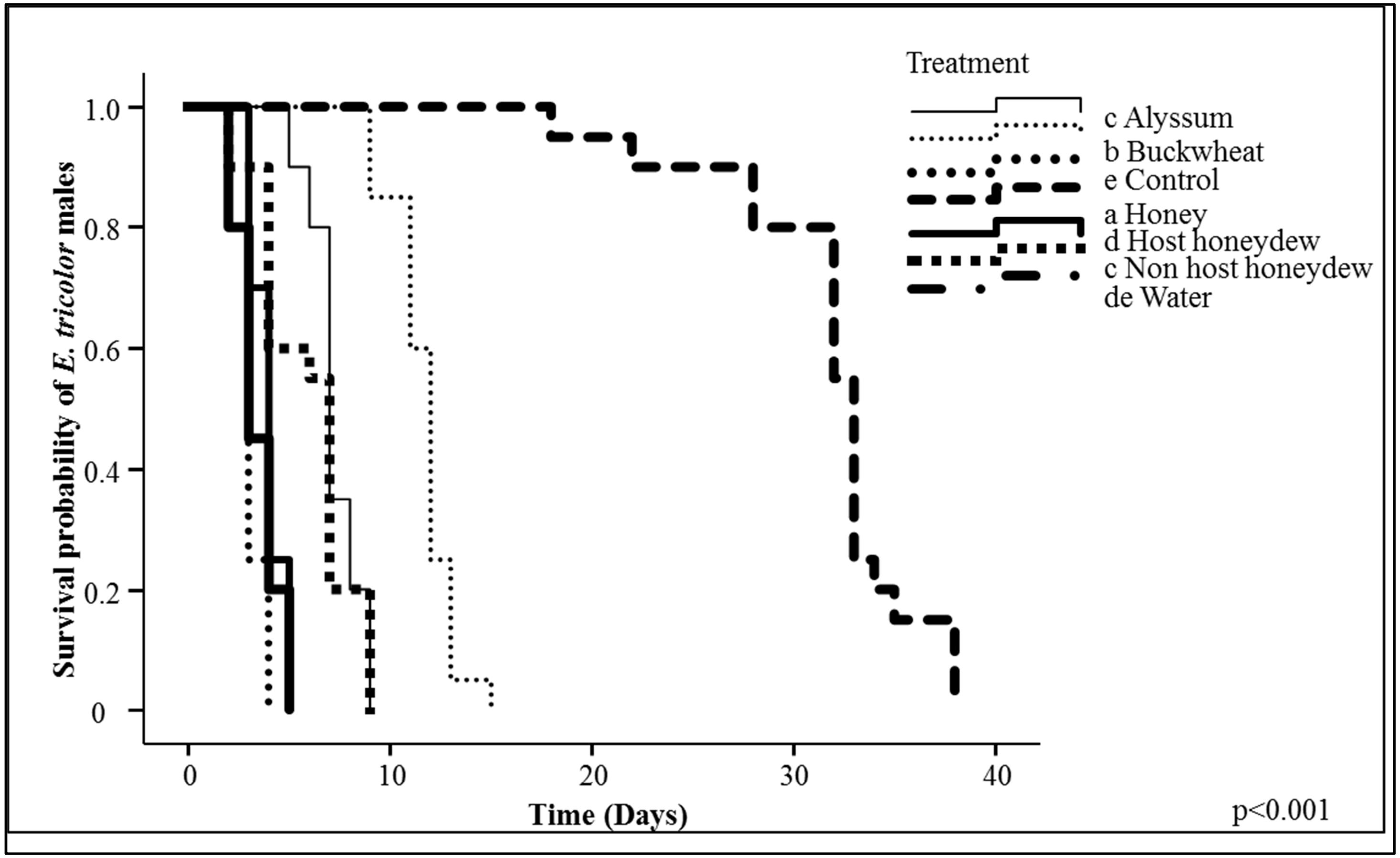

Fig. 3.18. Effect of several food sources on the survival of *E. tricolor* males. Different letters indicate significant differences according to Log Rank Test (Mantel-Cox) n=20.

3.3.3. Effect of the flowering plants on the realised fecundity of the target parasitoids

Access to the flowering plant nectar increased the realised fecundity of *D. rapae* (F=63.71, *df*=1, p<0.001) (Fig. 3.19). The percentage of mummies in the treatment with the flowering plants (alyssum + buckwheat) was approximately twice as high as that in the control. For *D. rapae* experiment, the percentage of mummies was 63.71 ± 10.03% in the flowering plant treatment and 34.57 ± 8.87% in the control. For *E. tricolor* experiment, no differences were observed (F=0.444, *df*=1, p=0.518) (Fig. 3.20). The percentage of mummies was 68.28 ± 10.50 in the flowering plant treatment and 64.42 ± 11.15 in the control.

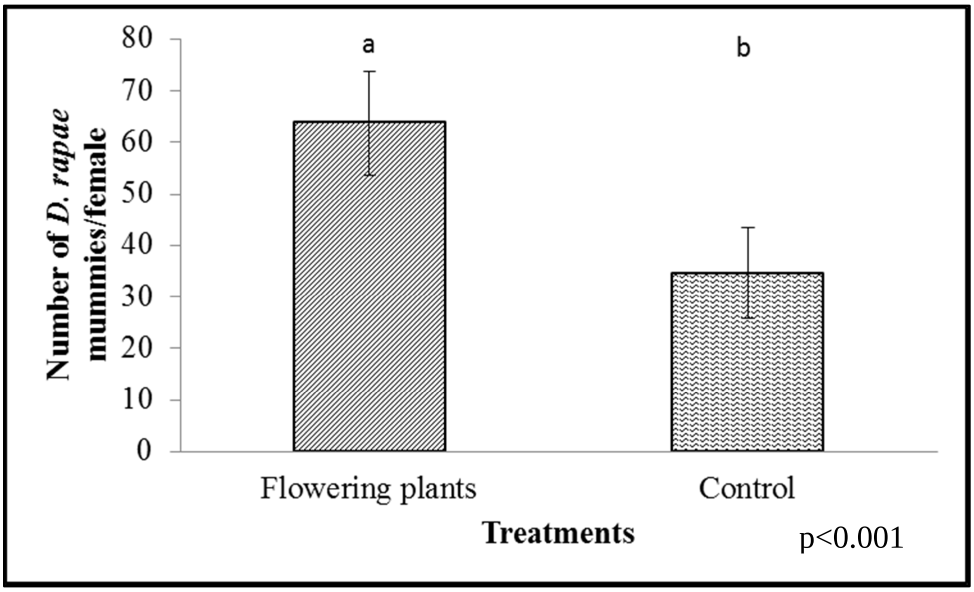

Fig. 3.19. Effect of flowering plants on the number of *D. rapae* mummies/female. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test (n=7).

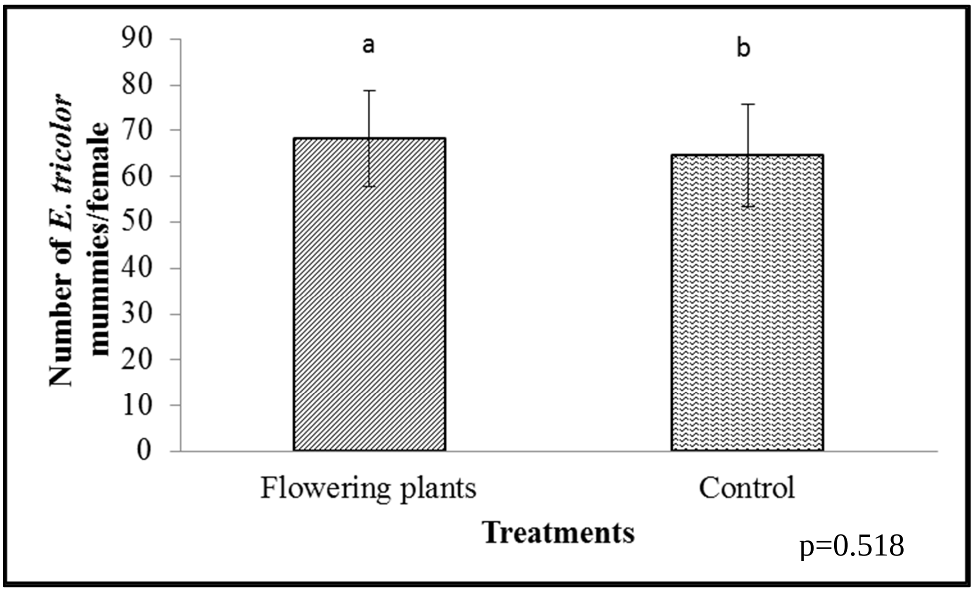

Fig. 3.20. Effect of flowering plants on the number of *E. tricolor* mummies. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test (n=7).

3.3.4. Effect of flowering plants on the performance of the target parasitoids under open field conditions

For *D. rapae* experiment, the number of *B. brassicae* individuals (F=69.257, *df*=1, p<0.001) (Fig. 3.21) and the percentage of *D. rapae* mummies (F=54.030, *df*=1, p=<0.001) showed significant differences between treatments (Fig. 3.22). In the variables regarding the number of *D. rapae* mummies (Fig. 3.23), percentage of *D. rapae* female parasitoids (Fig. 3.24), percentage of *D. rapae* male parasitoids (Fig. 3.25), hyperparasitoid percentage (Fig. 3.26) and percentage of parasitoid emergence (Fig. 3.27), no significant differences were observed. The number of aphids was three times lower in the flowering plant treatment compared to the control, and the parasitism percentage was twice times higher in the treatment with flowering plants compared to the control. The mean and standard error of every variable for *D. rapae* experiment are shown in Table 3.4.

Table 3.4: Mean and standard error of the measured variables in the experiment titled effect of flowering plants in the *D. rapae* performance in the field.

Variable	Treatment	Mean ± Standard error
Number of *B. brassicae* individuals/3 plants/plot	Flowering plants	84 ± 40.17 b
	Control	275 ± 39.05 a
Percentage of mummies of *D. rapae*	Flowering plants	68 ± 11.27 a
	Control	32 ± 4.12 b
Number of mummies/3 plants/plot	Flowering plants	176 ± 63.64 a
	Control	129 ± 21.37 a
Number of females/50 mummies of *D. rapae*	Flowering plants	16 ± 6.59 a
	Control	17 ± 6.98 a
Number of males/50 mummies of *D. rapae*	Flowering plants	10 ± 2.88 a
	Control	13 ± 5.2 a
Number of hyperparasitoids/50 mummies of *D. rapae*	Flowering plants	25 ± 7.94 a
	Control	21 ± 8.55 a
Percentage of *D. rapae* females	Flowering plants	31 ± 13.19 a
	Control	33 ± 13.95 a
Percentage of *D. rapae* males	Flowering plants	19 ± 5.76 a
	Control	25 ± 10.41 a
Hyperparasitoid percentage from *D. rapae* mummies	Flowering plants	50 ±15.87 a
	Control	41 ± 17.10 a
Percentage of parasitoid emergence	Flowering plants	60 ± 9.87 a
	Control	63 ± 16.10 a

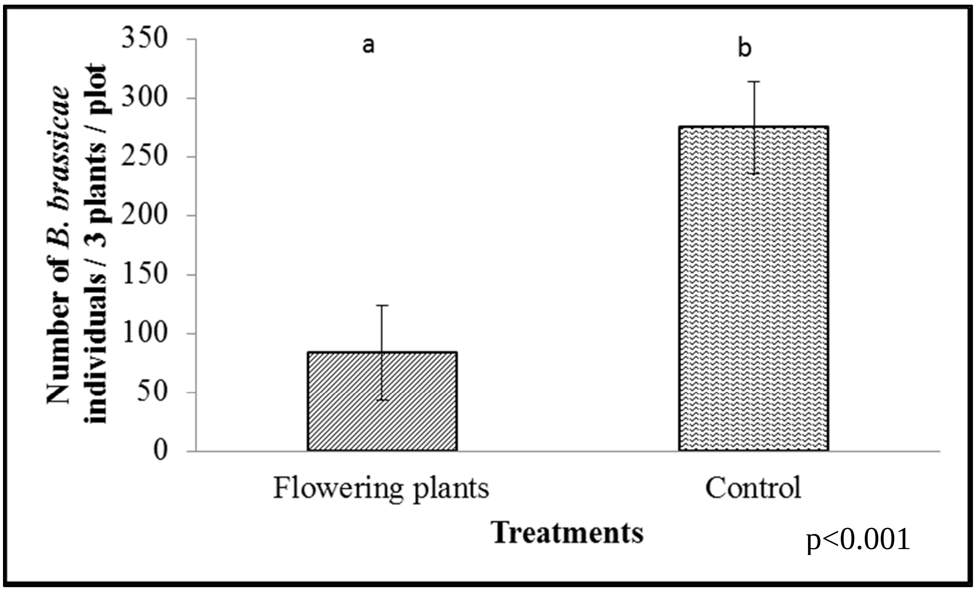

Fig. 3.21. Effect of flowering plants on the number of *B. brassicae* individuals per plot (3 plants) under open field conditions with the parasitoid *D. rapae*. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test.

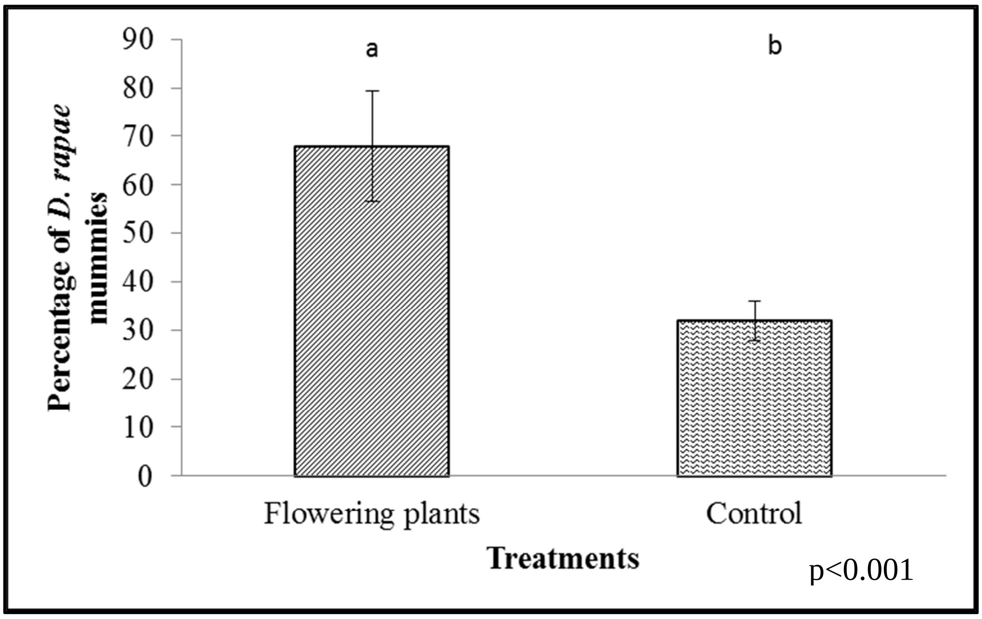

Fig. 3.22. Effect of flowering plants on the percentage of *D. rapae* mummies under open field conditions. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test.

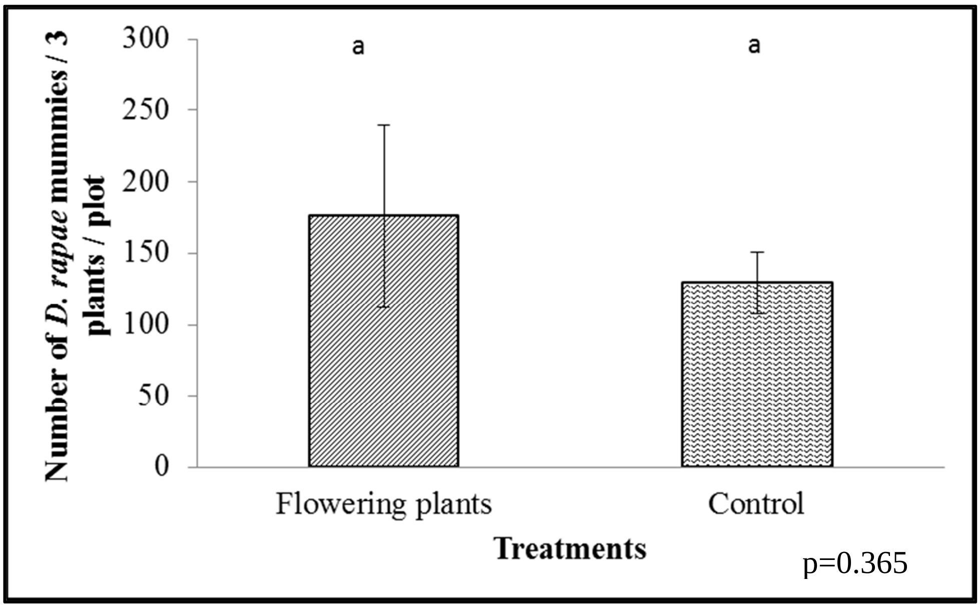

Fig. 3.23. Effect of flowering plants on the number of *D. rapae* mummies per plot (3 plants) under open field conditions. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test.

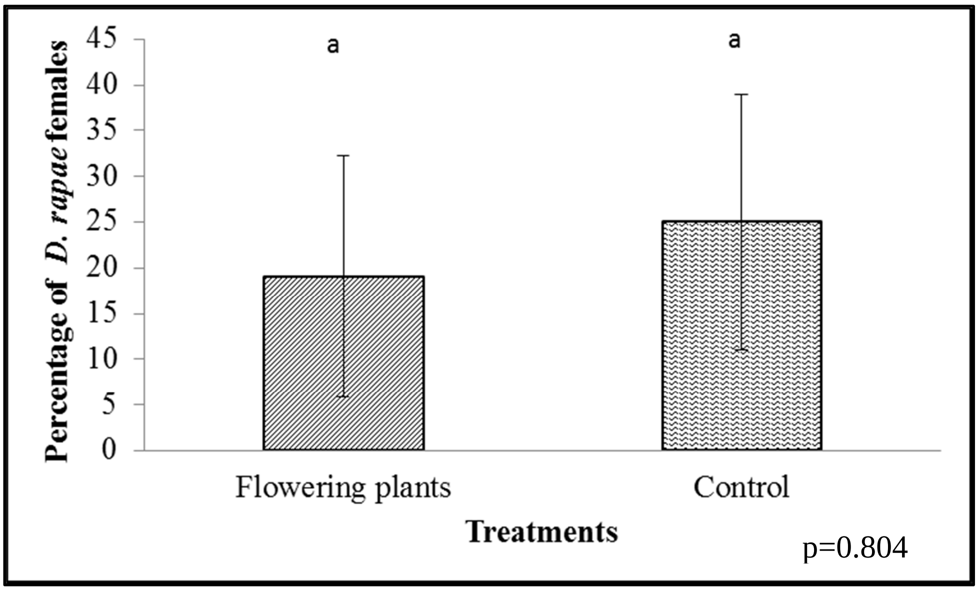

Fig. 3.24. Effect of flowering plants on the percentage of *D. rapae* females under open field conditions. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test.

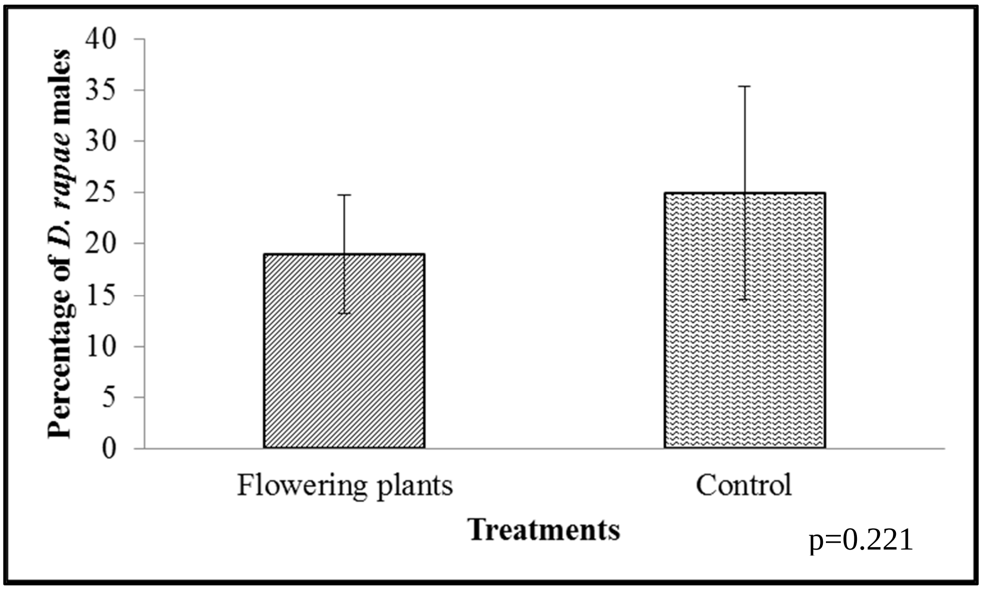

Fig. 3.25. Effect of flowering plants on the percentage of *D. rapae* males under open field conditions. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test.

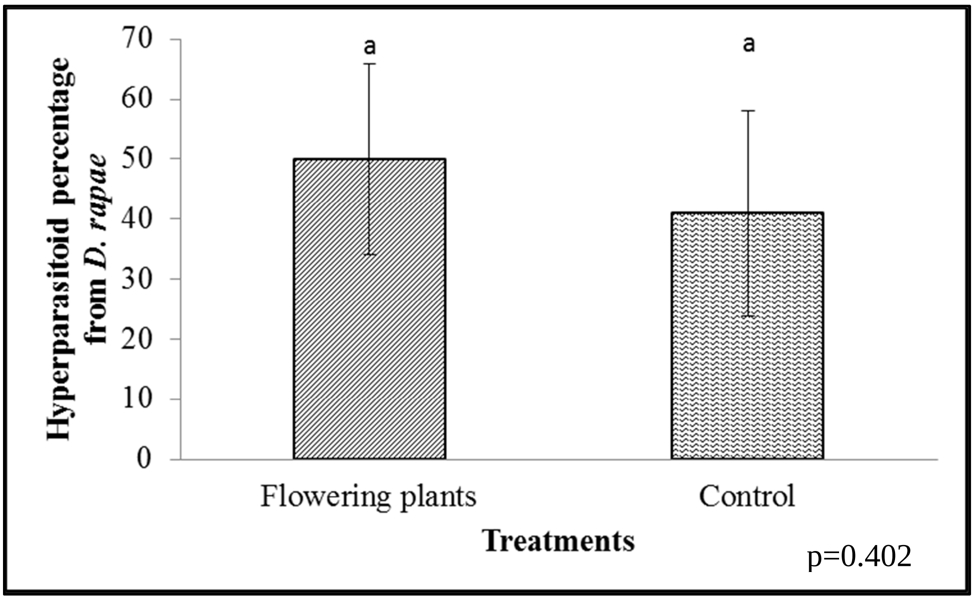

Fig. 3.26. Effect of flowering plants on the hyperparasitoid percentage from *D. rapae* mummies under open field conditions. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test.

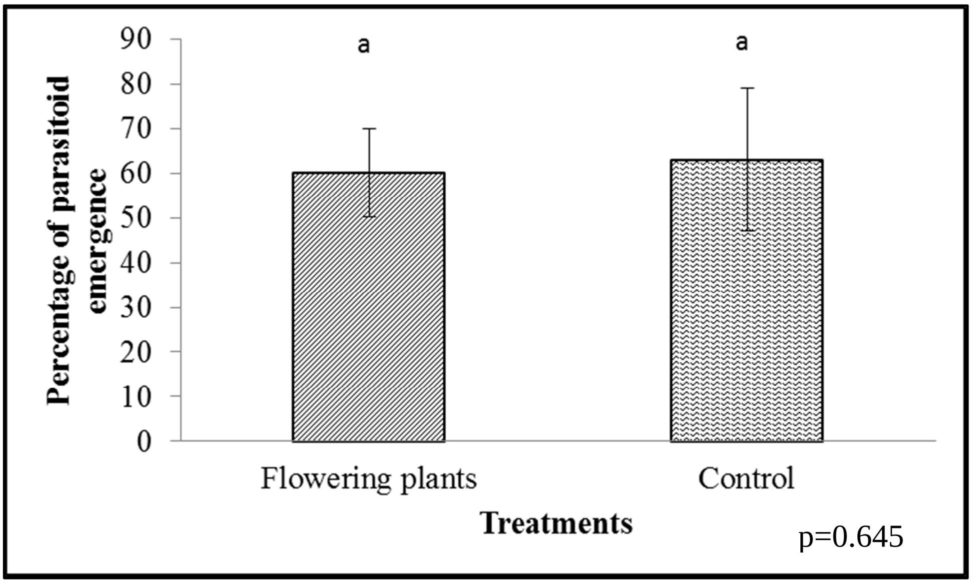

Fig. 3.27. Effect of flowering plants on the percentage of parasitoid emergence of *D. rapae* under open field conditions. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test.

For *E. tricolor* experiment, the treatment with flowering plants significantly increased the number of *A. proletella* larvae (F=32865 *df*=1 P<0.004) (Fig. 3. 28), percentage of *E. tricolor* mummies (F=132.567 *df*=1 P<0.001) (Fig. 3.29), and the number of *E. tricolor* mummies (F=660.086, *df*=1, P=0.014) (Fig. 3.30). The flowering plant treatment had 15 mummies per plant more than the control. The percentage of mummies in the flowering plant treatment was 1.46 times greater compared to the control. The variables percentage of *E. tricolor* females (Fig. 3.31), percentage of *E. tricolor* males (Fig. 3.32), and percentage of parasitoid emergence (Fig. 3.33) had no significant differences. The mean and standard error of every variable for *E. tricolor* experiment are shown in Table 3.5.

Table 3.5: Mean and standard error of the measured variables on the experiment titled effect of flowering plants in the *E. tricolor* performance in the field.

Variable	Treatment	Mean ± Standard error
Number of *A. proletella* larvae / 3 plants / plot	Flowering plants	340 ± 44.07 b
	Control	445 ± 60.56 a
Percentage of *E. tricolor* mummies	Flowering plants	22 ± 4.3 a
	Control	15 ± 2.03 b
Number of *E. tricolor* mummies / 3 plants / plot	Flowering plants	96 ± 13.77 a
	Control	81 ± 7.69 b
Number of females/20 mummies of *E. tricolor*	Flowering plants	20 ± 0.41 a
	Control	20 ± 0.52 a
Number of males/20 mummies of *E. tricolor*	Flowering plants	0.17 ± 0.41 a
	Control	0.33 ± 0.52 a
Number of hyperparasitoids of non-target species/20 mummies from *E. tricolor*	Flowering plants	0 ± 0 a
	Control	0 ± 0 a
Percentage of *E. tricolor* females	Flowering plants	99 ± 2.04 a
	Control	98 ± 2.58 a
Percentage of *E. tricolor* males	Flowering plants	1 ± 0
	Control	2 ± 0
Hyperparasitoid percentage of non-target species	Flowering plants	0 ± 0 a
	Control	0 ± 0 a
Percentage of parasitoid emergence of *E. tricolor*	Flowering plants	46 ± 5.85 a
	Control	43 ± 6.98 a

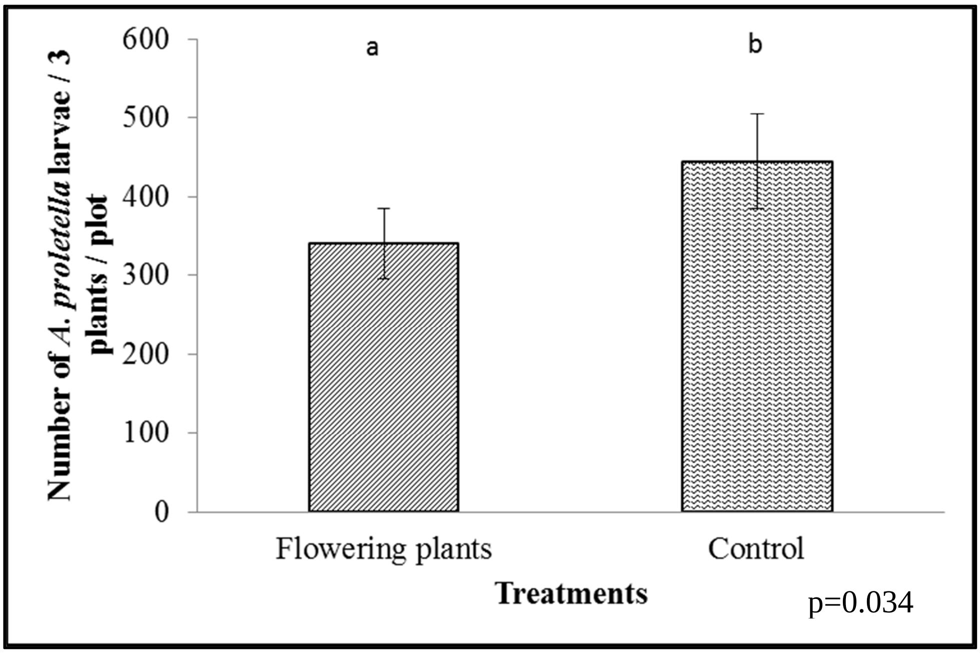

Fig. 3.28. Effect of flowering plants on the number of *A. proletella* larvae per plot (3 plants) under open field conditions with the parasitoid *E. tricolor*. Mean±SE are presented. Different letters indicates significant differences at 5% level according to Tukey´s Test.

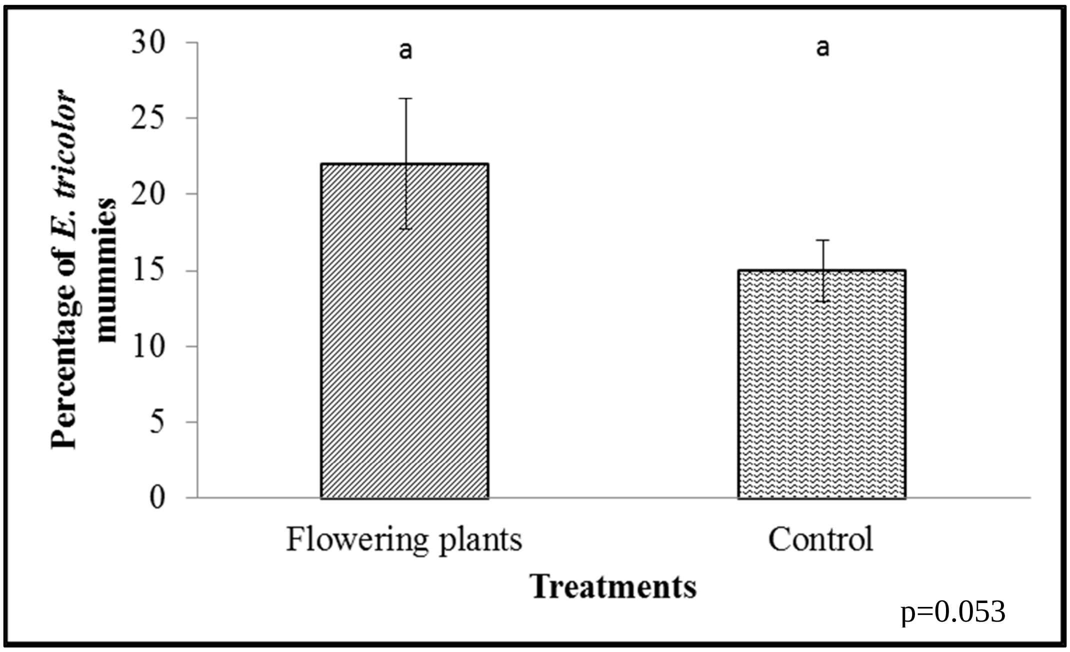

Fig. 3.29. Effect of flowering plants on the percentage of *E. tricolor* mummies under open field conditions. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test.

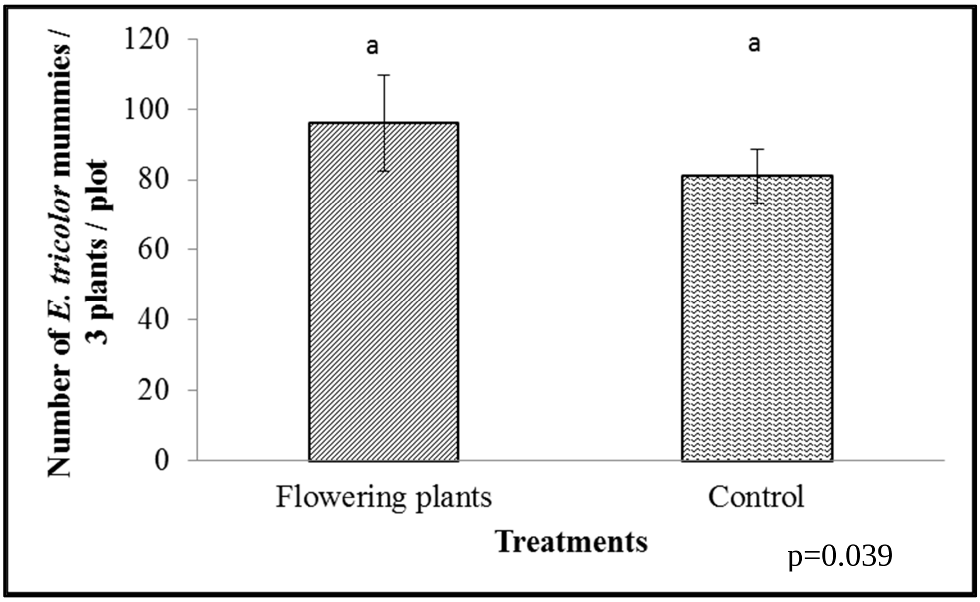

Fig. 3.30. Effect of flowering plants on the number of *E. tricolor* mummies per plot (3 plants) under open field conditions. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test.

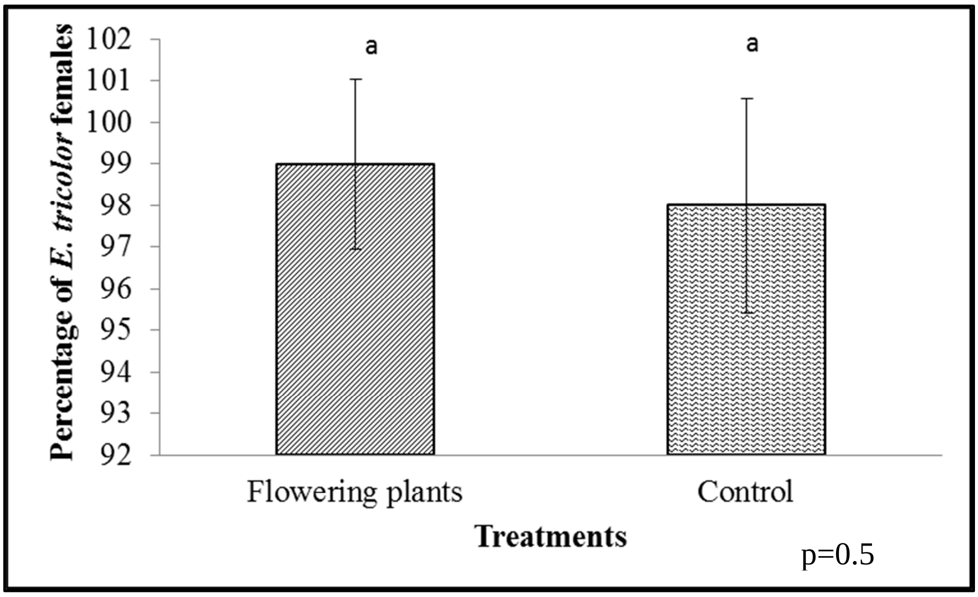

Fig. 3.31. Effect of flowering plants on the percentage of *E. tricolor* females under open field conditions. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test.

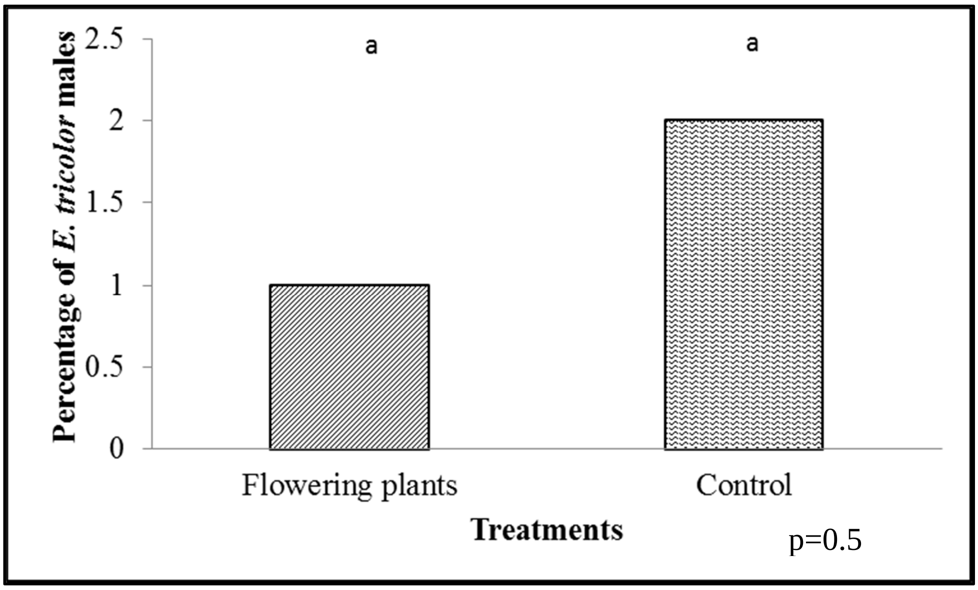

Fig. 3.32. Effect of flowering plants on the percentage of *E. tricolor* males under open field conditions. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test.

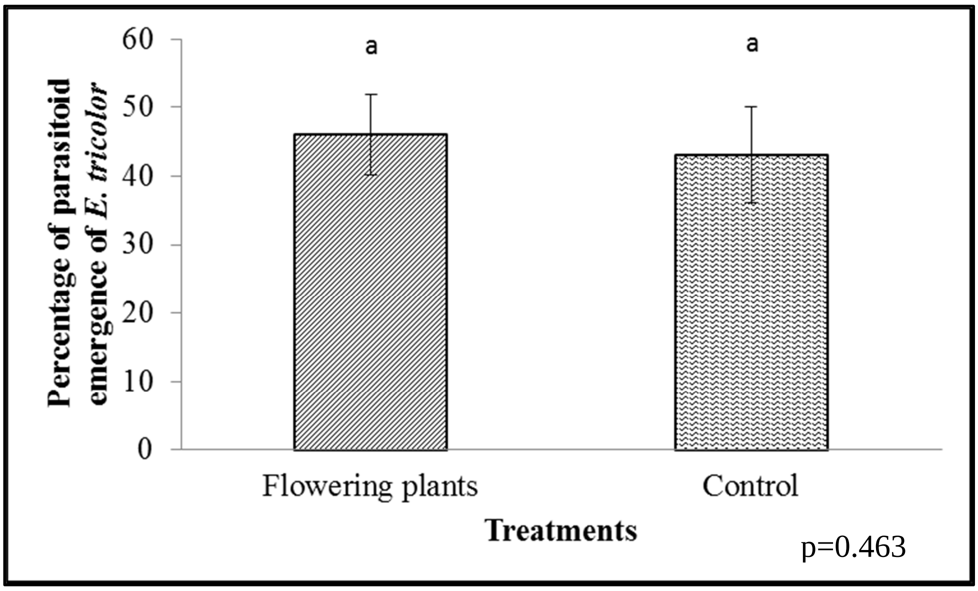

Fig. 3.33. Effect of flowering plants on the percentage of parasitoid emergence of *E. tricolor* under open field conditions. Mean±SE are presented. Different letters indicate significant differences at 5% level according to Tukey´s Test.

3.4. Discussion

Flowering plants play relevant roles in the fitness of pest antagonists. These plants lead to many positive effects such as improve the survival, reduce the mortality, enhance the potential and realised fecundity of pest antagonists among others. Flowering plants produce volatiles that attract crop beneficials and also offer nectar. Nectar constitutes a rich source of carbohydrates of high quality and water for crop beneficials (Leatemia *et al*., 1995; Rivero and Casas, 1999; Begum *et al*., 2006; Jervis *et al*., 2008; Varennes *et al*., 2015). Therefore, the purpose of this chapter was to evaluate the effect of selected flowering plant species on different aspects related to the performance of parasitoids.

Considering the access to the floral resources, the relations between sizes and forms of flowers parts with insect´s body parts (i.e. head, mouth) influence the foraging behaviour because these relations define the access of the insect to the nectar resource in flowers (Wäckers, 2004). Flowers with short corolla permit to insects with short mouthparts have easy access to floral resources (Gilbert, 1981) as that was observed in this study. Flowering plants where nectar is not accessible may have a detrimental effect on population levels of pest antagonists because these plants attract these insects without offering floral resources (Barth, 1991). In this study, parasitoids consumed the floral nectar of alyssum and buckwheat as well as the extra-floral nectar of faba bean, suggesting that these plant species could be used as possible nectar sources.

Regarding the attractiveness experiments, the differential effect of food resources on this variable was observed. The attractiveness of food resources and the mechanisms to recognise the food by organisms is the result of coevolution process mediated by selection process of mutations. Organisms with favourable mutations have a high probability to reproduce and conserve these characteristics. Insects achieve recognition mechanisms when find benefits in other organisms, and some plants coevolved cues to attract determined insects which have a vital role as pest antagonists or pollinators. Between organisms there are different types of relationships such as antagonism, mutualism, and comensalism among others (Solomon *et al.*, 2002). Results of this study showed that: the selected flowering plants have the potential to produce cues that parasitoids of *D. rapae* could easily recognise, *D. rapae* parasitoid can consume the nectar and this parasitoid do not affect negatively the flowering plant, showing an ecological relation of comensalism. For *E. tricolor,* the attractiveness of these sources was low however this parasitoid may also benefit from the nectar of flowering plants, demonstrating also an ecological relation of comensalism. It suggests that the farmer can sow these plants in high density to improve the parasitoid fitness and obtain adittional economical benefits.

In this research, there was evidence of the highest attractiveness of alyssum to *D. rapae*. Similar results were reported by Hogg *et al*., (2011), where alyssum significantly attracted more parasitoids compared with buckwheat. The highest attractiveness of alyssum to adult parasitic hymenoptera compared to other flowering plants has also been mentioned by other authors (Fiedler and Landis, 2007 and Sivinski *et al*., 2011).

Faba bean was not used for the subsequent experiments due to the following reasons, this species never had a higher attractiveness than the other flowering plants and the parasitoids had no access to the floral nectar. Additionally, during the preparation of materials for these experiments, in a first attempt, faba bean plants had a high incidence and damage severity by gray mold, powdery mildew, rust and thrips. Hence, these plants were sowed one time more and different strategies of integrated pest management were used to get plants free of pests, meaning that this plant is not suitable as flowering plant because the high agronomical management is no desirable on the use of flowering plants.

Some plants are able to produce cues to attract beneficial insects. Brassica plants produce herbivore-induced plant volatiles (Reed *et al*., 1995; Bradburne and Mithen, 2000; Hopkins *et al*., 2009; Najar-Rodriguez, 2015), attracting *D. rapae* parasitoids. These volatiles are called synomons - hormones from which both the producer and receiver benefit -. Other parasitoids of Braconidae family such as *Aphidius rhopalosiphi* and *Aphidius ervi* and *Glypta haesitator* have also been reported as being capable of recognize these volatiles blends (Wickremasinghe and van Emden, 1992; Guerrieri *et al*., 1993; Du *et al*., 1998; Dalen *et al*., 2015). Therefore, in this study, we wanted to know if these volatiles had more attractiveness than those from flowering plants and we observed for *D. rapae* experiment that any flowering plant species had a higher attractiveness that the herbivore-plant complex.

For *E. tricolor,* the non-competent searching behaviour was evident for the following reasons: The percentage of no-response was higher than that in *D. rapae* parasitoids, *E. tricolor* spent a maximum of two hours choosing a food source (n=200) in a petri-dish of 7 cm of diameter whereas *D. rapae* did a chose in the first 5 minutes (n=200). Additionally, for *E. tricolor* was utilised smaller Petri-dishes than for *D. rapae* because in preliminary experiments *E. tricolor* spent more of 2 hours for choosing an option in a 14 cm Petri-dish (n=200). In *E. formosa* - a very close species of *E. tricolor* - it was described that the host searching is randomised and when the parasitoid finds the herbivore it remains in the area, demonstrating that the recognition of visual and olfactory cues is low and this in tactile and gustatory cues is high. Indeed, this parasitoid spends more time walking and touching the surface in which walk with the antennas than flying and when the parasitoid walk touch with the antenas the surface of the ground (Hoddle *et al.,* 1998). Taking into account the no competent nectar searching by this parasitoid, the flowering plants should be sowed uniformly in the farm and not in strips, to increase the probability of encounter of the nectar while host finding. Considering, the no competent searching for the host, banker plants should be introduced close to the hot spots -initial points of infestation-, facilitating the host finding by parasitoids. However, the uniform sowing flowering plants and the introduction of banker plants close to the hot spots can be expensive. Hence, the policulture could be an idea because the farmer might sell alyssum seeds for sowing and buckwheat seeds for flour and it guarantee also the appropriate agronomical management of these plants which will be reflected in the quality of the nectar.

Regarding survival experiments, it was observed that the values of this variable significantly differed among treatments. It confirms the importance of selecting food sources because the different types and proportions of sugars in foods have an effect on the parasitoid survival (Wäckers, 2001).

Honey was used as a positive control, confirming that access to a source of carbohydrates improved the longevity of both parasitoids compared to the control and the water treatment. These results are demonstrated by other works with honey-fed parasitoids; for example, *Copidosoma koehleri* (Baggen and Gurr, 1998), *Cotesia rubecula* (Siekmann *et al*., 2001), *Meteorus comunis* (Costamagna and Landis, 2004), *Microctonus hyperodae* (Vattala *et al*., 2006), *Gonatocerus ashmeadi* (Irvin *et al*., 2007) and *D. rapae* (Jamont *et al*, 2013) among others. Honey-fed wasps of *E. tricolor* lived longer compared with those fed on nectar, because undiluted honey was used, which has a higher concentration of sugars, at around 80% (Buba *et al*., 2013) than nectar between 10-40% (Barth, 1991). The positive correlation between the sugar levels with the longevity of parasitoids has been verified by Wäckers (2001) with *Cotesia glomerata*, Siekmann *et al*., 2001 with *Cotesia rubeula* and Azzouz *et al*., (2004) with *A. ervi.*

Honey-fed *E. tricolor* females lived 70.65 days and males for 31.85 days at 18.52 ± 0.17 °C, which is high compared to a study by Williams (1995), who recorded an average of 16.9 days for honey-fed *E. tricolor* females and 13.8 for males at 25 °C, but this author did not mention the concentration of honey. The results of this study were consisted with the findings of Vet and Van Lenteren, (1981), who observed that on *E. formosa* wasps with pure honey at 15.6 °C lived 99.3 days. This suggests that for mass rearing of *Encarsia* sp. pure honey could be used without water at temperatures of 18 °C to achieve a high level of survival of parasitoids.

Considering the flowering plant treatments, nectar-fed parasitoids had higher results than those in control or water treatments, suggesting that flowering plants could be sown in the field to offer an alternative source of food during the host finding or to improve the realized fecundity of the target parasitoids. Furthermore, there were significant differences between flowering plants. Buckwheat-fed parasitoids lived longer than those fed on alyssum, confirming the results of studies with *Aphidius ervi* (Araj *et al*., 2006; Araj *et al*., 2008), *Gonotacerus ashmeadi* (Irvin *et al*., 2007) and *Microctonus hyperodae* (Vattala *et al*., 2006). According to Vattala *et al*, (2006), it is attributable to the sucrose: hexose ratio, being higher in buckwheat (1.5) compared to alyssum (0.4).

With respect to honeydew treatments, for *D. rapae,* no statistical differences were observed between the host honeydew and the non-host honeydew treatments. These results were consistent with the results of Newton *et al*., (2009ab), who found that glucosinolates have no effect on the *D. rapae* fitness. For *E. tricolor*, the differences were very low (1.6 days for females and 2 days for males), maybe, glucosinolates of the host honeydew have a small effect on the parasitoid survival.

Regarding the suitability of the honeydew as food source, despite, it was observed statistically significant differences in *D. rapae* respect to the control, the arithmetic differences were low (only, 0.8 days for females and 0.4 days for males), reflecting that honeydew is not a source with significant effect on the survival of this parasitoid. These results were similar to those obtained by Jamont *et al*., 2013. By contrast, honeydew-fed *E. tricolor* parasitoids had a high difference than those in the control (4.55 days for females and 1.9 days for males). The differential effect of honeydew on the survival of *D. rapae* and *E. tricolor* could be related to the nutritional requirements, which are less in *E. tricolor*.

Honeydew can have a higher, similar or lower effect than honey, nectar and/or common sugar on the survival of parasitoids. However, on the majority of the cases the result is lower (Wäckers *et al*., 2008), being consistent with our findings. Buckwheat and alyssum nectar-fed *D. rapae* lived longer than those fed on honeydew, which is possible because insects in the suborders sternorrhyncha and auchenorryncha - i.e. aphids and whiteflies, respectively - produce oligosaccharides as a defence mechanism against parasitoids (Wäckers, 2000). These types of sugars include maltose, erlose and trehalose, having a low nutritional value and rapidly crystallise in the parasitoid's gut. Low concentrations of these sugars can significantly diminish the suitability of the total sugar source (Wäckers, 2001; Lee *et al*., 2004). Studies with the parasitoids *Cotesia marginiventris, Campoletis sonorensis, Microplitis rufiventris* have reported a higher positive effect of sucrose on the parasitoid survival compared with honeydew (Faria *et al*., 2008).

Although it was not the objective of this study, it was observed that parasitoids also could survive for some days in the control and the water treatment. This result suggests that these insects have reserves of food consumed during the immature stages, permitting the initial searching for an appropriate habitat with food and herbivores.

For *D. rapae*, flowering plants significantly increased the number of mummies in both cage and in the field experiments. The *D. rapae* parasitoids are pro-ovigenic, meaning that the ovigenesis is during the immature state (Kant *et al*., 2013, Wajnberg *et al*., 1994). Therefore, they need sources of energy - i.e. sugars - for metabolism, mating, host location and egg laying. These sources of energy could be nectar and/or honeydew. The treatment of flowering plants possibly had a better performance because nectar had a better quality than honeydew.

For *E. tricolor*, flowering plants had no effect on the number of mummies in cages, although differences were observed in the field experiment. It may be due to the fact that the parasitoids were released in the infested plants and the parasitoid remains in the area when find the host, because haemolymph satisfy all the nutritional requirements. Haemolymph provides sources of water, inorganic salts, aminoacids, proteins, carbohydrates and lipids (Nation, 2007; Jervis *et al*., 2008), while nectar is the only source of water, sugars and small quantities of inorganic salts and aminoacids (Nicolson and Nepi, 2007; Stahl *et al*., 2012).

Proteins of haemolymph are important for *E. tricolor* because this parasitoid is synovigenic, meaning that ovigenesis is during the adult stage (Wajnberg *et al*., 1994 and William, 1995). Therefore, the consumption of nectar in the presence of the host would be an unnecessary waste of energy. Moreover, flowers do not provide a suitable source of protein because the species of the family aphiidinae as *E. tricolor* generally do not have mouthpart specialisations to consume pollen (Jervis, 1998). In the field, flowering plants had a positive effect on the number of mummies. It can be explained by the fact that nectar was the first food that they could consume before the host finding and nectar can improve the *E. tricolor* survival during the host finding.

The female percentage is an important variable for biological control programs because a higher number of females translates into a larger number of oviposition events and consequently a better control of the pest. In this study, the percentage of females was high for both parasitoids and there was no effect of flowering plant treatment. Possibly because other aspects of the biology of these parasitoids had a stronger influence than the nutritional need/supply; for instance, unmated *E. tricolor* females produce females (thelytoky) and the males are developed for hyperparasitism, meaning that the first generation of parasitoids always will be females. Unmated *D. rapae* females produce males (arrhenotoky) and females are produced after mating (Kant *et al*., 2013).

Nonetheless, there are studies observing that food resources increase the female sex-ratio, as reported by Berndt and Wratten (2005) with *Dolichogenidea tasmanica*. According to a review of this author, flowering plants may have an influence in terms of increasing the number of mating events and hence modify the sex ratio because these plant species can attract females and males to a common place.

For *D. rapae,* plots with flowering plants had on average 20% more hyperparasitoids compared to the control, despite no statistically significant differences, as it has been demonstrated by Araj *et al*., (2009), who observed that flowering plants have an effect on the populations of the fourth trophic level (hyperparasitoids).

Finally, it can be concluded from the conducted experiments that by providing nectar from flowering plants, the target parasitoids could improve the fitness. However, this approach had a better effect on the percentage of *D. rapae* mummies (68 ± 11.27) than for *E. tricolor* (22 ± 4.3). It can be explained by the differences in food requirements in each parasitoid. For *D. rapae*, nectar had a better quality than honeydew and for *E. tricolor*, nectar is only important in absence of the host because the host food (haemolymph) has a better effect than nectar. Hence, further research is necessary to elucidate how flowering plants and *D. rapae* could improve the integrated aphid management in order to reduce the use of agrochemicals. In view of the suitability of flowering plants to improve the performance of *E. tricolor* to manage *A. proletella* populations, the efficiency of this technique is very low.

However, it is important for farmers evaluating whether this technique is suitable for their particular cropping system. For instance, it is easier to implement the use of flowering plants/banker plant system in organic crops or with a satisfactory Integrated Pest Management system because farmers use agrochemicals of low toxicity, which could be compatible with the parasitoids. The effect will be higher if the flowering plants are sowed in high densities taking into account that they could have other uses. For instance, buckwheat can be used as human food, animal food or to increase the production of honey on beehives. It can also be used for weed management when it is used as mulch whereas alyssum can also be used for decoration as well as feeding bees.

4. Effect of habitat management techniques on the performance of *Diaeretiella rapae* and *Encarsia tricolor*

Abstract

The fast control of hot spots is a key indicator to measure efficacy of strategies of pest management. Therefore, the main goals were to know whether *Diaeretiella rapae* and *Encarsia tricolor* banker plants can control in the suitable time the *Brevicoryne brassicae* and *Aleyrodes proletella* hot spots, respectively, to determine whether flowering plants could improve the efficiency of banker plants, and to verify whether parasitoids move to infested plants from the banker plants in which they emerge. The opportune control was defined as a percentage of mummies higher than 70% related with the first five days after beginning of the experiments. Flowering and banker plants are techniques of habitat management for improving the performance of pest antagonists. Flowering plants produce volatiles that may attract natural enemies of pests and offer alternative food to parasitoids. The results showed that the percentage of mummies in *D. rapae* and *E. tricolor* banker plant treatments were 55.5% and 39.9% respectively. The percentage of mummies was estimated as the number of mummies related to the oviposition events during the five days of the experiment divided by the initial number of herbivores. Rather, this was observed that flowering plants did not improve the efficiency of banker plants and banker plants reduce the pest individuals but they were not enough to reach an efficient control. The role of banker and flowering plants was discussed as techniques for optimising the management of the target pests depending on the appropriate use.

Keywords: *Diaeretiella rapae; Encarsia tricolor; Brevycorine brassicae; Aleyrodes proletella;* Banker plants; Flowering plants

4.1. Introduction

The quick control of hot spots by any pest management strategy is a key indicator of its efficiency. The target pests of this study can disperse easily by the wind and increase its populations very rapidly. By contrast, the establishment of some pest antagonists in a place is very difficult and they need constantly reintroduction due to many biotic (Finke and Demo 2003; Freuler *et al*., 2003; Martinou *et al*., 2009; Horowitz *et al*., 2011), abiotic and management factors that affect them as well as factors related with the biology of these organisms (Martinou *et al*., 2009). Hence, if the parasitoid is not able to regulate pest populations of the initial hot spots and the environmental conditions favor the development of the pest, the population level of the pest can grow very fast and after any strategy of management will be able to regulate the target pests.

The target systems of this study were: i) *D. rapae* - *B. brassicae* and ii) *E. tricolor* - *A. proletella*. *B. brassicae* and *A. proletella* are pests of economic importance in many crops of the *Brassica* genus known as cruciferous vegetables. *Diaeretiella rapae* and *Encarsia tricolor* are parasitoids of the mentioned pests (Williams 1995; Kant, 2012). These parasitoids survive through all seasons and can reproduce in diverse ecozones constituting the Earth's, demonstrating their high biological efficiency in terms of survival (Carver and Stary, 1974; Evans 2002; Hernandez *et al*., 2003). However, the agronomic efficiency or parasitism percentage of the pests by these parasitoids is not enough to protect the crop fields. Hence, it was evaluated flowering and banker plants to improve the efficiency of these parasitoids.

Flowering plants can increase the potential (number of eggs) and realised (number of mummies) fecundity of parasitoids either directly by providing appropriate nutrients for the production and/or maturation of eggs (Leatemia *et al.*, 1995; Rivero and Casas, 1999; Tylianakis *et al*., 2004; Berndt and Wratten, 2005; Jervis *et al*., 2008; Varennes *et al*., 2015) or indirectly by attracting parasitoids by means of volatiles to increase the richness, abundance and survival of parasitoids (Landis *et al*., 2000; Tylianakis *et al*., 2004; Berndt and Wratten, 2005; Fiedler and Landis, 2007; Jervis *et al*., 2008; Wäckers *et al*., 2008; Zamora *et al*., 2011).

Banker plants are systems integrated by a plant and herbivores with their antagonists that the farmer introduces in the field to release and/or to establish biological control agents for pest suppression (Frank, 2010; Huang *et al*., 2012). Banker plants in some cases also can attract natural occurring pest antagonists, given that some plant species can to produce volatiles that attract these organisms when the pest attacks the crop (Du *et al*., 1998; Orre Gordon *et al*., 2003). For example, *D. rapae* can recognise the presence of these volatiles from the cabbage crop fields (Hopkins *et al*., 2009). Release of parasitoids with banker plants is easier, more efficient and cheap than multiple releases by hand with paper bags or plastic cards (Stacey, 1977; Goolsby and Ciomperlik, 1999; Conte *et al*., 2000; Frank, 2010). Herbivores of the banker plants offer honeydew and or hemolymph as a food source; mummies in banker plants are mostly located on the underside of the leaf where they are less susceptible to rain, wind, high temperature and sprayed pesticides or other factors. As a result of this, emergence percentage and the survival percentage of natural enemies of pest are higher in banker plants than in releases by hand (Pickett *et al*., 2004).

The exposition of the infested plants to the banker plants was five days because in *D. rapae* is reported that in the first five days after the emergence the female parasitoid lay the highest quantity of eggs (Qayyum, 2000) and for *E. tricolor* in the day third and fourth the female lay the highest quantity of eggs (Williams, 1995). Rather, in five days the growing of the pest can be significantly high, taking into account first that every *B. brassicae* female can lay around 1.6 eggs/day (Ulusoy and Olmez, 2006) and *A. proletella* female can lay around 5.7 eggs/ day and second that dispersion of these pests is by the wind (Musa, 2010). The infestation level of the pest was determined, taking into account that the use of pest antagonists could be efficient in the management of pest when the populations of the pest are not so high (Kuo-Sell, 1987; Frank, 2010).

Pest infestation is not homogenous (Van Helden, 2010) hence although banker plants can be placed from the crop establishment, it is better to place these plants at the beginning of hot spots of pest infestation based on a careful monitoring. Placing the banker plants near initial points of pest infestation also reduces the searching time of the parasitoid, permitting the optimisation of the management of energy of these organisms (Sirot and Bernstein, 1995) and reducing costs considering the number of banker plants utilised per hectare. To simulate the hot spots, in this study were infested broccoli plants with the target pests.

Banker plants can be produced in open rearing systems but according to Pickett *et al*., (2004) and Goolsby and Ciomperlik (1999), it is more efficient under controlled conditions because pest antagonists are protected from abiotic (rain, wind, etc.), biotic (hyperparasitoids or predators) (Collier and Steenwyk, 2004; Martinou *et al*., 2009; Finke and Demo 2003; Freuler *et al*., 2003) and management factors (chemicals). It is also possible to check the quality of the banker plant transplant before the introduction in the field and finally, the banker plant transplants can be ready for the beginning of the crop season. By contrast, open rearing systems require time for the establishment in the field, and during this period the population level of the pest may develop very fast. Therefore, banker plants produced in cages were used considering the results presented in chapter 2 of this thesis about approaches to improve the quality of banker transplants. These results suggested that broccoli plants with a special treatment of pruning and produced in large cage were most convenient to obtain *D. rapae* and *E. tricolor* banker plant systems of high quality. These approaches allow obtaining hosts of large size, parasitoids that live a long time, high production of mummies, plants without powdery mildew and satisfactory adaptation after transplanting.

Successful studies with banker plants have been documented with alternative hosts (Pineda and Marcos-García, 2008; Xiao *et al*., 2012) as well as with non-alternative hosts (Stacey, 1977; Freuler *et al*., 2003). Stacey (1977) evaluated the effect of banker plants on *Trialeurodes vaporariorum* in a crop of tomato *Solanum lycopersicum* L. The banker plant was produced with the same pest of the crop (non-alternative host) and this author observed a high parasitism percentage and a low presence of sooty mold –fungi that grow in honeydew of whiteflies-. A simple introduction was required, and the production of banker plants was easy because the same vegetable and pest species of the crop were used. Therefore, for this study, the non-alternative host system to produce the banker plants was used. According to this author this method was created by growers that wanted to produce their own rearing systems of parasitoids because they did not find these in the market. Moreover, non-alternative host was chosen for *D. rapae* banker plants because Pike *et al*., (1999) reported 60 host (herbivore species) for *D. rapae* recognized worldwide but on previous experiments of this study, this parasitoid was not able to lay eggs on *Aphis fabae, Sitobion avenae, Macrosiphum euphorbiae* and *Rhophalosiphum maidis* and only lay eggs on cabbage aphids like *B. brassicae* and *Myzus persicae*. These results are supported by Freuler *et al*., (2003), who reported that in central Europe, *D. rapae* lays eggs in cabbage aphids and only occasionally in others species of aphids. The range of host (herbivore species) parasitized by *D. rapae* can differ according to the geographic location. The genetic variability and flow of genes may influence the performance of parasitoids to utilise the herbivores (Baker *et al*., 2003) and hence the subpopulations in *D. rapae* are determined by the herbivore use (Antolin *et al*., 2006).

Experiments with both parasitoids were conducted independently because some studies have used banker plants with two or more pest antagonists on the same plant but the results were not satisfactory (Bennison, 1992).

In this chapter, the main aims were: 1) to know whether banker plants can give an opportune control at the beginning of the hot spots of the pest, 2) verify whether the flowering plants can improve the efficiency of banker plants and 3) observe whether parasitoids move to infested plants from the banker plants in which they emerge, offering food and host. The opportune control was defined as a percentage of mummies higher than 70% in the first five days after beginning of the experiments. The hypothesis were: 1) when the banker plants are introduced to the target system in the field is possible to get an opportune control (reduce in a 70% the herbivore population) in the hot spots of pest infestation, 2) flowering plants can improve the efficiency of banker plants and 3) parasitoids that emerged in the banker plant move to infested plants.

4.2. Materials and methods

4.2.1. Plants and insects

To produce banker plants, twelve-week-old broccoli plants were infested with 20 herbivores. Infested plants were exposed to five females during five days, and after 10 and 20 days banker plants were used in the experiments for *D. rapae* and *E. tricolor,* respectively. Infested plants also were used to simulate initial points of infestation. Infested plants simulating hot spots or initial points of infestation had 100 individuals of *B. brassicae* and 100 larvae of 3rd instar of *A. proletella* for the experiments with *D. rapae* and *E. tricolor* respectively. Roots, old leaves and leaves higher than 15 cm in length were pruned to avoid contamination of non-target pests, improve the vigour and duration of infested and banker plants taking into account the outcomes of the chapter 2. To produce infested and banker plants, mesh cages measuring (51 x 56 x 76) cm and 12 cm diameter plastic pots were used. Seeds of flowering plants, i.e. alyssum variety snows crystals *(Lobularia maritima)* Brassicaceae, buckwheat variety Bamby *(Fagopyrum esculentum)* Polygonaceae were sown in plots of 12 cm diameter and maintained for twelve weeks in the nursery to get between 20-30 flowers per plant. The flowering plants were chosen considering the results from the third chapter.

4.2.2. Description of the experiment

A completely randomised design was conducted with three treatments for both parasitoids. The treatments were banker plants (Fig. 4.1), banker + flowering plants (Fig. 4.2) and the control (Fig. 4.3). The experiments for *D. rapae* and *E. tricolor* were conducted in May 2016 and July 2015, respectively. For *D. rapae*, the experiment had 3 replicates in the space and 3 repetitions in the time to get 9 data sets per treatment. The plots or experimental units measured (0.9 x 0.9 x 0.9) m (Fig. 4.4). For *E. tricolor*, the experiment had 6 replicates and 3 repetitions to get 18 data sets per treatment. The plots measured (4 x 3) m and the distance between plots was 6 m (Fig. 4.5). Every banker plant had 50 mummies and flowering plants contained between 20-30 flowers. Every day, the quality of banker, flowering and infested plants was checked to guarantee the optimal state of the materials in the field. In both experiments, the experimental units in all the treatments had two infested Broccoli plants.

For *E. tricolor,* the plants were cover with black mesh to avoid damage by birds or strong winds and an electric fence was used to avoid damage by rabbits. Additionally, plots were covered by a poly tunnel of 2 m hight to reduce the negative effect from wind and rain. Pesticides were not applied during the experiment. The *D. rapae* experiment took place in a greenhouse due to logistic constrains. In both experiments, the infested plants were exposed to parasitoids during five days because according to Quayyum, (2000) the 88% of oviposition occurred in the first five days of adult life in *D. rapae* and the highest number of eggs in *E. tricolor* were laid in the fourth and fifth days of adult life in *E. tricolor* (Williams, 1995). After that time, the number of pest individuals of the herbivores was counted, and experimental plants with herbivores were stored in cages. The number of mummies was counted 8 and 21 days after for the *D. rapae* and *E. tricolor* experiments, respectively because in these times it is possible to see the majority of mummies related to oviposition events during the five days in which the herbivores were exposed to the parasitoids.

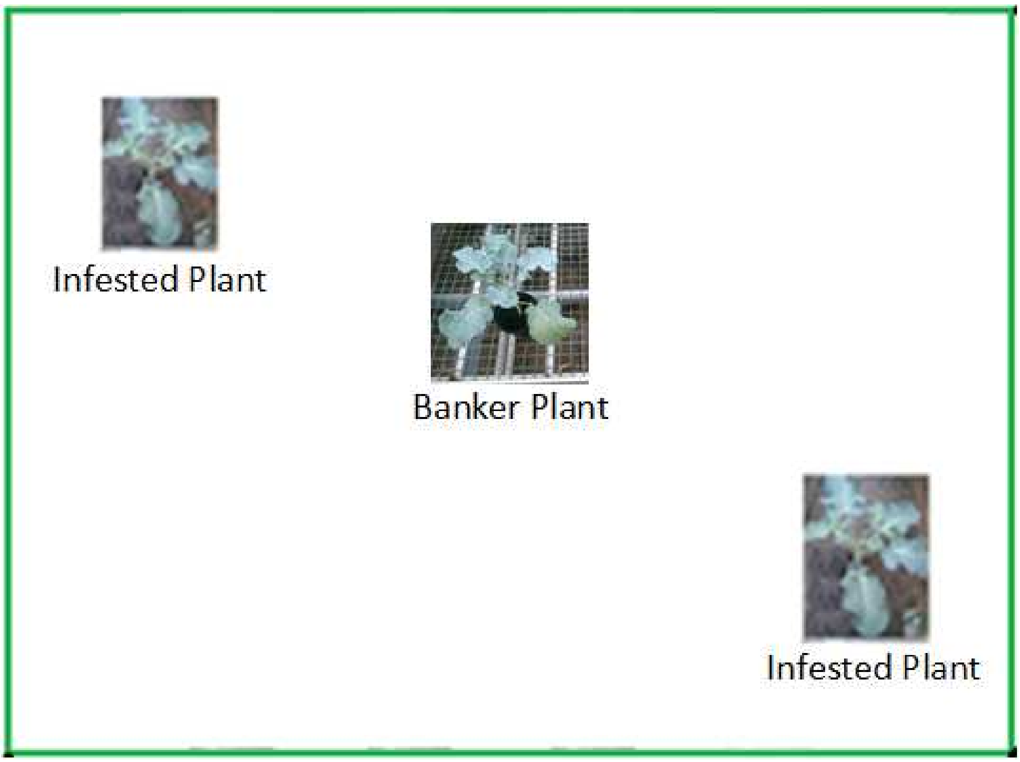

Fig. 4.1. Set-up of the banker plant treatment

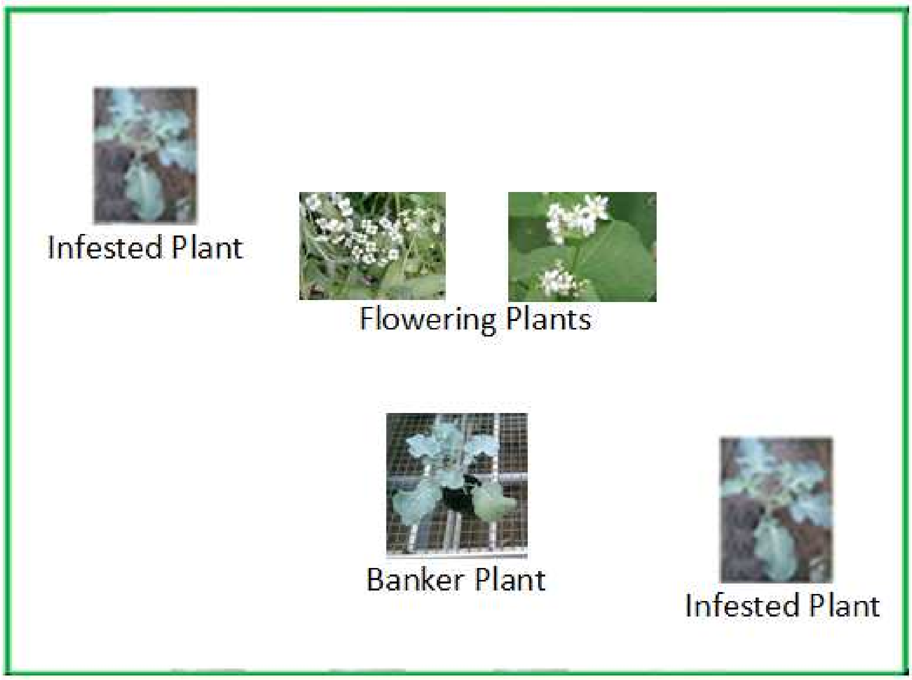

Fig. 4.2. Set-up of banker + flowering plant treatment

Fig. 4.3. Control treatment.

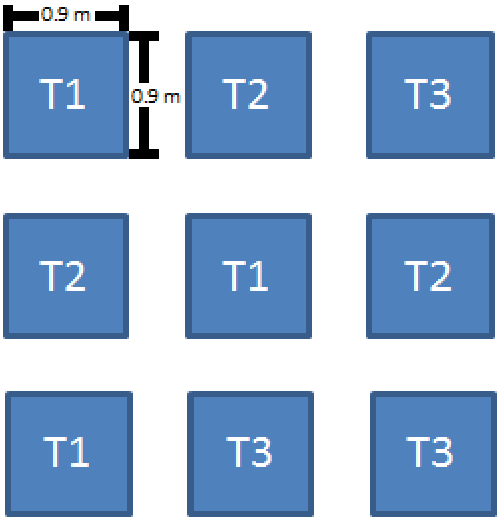

Fig. 4.4. Set-up of the *D. rapae* experiment. Completely Randomised Design with three replicates and three treatments T1: banker plants, T2: banker+flowering plant treatment and T3: control treatment.

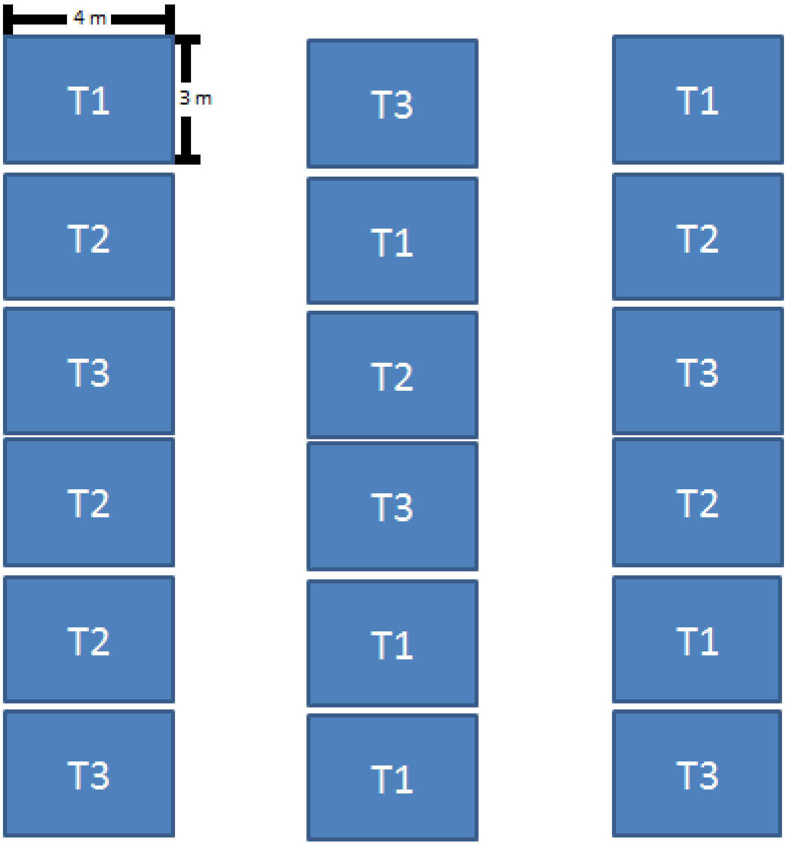

Fig. 4.5. Set-up of the *E. tricolor* experiment. Completely Randomised Design with six replicates and three treatments T1: banker plants, T2: banker+flowering plant treatment and T3: control treatment. This experiment was repeated three times.

4.2.3. Statistical analysis

To analyse the data, the Generalized Linear Model GLM was used if the data were normally distributed, whereas the Kruskal-Wallis test was used when the data had no normality. The treatment means were compared using a Tukey's test (HSD). When the data had no homogeneity of variances, Tamhane's T2 was used. The level of significance was $p<0.05$ for all tests. All analyses were carried with SPSS 23 IBM.

4.3. Results

4.3.1. Improving the performance of *D. rapae* by habitat management techniques

For the experiment with *D. rapae,* there were significant differences in the number of mummies (F=103.195, *df*=2, $p<0.001$) (Fig. 4.6) and number of aphids (X=18.212, *df*=2, $p<0.001$) (Fig. 4.7). According to the Tamhane-T2 test, for these variables, the difference between banker plants treatment and banker + flowering plants were no significant, but both treatments differed from the control treatment. The number of mummies was 55.5 ± 3.91 in the treatment of banker plants, 64 ± 5.26 in banker + flowering plants. The number of aphids was 80 ± 3.46 in the treatment of banker plants, 68.4 ± 5.54 in banker + flowering plants and 146.4 ± 15.21 in the control. The banker plant treatment had a 45.35% fewer individuals of the pest compared to the control.

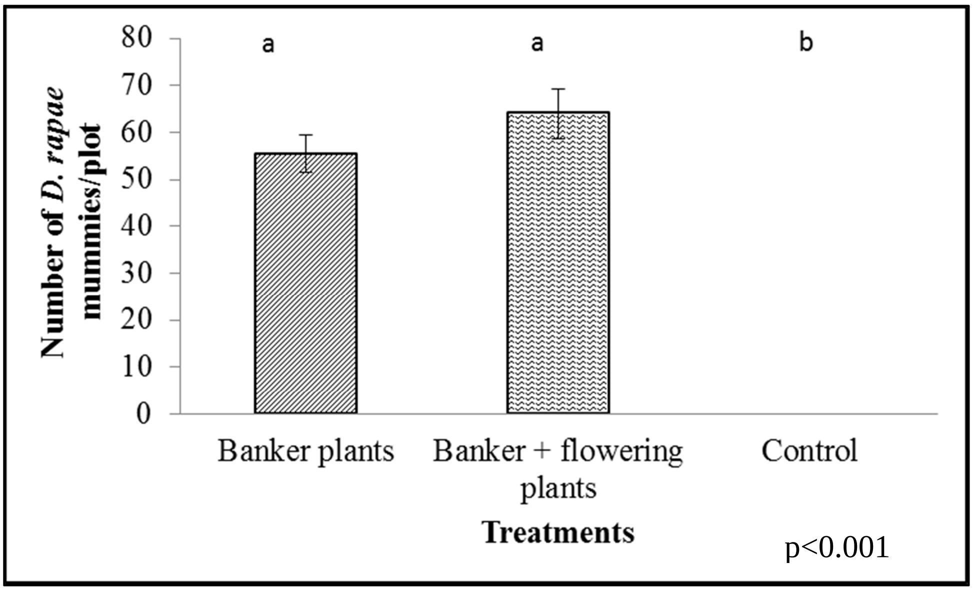

Fig. 4.6. The effect of habitat management techniques on the *D. rapae* number of mummies per plot. Mean±SE are presented. Different letters indicate significant differences at 5% level according to theTamahne's Test n=18.

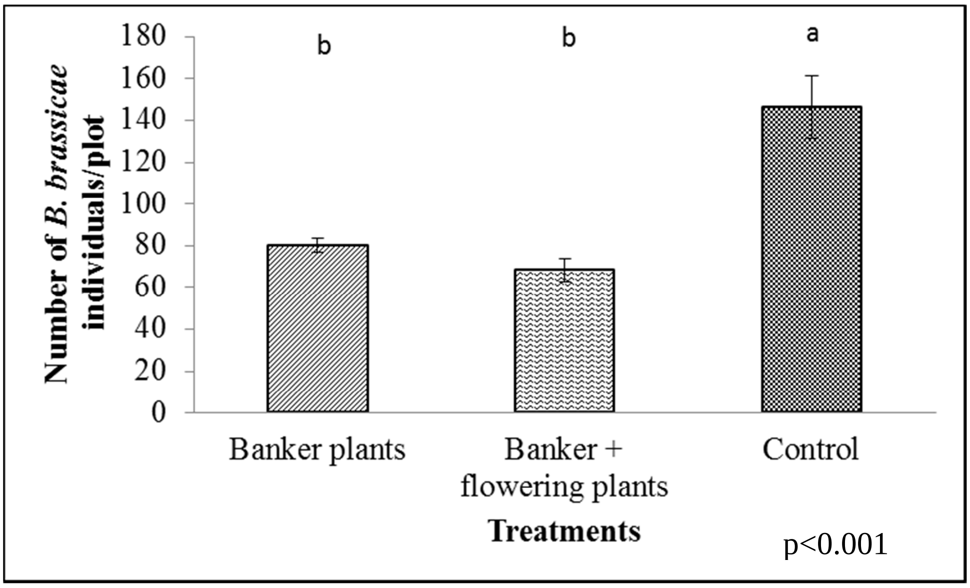

Fig. 4.7. The effect of habitat management techniques on the number of cabbage aphids per plot. Mean±SE are presented. Different letters indicate significant differences at 5% level according to the Tamahne's Test n=18.

4.3.2. Improving the performance of *E. tricolor* by habitat management techniques

The number of mummies is an indicator of the efficiency of parasitoids for the management of pests. For the experiment of *E. tricolor*, there were significant differences in the number of mummies (F=44.259, *df*=2, p=0.002) (Fig. 4.8). According to the Tamhane-T2 test, there were differences between the control and the other treatments and there were no differences between banker and banker + flowering plants. The *E. tricolor* banker plants contributed in the reduction of the hot spots of *A. proletella* infestation by 39.9%. The number of *E. tricolor* mummies was 37.67 ± 2.38 in the treatment of banker plants, 39.94 ± 3.03 in banker + flowering plants and 13.81 ± 1.49 in the control. Although, it was not the objective of this study, it is important to mention that banker and infested plants attract other insects such as Hoverflies, lady beetles, lacewings, wasps and spiders (Fig. 4.9).

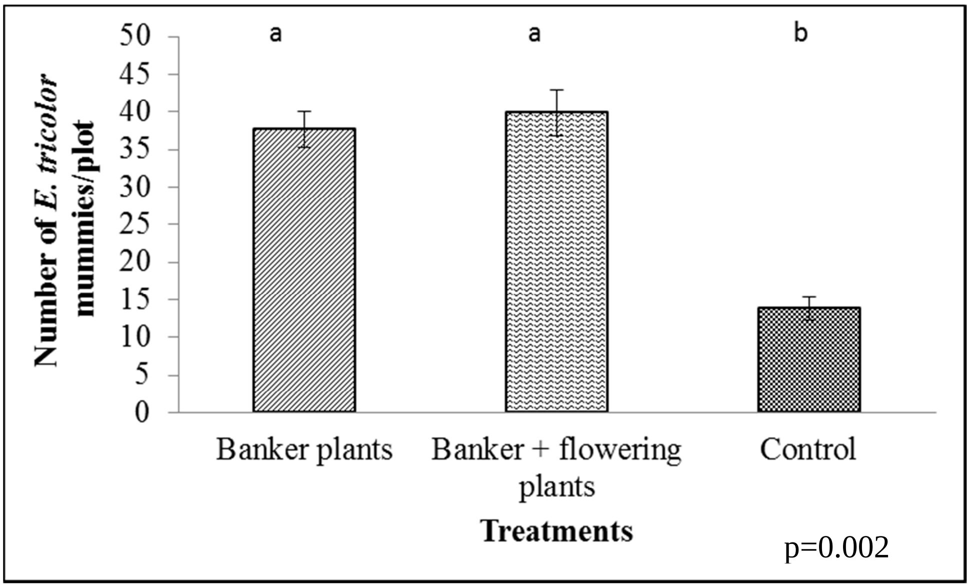

Fig. 4.8. The effect of habitat management techniques on the number of *E. tricolor* mummies per plot. Mean±SE are presented. Plot box with different letters indicate significant differences at 5% level according to the Tamahne's Test n=9.

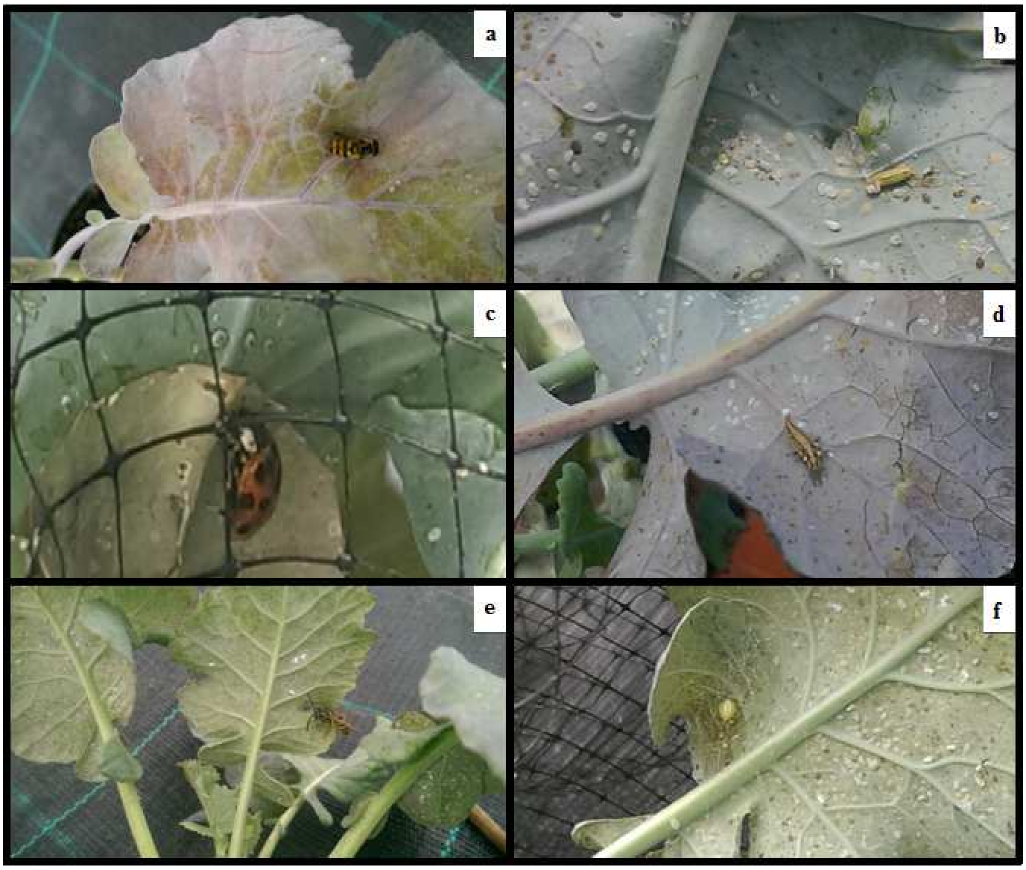

Fig. 4.9. Other insects observed in the *E. tricolor* experiment: a) Hoverfly, b) Larvae of hoverfly, c) Lady beetle, d) Larvae of lacewings, e) Wasp and f) Spider

4.4. Discussion

Aphidiidae and Aphelinidae parasitoids are the most widely used biological control agents against aphids and whiteflies (Carver and Stary, 1974; Bennison, 1992; Williams, 1995; Blumel and Hausdorf, 1996; Sampaio *et al*., 2001; Evans, 2002). This study focused on *D. rapae* and *E. tricolor*, the only parasitoids of *B. brassicae* and *A. proletella* cabbage pests, respectively. These parasitoids have a wide host range, but the highest preference is related to cabbage aphids and whiteflies (Williams, 1995; Kant, 2012).

To improve the efficiency of these parasitoids in terms of opportune control of the hot spots, this work focused on the use of banker and flowering plants because one of the most important subjects in biological control concerns to the use of habitat management techniques to generate an environment that favour the development of pest antagonists in the agroecosystems.

This work did emphasis in the opportune control of the pest during the initial formation of hot spots. Many studies have demonstrated that when the pest populations are low, fewer pest antagonists are needed (Ridgeway and Vinson, 1976; Popov, 1987). Results from one research of Simmons *et al*., (1995) showed a satisfactory management of *B. tabaci* when the parasitoid *Eretmocerus* spp was released in the early season of the crop. Additionally, Hassanali *et al*., (2008) mentioned that the efficiency of any technique of integrate pest management depend on identifying the hot spots opportunely and make a suitable management when the population of the pest is in low levels because when the population level of the pests is in high level even with agrochemicals is difficult a suitable management.

Considering the topic of banker plants, Pickett *et al*., (2004) observed that banker plants produced under control conditions -transplants- is an efficient technique to spread parasitoids into fields for the pest management. The first researchers that used transplants produced under controlled conditions for the regulation of pest populations were Stacey (1977) and Goolsby and Ciomperlik, (1999). Stacey (1977), used host banker plants –plants with the same pest of the crop and the pest antagonist- at the beginning of the crop season to control glasshouse whitefly on tomatoes and the outcomes showed that the impact of the *Encarsia formosa* banker transplants was satisfactory. Goolsby and Ciomperlik (1999) utilised inoculated seedlings transplants to spreading the parasitoid *Eretmocerus hayati* against silverleaf whitefly *Bemisia argentifolii* and they found that this was a reliable technique for delivery of this pest antagonist. In this study, banker plants contributed to the reduction of the hot spots but this technique was not enough. The reduction for *B. brassicae* infestation was 55.5% and for *A. proletella* was 39.9%.

Regarding the subject of flowering plants, studies on conservational biological control have shown evidence that flowering plants could play a paramount role to produce attractive volatiles for parasitoids and offer food to natural enemies and hence sustain or increase the abundance, richness and life history of pest antagonists (Frank, 2010; Kopta *et al*., 2012; Wong, 2012). Farmers have long observed that agroecosystems with a high diversity of flowering plants in the adjacent fields are related with high richness and abundance of pest antagonists (Personal observation). In this chapter, the efficiency of flowering plants was investigated with respect to its suitability to improve the effect of banker plants to control the target pests.

The outcomes showed that flowering plants did not improve the efficiency of banker plants. Perhaps, it is due to first the biology of the natural enemy, and second to the distance between the banker plants and the flowering plants. Parasitoid *E. tricolor* is synovigenic i.e. only produces eggs in the adult stage. Therefore, this parasitoid need sources of protein for production of eggs and carbohydrates as sources of energy. These nutrients can be found in the haemolymph of the host, including water, inorganic salts, amino acids, proteins, carbohydrates and lipids (Nation, 2007; Jervis *et al*., 2008). Consequently, the parasitoid does not need alternative food sources because the banker plants was placed close to infested plants with the pest and the haemolymph of the pest has better nutritional content that nectar. Haemolymph is a source of protein and carbohydrates while nectar is only source of carbohydrates. These results are in agreement with those obtained by Vet and Van Lenteren (1981) and Hoddle *et al*., (1998), who observed that in *E. formosa*, the searching of the herbivore is randomised, and when the parasitoid finds the herbivore it remains close this which provides haemolymph. For *D. rapae*, the high number of parasitoids in the banker plants could mask the effect of flowering plants. Therefore, to ensure the functionality of flowering plants for the next studies, these plants should be placed in high density, randomised in the crop far away from the banker plants. In a randomised spatial arrangement natural occurring parasitoids, parasitoids of banker plants moved by the wind or parasitoids that go out from banker plants due to high interspecific competence will have nectar as alternatives food during the host searching.

Mobility of parasitoids from the banker plant to infested plants is an essential factor in the evaluation of banker plants. Banker plant treatment in this research had a better effect than the control, demonstrating that parasitoids of banker plants moved to infested plants. Perhaps, after emergence of parasitoids, those do not remain in the banker plant due to that the interspecific competence is increased when the host number is reduced, and hence parasitoids migrate looking for new hosts.

Additionally, for protection purposes, the plots in the field experiment were inside plastic tunnels. This is because some environmental conditions like wind or rain can dislodge the mummies from the leaves (Wong, 2012; Wong and Frank, 2012). The tunnels reduce the negative effect of these environmental factors but permit the movement of the parasitoids outside of them. In commercial systems on crops under open field conditions, these tunnels could be smaller.

Banker plants can also attract other natural enemies (Huang *et al.*, 2011). Despite, it was no the aim of this study, it was also observed that habitat management treatment (flowering and banker plants) had 2.6 times the presence of other pest antagonists as much as the control. These were hoverflies, lady beetles, lacewings, wasps and spiders.

In conclusion, this technique can be used as a component that contributes to the appropriate integrated pest management of cabbage aphids and whiteflies. Nevertheless, the efficiency of these approaches depends on the appropriate management. It is relevant, use banker plants of high quality, identify opportunely hot spots or initial point of pest infestation to place the banker plants close to the pest, to maintain these plants in the optimal conditions, and renovates these and if it is necessary. It is suggested for the next studies evaluate the effect of flowering plant distances from banker plants.

5. General discussion

Failures of biological control agents have been associated with factors, such as rearing systems of low quality (van Lenteren 1991, 1993, 2003), a lack of food for pest antagonists (Landis *et al*., 2000), low capacity of parasitoids for recognizing herbivore or food, and low efficiency of releasing methods (Collier and Van Steenwyk, 2004). To contribute to the solution of these gaps, this work focused on to evaluate techniques to improve the quality of rearing systems as well as to study strategies of habitat management to enhance the control of the target pests in the field. The examined strategies to improve the quality of rearing systems were host plant, plant architecture and cage size. The evaluated habitat management techniques were flowering and banker plants. Flowering plants produce volatiles, attracting naturally occurring parasitoids, minimising migration of released parasitoids (Orre Gordon *et al*., 2003; Bianchi and Wäckers, 2008; Pineda and Marcos García, 2008), and offering food to parasitoids. Banker plant is a release technique more efficient that multiple releases by hand with paper bags or cards produced in companies of biological control (Stacey, 1977; Goolsby and Ciomperlik, 1999; Conte *et al*., 2000; Frank, 2010).

This research deals with the koinobiont, solitary, endoparasitoids *Diaeretella rapae* and *Encarsia tricolor.* These insects are the only parasitoids of *B. brassicae* and *A. proletella*, respectively. They were chosen because they have a high capacity to survive in diverse ecozones constituting the Earth's, demonstrating their high plasticity in terms of survival (Evans, 2002; Hernandez *et al*., 2003). However, their agronomic efficiency or parasitism percentage in crops is not enough (Williams, 1995; Kant, 2012).

Regarding the first topic of this research about rearing quality, normally studies about biological control are carried out without considering aspects related to their rearing systems. It is crucial to evaluate techniques to obtain a high quality of rearing systems. The quality of those systems should be regarded to reduce the standard error of the experiments due to intraspecific differences of pest antagonist species and optimise the performance of these in researching programs. After getting rearing systems of high quality, it is relevant to evaluate techniques of habitat management i.e. flowering and banker plants, to improve the performance of parasitoids in the field.

In Chapter 2, it was confirmed the hypothesis that the host plant (cultivar groups) and the management of rearing systems have an influence on the vigour of the rearing plant, the fitness of the herbivores, and the performance of the parasitoids. The most suitable management technique was pruned broccoli plants in large cages compared to the other options. With this technique, the plants were smaller and with a higher percentage of dry matter, parasitoids lived longer, the herbivore size was larger, the number of mummies was higher, and the incidence of powdery mildew was less than in the control. Additionally, the adaptation after transplanting was satisfactory when the rearing plants were utilised as banker plants.

The obtaining large pest individuals is a relevant topic because it influences the quantity of haemolymph -the food of the larvae of the parasitoids-. The quantity of haemolymph has a positive relation with the development of the parasitoid larvae and the potential longevity of the adult parasitoids (Hardy *et al*., 1992; Cloutier *et al*., 2000; Jervis *et al*., 2008), as this has also been confirmed by Williams (1999) and Kant *et al*., (2012) respectively for *E. tricolor* and *D. rapae.* Although in other species, such as *Apanteles carpatus* or *Microplitis demolitor,* has been observed that herbivore size has no effect on the parasitoid fitness (Harvey *et al*., 2000). In this study, the host plant had an effect on the herbivore size which is consistent with other studies and justified due to the host plant resistance by antibiosis (Ellis *et al*., 1996, Frank, 2010). Also, the architecture and cage size influenced this variable because the management of the rearing plants has effect on the expression of the genetic potential of the plants. Considering, the number of mummies per plant, it is other relevant indicator of the quality of rearing plants, which are used as banker plants, given that a high quantity of mummies per plant reduce the number of banker plants used per ha/cycle of the crop field, frequency of renovation, as well as work and costs linked with these activities (Frank, 2010). In this study, the factor host plant and management influenced the number of mummies and this was observed also in the findings of other authors (Freuler *et al.,* 2003, Jandricic *et al.*, 2014).

The contamination in rearing systems by non-target pests reduced the life span of banker plants (Frank, 2010). In our case, the non-target pest was powdery mildew that according to the literature this fungus develops in conditions of high relative humidity. According to Reich, 1997, plants with root pruning need less quantity of water, and the transpiration is less. Therefore, it was possible to deduce that the relative humidity inside of the cage would be less compared to the control. In an additional experiment, it was measured the relative humidity inside of the experimental cages as a variable for giving a possible explanation for the absence of powdery mildew in pruned plants, and effectively the relative humidity was less (42.84 ± 8.41 in pruned plants vs. 50.81 ± 10.3 in the control). Pruned plants had 15.68% less of relative humidity as compared to the control. The values of this study are supported by Guzman-plazola *et al*., (2003), who conducted an experiment in a crop of tomato. They observed that relative humidity levels of 50-70% were favourable for developing of the powdery mildew (*Leveillula taurica*).Two months after releasing the parasitoids, pruned plants were still strong while the control plants were already dead, indicating that the pruning technique elongates the lifespan of the plants. It allows deducing that the satisfactory adaptation in the field was due to the fact that pruned plants were stronger, and this was confirmed with the experiment outcomes about effect of different architectures on some variables of the broccoli plant physiology. Pruned plants were smaller and they had a higher percentage of dry matter than the control.

Taking into account the outcomes of this research, pruning was also used to produce vigorous infested plants to simulate hot spots in the field in our experiments because normally plants sowed in pots are contaminated by powdery mildew or other non-target organisms, and they are weaker than plants sowed in the real conditions affecting the homogeneity of plots, increasing intraspecific differences between parasitoids, increasing the experimental error, and affecting the quality of the results in general.

Despite it was not measured, it is relevant to mention that other observed advantages of the pruning technique to produce banker plant are that the tissues are thicker and besides the broccoli plants never get the reproductive physiological stage. Broccoli plants that get the reproductive physiological stage reduce the quality as rearing plants. It is because the photoassimilates produced in the leaves go to the fruits and therefore the leaves are weakened and they fall, which are the base for the development of herbivore and parasitoid populations in rearing systems.

The results of cage size experiments showed that size of the herbivores, the number of *D. rapae* mummies, the number of *E. tricolor* mummies and the *E. tricolor* longevity was higher inside large rather than small cages. This is possible given that the quality of light is a factor linked to vigorous plants, and the strength of the plant has an important effect on the herbivore and parastoid fitness (Ballaré, 1994; Lambers *et al*., 2008). It is in agreement with the results of this study because plants inside larger cages had more light and a better growing.

The Chapter 3 dealt with flowering plants topic because recently in various reported cases this approach has been notably effective to optimise the performance of pest antagonists and hence this technique merits a careful study. In this way, several factors were monitored to determine which plants could be appropriate for *D. rapae* and *E. tricolor*, as explained below. The flowering plant attractiveness and nectar accessibility are the first aspects to know whether a flowering plant species is suitable as alternative food because the presence of flowering plants in agroecosystems is not enough to provide food for parasitoids. Wäckers, (2004), observed that the attractiveness of *Aegopodium podagraria*, *Origanum vulgare* to the parasitoids *Cotesia glomerata*, *Heterospilus prosoidis* and *Pimpla turionellae* was satisfactory, and the parasitoids also could consume the nectar of these plants. While the attractiveness of *Galium mollugo* and *Leucanthemum vulgare* to the mentioned parasitoids was also high but the parasitoids had no access to the nectar of these plants. In this study, the parasitoids had access to the nectar of alyssum and buckwheat but no the floral nectar of *V. faba*. Flowers of alyssum and buckwheat are small and open, permitting the easy access of these insects to the floral nectar. Flowers of *V. faba* are long and close hence they have coevolved with bees which are efficient in the pollination of this plant (Barth, 1991). In a no-choice experiment of Jamont *et al*, 2013 was observed that extra-floral nectar of *V. faba* stipules increases the longevity of *D. rapae*, but in our research, this species was discarded in the next experiments because: it was observed that *F. esculentum* and *L. maritima* flowers had a higher attractiveness than *V. fabae* stipules and during the preparation of materials *V. faba* required a high agronomical management, which is not desirable in the suitability of flowering plant species. Moreover, according to Kwok and Laird (2002), the production of extra-floral nectar is not constant, and this nectar attracts ants which protect aphids. Considering the pollen as a source of food, these insects did not consume this resource. According to Jervis, (1998) the families of these parasitoids have not mouthpart specialisations to consume this food.

The measurement of the response times of parasitoids to plants offering food allows having guidelines about how should be the distribution of flowering and banker plants in the field. For *D. rapae*, it was evident the ability to recognise quickly flowering plants and the host-plant complex representing the pest-infested cabbage plants, as it has been documented on other studies (Cole,1980; Hopkins *et al*., 2009). Most times, *D. rapae* showed a decision in the first five minutes. By contrast, for *E. tricolor* parasitoids, the response times ranged from thirty minutes to two hours, the Petri-dish were smaller than that for *D. rapae* because in preliminary experiments was observed that the times of response in petri-dishes of the size used for *D. rapae* were more of 2 hours. It means that *D. rapae* is a foraging insect with high ability to find resources while *E. tricolor* searchs the food resources randomly. According to Wäckers, (2004), larger parasitoids can recognise volatiles of flowering plants because the size allows them moving and locating nectar sources in the field which is in agreement with this study because *D. rapae* is larger than *E. tricolor*. For *E. formosa* -a very close species of *E. tricolor*-, the herbivore searching is randomised and when the parasitoid finds the herbivore, it remains in the area. The recognition of visual and olfactory cues is low and this in tactile and gustatory cues is high. This parasitoid spends more time walking and touching the surface in which walk with the antennas than flying (Hoddle *et al*., 1998; personal observations).

Therefore, the low capacity for recognising the food and herbivore-plant complex may help explain some reasons about why this parasitoid has a low percentage of mummies in the field experiments compared to *D. rapae*. Taking into account the no competent nectar searching by this parasitoid, the flowering plants should be sowed uniformly in the farm and not in strips, to increase the probability of encounter of the nectar while host finding. Considering, the no competent searching for the host plant complex, banker plants should be introduced close to the hot spots -initial points of infestation-, facilitating the host finding by parasitoids. However, to sow uniformly in the farm and no in strips flowering plants and introduce banker plants closed to the hot spots is expensive and the economical viability would be low. It also underscores that micro cosmos experiments can be an important tool to have guidelines concerning the appropriate implementation of programs of biological control in the field because every parasitoid species can have differences in the biology and behavior.

In this study, the results showed that alyssum and buckwheat had the same pattern in both *D. rapae* and *E. tricolor* because alyssum had a better attractiveness than buckwheat and buckwheat had a higher effect on parasitoid longevity than alyssum for both parasitoids. This is consistent with the findings of Hogg *et al*., (2011), who found that alyssum had the highest attractiveness to other arthropods compared with buckwheat and Araj *et al*., 2006, who observed that buckwheat-fed *Aphidius ervi* parasitois lived longer than those fed on alyssum. It is also according to many works, reporting that the high diversity of selected flowering plants favours the richness and abundance of beneficial insects (Barret and Harder, 1996; Frank, 1999; Sjödin *et al*., 2008; Zamora *et al*., 2011) due to the fact that every flowering plant species may have the highest performance depending of the evaluated benefit. Thus, this confirms that selection of flowering plants should be based on different criteria because some flowering plants had a better effect than others depending on the considered variable.

For *D. rapae*, flowering plant treatments improved significantly the percentage of mummies in the climate chamber and in the field experiments (Chapter 3). This can be explained taking into consideration that *D. rapae* can feed on nectar and honeydew. Nectar had a better quality than honeydew as it could also be confirmed in the longevity experiments of the chapter 3.

For *E. tricolor*, flowering plants had no effect on percentage of mummies when parasitoids were released close to the host (experiment in cages of the chapter 3 and experiment in field of the chapter 4) and those had effect on this variable when parasitoids were released far of the host (Field experiment of the chapter 3). This could be because in the field experiment, parasitoids could consume nectar while the host finding but in the other experiments, the parasitoids were released close to the host which provide haemolymph and hence they did not need nectar. It is because haemolymph has better quality than nectar. This suggests that flowering plants should be sowed far of banker plants to offer a source of energy to natural occurring or released parasitoids during the host searching. Haemolymph is more suitable than nectar because provides sources of protein and sugars (Jervis *et al.*, 2008) while nectar only offers sugars and small quantities of aminoacids and proteins. The parasitoid *E. tricolor* is synovigenic, meaning that the ovigenesis is during the adult stage and hence protein sources are indispensable (Wajnberg *et al.*, 1994; William, 1995). Therefore, the consumption of nectar in the presence of the herbivore would be an unnecessary waste of energy and secondly, flowers don't provide a suitable source of protein because in general the species of the family aphiidinae as *E. tricolor* do not have mouthpart specialisations to consume pollen (Jervis, 1998).

For *D. rapae,* flowering plants did not improve the efficiency of banker plants and it was no observed significant differences with the control. Maybe, the high number of parasitoids in the banker plants could mask the effect of flowering plants. Therefore, to ensure the functionality of flowering plants, these plants should be placed randomised in the crop far way from the banker plants. In this form natural occurring parasitoids or parasitoids of banker plants moved by the wind during the host searching will have alternatives food while the host finding. The percentage of mummies in the last experiment for *D. rapae* demonstrates that the movement from banker plants to infested plants is evident due to the overcrowding or high intraspecific competence.

In this work was also seen that the percentage of *D. rapae* mummies (26-86%) was higher than in *E. tricolor* (13.5-30%) (Field Experiments on chapter 3). Popov (1987), mentioned that if the reproductive rate of the pest is higher than that in the parasitoids, it could affect the efficiency of the parasitoids. Therefore, the differential reproductive rate between pests and parasitoids could elucidate one reason about the fact that *D. rapae* had higher efficiency than *E. tricolor*. In fact, the reproductive rate of *D. rapae* (49 mummies/female) (This study) is higher than this on *B. brassicae* (1.8-19.26 offspring/female) (Ulusoy and Olmez, 2006) but in *E. tricolor* (66 eggs/female) (This study) the reproductive rate is lower than this on *A. proletella* (144 eggs/female) (Musa, 2010).

Consequently, the use of parasitoids is not enough to maintain the pest below acceptable levels. This could be possible because life systems have mechanism of regulation to guarantee the survival of the offspring and to optimise the fitness of next generations. Aphidiinae parasitoids use a number of host-derived cues, such as shape, colour, odour, taste and movements to assess host suitability (Mackauer *et al*., 1996) and finally, accept herbivores for oviposition. Hence the other herbivores of less quality continue reproducing (Henry *et al*., 2005). According to Henry *et al*., 2005 the oviposition preferences is a factor that affects the efficiency of parasitoids.

In conclusion, the findings from the preceding experiments have revealed the importance of the management techniques for improving the quality of rearing systems and enhancing the performance of the target parasitoids in the field. Pruned broccoli plants in large cages (0.51 x 0.76 x 0.56) m) had the best performance to get banker plants of satisfactory quality. For *D. rapae* females, experiments showed that the addition of selected flowering plant species can attract these parasitoids, optimise their survival and production of mummies. By contrast, for *E. tricolor*, flowering plants sometimes had an effect on the measured variables, but that was very low. A possible explanation to this could be that *E. tricolor* can supply all the nutritional requirements with haemolymph and nectar is only necessary in the absence of the host (results of chapter 3).

Undoubtedly, the assessed habitat management techniques contribute to improving the performance of the parasitoids especially for *D. rapae* and they can moderate the population level of the target pests but they are not able to maintain the pest in acceptable levels, implying this is needed other techniques of integrated pest management, involving physical, cultural, chemical, preventive techniques among other. Understanding about the appropriate use of techniques to achieve the maximum potential of pest antagonists in the integrated pest management is one of the most relevant topics. Hence, further studies should emphasise the possibility of studying as flowering and banker plants in combination with other could minimise the doses and frequencies of chemical applications. Buckwheat should be evaluated in policulture with cabbage plants taking into account that the fruit of this plant is food for humans and parasitoids no affect this plant. This would allow having a high density of this plant in cabbage and the intensive agronomical management to buckwheat would allow to have nectar of high quality for the parasitoids and the concentration of volatiles in the air will be high. This is also important to emphasise that the flowering and banker plants evaluated were attractive to other pest antagonist species (hoverflies and lady beetles) and therefore they should be used in other crops with pests as whiteflies or aphids.

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Curriculum Vitae

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Profile:
Agronomist and scientific with experience in: 1) improving efficiency of parasitoids during four years, 2) teaching topics related with entomology and research methodology during six months, 3) effectiveness of pollinators during three years, 4) use of vegetable extracts in the integrated pest management during two years and 5) effect of mycorrhizas on the productivity and quality of lettuce and seedling plants during two years.

Educational Qualifications:

- **Ph. D. Horticultural Sciences (Dr. rer. Hort.) (October 2012- January 2017)**
  Institut für Gartenbauliche Produktionssysteme - Leibniz Universität Hannover, Germany
- **Magister in Agricultural Sciences (Emphasis in Entomology) (January 2008- March 2011)**
  Faculty of Agronomy - National University of Colombia, Bogota
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Research and Professional Experience:

- **Institution: Institut für Gartenbauliche Produktionssysteme - Leibniz Universität Hannover, Germany DAAD Scholarship (October 2012-Present).**
  Function: Improving efficiency of parasitoids: Case studies with *Diaeretiella rapae* and *Encarsia tricolor*.
- **Institution: University of Cundinamarca (First semester 2012)**
  Functions: Teaching Integrated Pest Management, Research Methodology, Taxonomy of Insects and Experimental Design.
- **Institution: National University of Colombia, Institute of Natural Sciences, Bogota (2009-2011)**
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- **Institution: National University of Colombia, Faculty of Agronomy, Bogotá (2008-2009).**
  Function: Coordination of integrated crop management of basil and mint.
- **Institution: National University of Colombia, Faculty of Agronomy, Bogotá (2007-2008).**

Functions: Formulation of projects about integrated crop managements of basil, mint and stevia.

- **Institution: Abonos Superior (Chia-Cundinamarca), Colombia (2004-2006).**
  Functions: Testing plant extracts in integration with other practices of pest management.
- **Institution: National University of Colombia, Faculty of Agronomy, Colombia (2002-2004).**
  Thesis Title: Effect of arbuscular mycorrhizas on the productivity of a commercial system of lettuce *(Lactuca sativa)* in the municipality of Tenjo (Cundinamarca, Colombia).
- **Institution: Research Centre and Agribusiness Consultancies of the Jorge Tadeo Lozano University, Colombia. Student practice (2001-2002)**
  Functions: Coordination and implementation of activities related with experiments of integrated crop management in Cape gooseberry, Tomato and Lulo.

Publications

- Zamora, M. M., G. D. Amat, and J. L. Fernández (2011) Studies on flowers flies (Diptera: Syrphidae) in *Salvia bogotensis* (Lamiaceae) in the Botanical Garden "José Celestino Mutis" (Bogotá, D.C.). Caldasia 33:453-470.
- Zamora Carrillo M.M. and Meyhöfer R. (2015) Improving mass rearing systems for micro-hymenoptera: Case studies with *Diaeretiella rapae* and *Encarsia tricolor*. Integrated Protection in Field Vegetables. IOBC-WPRS Bulletin Vol. 118, 2016. pp.37-45

In conference proceedings

- Zamora Carrillo M.M., Meyhoefer R. (2015) Preferences of the aphid parasitoid *Diaeretiella Rapae* (Braconidae) for different aphid-host plant combinations, Tropentag 2015, Management of land use systems for enhanced food security-conflicts, controversies and resolutions - Humboldt Universität, Berlin, Germany pp. 195, Cullvier Verlag
- Zamora Carrillo M.M., Meyhöfer R. (2016) The role of flowering plants in improving biological control of cabbage whiteflies and aphids by parasitoids. In: Herz, Annette and Ehlers, Ralf-Udo. Deutsche Gesellschaft für allgemeine und angewandte Entomologie e. V.34. Tagung des AK: "Nutzarthropoden und Entomopathogene Nematoden" 02 November. 2015 Hannover-Germany.
- Zamora Carrillo M.M., Amat-García G.D. and Fernández-Alonso J.L. (2016) Ecological relationship between hoverflies (Diptera: Syrphidae) and *Salvia bogotensis* in an urban environment. In: Herz, Annette and Ehlers, Ralf-Udo. Deutsche Gesellschaft für allgemeine und angewandte entomologie e. V.34. Tagung des AK: "Nutzarthropoden und Entomopathogene Nematoden" 01 November. 2015 Hannover-Germany.

Awards and Research Grants:

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Presentations:

- Zamora Carrillo M.M., Meyhoefer R. (2015) Preferences of the aphid parasitoid *Diaeretiella Rapae* (Braconidae) for different aphid-host plant combinations, Tropentag 2015, Management of land use systems for enhanced food security- conflicts, controversies and resolutions - Humboldt Universität, Berlin, Germany pp. 195, Cullvier Verlag ISBN: 978-3-7369-9092-0 (Poster).
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References

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