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The delay in arrival of the parasitoid *Diaeretiella rapae* influences the efficiency of cabbage aphid biological control

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Abstract The efficiency of the biological control strategies based on parasitoids, either as a release or a conservative measure, depends on the timely arrival or release of the parasitoids after colonization of the field by the pest. In this study, the effects of the delay between the cabbage aphid *Brevicoryne brassicae* (Hemiptera: Aphididae) infestation and the release of its parasitoid *Diaeretiella rapae* (Hymenoptera: Braconidae) and of the number of parasitoid releases on aphid population dynamics and host plant growth were investigated. When the parasitoids were released at the start of the infestation, 89.6 % of the aphids were parasitized one month later, and the damage to cabbage was low. The identical number of parasitoids released two weeks after the start of the infestation partially controlled the aphid population, but the growth of the cabbage was altered. The earliest introduction of the parasitoid to the crop led to an efficient host regulation, and the release in three times rather than in one time did not improve the control efficiency at this release delay. The implications for biological control strategies that use parasitoids of aphids are discussed.

Keywords Biological control · Release delay · Functional response · Hymenoptera · Braconidae

Introduction

The host-parasitoid synchronization is a key evolutionary factor that determines the survival and reproductive success of both the host and the parasitoid (Godfray et al. 1994; van Nouhuys and Lei 2004). If the emergence pattern of the host is dependent on various factors, such as the host plant phenology and the time of emergence of the entire natural enemy community, the pattern of the parasitoid's emergence will be strongly selected to maximize the synchronization with its host, particularly for specialist species and when the host is abundant for only a few days or weeks in a year (Hackett-Jones et al. 2011). To avoid mortality risks, the parasitoid should not emerge too early before the peak host abundance, but the strategy of emergence should also consider the costs of competition with other parasitoids or predators that emerge when the hosts are abundant (Godfray 1994). With the host emerging first, a delay between host and parasitoid emergence is generally observed for most parasitoid species.

The control of insect herbivores using parasitoids has been successfully applied in the protection of cropping systems, but the success of parasitoids in

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annual field crops has been more limited largely because of the delay between pest and parasitoid colonization of the crop (Chua 1977; Wiedenmann and Smith 1997; Wissinger 1997). This phenomenon of delayed colonization, observed in many crops and for most of the natural enemies, is more significant in annual crops in which rotation, tillage, harvest, and pesticide application are frequent perturbations, which force natural enemies to recolonize periodically the crop fields (Rauwald and Ives 2001). The parasitoids are often more specialized and therefore are affected more by these perturbations than predators, which can maintain populations in the field or in the field hedges when the pest is absent (Doutt and Debach 1964). In annual cropping systems, the density of the parasitoids early in the season is often not sufficient to control the pest before it causes economic damages. Thus, the timely arrival of parasitoids in crops is an important prerequisite for successful pest control (Landis and van der Werf 1997). Even when the parasitoids reach the field sufficiently early, the parasitoid-host interaction involves several delays (Murdoch et al. 1987), which lead to a slower and often a less effective control of the host population than with the use of predators (Snyder and Ives 2003). Before the attack of the host by the parasitoid, mating and oviposition delay the parasitism activities of newly emerged females (Kant et al. 2013). After the host is attacked, host death is delayed with koinobiont parasitoid species (Askew and Shaw 1986), and therefore, offspring development time (Rochat 1997) must be considered in host-parasitoid population dynamics, which could limit the control of the host population, particularly in short-term crops. Some biological control strategies, such as inundative releases or conservative measures, are used to increase the density of parasitoids in the crops early in the season to reduce the delay in growth between host and parasitoid populations and to increase the control of the pest (Chang and Kareiva 1999; Rauwald and Ives 2001).

Inundative biological control strategies rely on preventive or curative releases of antagonist organisms that are reared in the laboratory and that are largely applied to protected crops. To determine the optimal parasitoid release strategy, several questions remain to be answered, including the number of parasitoids to release and the timing and frequency of the releases of the parasitoids. The parasitoids can be released once or several times, either before the pest is detected (a preventive strategy) or after detection (a curative strategy), and the number of parasitoids released can also be varied (Lopes et al. 2009).

Conservative biological control relies on habitat management to promote the reproduction, longevity and immigration of indigenous natural enemies in the field. One of the primary goals is to ensure a timely colonization of the crops by parasitoids in the spring, which can boost pest control (Chang and Kareiva 1999; Rauwald and Ives 2001). Although positive effects of landscape complexity or of specific structures, such as weeds or flower strips, on parasitoid winter survival (Dennis et al. 1994; Pfiffner and Luka 2000) and time to colonization (Bianchi et al. 2009) has been well studied and sometimes demonstrated, whether these mechanisms influence pest populations, pest damage and crop yields remains unclear (Bianchi et al. 2006; Chaplin-Kramer et al. 2011).

Many authors and practitioners argue that the delay between pest infestation and parasitoid release is the key parameter underlying efficient parasitoid release (Helzen and King 1999). Several models (Rochat 1997; Shea and Possingham 2000; Lopes et al. 2009) were developed to study the influence of this delay on host population control to determine the optimal release date, but very few studies experimentally demonstrated the effect of this delay in comparison with other parameters, such as the host-parasitoid ratio (Jones et al. 1999) or the number of releases (Shea and Possingham 2000; Lopes et al. 2009). Rochat (1997) and Lopes et al. (2009) suggested that the strategy used should depend on the functional response (Holling 1959) of the parasitoid species, and in particular, the strategy used for the delay between pest infestation and parasitoid release. The two major types of functional response that are encountered in parasitoids are types II and III. In a type II functional response, the proportion of attacked hosts decreases with an increase in the density of available hosts, and in a type III functional response, the proportion of attacked hosts increases with the host density when hosts are scarce and then decreases at high host density. The release of a parasitoid with a type II functional response is predicted to be optimal instantly for a pest infestation, whereas a type III parasitoid should be released at some days (Lopes et al. 2009) or at half the time of a generation after host population establishment (Rochat 1997). Whether a parasitoid has a type II or a type III functional response is difficult to determine without detailed behavioral studies (van Lenteren and Bakker 1976; Hassell 2000; Fernandez-Arhex and Corley 2003).

Brevicoryne brassicae (Hemiptera: Aphididae) is one of the most important pests of cruciferous crops, affecting plant growth and quality and transmitting several phytopathogenic viruses (Blackman and Eastop, 2000). *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae) is a solitary endoparasitoid of several aphid species and is the main primary parasitoid of *Brevicoryne brassicae*, frequently described as the only primary parasitoid of this aphid (Hafez 1961; Chua 1977; Wilson and Lambdin 1987; Pike et al. 1999). The early parasitism by this wasp prevents the establishment of aphid colonies (Bradburne and Mithen 2000) and could prevent aphid outbreaks in cruciferous crops (Bahana and Karhioc 1986; Souza et al. 1992). The behavioral studies of host foraging by this parasitoid species showed a higher search activity and parasitism rate when the hosts were dispersed instead of aggregated (Lopez et al. 1990; Powell and Nickless 1996). In laboratory studies of the host-parasitoid populations, a type II functional response for the foraging behavior of *D. rapae* on *B. brassicae* was identified (Fathipour et al. 2006; Moayeri et al. 2013).

Several field studies investigated the degree of natural control of the cabbage aphid by *D. rapae* on Brassicaceae crops in the field (Chua 1977; Bahana and Karhioc 1986; Gadomski 1994). The parasitoid did not control the cabbage aphid populations much during the initial stage of plant infestation, a result that can primarily be explained by the lack of synchronization between the parasitoid and its host. Although *D. rapae* remained active at low temperatures, the parasitoid appeared later and in lower numbers than the host (Chua 1979; Bahana and Karhioc 1986; Gabrys et al. 1998). Moreover, the hyperparasitoids that rapidly colonized the cabbage crop after arrival of the parasitoids could strongly affect the potential control by *D. rapae* on the *B. brassicae* population (Chua 1977; Souza et al. 1992). Release experiments in the field were used to test whether the control of *B. brassicae* by the parasitoid increased by varying the parasitoid:aphid ratios and the release modalities. These experiments led to variable control of the aphids, even with a high parasitoid:aphid ratio, which suggested that the early timing of the release of the parasitoids could play a major role in the effective control of *B. brassicae* by *D. rapae* (Zaki et al. 1999; Zhang and Hassan 2003).

We investigated the control of cabbage aphid populations by *D. rapae* under nearly optimal conditions, without consideration of any biotic or abiotic interference that might alter the parasitoid efficiency in the field, and with a high parasitoid female to host ratio of 1:5. The aims of this study were (i) to determine the effect of the delay between *B. brassicae* aphid infestation and *D. rapae* parasitoid release on aphid population dynamics and on cabbage damage and yields, (ii) to determine if the release modality, i.e. multiple rather than single release, could improve the efficiency of early release, and (iii) to test if the most efficient release date for a parasitoid species presenting a functional response type II as *D. rapae* should be from the start of host infestation as predicted by mathematical models (Rochat 1997; Lopes et al. 2009). The significance of the results for parasitoid use in biological control strategies are discussed.

Materials and methods

Cultures

Laboratory mass rearing of the cabbage aphid *B. brassicae* was established from clonal individuals of a single clonal colony collected in a cabbage field near Angers, France (47°28′42″ N, 0°33′47″ O). The aphid cultures were maintained in large cages (50 × 200 × 50 cm) on 5–6 leaves of the cabbage, *Brassica oleracea* convar. *Acephala*. The cages were placed in a climate-controlled room at 21 °C and 70 % RH, with a L16:D8 h photoperiod. Collected from a cabbage field near Angers from *B. brassicae* mummies, the parasitoid *D. rapae* was reared on *B. brassicae* on cabbage in smaller cages (30 × 30 × 50 cm) that were placed in a climate-controlled room under identical conditions. The infested cabbage was renewed every two days, and the cabbages leaves with the parasitized aphids were placed in dark emergence boxes in the same conditions as the rearing cages. The adult parasitoids were collected daily in a transparent collector tube, which exploited the phototropism of the *D. rapae* adults.

Experiments

This study was conducted in a greenhouse compartment that contained 12 culture tables (150 × 150 cm)

Table 1 Release parameters of the release treatments. Estimated number of aphids on the 25 plants of each plot on the day of the release (R_0, R_{0+}) or on the day before parasitoid release (R_7, R_{7+}, R_{14}), date of the releases, and number of male and female parasitoids in each release

	Number of aphids on the 25 plants	Date of the release in days after aphid infestation	Number of parasitoids released	
			Females	Males
R_0				
Plot 1	25	D0	6	3
Plot 2	25	D0	6	3
R_{0+}				
Plot 1	25	D0, D3 and D6	2	1
Plot 2	25	D0, D3 and D7	2	1
R_7				
Plot 1	889	D7	177	102
Plot 2	335	D7	68	34
R_{7+}				
Plot 1	500	D7, D10 and D13	33	20
Plot 2	310	D7, D10 and D13	21	11
R_{14}				
Plot 1	2635	D14	527	338
Plot 2	2405	D14	481	332

at the Agrocampus Ouest (Angers, France) for two months. Twenty-five potted cabbage plants (with four or five leaves) were placed on each table, and each table was then covered with an insect-proof net tunnel. Throughout the experiment, the temperature in the compartment was maintained at 23 °C, which was the near optimal temperature for the plants, aphids and growth of the parasitoids, as described in the literature (Bernal and Gonzalez 1997; Satar et al. 2005). On day 0, one seven-day-old parthenogenic adult *B. brassicae* female was transferred onto each cabbage plant. Under these conditions in the laboratory, the larval development of *B. brassicae* is the shortest of those reported and requires approximately seven days (Zhang and Hassan 2003; Satar et al. 2005). An initial homogeneous distribution of one aphid per plant was chosen to provide aphid colonies with a similar density on each plant in all repetitions. The females that were produced parthenogenetically then quickly aggregated into colonies, which mimicked an aphid infestation by alate aphids in the field.

Six treatments were tested and assigned to the experimental plots (one plot = one table) in a completely randomized design, and each treatment was replicated once. Three delays in the release of *D. rapae* at day 0, day 7 and day 14 after aphid infestation and two release modalities in one time and in three times were tested and compared to a control, i.e. without parasitoid release. The three treatments R_0, R_7, and R_{14}, consisted in parasitoid release at one time at 5 h, and at 7 and 14 days, respectively, after the infestation with *B. brassicae*. Two additional treatments R_{0+} and R_{7+} corresponded to multiple release treatments on the two earliest delays. In these treatments, the total number of parasitoids was released in three equal proportions at 5 h, and at three and six days for the R_{0+} treatment and at seven, ten and 13 days after infestation with *B. brassicae* for the R_{7+} treatment. Two of the plots were not exposed to the parasitoids and were used as a control. Identical parasitoid to host ratios were used for each type of treatment, i.e., one 24–48 h-old *D. rapae* female for five aphids and one *D. rapae* male for two *D. rapae* females (Zhang and Hassan 2003). For the R_0 and R_{0+} treatments, the number of parasitoids released was determined from the number of aphids transferred to the 25 cabbage plants. For the later releases, the number of *D. rapae* was determined from an estimation of the entire population of aphids in the plot the day before the parasitoid release, which was based on a count of the aphids on 12 cabbage plants per plot (Table 1).

Measurements

The surveys of the aphid population and the level of parasitism were performed on the 12 cabbage plants in the center of each plot, to avoid bias due the microclimatic differences between the center and the border of the cage. The population survey began three days after the inoculation of *B. brassicae*. The aphids that were alive or mummies, whether emerged or not, were counted directly on each plant at 3–4 day intervals during the first 15 days and at one week intervals during the last 15 days. The numbers of mummies were cumulative because the mummies of this species remain on the plant after parasitoid emergence (Dhiman and Goel 2006). Those winged aphids that left the cabbage plants were not accounted for. The rate of parasitism was assessed for each sampled plant based on the number of mummified aphids: percentage parasitism = total parasitized aphids in the population (cumulative) × 100/total aphids in the population (parasitized and not parasitized). At the end of the experiment (31 days after the aphid infestation), the wet weight of the cabbage plants (cut at ground level) was determined using an electronic balance (±1 g) for the 25 plants of each plot (Ponti et al. 2007). The number of curved and necrotic leaves per cabbage plant and the presence of an aphid colony (estimated by more than ten aphids) on the apical bud were noted.

Statistical analyses

The aphid population and the parasitoid population dynamics, estimated by the number of mummies, were analyzed using a Generalized Estimated Equation model with a Poisson distribution and a log link function (GEE, Liang and Zeger 1986). The parasitism data, expressed as the percentage of mummified aphids, was analyzed using a generalized linear model (GLM, McCullagh and Nelder 1989) with a binomial distribution and a logit link and followed by a χ^2 test. The cabbage plant fresh weight at the end of the experiment was analyzed using a Kruskal–Wallis or a Mann–Whitney and a Dunn test as the post-hoc test when appropriate. The number of aphids, necrotic leaves and deformed leaves at the end of the experiment were analyzed using a GLM with a Poisson distribution and a log link function and a contrast method (esticon function) as the post-hoc test when appropriate. The proportion of plants that presented more than ten aphids on the apical bud was compared among treatments using a χ^2 test and a Fischer exact test. All data were analyzed using the R statistical software package (R Development Core Team 2008).

Results

Aphid dynamics (Fig. 1)

The population dynamics of the aphids were significantly affected by the release of the parasitoids ($\chi^2 = 189.0$, df = 5, $p < 0.01$). In the controls, the aphid population approached an exponential growth, whereas the mean number of aphids in all the treatments reached a maximum between 15 and 25 days after infestation and then decreased. Moreover, the delay between aphid infestation and parasitoid release had a significant influence on the aphid population dynamics. With the same parasitoid:aphid ratio, a release on day 0, either in one release or in three separate releases, strongly arrested aphid population growth compared with the control (R_0: $p < 0.01$; R_0+: $p < 0.01$). A parasitoid release seven days after the aphid infestation also had a significant effect on the aphid population (R_7: $p < 0.01$; R_7+: $p = 0.03$). With the last release of parasitoids after 14 days, the dynamics of the aphid population were not significantly affected (R_{14}: $p = 0.26$). At the end of the experiment, the mean number of aphids per plant (± SE) was significantly different depending on the modality ($\chi^2 = 135.0$, df = 5, $p < 0.01$; contrast method; $p < 0.01$), reaching 3089 ± 294 (mean ± SE) aphids per cabbage for the control treatment, 528 ± 116 for the R_{14}, 271 ± 106 for the R_7+, 258 ± 53 for the R_7, 181 ± 33 for the R_0+ and only 38 ± 15 for the R_0 treatment.

Parasitoid dynamics (Fig. 1)

When parasitoids were released in one time, their population dynamics, estimated by the number of mummies, were significantly influenced by the release delay ($\chi^2 = 9.1$, df = 2, $p < 0.05$). The higher number of mummies was observed on the last day of the experiment, whatever the release delay, reaching

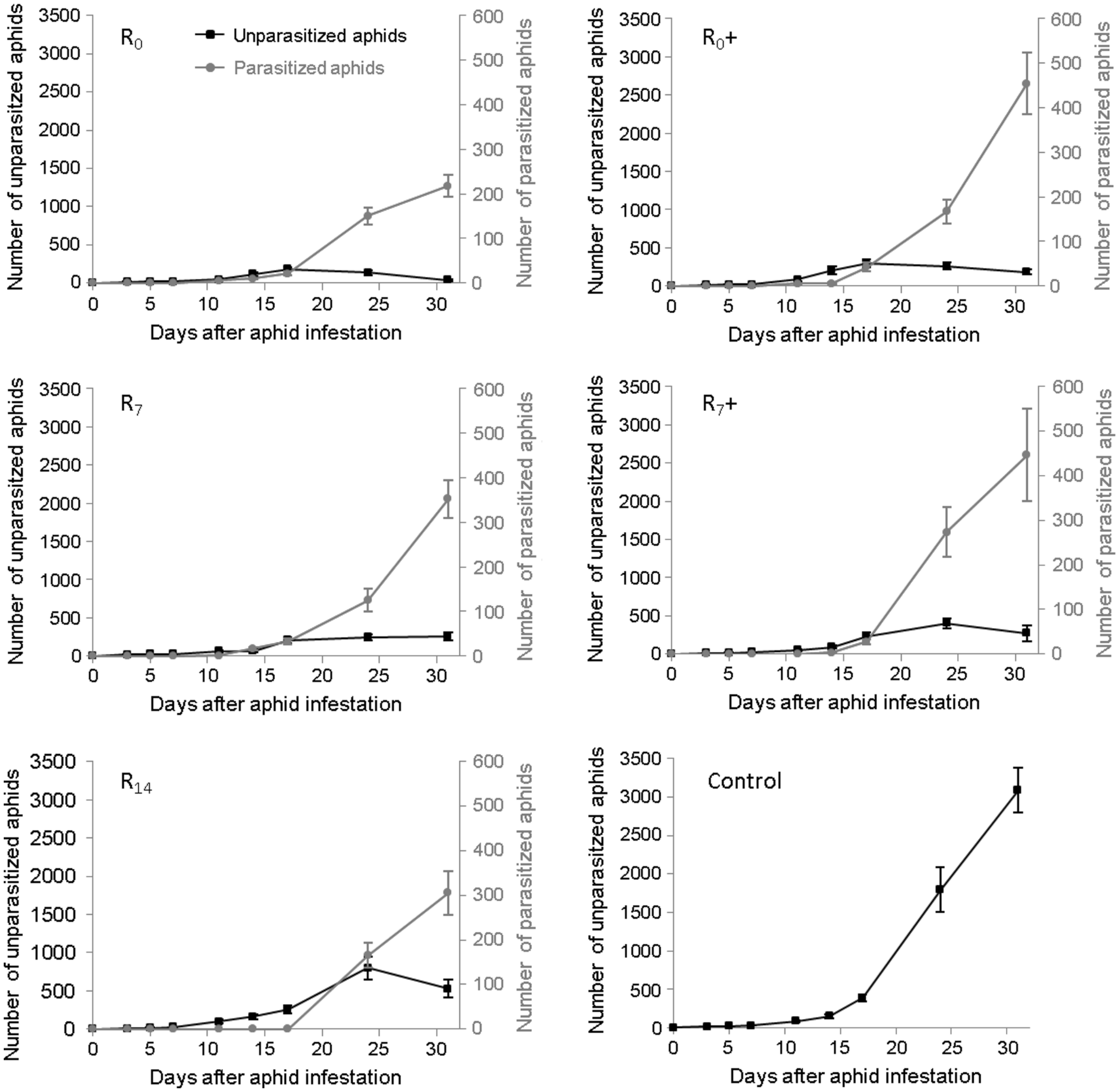

Fig. 1 Mean number of unparasitized (black) and parasitized (gray) *Brevicoryne brassicae* per plant (mean ± SE) with varying delays between aphid infestation and *Diaeretiella rapae* release, using two release methods. *Diaeretiella rapae* was released the day of the *B. brassicae* infestation at one time (R_0) or at three times from the first day (R_{0+}), *D. rapae* was released seven days after the aphid infestation at one time (R_7) or at three times from the 7th day (R_{7+}), *D. rapae* was released 14 days the after aphid infestation (R_{14}), and *D. rapae* was not released (control)

218 ± 24, 352 ± 42 and 306 ± 49 mummies per plant for R_0, R_7 and R_{14} respectively. When parasitoid were released from the start of the infestation or seven days later, the release modality, i.e. in one time or in three times, significantly influenced the dynamics of the parasitoid population ($\chi^2 = 13.0$, df = 1, $p < 0.05$).

Parasitism rate

The cumulative parasitism rate 31 days after aphid infestation was significantly influenced by the delay between aphid infestation and parasitoid release ($\chi^2 = 3555.8$, df = 2, $p < 0.01$; Fig. 2): the parasitism rate was higher when parasitoids were released earlier than later. The rate reached 89.6 ± 2.7 % for the R_0 treatment, 63 ± 3.5 % for the R_7, and only 39.7 ± 2.6 % for the R_{14} treatment. The release method of the parasitoid (one release or three separate releases) influenced the final parasitism rate observed at the end of the experiment, but the effect changed with the date of release. The mean percentage of parasitized aphids at the end of the experiment was significantly higher when parasitoids were released at one time on the day of the infestation (89.6 ± 2.7 % for R_0) than when they were released at three separate times (71.4 ± 2.9 % for R_{0+}) ($\chi^2 = 2065.3$, df = 1, $p < 0{,}01$). When the parasitoids were released seven days after aphid infestation, the parasitism rate was significantly higher when they were released in three times than when they were released in one time (63.0 ± 3.5 % for R_7 and 69.4 ± 2.8 % for R_{7+}) ($\chi^2 = 3161.5$, df = 1, $p < 0.01$).

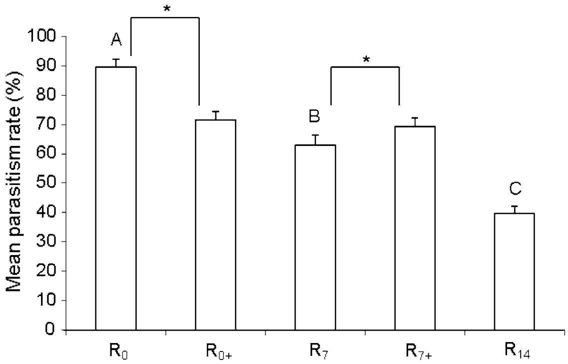

Fig. 2 Percentage of parasitized aphids (mean + SE) 31 days after infestation following the different treatments. In the treatments R_0, R_7 and R_{14}, *D. rapae* was released the day of *B. brassicae* infestation, and seven and 14 days after aphid infestation, respectively. In the R_{0+} and R_{7+} treatments, the parasitoid was release in three separate and equal proportions on the day of aphid infestation, and three and six days after infestation (R_{0+}) or seven, ten and 13 days after infestation (R_{7+}). Different letters on the column indicate significant difference ($\chi^2 = 3555.8$, df = 2, $p < 0.01$) between release delays according to contrast method. An asterisk indicates significant difference ($\chi^2_{R_0-R_{0+}} = 2065.3$, $\chi^2_{R_7-R_{7+}} = 3161.5$, df = 1, $p < 0.01$) between release modalities at the two first release delays

Cabbage plant health parameters (Table 2)

Independent of the delay or the release method, the early release (on day 0 or on day 7) of parasitoids significantly reduced the loss of cabbage fresh weight caused by the aphid infestation ($\chi^2 = 29.2$, df = 5, $p < 0.01$). The mean cabbage fresh weight 31 days after the aphid infestation was approximately 100 g, whereas in the control, the mean fresh weight decreased to 67 g. When the parasitoids were released 14 days after the infestation, the cabbage fresh mean weight was about 91 g, which was not significantly different from the control. For each treatment, the numbers of necrotic and deformed leaves per plant were significantly different between the control and the treatments and also within the treatments, with these damages increased with the delay between aphid infestation and parasitoid release (necrosed: $\chi^2 = 63.2$, df = 5, $p < 0.01$; deformed: $\chi^2 = 77.1$, df = 5, $p < 0.01$). For these parameters, the separate releases resulted in more damage to the leaves than the single release of the parasitoids. When the parasitoids were released 14 days after the aphid infestation, the

Table 2 Health parameters of cabbage plants 31 days after aphid infestation, following the treatments R_0, R_7 and R_{14}, which were the release of *D. rapae* on the day of *B. brassicae* infestation, and seven and 14 days after aphid infestation, respectively

	Mean plant fresh weight in g	Mean number of necrotic leaves per plant	Mean number of deformed leaves per plant	Mean percentage of plants with at least ten aphids on the apex
Control	67.41 ± 4.84 B	3.70 ± 0.36 D	2.04 ± 0.29 C	91.3 ± 6.0 % A
R_0	104.17 ± 7.78 A	0.79 ± 0.43 A	0.08 ± 0.06 A	0.0 ± 0.0 % C
R_{0+}	100.10 ± 6.35 A	1.50 ± 0.22 B	0.42 ± 0.25 B	0.0 ± 0.0 % C
R_7	103.14 ± 5.64 A	1.35 ± 0.23 AB	0.30 ± 0.19 A	0.0 ± 0.0 % C
R_{7+}	101.04 ± 4.13 A	1.63 ± 0.24 B	0.29 ± 0.15 A	0.0 ± 0.0 % C
R_{14}	91.71 ± 5.51 AB	2.61 ± 0.35 C	0.83 ± 0.25 B	21.7 ± 8.8 % B

In the treatments R_{0+} and R_{7+}, the parasitoid was released in three separate and equal proportions on the day of aphid infestation, and three and six days after infestation (R_{0+}) or seven, ten and 13 days after infestation (R_{7+}). Different letters in each column (mean ± SE) indicate significant differences ($p < 0.05$) between treatments according to the Dunn test (mean plant fresh weight), contrast method (mean number of necrotic and deformed leaves), or Fisher exact test (mean percentage of plants with at least ten aphids on the apex) damages were reduced compared to the control but were approximately twice as large as in the other treatments.

The presence of aphids on the plant apex was significantly affected by the release of the parasitoids ($\chi^2 = 220.0$, df = 5, $p < 0.01$), and in the control, 91.3 ± 6.0 % of the cabbage apexes were covered by more than ten aphids compared with 21.7 ± 8.8 % in the R_{14} treatment. Additionally, in the other treatments, the aphid colonies were restricted to the leaves. When the aphid colonies reached the apical bud, the production of new leaves and the growth of the cabbage were severely affected (personal observations). In the control and R_{14} modalities, aphids migrated from the lower leaves to the cabbage apex, which strongly altered the development and growth potential of the cabbage.

Discussion

Our study illustrated the importance of the delay between *B. brassicae* infestation and *D. rapae* release for the efficient control of the aphid population. Significantly, the parasitoids that were early released had the greatest effect on the aphid population, which indicated that for optimal control the parasitoids must be present in the field at the beginning of the aphid infestation. The rate of parasitism was higher when the parasitoids were released immediately after the infestation than when released later. These results were consistent with the predictions of parasitoid-host population dynamics models (Rochat 1997; Lopes et al. 2009). Similar to most of the parasitoid species studied (Fernandez-Arhex and Corley 2003), *D. rapae* presented a type II functional response (Fathipour et al. 2006; Moayeri et al. 2013), and, for optimal control, these models predicted that the release should occur simultaneously with the start of the host infestation. However, the time steps for the three delays investigated in this study did not allow us to determine the precise optimal release delay for this species. As the only way to test this hypothesis, a comparison of the control efficiency of different parasitoid species with known functional responses that are released at different delay times must be conducted.

The requirement of quasi-synchronicity between the aphid colonization of the plant and the parasitoid colonization of the host for effective control might be explained by the differences in the biological cycles of these organisms. For the parthenogenic phase, aphids developed adaptations to minimize generation time, to avoid mating and ovipositional delays and to minimize larval developmental delays. In contrast to aphids, the parasitoid life cycle imposes some mating and ovipositional delays, particularly for synovigenic species that must feed for the eggs to mature before oviposition (Jervis et al. 2001; Kant et al. 2013), as well as developmental delays (Murdoch et al. 1987) and delays in the host death, particularly for koinobiont species (Godfray 1994). The better control observed when the parasitoids were released at the start of aphid infestation could also be caused by, at least in part, the foraging efficiency of *D. rapae* in relation to the spatial distribution of the host. At low host densities when the aphids were dispersed on individual plants, *D. rapae* was a better forager (Lopez et al. 1990; Powell and Nickless 1996). When the delay in time between the aphid infestation and the parasitoid release increased, the *D. rapae* females found larger aphid colonies, with highly aggregated aphids, which led to a decrease in its foraging efficiency and parasitism rate. Cabbage aphids form highly aggregated colonies, with synchronized kicking defense behavior, which could prevent parasitoid exploitation of the aphids in the heart of the colony (personal observation). Moreover, at early stage of infestation, parasitoid encounter almost only adult colonizer aphids, parthenogenic in our experiment or alate in the field. Kant et al. (2008) showed that *D. rapae* parasitism efficiency depends on the host stage and reaches its maximum when attacking seven-days-old adult *B. brassicae*, (but see Zhang and Hassan 2003). *D. rapae* higher efficiency observed at early stage of infestation could be due in part to the age structure of the host with over-representation of adults.

In our experiment, *D. rapae* was released at a ratio of one parasitoid female per five aphids, which had a strong effect on the growth of the aphid population independent of the delay between the aphid infestation and the parasitoid release or the release strategy used. The 1:5 parasitoid:host ratio, however, was consistent with the parasitoid density observed in the spring in cabbage fields and was economically realistic for a release when the aphid population density remained low. Moreover, unlike Zhang and Hassan (2003), we observed control of the aphid population with the application of this ratio, even for the later releases. These conflicting results could be because of the number of aphids deposited per plant at the start of the experiment by Zhang and Hassan (2003), which was ten seven-day-old aphids, and these aphids could represent the first offspring generation of a colonizing alate female and therefore a delay of at least one generation between aphid colonization and parasitoid release would occur. A very short delay between aphid infestation and parasitoid release had a greater effect on aphid population control than a high parasitoid:host ratio.

When releasing parasitoid early, from the start of the infestation, multiple releases of the parasitoid did not improve the control efficiency, whereas seven days after multiple releases improved the control efficiency as observed by Zhang and Hassan (2003) or predicted by the models of Rochat (1997), Shea and Possingham (2000) and (Lopes et al. 2009). Immediately after the aphid infestation, the low number of adult parasitoids released when the total quantity was released three separate times (two parasitoid females per release) could have increased the risk of the disappearance of such a small population of parasitoids. In these conditions, the density of the aphids might have been too low and too dispersed to be discovered by the parasitoids, which prevented few parasitoids from becoming well established.

The early releases of the parasitoids significantly reduced the loss of plant weight caused by aphid feeding, while the latter release showed only a non-significant reduction. Moreover when the release occurred 14 days after the infestation, the plant health was affected. On the plant, the location of the *B. brassicae* colonies varied during the experiment and was different among the treatments. When the parasitoids were released immediately or seven days after the aphid infestation, the aphid colonies were restricted to the lower leaves and very few damage symptoms were observed. When the parasitoids were released after 14 days, the aphid populations had time to reach a density sufficiently high before parasitoid control to cause necrosis and deformations of the leaves and to induce migration of some aphids from the basal leaves to the apical leaves or to the heart of the cabbage plant (Hullé et al. 1999; Cividanes and Santos 2003). Unless the plant weight remained unaffected one month after the infestation, the presence of aphid colonies on the plant apex altered the later growth and the subsequent crop quality and value (Liu et al. 1994; Ceron-Hernandez and Salguero 1995; Nieto et al. 2006).

Implications for biological control strategy

For the optimal control of the cabbage aphid, the primary conclusion of this study is that the parasitoid must be present very early in the system, ideally from the start of the infestation. The high foraging efficiency of *D. rapae* at a low host density combined with the exponential growth of the aphids and the higher damage due to the migration of *B. brassicae* from the outer leaves to the apex of the plant when the density of the aphids exceeded a certain threshold all meant that better control of the aphid populations would be expected with parasitoid release at an early stage of the infestation. This phenomenon represents a large constraint on the use of many parasitoid species that have a type II functional response in Integrated Pest Management programs, which typically make a decision to release the parasitoid after the pest is observed on the crop. The logistical delays from the observation of the pest to the release of the parasitoid could strongly affect the efficiency of the parasitoid control of the pest. The release modality or the aphid:parasitoid ratio (Zhang and Hassan 2003) had less influence on the control efficiency of *D. rapae* compared with the early presence of the parasitoids. In conjunction with the results of previous research (Zaki et al. 1999; Zhang and Hassan 2003), the results of this study strongly indicate that a biological control program that uses such parasitoids to control aphids must favor strategies that improve the early arrival of the parasitoid in the system instead of the mass release of the parasitoid. Because the survival of the parasitoids is low in the field without the hosts, the preventive release of parasitoids before the detection of aphids in the field would be risky. In protected culture systems, i.e., tunnel or greenhouse systems, banker plant strategies (Freuler et al. 2001) that are based on the polyphagy of some beneficial species could avoid this mortality risk before host arrival and represent a promising way to improve the early presence of *D. rapae*. However, for *D. rapae*, although many aphid species were described as potential hosts (Pike et al. 1999), the evidence of local specialization and the occurrence of host races in this species (Nemec and Stary 1984; Vaughn and Antolin 1998; Le Ralec et al. 2011) could complicate the development of this strategy. In the field, landscape management strategies (reviewed in Gurr et al. 2004; Geiger et al. 2005) designed to improve the overwintering of *D. rapae* close to the field and the early natural colonization of Brassica crops in the spring require further investigation. The use of non-crop areas in the agroecosystem and at the field margins often accommodates various wild species of Brassicaceae and other potential alternative plant-host complexes for *D. rapae* (Le Guigo et al. 2012). The effects of the management of these areas, particularly the mowing practices, herbicide spraying, or destruction of these areas at the end of the season on overwintering mummies requires investigation. In summary, our results confirmed previous studies on this species and showed that earliest presence of the parasitoid *D. rapae* in the system should be the main aim of cabbage aphid biological control program, whatever the strategy used to reach it. Other parameters, such as parasitoid:host ratio or release modality seem to have less impact on aphid population than the delay in parasitoid arrival.

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