Parasitization of Commercially Available Parasitoid Species Against the Lettuce Aphid, *Nasonovia ribisnigri* (Hemiptera: Aphididae)

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ABSTRACT The lettuce aphid, *Nasonovia ribisnigri* (Mosley), is an economically important pest of lettuce worldwide. Little documentation exists for the control efficacy of aphid parasitoids against N. ribisnigri. This laboratory study evaluated three commercially available parasitoid species: Aphidius colemani (Viereck), Lysiphlebus testaceipes (Cresson), and Aphelinus abdominalis (Dalman) for their mortality impact on N. ribisnigri. The green peach aphid Myzus persicae (Sulzer) was included as a reference aphid. The study showed that A. abdominalis successfully parasitized 39 and 13% of the offered N. ribisnigri and M. persicae, respectively, within a 24-h exposure period. In contrast, none of the lettuce aphids exposed to Ap. colemani or L. testaceipes were successfully parasitized, whereas 60 and 3.5% of *M. persicae*, respectively, were successfully parasitized within a 6-h exposure period. Lettuce aphid mortality due to incomplete parasitization was 26 and 31% when exposed to Ap. colemani and *L. testaceipes*, respectively, with corresponding values for *M. persicae* being 5 and 10%, respectively. Mortality as a result of incomplete parasitization when aphids were exposed to A. abdominalis was low for both aphid species. The total mortality inflicted by A. abdominalis within a 24-h exposure period was 51% for the lettuce aphids and significantly less (19%) for green peach aphids. In contrast, Ap. colemani inflicted a higher mortality in M. persicae (65%) compared with N. ribisnigri (26%) within a 6-h exposure period. L. testaceipes caused a greater mortality in N. ribisnigri as compared with M. persicae. This study concludes that A. abdominalis has the potential to be used against N. ribisnigri in inoculative biocontrol programs as compared with the other parasitoid species based on successful parasitization.

KEY WORDS Aphelinidae, Braconidae, Aphelinus abdominalis, mortality, biocontrol

The lettuce aphid, *Nasonovia ribisnigri* (Mosley) (Hemiptera: Aphididae), is an economically important pest of lettuce, *Lactuca sativa* L. worldwide (Blackman and Eastop 2000). It is an r-selected species with high reproductive capacity, parthenogenesis, and short generation time (Diaz and Fereres 2005). *N. ribisnigri* reduces lettuce yield directly by causing leaf distortion, reducing seedling vigour, and deforming the lettuce heads (Stufkens and Teulon 2003). It is also considered a cosmetic pest when present in harvested lettuce heads, as it will reduce the marketable value for the growers (Kift et al. 2004).

The feeding preference of *N. ribisnigri* in lettuce heart leaves (Mackenzie and Vernon 1988) makes it difficult to control with insecticides. Consequently it forces most growers to frequently apply chemicals to prevent infestations from developing (Mackenzie et al. 1988, Ester et al. 1993, Parker et al. 2002). However, complete reliance on chemical products has been criticized in recent years. Not only because pest control in lettuce, subjected primarily to chemical control tactics, is unacceptable at the consumer level, but also due to growing awareness about the residual effects of insecticides on human health and the environmental concerns (Sances et al. 1993). Furthermore, using insecticides may also increase the risk of development of resistant aphid populations (Rufingier et al. 1997, Barber et al. 1999). Therefore, in this context, there is an urgent need of alternative measures, e.g., biological control which could contribute to the development of a likely more sustainable and effective management strategy toward control of *N. ribisnigri*.

Parasitoids along with predators and fungal entomopathogens are potential biocontrol agents against lettuce aphids (e.g., Quentin et al. 1995, Fournier and Brodeur 2000, Nebreda et al. 2005, Shrestha and Enkegaard 2013, Shrestha et al. 2013). Especially aphid parasitoids are presently regarded as the most effective and reliable biocontrol agents due to their high population growth rates and ability to respond density dependently to increasing aphid densities (Hofsvang 1990, van Steenis 1995).

Regarding parasitoids of the lettuce aphid, *Aphidius hieraciorum* (Stray) (Hymenoptera: Braconidae) has

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recently been described as a promising candidate against N. ribisnigri in the United Kingdom and Spain (Department for Environment Food and Rural Affairs [DEFRA] 2005, Nebreda et al. 2005). However, this species is still not made commercially available, and its natural occurrence in lettuce fields still has to be documented (A. Fereres, personal communication). Among the aphid parasitoid species commercially available (van Lenteren 2000) for use in greenhouses (van Lenteren et al. 1997) and fields (Levie et al. 2005), little is known about their potential against the lettuce aphid, except in the case of Aphidius matricariae (Haliday) and Praon volucre (Haliday), which have been found ineffective toward this aphid species (Quentin et al. 1995, DEFRA 2005). However, the possibility exists that other commercially available aphid parasitoids are able to parasitize and control N. ribisnigri.

The aim of this study was consequently to evaluate three commercially available parasitoids for their ability to parasitize and complete development in lettuce aphids as a first step in an attempt to identify suitable candidates for lettuce aphid biocontrol. The aphid parasitoids selected were Aphidius colemani (Viereck), Lysiphlebus testaceipes (Cresson) (Hymenoptera: Braconidae), and Aphelinus abdominalis (Dalman) (Hymenoptera: Aphelinidae). Ap. colemani was included, as mummified lettuce aphids have been detected in lettuce fields (Nebreda et al. 2005), while A. abdominalis and L. testaceipes were selected because they have comparatively wider host ranges and have been used earlier with success to control various other aphid pest species (Haardt and Höller 1992, Blümel and Hausdorf 1996, Jarošik et al. 1996, Starý et al. 2004, Silva et al. 2008, Hughes et al. 2011). The green peach aphid Myzus persicae (Sulzer) (Hemiptera: Aphididae) was included as a reference species because it is well known as a suitable host for all three parasitoids (Zamani et al. 2007, Silva et al. 2008, Acheampong et al. 2012).

Materials and Methods

Aphid Culture. The lettuce aphid N. ribisnigri and the green peach aphid *M. persicae* were reared separately on Iceberg lettuce (Lactuca sativa L. variety "Mirette") and pepper (Capsicum annuum L. variety "California wonder") plants, respectively. They were maintained inside insect-proof net-covered cages (68 by 75 by 82 cm³) in a controlled environment glasshouse compartment (20 \pm 1°C, 55–70% relative humidity [RH], and a photoperiod of 16:8 [L:D] h) at Research Centre Flakkebjerg, Department of Agroecology, University of Aarhus, Denmark. N. ribisnigri was originally supplied from Dr. Gemma Hough (Warwick Crop Centre, University of Warwick, UK). The culture of *M. persicae* has been maintained at Research Centre Flakkebjerg for >7 yr and regularly supplied with new individuals.

Second-instar aphids were used in the study, as this stage is generally considered suitable for parasitization by most aphid parasitoid species (Perdikis et al. 2004,

Wyckhuys et al. 2008, Rehman and Powell 2010). To obtain synchronized cohorts of the two aphid species, 20-25 adults were carefully moved from the stock culture to uninfested leaves of lettuce or pepper using a fine camel hair brush. Each leaf was placed at the bottom of a plastic petri dish (15 cm in diameter) with a vented lid (ventilation secured by build-in notches) on the inside edge of lid) lined with moist filter paper. The petri dishes were afterwards moved to a climate cabinet at $20 \pm 1^{\circ}$ C, 70–75% RH, and a photoperiod of 16:8 (L:D) h. After 24 h, the produced first-instar nymphs were gently transferred to new clean plants and kept in a separate cage in the glasshouse compartment at similar conditions as described above for additionally 3 d for the nymphs to develop into second instars (Hagyar and Hofsvang 1986, Diaz and Fereres 2005).

Parasitoid Culture. A. abdominalis and Ap. colemani were supplied as mummies from EWH Bio-Production, Tappernøje, Denmark, and L. testaceipes as mummies from Hatto Welte, Biologischer Pflanzenschutz, Germany. The mummies were placed, according to species, in a plastic petri dish with a vented lid (15 cm in diameter) and kept in a climate cabinet at 22 \pm 1°C, 70–75% RH, and a photoperiod of 16:8 (L:D) h. Mummies were checked every day for adult emergence. Adults were sexed based on morphological characteristics (Biological Services 2009, Biobest 2011) under a stereo microscope. Females and males were subsequently transferred pairwise to a new petri dish (5 cm in diameter) with a piece of cotton pad soaked with a solution of 10% of honey in water and left for mating in the same climatic conditions as mentioned in the beginning of this subsection. Parasitoids of the following age classes were used in the experiment: Ap. colemani and L. testaceipes $(24 \pm 6 \text{ h old})$ and A. abdominalis $(48 \pm 6 \text{ h old})$. All parasitoids were naive i.e., without prior contact with hosts when used in the experiment.

Experimental Procedure. The experiment was performed in plastic petri dishes with vented lids (9 cm in diameter) lined with a moist filter paper. A circular lettuce or pepper leaf disc (4 cm in diameter) was placed at the bottom of each dish. A total of 25 second-instar aphids (*N. ribisnigri* or *M. persicae*) were gently transferred to each dish by a fine camel hair brush from the plants harboring cohorts of aphids of standardized age. Aphids were allowed to settle on the leaf disc for 1 h before the introduction of parasitoids.

One mated female parasitoid was released into the petri dish arena occupied by second-instar aphids and allowed to parasitize for 6 h in constant conditions (*Ap. colemani* or *L. testaceipes*) or 24 h (*A. abdominalis*) at $22 \pm 1^{\circ}$ C, $70 \pm 5\%$ RH, and a photoperiod of 16:8 (L:D) h. The different exposure periods were chosen because Braconidae parasitoids have the capacity to parasitize a high number of hosts within a short period of time whereas the opposite is the case for most Aphelinidae species. The female parasitoids were removed after the parasitization period and dead or live aphids counted under a stereo microscope. The numbers of aphids host fed by *A. abdominalis* (the

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only of the species engaging in host feeding) was also recorded based on the shrunken appearance of hostfed individuals (Röhne 2002, Tatsumi and Takada 2005). The live aphids of each leaf disc were transferred to two clean leaves with the petiole or base wrapped with moist cotton and inserted into a 1.5 ml Eppendorf tube with demineralized water. This was done to avoid leaf degradation. Each leaf was placed in a plastic petri dish with a vented lid (15 cm in diameter) lined with filter paper and incubated in a climate cabinet at similar conditions as described above. After 4-5 d of incubation, the filter paper was replaced and a new fresh leaf in an Eppendorf tube was placed close to the older ones to allow the aphids to self-transfer. Lettuce and pepper leaves remained fresh for at least 6 d using this setup. For each treatment, 15 replicates were performed simultaneously. For the control treatment, the same procedure was followed, though in absence of any parasitoids. Aphids were monitored up to 10 d after incubation with 2-3 d intervals for recording of mummified and dead aphids.

Statistical Analysis. In this experiment, aphids died of four causes: natural death, host feeding (Hfed; by A. *abdominalis* only), successful parasitization (*Spar*) resulting in mummy formation, or incomplete parasitization (Ipar; Starý1989, Rodrigues and Bueno 2001) where both aphid and immature parasitoid died before parasitoid pupation and mummy formation. The formulas below were used to calculate the percentage of aphids that died for each of the three parasitoidrelated mortalities outlined above. Dead aphids recorded in the incomplete parasitization group were corrected for control mortality (Abbott 1925) before calculation and statistical analysis (Colinet et al. 2005). *Hfed* % = (Number of host fed aphids/Total number of aphids exposed) \times 100. Spar % = (Number of mummified aphids/Total number of aphids exposed) \times 100. *Ipar* % = (Number of corrected dead aphids without signs of mummification/Total number of aphids exposed) \times 100. Total mortality (*Tmort* %) = ((Hfed + Spar + Ipar)/Total number of aphids exposed) \times 100. Data for A. abdominalis were adjusted to a 6-h exposure period (assuming that parasitization proceeded at a constant rate during the 16-h light regime) to be able to compare the three parasitoid species.

Nonparametric one-way analysis of variance, Kruskal–Wallis test, was performed to compare between aphid species and between parasitoid species. A Mann–Whitney U test was used as a post hoc test if significant differences were found in the Kruskal– Wallis tests. A P value of 0.05 was used for the level of significance. The data were analyzed using the free software statistical package R 2.15.1 (R Development Core Team 2011).

Results

Host Feeding. This study showed that *A. abdominalis* was the only species to use a few of the exposed hosts for host feeding. The mean number of aphids

host fed within the 24 h of exposure by *A. abdominalis* was less than one individual aphid, 0.67 \pm 0.19 (mean \pm SE; \approx 3%) for *N. ribisnigri* and 0.53 \pm 0.17 (2%) for *M. persicae*, respectively (Table 1). There was no significant difference in the number of individuals used for host feeding by *A. abdominalis* when the two aphids species were compared ($\chi^2 = 0.23$; df = 1; *P* > 0.05, Kruskal–Wallis test).

Successful Parasitization. The three parasitoids differed in their ability to parasitize the two aphid species. A. abdominalis successfully parasitized 39% of the lettuce aphids offered within the 24-h exposure period (equivalent to 15% in 6 h) whereas a significantly lower proportion ($\chi^2 = 12.69$; df = 1; P < 0.001, Kruskal-Wallis test; ≈13% at 24-h exposure equivalent to 5% in 6 h) of *M. persicae* were successfully parasitized (Table 1). Based on nonhost fed aphids, these parasitization rates correspond to $39.74\% \pm 4.60$ (mean \pm SE) and 13.02% \pm 3.02, respectively. In contrast, exposure of the lettuce aphid to Ap. colemani or L. testaceipes did not result in successful parasitization whereas 60% ($\chi^2 = 24.39$; df = 1; P < 0.0001, Kruskal–Wallis test) and 3.5% ($\chi^2 = 8.66$; df = 1; P < 0.01, Kruskal-Wallis test), respectively, of M. persicae were parasitized within the 6-h exposure period (Table 1).

The overall comparison of the three parasitoids with respect to successful parasitization shows that *A. abdominalis* had a significant effect on *N. ribisnigri* ($\chi^2 = 37.99$; df = 2; P < 0.0001, Kruskal–Wallis test) whereas *Ap. colemani* had significant effect on *M. persicae* ($\chi^2 = 29.39$; df = 2; P < 0.0001, Kruskal–Wallis test; Table 1).

Incomplete Parasitization. There was a significant difference in the degree of incomplete parasitization between aphid species when exposed to either *Ap. colemani* ($\chi^2 = 12.31$; df = 1; P < 0.001, Kruskal-Wallis test) or *L. testaceipes* ($\chi^2 = 8.14$; df = 1; P < 0.001, Kruskal-Wallis test; Table 1). Compared with *M. persicae*, the incomplete parasitization in the lettuce aphid was greater for *Ap. colemani* and *L. testaceipes*, with a mean mortality of 26 and 31%, respectively (Table 1). No significant difference in mortality due to incomplete parasitization was found between aphid species when exposed to *A. abdominalis* ($\chi^2 = 1.11$; df = 1; P > 0.05, Kruskal-Wallis test; Table 1).

A comparison of all three parasitoids showed that *Ap. colemani* and *L. testaceipes* induced significantly higher mortality due to incomplete parasitization in *N. ribisnigri* as compared with *A. abdominalis* ($\chi^2 = 20.23$; df = 2; *P* < 0.0001, Kruskal–Wallis test), with no significant difference found between *Ap. colemani* and *L. testaceipes*. In *M. persicae*, a significantly lower incomplete parasitization was found after exposure to *Ap. colemani* or *A. abdominalis* as compared with *L. testaceipes* ($\chi^2 = 10.27$; df = 2; *P* < 0.01, Kruskal–Wallis test; Table 1).

Total Mortality. When combining the above three mortalities, significant differences were found between the two aphid species in the total mortality caused by the three parasitoids: A. *abdominalis* ($\chi^2 = 18.11$; df = 1; P < 0.0001, Kruskal-Wallis test), L.

Bergen Host feeding Incomplete parasitization Successful parasitization Total mortality	Exposure	Host f	Host feeding	Incomplete 1	Incomplete parasitization	Successful parasitization	urasitization	Total mortality	nortality
rasitoids	periods	Nr	Mp	Nr	Mp	Nr	Mp	Nr	Mp
AB	24 h	$2.67\pm0.75a$	$2.13 \pm 0.66a$	$9.17\pm2.75a$	$4.29\pm1.39a$	$38.67 \pm 4.48a$	$12.53 \pm 2.89b$	$50.50 \pm 3.04a$	$18.95\pm3.58\mathrm{b}$
	6 h (adjusted)	I	I	$3.44 \pm 1.03B$	$1.61 \pm 0.52B$	$14.50 \pm 1.68 \mathrm{A}$	$4.70 \pm 1.08B$	$18.94 \pm 1.14 \mathrm{A}$	$7.11 \pm 1.34B$
TT	2.5			0004 + 40KA	$10.00 \pm 0.60 \mathrm{k}$	0.00 ± 0.00	2.47 ± 1.000	-4.524 ± 4.024	10 LU + 2 LU - 1

AC

 $13.75 \pm 3.07B0$ $64.90 \pm 3.85Aa$ 30.94 ± 4.65 Aa 26.35 ± 4.64 Ab L.ZUDA $\begin{array}{l} 0.00 \pm 0.00 Bb \\ 0.00 \pm 0.00 Bb \end{array}$ $0.29 \pm 2.69 \text{Ab}$ $4.90 \pm 1.31 \text{ABb}$ 30.94 ± 4.65 Aa 26.35 ± 4.64 Aa r. 2.67 6 h (adjusted) $24 \,\mathrm{h}$ 6 h 6 AB 5

 $60.00 \pm 4.55 \text{Aa}$

Mean values within columns bearing the same upper case letter and within rows bearing the same lower case letters are not significantly different (Mann-Whitney test, P = 0.05)

testaceipes ($\chi^2 = 5.71$; df = 1; P < 0.05, Kruskal–Wallis test), and Ap. colemani ($\chi^2 = 17.21$; df = 1; P < 0.0001, Kruskal-Wallis test). A. abdominalis killed almost 51% of the lettuce aphids offered within the 24-h exposure period whereas only 19% of the offered M. persicae were killed. L. testaceipes killed a higher percentage of N. ribisnigri (31%) as compared with M. persicae (14%) within the 6-h exposure periods. In contrast, Ap. cole*mani* killed a significantly greater percentage of M. persicae (65%) compared with N. ribisnigri (26%) within the 6-h exposure periods.

A comparison of the total mortality among the three parasitoids showed no significant differences for lettuce aphids ($\chi^2 = 4.63$; df = 2; P > 0.05, Kruskal–Wallis test) whereas for *M. persicae*, *Ap. colemani* ($\chi^2 = 28.79$; df = 2; P < 0.0001, Kruskal–Wallis test) inflicted the highest mortality.

Discussion

This study is the first to show that A. abdominalis can parasitize and develop in N. ribisnigri and that L. testaceipes will parasitize the lettuce aphid but is unable to complete its juvenile development beyond the egg or larval stage. The study also showed that the outcome of exposure of lettuce aphid to Ap. colemani was the same as for exposures to L. testaceipes. Although not recorded in the current study, it is expected that \approx 89% of A. abdominalis reaching the pupal stage will survive to adulthood (Shrestha, personal observation). Even though no viable offsprings were produced in N. ribisnigri by Ap. colemani and L. testaceipes, both species killed a high number of N. ri*bisnigri* through the process of incomplete parasitization, resulting in no significant differences in the total mortality inflicted by all three parasitoid species.

Among the three examined parasitoids, A. abdominalis was, as expected (e.g., Enkegaard et al. 2013), the only one to engage in host feeding, a common trait within the family Aphelinidae. The percentage of both aphids species killed by the host feeding activity of A. abdominalis in the current study was rather low. This is in accordance with results reported for the closely related species: Aphelinus albipodus (Hayat and Fatima) and Aphelinus asychis (Walker), which daily host fed on an average of 2-2.5% of cotton aphid (Aphis gossypii Glover) and pea aphid Acyrthosiphon pisum (Harris), respectively (Bai and Makauer 1990, Tatsumi and Takada 2005). However, our finding is in contrast to the observation that A. abdominalis host fed on \approx 50% shallot aphids, Myzus ascalonicus (Doncaster) per day (Enkegaard et al. 2013). These differences in the extent of host feeding suggest that A. abdominalis switch from oviposition to host feeding when the host is of poor quality for parasitism.

The exposure of both aphid species to any of the three parasitoids resulted in aphids dying due to incomplete parasitization. These aphids were accepted for oviposition but later died together with the juvenile parasitoid due to physiological incompatibilities (Vinson and Iwantsch 1980, Hagvar and Hofsvang 1991). Physiological compatibility beDecember 2014

tween host and parasitoid is essential before immature development can become successful. The development of a parasitoid to the adult stage depends on several aspects, for instance, the host's internal defensive system where toxins can be detrimental to parasitoid eggs or larvae (Vinson and Iwantsch 1980). The level of incomplete parasitization in lettuce aphids differed among the three parasitoid species, being much higher for Ap. colemani and L. testaceipes than for A. abdominalis. This reflects a poor host quality of lettuce aphids to the two former parasitoid species as also evidenced by their complete lack of successful parasitization in lettuce aphids and is in accordance with the study of Messing and Rabasse (1995) demonstrating that many aphid species easily accepted for oviposition by Ap. colemani later die due to incomplete parasitization thereby attaining the status of unsuitable host species.

The capability of any parasitoid species to parasitize and produce viable offspring in host species is crucial in inoculative biological control. The level of successful parasitization by A. abdominalis on N. ribisnigri obtained in this study (38% in 24 h) is somewhat higher than found for its parasitization on M. ascalonicus (23%; Enkegaard et al. 2013) but very similar to the findings reported for its parasitization of the potato aphid Macrosiphum euphorbiae (Thomas). Thus, in lab studies similar to the present, Jarošik et al. (1996) and Couty and Poppy et al. (2001) found parasitization rates on *M. euphorbiae* of 29 and 37.5%, respectively. Because A. abdominalis has been shown to successfully control M. euphorbiae under greenhouse conditions (Blümel and Hausdorf 1996), this indicates that a parasitization rate of 30-38% can lead to successful biocontrol and that A. abdominalis therefore might be able to control N. ribisnigri.

Based on the current study, the prospects for using L. testaceipes or Ap. colemani for inoculative biocontrol against N. ribisnigri seems nil, as neither parasitoid were able to produce viable offsprings in this aphid species. N. ribisnigri must be characterized as an unsuitable host to both these parasitoids. This is corroborated by the fact that no reports have been found in the literature on the outcome of parasitization of L. testaceipes on N. ribisnigri and that only one study has reported on Ap. colemani in relation to N. ribisnigri (Nebreda et al. 2005) who collected a few N. ribisnigri mummified by Ap. colemani during lettuce cropping seasons. The fact that successful parasitization of N. ribisnigri by Ap. colemani, albeit low, was demonstrated by Nebreda et al. (2005) when none could be demonstrated in the current study might be explained by genetic variations in lettuce aphid populations or parasitoid populations (Henter and Via 1995). Even though L. testaceipes and Ap. colemani can be disregarded as useful for inoculative biocontrol of the lettuce aphid, the relatively high total mortality inflicted by these parasitoids, which was not different from that inflicted by A. abdominalis, indicate a possible use in an inundative biocontrol approach in which no parasitoid reproduction is required.

The reference aphid, *M. persicae*, was successfully parasitized by all three parasitoid species, although *Ap. colemani* clearly inflicted the highest total mortality in consistency with previous studies (Martinou and Wright 2007, Zamani et al. 2007, Silva et al. 2008, Byeon et al. 2011, Hughes et al. 2011, Syngenta 2012). The total mortality inflicted on the green peach aphid by *A. abdominalis* was relatively low. Consequently, a future use of *A. abdominalis* as a biocontrol agent of lettuce aphids will require supplement with other biocontrol agents, e.g., *Ap. colemani*, against green peach aphids should this occur simultaneously (Parker et al. 2002, Fagan et al. 2010).

In conclusion, this study shows that A. abdominalis has the potential to be used against N. ribisnigri in inoculative biocontrol program as compared with the other parasitoid species examined here. However, before A. abdominalis can be recommended to lettuce growers against N. ribisnigri, additional investigation on the influence of plant architecture on host finding is crucial. Because N. ribisnigri has a preference of feeding on heart leaves of lettuce, it may be difficult to access for A. abdominalis. Additional aspects to investigate include host stage selection of A. abdominalis, as it influences parasitoid fitness, as well as the vulnerability of A. abdominalis to competition from other parasitoids and to intraguild predation, as such interactions may change the outcome of biocontrol in cases where more than one biocontrol agents are integrated for control of aphids in lettuce.

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