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Intraguild interactions between generalist predators and an introduced parasitoid of *Glycaspis brimblecombei* (Homoptera: Psylloidea)

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Abstract

Studies were conducted to evaluate potential impacts of generalist predators on the biological control of *Glycaspis brimblecombei* Moore (Homoptera: Psylloidea), a pest of eucalyptus trees in California, and its introduced parasitoid *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae). Based on the results of the monitoring populations of *G. brimblecombei* and their natural enemies in inland and coastal California in the field, *P. bliteus* or *Anthocoris nemoralis* (Fabricius) (Heteroptera: Anthocoridae), alone or both species together were released into cages with *G. brimblecombei* to simulate predatory interference on the introduced parasitoid in the laboratory. Although coastal sites had higher densities of natural enemies as were percent parasitism and predation, the mean number of *G. brimblecombei* did not vary between inland and coastal sites. *P. bliteus* or *A. nemoralis* significantly reduced the psyllid densities in cages. However, when both species were together, the presence of *A. nemoralis* increased the parasitoid mortality relative to the mortality observed in the parasitoid-alone treatment. Moreover, the increase in parasitoid mortality was followed by the decrease in mortality of the psyllids. The current study also indicated that predation risk of parasitized hosts varies depending on the developmental stages of the psyllids.

Keywords: Psyllids; *Glycaspis brimblecombei*; Specialist parasitoid; *Psyllaephagus bliteus*; Generalist predators; *Anthocoris nemoralis*; Intraguild predation

1. Introduction

In the over 100-year history of biological control, many species of exotic natural enemies have been reared, released, and resulted in successful control of many pest species (e.g., Greathead, 1995; van Lenteren et al., 2003; Wratten and Gurr, 2000). However, some biological control programs failed to predict the impacts of natural enemies on the target pest populations, because, in part, they simply focused on interactions between biocontrol agents and individual pests (Kareiva, 1994). Growing evidence suggests that predation by natural enemies on biological control agents, an interaction known as intraguild predation (IGP), can significantly affect the success of biological control (Polis, 1991; Polis and Strong, 1996; Rosenheim, 1998; Rosenheim et al., 1995).

The effects of such interaction in a guild may produce diverse impacts on biological control of herbivorous pests. In some cases, IGP is found to reduce the efficacy of biological control (Croft and MacRae, 1992; Ferguson and Stiling, 1996; Rosenheim et al., 1993, 1999), or in other cases the combination of several natural

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enemies improved the pest suppression (Chang and Kareiva, 1999; Ferguson and Stiling, 1996; Losey and Denno, 1998; Provencher and Riechert, 1994). Considering the crucial importance of interspecific interactions among natural enemies and their consequences for pest suppression, we need to identify the important biological factors that promote or impair biological control. Studies on interactions between generalist and introduced natural enemies would contribute to our understanding of not only the methods of effective biological control for pest insects by multiple natural enemies, but also the mechanisms responsible for reduction of exotic natural enemies. Presently, such studies are lacking in forestry.

Eucalyptus is a fast-growing tree originally from Australia and planted widely in California as an urban tree. *Glycaspis brimblecombei* Moore (Homoptera: Psylloidea) was first reported on eucalyptus trees in Los Angeles in 1998 (CPPDR, 1998). Psyllids may have as many as seven generations per year (Morgan, 1984). This psyllid is known to feed on several species of eucalyptus (Moore, 1970). The lifespan of adult psyllids varies between 3–10 days, and it usually takes 7–10 days for eggs to hatch. The psyllid forms a lerp, which is a white, conical, sweet-testing structure secreted by nymphs as a protective cover. High psyllid populations can cause extensive defoliation on eucalyptus as heavily infested leaves wither and drop off. For ecological and economic reasons, classical biological control was the most appropriate management strategy to control the psyllid in its new range. The parasitoid *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) was introduced from Australia as a biological control agent against *G. brimblecombei* into California in the late 1999s. Information from the initial screening trials indicated that *P. bliteus* prefers mid to large psyllids for oviposition and feeds on smaller psyllids. The parasitoid can produce eggs for several weeks.

In almost every habitat with the psyllid, a wide range of generalist predators is present (Table 1). Considering the possible intraguild interactions of generalist predators with the introduced parasitoid, predators may potentially affect parasitoid populations by directly consuming parasitized hosts, and by competing with them for a shared resource. Since pre-adult stages of *P. bliteus* develop within immature psyllids, this makes them particularly vulnerable to predation. There are many examples of parasitized hosts being consumed by many different predators (Brodeur and McNeil, 1992; Ferguson and Stiling, 1996; Frazer and van den Bosch, 1973; Rosenheim et al., 1997; Wheeler, 1977). Clearly, an understanding of the consequences of the interactions between generalist predators and the introduced specialist parasitoid is essential for the success of biological control of psyllids in California.

Our field observations indicated disparities in the abundance of *G. brimblecombei* and its natural enemies between coastal and inland sites. The numbers of the psyllids were similar between locations (coastal vs. inland), while both *P. bliteus* and foraging predators were more abundant in coastal sites (Table 1). Thus, we hypothesize that IGP between generalist predators and

Table 1
Phytophagous and predaceous insects occurring on foliage of eucalyptus trees colonized by *G. brimblecombei* in coastal and inland California

Sampling period	Mean No. (±SE) of Insects[a]		Statistical values	
Insect species	Coastal	Inland	*F*	*P*
March–June				
Phytophagous				
G. brimblecombei	132.8 ± 15.4	195.2 ± 26.8	$F_{1,28} = 3.03$	0.0927
Predaceous				
P. bliteus	1.27 ± 0.3 a	0.3 ± 0.1 b	$F_{1,28} = 8.01$	0.0085
A. nemoralis	13.7 ± 3.1 a	4.8 ± 1.5 b	$F_{1,28} = 5.03$	0.0331
Harmonia axyridis	1.9 ± 0.5	1.4 ± 0.3	$F_{1,28} = 0.77$	0.3875
Hippodamia convergens	2.2 ± 0.9 a	0.2 ± 0.1 b	$F_{1,28} = 4.28$	0.048
Cantharis carolinus	1.8 ± 0.5 a	0.00 ± 0.0 b	$F_{1,28} = 6.97$	0.0134
July–September				
Phytophagous				
G. brimblecombei	64.5 ± 10.2	119.1 ± 20.9	$F_{1,18} = 3.69$	0.0709
Predaceous				
P. bliteus	10.8 ± 2.4 a	0.07 ± 0.1 b	$F_{1,18} = 8.03$	0.0110
A. nemoralis	40.3 ± 6.4 a	7.84 ± 1.3 b	$F_{1,18} = 24.5$	<0.0001
Harmonia axyridis	1.9 ± 0.2 a	0.07 ± 0.1 b	$F_{1,18} = 46.1$	<0.0001
Hippodamia convergens	0.7 ± 0.3 a	0.04 ± 0.1 b	$F_{1,18} = 4.93$	0.0395
Cantharis carolinus	0.00 ± 0.0	0.00 ± 0.0	N/A	N/A

[a] Data show mean numbers (±SE) of insects per tree. Means followed by the same letter within a row are not significantly different (Repeated Measure Analysis in Proc Mixed, SAS; Fisher's Protected LSM test ($P < 0.05$) was used for multiple comparisons).

P. bliteus might affect the survival of *G. brimblecombei*. We further conducted a complimentary behavioral experiment in the laboratory to investigate how generalist predators affect the population dynamics of the psyllid–parasitoid interactions over the course of one or more host and parasitoid generations.

Our experimental system comprised of greenhouse-grown eucalyptus trees, *G. brimblecombei*, *P. bliteus*, and a generalist predator. The field monitoring conducted in the current study revealed that the most abundant predator of psyllids on eucalyptus trees was *Anthocoris nemoralis* (Fabricius) (Heteroptera: Anthocoridae) (app. 91% of all predator populations) (Table 1). *A. nemoralis* was introduced into North America within the last 50 years (Horton et al., 2000; and references therein). This species is now found both western and eastern United States (Dreistadt and Hagen, 1994; Hagley and Simpson, 1983), and feed on psyllids and other soft bodied insects (Campbell, 1977; Hodgson and Aveling, 1988; Solomon et al., 2000).

Our objective was to evaluate potential impacts of generalist predators on the biological control of *G. brimblecombei* and its introduced parasitoid *P. bliteus*. In the current paper, we examined three aspects of IGP by foraging predators on the introduced parasitoid: (1) the degree of psyllid suppression produced by *P. bliteus* alone or in combination with a predator (intraguild interference), (2) the percent psyllid parasitized by *P. bliteus* in the absence or presence of a predator, and (3) the percentage of psyllid mummies consumed by a predator.

2. Materials and methods

Field observations were initiated to monitor densities of psyllids, the introduced parasitoid and foraging predators in inland and coastal California. We further conducted a complimentary laboratory experiment to simulate interference of the introduced parasitoid by a generalist predator.

2.1. Field observations

Field monitoring was conducted in 3 pairs of coastal-inland sites (total 6 sites) from March to September 2002: Sonoma-Glenn, Alameda-Sacramento, and San Luis Obispo-Fresno. The numbers of paired sites were reduced to two after June 2002 due to removal of host trees in San Luis Obispo and Fresno. Two sampling techniques were applied in the current study: beating sheet and foliage sampling.

2.1.1. Beating sheet

A sheet (1 m by 1 m) was used to collect adults of psyllids and parasitoids and both adult and immatures predators. The sheet was held under a branch, and then the branch was beaten firmly with a club systematically (10 beats per branch). Insects were collected with aspirator into glass vials. This sampling protocol was repeated for 3 branches per tree for 5 trees for each site every 4 weeks.

2.1.2. Foliage sampling

This sampling technique is very effective for sampling immature psyllids and parasitoids (Dahlsten et al., 2000). Branch tips containing the first seven leaves from 3 branches of each of 7 trees per site were randomly sampled every 4 weeks. Tips were cut by hand clippers into plastic bags and taken to the laboratory. Numbers and current stages of psyllids and parasitoids on each leaf were counted and recorded.

Both sampling techniques were conducted shortly after sunrise in all sites in order to collect live parasitoids and psyllids before they become active. All samples were stored in the freezer until identifications could be made.

2.2. Cage experiments

Rearing cages (46W × 31L × 92H cm) were utilized for the laboratory experiment. Glassy, door-like front panel of these tent-like cages has two access points to water plants and release insects. The two side panels are fine mesh polyester netting to provide ventilation. One 1-year-old, uninfested eucalyptus seedling individually planted in a standard plant pot (15 cm bottom and 30 cm upper diameter and 30 cm high) was placed in the cages. We released 100 adult *G. brimblecombei* into all of 36 cages. The psyllids were kept in the cages for 10 days and then removed. We recovered more than 97% of adult psyllids (live or dead). Ten predators (7 adults, 3 nymphs) and/or four parasitoids (3 females + 1 male) were introduced into the cages short after the removal of the psyllids. Predators and parasitoids were held in cages until the end of the experiment. Each cage was subjected to one of the following six treatments: (1) Release *P. bliteus* into cages 3 days earlier than *A. nemoralis*, (2) Release *A. nemoralis* into cages 3 days earlier than *P. bliteus*, (3) Release *A. nemoralis* and *P. bliteus* simultaneously, (4) Release only *P. bliteus*, (5) Release only *A. nemoralis*, and (6) Control. Each treatment was replicated six times.

Development of parasitoids from egg to adulthood usually takes 14–20 days, thus the experiment was maintained for additional 20 days. Emerging adult psyllids and parasitoids were collected daily. At the end of the experiment, all leaves from each plant were cut and stored in the freezer for further analysis. Numbers of psyllid eggs and immatures on each leaf were counted. In addition, all immature psyllids collected in the field and laboratory experiments were treated with chloral–phenol (10 g phenol crystals, 10 g chloral hydrate, and 3 ml distilled water) solutions to determine the presence of parasitoids contained within psyllids. The laboratory experiment was carried out at a constant temperature of 25 °C and a photoperiod of L:D = 14:10 in a greenhouse during September–October of 2002.

All insects used in the cage experiment were obtained from field infested branches. The numbers of insects tested in this study were within the range of numbers observed in the field. Using data from beating sheet sampling only, the following proportions were estimated: *G. brimblecombei A. nemoralis*: 9.86; *G. brimblecombei P. bliteus*: 59.0.

2.3. Data analyses

Data were analyzed over time using repeated measures analysis of variance (Proc Mixed, SAS Institute, 1996); with 'tree,' 'time,' 'site,' and 'location' defined as classes, 'site (tree)' defined as a random statement (blocking factor), and 'numbers of insects' as a continuous variable (treatment effect) for the field data. For the laboratory study, 'time,' 'cage,' and 'treatment' defined as classes, 'cage (treatment)' defined as random statement (blocking factor), and 'numbers of insects and eggs' as a continuous variable (treatment effect). In all cases, degrees of freedom were estimated by 'Satterthwaite' method. A two-factor repeated measures ANOVA (presence or absence of *A. nemoralis* and/or *P. bliteus*) was used to test for non-additive effects. When significant differences in treatments were found, we used Tukey's (LSM) to compare the effects among treatments. Psyllid nymphs were classified into four categories: intact (neither consumed nor parasitized), parasitized (parasitized, but not consumed), consumed (consumed, but not parasitized), and parasitized and consumed (both parasitized and, before the parasitoid emerged, consumed). Percentages of these categories were transformed to arcsin (square root) prior to the Chi-Square-analysis.

3. Results

3.1. Field observations

3.1.1. Beating sheet

We sampled a total of 29,397 insects, among which 25,816 were herbivores and 3581 were natural enemies. More than 97% of all herbivores were *G. brimblecombei* (total 25,188: female:male sex ratio: 0.46:0.54). High population densities of the psyllids occurred between May and July with a peak at the end of June. Other herbivores were other exotic psyllids (2%) and eucalyptus tortoise beetle, *Trachymela sloanei* (Blackburn) (Chrysomelidae) (<1%). The most abundant natural enemies on eucalyptus trees was *A. nemoralis* with an average population frequency of 73%, of the total of natural enemies found, followed by *P. bliteus* 12% (female:male sex ratio: 0.69:0.31), and coccinellid predators (Coleoptera: Coccinellidae) 12%. Of the Coccinellidae species, those identified were: *Harmonia axyridis* with 7%, *Hippodamia convergens* with 4%, *Coccinella semtempunctata* with 0.6% and *Adalia bipunctata* with 0.4%. We also caught *Cantharis carolinus* (Coleoptera: Cantharidae) (2%), green and brown lacewings (Neuroptera: Chrysopidae) (<1%), and other generalist predators (<1%).

Mean numbers of *G. brimblecombei* were not significantly different between inland and coastal sites (Table 1). In contrast, there were significant location effects on the mean numbers of *P. bliteus*, *A. nemoralis*, *H. convergens*, and *C. carolinus* in both March–June and July–September samplings and on *H. axyridis* only during July–September period. In all cases, coastal sites had higher densities of these insects than inland sites.

Location × time interaction effect on the mean number of *G. brimblecombei* was not significant ($F_{3,28} = 1.216$, $P = 0.32$ for March–June, $F_{2,18} = 3.311$, $P = 0.06$ for July–September). However, the abundance of predators and *P. bliteus* varied by location × time interaction effects. Both *P. bliteus* and *A. nemoralis* were more abundant in coastal than inland sites in each of the seven collection periods (*P. bliteus*: $F_{3,28} = 10.06$, $P = 0.0001$ for March–June, $F_{2,18} = 12.15$, $P = 0.0005$ for July–September; *A. nemoralis*: $F_{3,28} = 4.14$, $P = 0.015$ for March–June, $F_{2,18} = 20.289$, $P < 0.0001$ for July–September). Mean numbers of *H. axyridis* and *H. convergens* were significantly higher in coastal than inland sites for July–September sampling (*H. axyridis*: $F_{2,18} = 4.350$, $P = 0.028$; *H. convergens*: $F_{2,18} = 3.831$, $P = 0.04$), but not for March–June sampling (*H. axyridis*: $F_{3,28} = 0.207$, $P = 0.89$; *H. convergens*: $F_{3,28} = 2.037$, $P = 0.13$). Mean number of *C. carolinus* was significantly higher in coastal than inland sites ($F_{3,28} = 4.17$, $P = 0.015$) for only March–June sampling, and their numbers were too low for analysis of location × time interaction effect for July–September sampling. Overall, predator populations started to increase in numbers by May in both coastal and inland sites, however coastal sites had longer peak periods (June–September) than inland sites (May–June), especially for *A. nemoralis*. Furthermore, *P. bliteus* was not even observed at all in the period between July and August in inland sites, whereas it appeared in great numbers after May and peaked at the end of July and August in coastal sites.

3.1.2. Foliage sampling

A total of 43,135 immature *G. brimblecombei* was sampled. Among them, 41% was $1^{st}/2^{nd}$ instars (no morphological distinction possible between these two instars), 15% was the 3^{rd} instar, 18% was the 4^{th} instar, and 26% was the 5^{th} instar. Total numbers of eggs and parasitized psyllids were 106,667 and 496, respectively. A total of 12% of the $1^{st}/2^{nd}$ instar, 12% of the 3^{rd} instar,

Table 2
Immature stages of *G. brimblecombei* on foliage of eucalyptus trees in coastal and inland California

Sampling period	Mean No. (±SE)		Mean proportions (±SE) of Nymphs[a]			
	Eggs	Total nymphs	Intact	Parasitized	Consumed	Parasitized and Consumed
March–June						
Coastal	405.3 ± 56.4 b	150.92 ± 22.1	0.93 ± 0.14	0.01 ± 0.00 a	0.04 ± 0.01 a	0.01 ± 0.00 a
Inland	676.1 ± 94.9 a	188.52 ± 20.1	0.95 ± 0.12	0.004 ± 0.00 b	0.02 ± 0.00 b	0.005 ± 0.00 b
$F_{1,40}$	6.02	2.12	1.54	15.69	5.19	13.42
P	0.01860	0.1534	0.22185	0.0003	0.02813	0.0007
July–September						
Coastal	110.5 ± 13.9 b	104.0 ± 12.1 b	0.45 ± 0.07 b	0.03 ± 0.00	0.43 ± 0.09 a	0.06 ± 0.01 a
Inland	272.7 ± 28.4 a	264.3 ± 33.8 a	0.9 ± 0.15 a	0.01 ± 0.00	0.06 ± 0.09 b	0.003 ± 0.00 b
$F_{1,26}$	13.43	45.36	27.29	0.85	8.48	9.21
P	0.00111	<0.0001	0.00002	0.3652	0.00728	0.005

[a] Data show mean number (±SE) of insects per tree. Nymphs were classified as: (1) 'total nymphs' consisted of sum of all nymphs (including nymphs died as a result of unknown causes); (2) 'intact nymphs' were neither consumed nor parasitized; (3) 'parasitized nymphs' were parasitized, but not consumed; (4) 'consumed nymphs' were consumed, but not parasitized. Means (±SE) followed by the same letter within a column for each sampling period are not significantly different (Repeated Measure Analysis in Proc Mixed, SAS; Fisher's Protected LSM test ($P < 0.05$) was used for multiple comparisons).

7% of the 4th instar, and 6% of the 5th instar of *G. brimblecombei* was consumed by natural enemies.

Significantly more eggs were laid by *G. brimblecombei* in inland than coastal sites during from March to September (Table 2). Mean number of psyllid immatures did not vary significantly between locations from March to June, but was statistically higher in inland sites from July to September. Mean proportion of intact immatures was similar between locations for March and June sampling, but significantly lower in coastal sites for July–September sampling. Coastal sites had higher proportion of immatures parasitized than inland sites for March–June, but proportions were not significantly different between locations for July–September sampling. Mean proportion of immatures consumed was higher in coastal than inland sites during both sampling periods. Likewise, there were significantly higher parasitized and consumed immatures in coastal sites for both sampling periods.

Table 3
Emergence of adult *G. brimblecombei* and *P. bliteus* under the presence or absence of *P. bliteus* with or without *A. nemoralis* in cage experiments in the laboratory

Treatments	Mean No. (±SE) of Insects[a]	
	G. brimblecombei	*P. bliteus*
P. bliteus + 3-day *A. nemoralis*[b]	32.17 ± 3.08 c	4.83 ± 1.19 b
A. nemoralis + 3-day *P. bliteus*[c]	36.67 ± 2.36 c	5.00 ± 0.58 b
P. bliteus + *A. nemoralis*[d]	25.00 ± 1.51 c	1.83 ± 0.65 c
P. bliteus alone	34.33 ± 9.19 c	8.33 ± 1.12 a
A. nemoralis alone	68.67 ± 12.57 b	N/A
Control	112.33 ± 11.16 a	N/A
F	$F_{5,28} = 17.44$	$F_{3,18} = 8.22$
P	<0.0001	0.001

[a] Data show mean number (±SE) of insects per cage. Means (±SE) followed by the same letter within a column are not significantly different (Repeated Measure Analysis in Proc Mixed, SAS; Fisher's Protected LSM test ($P < 0.05$) was used for multiple comparisons).

[b] *P. bliteus* were released 3-day earlier than *A. nemoralis*.

[c] *A. nemoralis* were released 3-day earlier than *P. bliteus*.

[d] *P. bliteus* and *A. nemoralis* were released simultaneously.

3.2. Cage experiments

Mean number of eggs laid by psyllids per cage was similar among six treatments, thus any statistical differences observed in mean number of mature or immature psyllids may not be associated with the number of eggs.

Emergence of *G. brimblecombei* and *P. bliteus* greatly varied among treatments (Table 3). Number of psyllids was significantly reduced by *P. bliteus* or *A. nemoralis* alone or both in combination, relative to the psyllid emergence from control treatments. Psyllid emergence from control cages was two times higher than cages with the predator alone or four times higher than cages with the parasitoid alone or both present. The mean psyllid emergence did not vary among cages containing either the parasitoid alone or various predator–parasitoid combinations.

The highest number of *P. bliteus* emerged from cages with the parasitoid alone. Three-day time lag between introductions of parasitoids or predators in treatment numbers 1 and 2 influenced the outcome of the predator–parasitoid interaction. The time lag increased the parasitoid emergence relative to the simultaneous release of both the parasitoid and predator, but reduced its emergence relative to the parasitoid alone treatment. Furthermore, the interactions between *A. nemoralis* and *P. bliteus* were significant (Table 4), indicating that there was a non-additive effect of the natural enemies.

Cages treated had significantly lower numbers of psyllid immatures than untreated cages (Table 5). This

Table 4
Repeated-measures ANOVA two-way analysis of variance on psyllid densities for parasitoid (present or absent) and *A. nemoralis* (present or absent) treatments (last four treatments listed in Table 3)

Source of variation	*df*[a]	*F*	*P*
P. bliteus	1,30	47.49	<0.0001
A. nemoralis	1,30	14.88	0.0006
P. bliteus × *A. nemoralis*	1,30	51.57	<0.0001

[a] Numerator, denominator degrees of freedom (Proc Mixed, SAS 1996).

suggests that the presence of predators or parasitoids, or both influenced the emergence of nymphs from eggs. The mean number of immatures was similar among cages treated.

We categorized psyllid immatures as 'intact,' 'parasitized,' 'consumed,' and 'parasitized and consumed.' 'Intact nymph' was killed by neither predator nor parasitoid. 'Parasitized nymph' was parasitized, but not consumed. 'Consumed nymph' was consumed, but not parasitized. The presence of a carcass of a psyllid immature with a dark stained puncture(s) indicated that it had been killed by a predator or parasitoid (no clear distinction made between these two), whereas a shrunken carcass without a small hole indicated desiccation or any other unknown causes of mortality. 'Parasitized and consumed nymph' was parasitized and, before the parasitoid emergence, consumed.

Mean proportion of intact immatures varied significantly among treatments (Table 5). Overall, control cages or cages with the parasitoid alone had the highest or lowest proportion of intact nymphs, respectively. The proportions among the remaining treatments were similar. Mean proportion of immatures parasitized also showed differences among treatments (Table 5). The parasitoid alone treatment had higher proportion than the remaining treatments. There was no difference among predator–parasitoid combined treatments.

Mean proportion for immatures consumed varied only between control and treated cages, and the control cages had the lower proportion (Table 5). The proportion for unknown causes of mortality on average was 2%. The statistically lowest proportion for immatures both parasitized and consumed occurred in cages with the parasitoid alone treatment (Table 5). There was no difference among predator–parasitoid combined treatments.

We also calculated the overall percent morality of the parasitoids and psyllids. The percent morality of parasitoids was defined as: (number of nymphs parasitized and consumed)/(number of parasitoids emerged + number nymphs parasitized + number of nymphs both parasitized and consumed) × 100. *A. nemoralis* had a large, negative impact on the percent mortality of the parasitoids ($\chi^2_3 = 15.37$, $P = 0.0015$), ranged from 54.2 ± 13.05 to 79.3 ± 6.46 %. When kept without *A. nemoralis*, the percent mortality of the parasitoid was 2.8 ± 0.90%.

The percentage mortality of psyllids was defined as: (number of parasitoids emerged + number of nymphs parasitized + number of nymphs consumed + number of nymphs both parasitized and consumed)/(number of psyllids emerged + total number of nymphs)/ × 100. Overall, the statistically lowest and highest percent mortality of psyllids occurred in control (1.5 ± 0.87%) and the parasitoid alone (85.1 ± 6.41%) treatments, respectively ($\chi^2_5 = 25.69$, $P < 0.0001$). The percent mortality in cages containing *A. nemoralis* alone, or *A. nemoralis* with *P. bliteus* together ranged from 51.7 ± 9.57 to 72.0 ± 5.60%, and was not significantly different.

Since the mean consumption rate of parasitized psyllids was high in cages with *P. bliteus* and *A. nemoralis* together, we further evaluated which instar(s) of parasitized hosts were particularly vulnerable to the predation or cannibalism. This analysis indicated that the percent mortality of the parasitized early instars, ranged from 85.1 ± 4.1 to 94.0 ± 5.3%, was significantly greater than that in the 5th instar (10.2 ± 0.9%) ($F_{3,18} = 10.45$,

Table 5
Proportions of *G. brimblecombei* nymphs in each category under the presence or absence of *P. bliteus* with or without *A. nemoralis*

Treatments	Mean No. (±SE)		Mean proportion of nymphs of *G. brimblecombei*[a]			
	Eggs	Total nymphs	Intact	Parasitized	Consumed	Parasitized and consumed
P. bliteus + 3-day *A. nem.*	417.2 ± 155.7	112.8 ± 21.7 b	0.10 ± 0.06 b	0.02 ± 0.01 b	0.62 ± 0.04 a	0.24 ± 0.01 a
A. nem.+3-day *P. bliteus*	657.2 ± 127.2	129.7 ± 24.4 b	0.17 ± 0.13 b	0.08 ± 0.03 b	0.59 ± 0.13 a	0.14 ± 0.02 a
P. bliteus + *A. nemoralis*	361.5 ± 78.3	140.8 ± 22.4 b	0.15 ± 0.02 b	0.05 ± 0.01 b	0.56 ± 0.18 a	0.22 ± 0.01 a
P. bliteus alone	642.7 ± 103.1	191.5 ± 19.7 b	0.03 ± 0.02 c	0.30 ± 0.05 a	0.65 ± 0.07 a	0.01 ± 0.00 b
A. nemoralis alone	393.3 ± 97.5	130.2 ± 25.6 b	0.19 ± 0.09 b	N/A	0.79 ± 0.09 a	N/A
Control	702.8 ± 132.5	360.0 ± 73.7 a	0.98 ± 0.01 a	N/A	0.02 ± 0.01 b	N/A
Statistics	$F_{5,28} = 1.69$	$F_{5,28} = 5.8$	$\chi^2_5 = 17.47$	$\chi^2_3 = 14.15$	$\chi^2_5 = 13.17$	$\chi^2_3 = 17.42$
P	0.1698	0.029	0.00369	0.00271	0.02184	0.00058

[a] Nymphs were classified as: (1) 'total nymphs' included sum of all nymphs (excluding nymphs died as a result of unknown causes); (2) 'intact nymphs' were neither consumed nor parasitized; (3) 'parasitized nymphs' were parasitized, but not consumed; (4) 'consumed nymphs' were consumed, but not parasitized; (5) 'parasitized and consumed nymphs' were parasitized and, before the parasitoid emergence, consumed. Data show mean numbers or percentages (±SE) of individuals per cage. Means (±SE) followed by the same letter within a column are not significantly different ($P < 0.05$, χ^2).

$P = 0.0003$). Although there was a slight preference of predation or cannibalism towards the 3rd instar, it was not different from those of the 1st, 2nd, and 4th instars.

4. Discussion

In psyllid–eucalyptus system, the presence of generalist predators in addition to the introduced parasitoid influenced the parasitoid fitness and the target herbivore populations. When *P. bliteus* was combined with *A. nemoralis*, a high mortality rate on parasitized hosts was observed. This might suggest that generalist predators have a great potential to reduce the efficacy of *P. bliteus* as a biocontrol agent. Moreover, an increase in parasitoid mortality caused by *A. nemoralis* was followed by a decrease in the suppression of *G. brimblecombei*. This interaction between a generalist predator and a specialist parasitoid indicates a non-additive effect of natural enemies on psyllid populations. Influence of generalist predators on the efficiency of biological control agents was similar to results reported in earlier studies (Colfer and Rosenheim, 1995, 2001; Ferguson and Stiling, 1996; Lucas et al., 1998; Snyder and Ives, 2001).

Predator preference for hosts (unparasitized vs. parasitized) may be a key factor determining the net effect of predation in the current study. We did not particularly investigate whether *A. nemoralis* preferentially consumes parasitized hosts, but our laboratory experiment suggested that predation risk of parasitized hosts varies depending on the developmental stages of the psyllids. When nymphs of *G. brimblecombei* were parasitized, *A. nemoralis* preyed on only the first 4 instars, and nymphs concealing parasitoid pupae (the 5th instar) were largely free from attack. This is parallel to the earlier observations which suggested that some predators discriminate increasingly against parasitized prey as the parasitoid develops (Heinz et al., 1994; Kindlmann and Ruzicka, 1992). Preference for parasitized versus unparasitized hosts can be quite variable for other parasitoid–predator species. Predators may attack only unparasitized hosts, only parasitized hosts, or may show a partial preference for non-parasitized hosts (Brodeur and Rosenheim, 2000; Colfer and Rosenheim, 2001; Fritz, 1982; Rosenheim et al., 1995; Sunderland et al., 1997). More specific studies on the foraging behavior of predators in the psyllid–eucalyptus community are necessary to determine prey preference by generalist predators.

While the experimental time lag between parasitoids and predators yielded mixed results, the mechanism of predator–parasitoid interactions is not well understood. In one hand, differences in parasitoid emergence from cages were possibly a consequence of the delayed interactions between the parasitoid and the predator, on the other hand, the overall, similar percent mortality of the parasitoids suggested the vulnerability of parasitized hosts to predation by foraging predators. We suspect that encounter rate of *A. nemoralis* with the parasitoid or parasitized hosts is very high and influenced by mobility of the predator, as suggested in other systems (Agarwala et al., 2003; Lucas et al., 1998; Polis et al., 1989; Rosenheim et al., 1995). In fact high abundance of *A. nemoralis* in the psyllid-infested eucalyptus trees may indicate the competitive ability of *A. nemoralis* in this guild. Rapid maturation of adults, a short pre-oviposition period and nymphal development time, and non-discriminatory mating behaviors (Anderson, 1962; Campbell, 1977; Horton et al., 2000) ensure that *A. nemoralis* may successfully compete with *P. bliteus* for the same resources.

The current study raises questions about the long-term consequences of IGP on psyllids suppression. Predation by generalist predators could be a major factor limiting the parasitoid population growth. From our field data, other predator species were also identified, and thus our estimates of *P. bliteus* mortality were conservative. Our study suggests two possible outcomes of the current biological control programs against *G. brimblecombei*. The effective control of the psyllids by the specialist parasitoid may not occur in areas with high populations of predators. Alternatively, once parasitoids were established, particularly in those areas with no or low predator populations as found in this study in inland sites, we might expect that *P. bliteus* will suppress the population growth of *G. brimblecombei*. Our future efforts should be directed towards quantification of multi-natural enemy interactions on target prey populations both in the laboratory and field.

Results of any studies conducted in the confined environment must always be viewed cautiously, because the controlled environment may not always predict long term patterns of interactions between species. In some respects conditions in our laboratory experiments closely approximated those in open fields because the insect densities used in our cages were a typical of that seen in the field. Furthermore, our cage experiments reflect both direct and indirect effects of a generalist predator on the populations of pests and its specialist parasitoids, because direct predation by the predators on psyllids resulted in a detectable, immediate change in parasitoid and psyllid densities, and indirect effect on parasitized hosts manifested itself as a reduction in the number of psyllids parasitized by the following generation of parasitoids.

We have some understanding of the IGP between a generalist predator and a specialist parasitoid reported here, however more complicated interspecific interactions are likely to occur in nature (Moran and Hurd, 1994, 1997; Moran et al., 1996; Polis et al., 1989). For example, it remains unclear what factor(s) promote the distribution and abundance of these predators in coastal, but not in inland, California. Perhaps temperature influences their behaviors, since inland sites on average is 9 °C warmer than the coastal sites during 7 months of data collection. For a better understanding of the biological control of psyllids, the next step of study should concentrate on the relationship between the parasitoid and multiple predators with the objective of understanding the dynamics of food webs in this community under different conditions.

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