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Establishment of the red gum psyllid parasitoid *Psyllaephagus bliteus* on Eucalyptus in Santiago, Chile

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Abstract

The red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae), was detected in *Eucalyptus* sp. foliage in Santiago, Chile, in 2002. The successful biological control results obtained in the US and México using *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) prompted its use in Chile. After its introduction during 2003, it became established in two years. To assess its effectiveness on *G. brimblecombei*, the level of parasitism by *P. bliteus* was determined in four sampling sites in Santiago, where random samples of 50 lerps (carbohydrates and protein shelters) were collected per site on trees infested with the psyllid every other week, during a 4 month period in the spring-summer of 2008-2009. Also, natural enemies were sampled with sticky traps on ten trees at two sites, which were evaluated every 15 days. Results were compared with a previous evaluation. The density of nymphs of the psyllid was ostensibly reduced by the action of *P. bliteus* throughout the season. The biological control program of *G. brimblecombei* with *P. bliteus* is developing well when comparing results with the previous evaluation in 2005 in Santiago, with a significant increase in the rate of parasitism. Generalist predators had also interacting with *P. bliteus* and *G. brimblecombei*. These results indicate a promising use of this parasitoid to strengthen the integrated management of the red gum lerp psyllid in the central zone of Chile.

Key words: *Glycaspis brimblecombei*; lerp; biological control; *Eucalyptus*.

Resumen

Establecimiento de *Psyllaephagus bliteus,* parasitoide del psílido de los eucaliptos rojos en Santiago, Chile

El psílido de los eucaliptos rojos, *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae), se detectó en follaje de *Eucalyptus* sp. en Santiago de Chile en 2002. Los resultados exitosos obtenidos en Estados Unidos y en México con el control biológico usando *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) motivaron su aplicación en Chile. Luego de su internación en agosto de 2003, se estableció durante los dos primeros años. Se determinó el nivel de parasitismo de *P. bliteus* en cuatro sitios de muestreo en Santiago para evaluar su efectividad sobre *G. brimblecombei.* Quincenalmente se extrajo muestras de follaje de árboles infestados donde se seleccionaron 50 lerps (refugios de carbohidratos y proteínas) al azar por sitio durante cuatro meses en la temporada primavera-verano 2008-2009. También se muestrearon enemigos naturales con trampas pegajosas instaladas en 10 árboles en dos sitios de muestreo, que se evaluaron cada 15 días. Los resultados se compararon con una evaluación previa. La densidad de población de ninfas del psílido se redujo ostensiblemente por la acción de *P. bliteus* en el transcurso de la temporada. El programa de control biológico de *G. brimblecombei* con *P. bliteus* se desarrolla positivamente en Santiago, con un incremento significativo en la tasa de parasitismo. Los depredadores generalistas tuvieron también interacciones con *P. bliteus* y *G. brimblecombei*. Los resultados indican un promisorio uso de este parasitoide para fortalecer el manejo integrado del psílido de los eucaliptos rojos en la zona central de Chile.

Palabras clave: *Glycaspis brimblecombei;* lerp, control biológico; *Eucalyptus*.

Introduction

Chile has been invaded in recent years by foreign pests of great impact on eucalypts (Poisson and Sandoval, 1998; Meza and Baldini, 2001), one of them the red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae), a species native to Australia which was detected in 2001 in the vicinity of the International Airport in Santiago, onto *Eucalyptus camaldulensis* Dehn (SAG, 2005). According to Ide *et al.* (2006), this insect is distributed from the regions of Coquimbo (north-central)

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through La Araucanía (south-central), where over 600 thousand ha are planted with species of *Eucalyptus* susceptible of damage by this pest (INFOR, 2010).

Adults of *G. brimblecombei* are about 3 mm long; they move rapidly and are capable of flying great distances (Artigas, 1994; Laudonia and Garonna, 2010). All stages of development of this multivoltine species can be found both on new and mature foliage (Olivares *et al*., 2003; Paine *et al*., 2006). It has a gradual metamorphosis after egg hatch, including several nymph stages increasing gradually in size until the adult stage. These have a light green elongated body, slightly larger in females. The unprotected ovoid yellowish eggs are placed singly or grouped, generally on succulent leaves and young twigs. The young nymphs build a lerp or white shelters. These lerps are crystal sugary structures that protect the orange nymphs under them, and may reach approximately 3 mm wide and 2 mm height (Paine *et al*., 2000; Sullivan *et al*., 2006).

Both adults and nymphs weaken the host plant gradually by sucking sap from the leaves, particularly the nymphs (Diodato and Venturini, 2007). In severe infestations, dozens of lerps can be observed on the leaves, which also favor the development of black sooty mold. When growing together with the lerps, these black fungi invade the surface of leaves, which decreases tree photosynthesis (Olivares *et al.*, 2003). In consequence, foliage decrease, growth reduction, and a general weakening of the plants develop, leaving them more susceptible to other insects and fungi that may cause their death. Although it is not clear whether repeated defoliation is the direct cause, infestations of the red gum lerp psyllid have killed tens to thousands of tree in southern California since it was introduced (Dahlsten *et al*., 2005).

Psyllaephagus bliteus Riek (Hymenoptera: Encyrtidae) is a known redgum lerp psyllid parasitoid wasp native from Australia. The female inserts the eggs, generally one, and exceptionally up to 4, into the abdomen of nymphs of any stage, although it prefers the 3rd and 4th instars of the host (Daane *et al*., 2005). The larva has an oval shape and a long breathing tube, as much as 60% the length of the body; it lives within the abdominal cavity of its host, consuming all the internal organs to death; the parasitized nymph, or «mummy», is immobilized and has the body expanded. This vermiform larva has differentiated head and sclerotized mandibles (Plascencia *et al*., 2005), and its development is not initiated until the host reaches the late fourth or early fifth instar (Daane *et al*., 2005). The black pupa is exarate, has a spindle body, and develops within the body of the nymph host. The adult emerges through a hole on the end part of the nymph abdomen of the nymphs. Adults have a metallic green body, the female being larger, with darker antennae, and more pubescent than the male. Their lifespan depends on the temperature. In the laboratory, at 32°C they can live only 13 to 15 days, while at 17°C they live from 37 to 43 days (Sime *et al*., 2004). However, females may live considerably longer, from 54 to 66 days under favorable conditions. Most eggs are deposited during the first 22 days of the female life (Daane *et al*., 2005). The biological cycle completes between 16 and 41 days, depending greatly on weather conditions (Plascencia *et al.*, 2005).

To mitigate damage by this insect, SAG («Servicio Agrícola y Ganadero», Agriculture and Cattle Service), Ministry of Agriculture, acting together with CPF («Controladora de Plagas Forestales S.A.», Forest Pest Controls), introduced in 2003 *P. bliteus* in Chile (Baldini *et al*., 2005) obtained from the state of Jalisco, Mexico. Liberation of the reared parasitoids occurred the same year in Valparaiso and Metropolitan Regions (SAG, 2004).

After *P. bliteus* release, SAG began in 2004 to evaluate the biological control program in several sampling points in Santiago, Chile. The results showed that establishment was effective, and also, that December was the month with more collects of *P. bliteus*. In general, levels of parasitism were low, as expected due to the short time elapsed since liberation. It was also estimated that this erratic behaviour of the parasitoid wasp was due to the short period of time since establishment, only three years (SAG, 2006). Currently, forestry surveys (Regional SAG Office in Santiago) show that *G. brimblecombei* affects aggressively each year all kinds of red eucalypt forests (stands, woods, or trees in rows or isolated) during spring-summer in the Metropolitan Region. This problem is permanent and increasing, and the real impact of the parasitoid on the pest remains uncertain.

The objective of this study was to evaluate the control exerted by *P. bliteus* on *G. brimblecombei* in the 2008-2009 spring-summer seasons in Santiago, Metropolitan Region of Chile, and to check evolution of parasitism levels comparing with those in the 2004-2005 seasons.

Material and methods

A total 224 individuals (117 males and 107 females) of the parasitoid were introduced, in the MPS (Metro-

politan Park of Santiago), on September 9 and October 1st, 2003. This introduction followed procedures as indicated by FAO (1996) and SAG resolution Nº 2229/01, and produced an effective colony of the parasitoid (SAG, 2004). The sampling area consisted of four sites in Santiago, Chile: MPS (33° 23' S, 70° 36' W), Spatial Studies Centre (SSC) (33° 12' S, 70° 41' W), Lo Espejo (33° 32' S, 70° 43' W), and Rinconada de Maipú (33° 53' S, 71° 21' W). The study comprised determination of the level of parasitism of *P. bliteus* and comparison of the levels of parasitism between the spring-summer seasons of 2004-2005 and 2008-2009 and determination of natural enemies.

Level of parasitism of *P. bliteus*

Three samples per tree of branch tips containing the first seven leaves from ten eucalypt young tree randomly chosen (Erbilgin *et al.*, 2004) were obtained in the stands or rows at each sampling site every 15 days during the spring-summer season, from November 2008 through December 2009. Once samples were collected, they were taken for analysis to the laboratory at the Subdepartment of Forest Vigilance and Official Control of SAG, Santiago. For the analysis of the material collected, 50 developed lerps were selected from each sampling site each time. From these, five clean lerps (without sooty mold) were randomly selected for each tree sampled. Each of these lerps was considered a sample unit. Parasitism was determined under stereoscopic magnification following the categories in Erbilgin *et al.* (2004): parasitized (mummified, greyish nymphs without mobility), consumed (presence of a small round emergence orifice on the nymph parasitized and the lerp, generally on its side), and intact (orange nymphs with darker points and mobile depending on the nymph stage). The parasitized and consumed categories corresponded to parasitism by *P. bliteus* in the lerps selected. The establishment of the parasitoid was expressed as % of parasitism (n° of *P. bliteus*/total n° of lerps) in the 50 lerp sample.

The seasonal action of *P. bliteus* on the pest was determined using data from the four sampling sites obtained every 15 days during the 2008-2009 spring-summer seasons. Percentages of nymphs parasitized, consumed or intact were calculated, together with the population fluctuations of the parasitoid (percentage of *P. bliteus* in mummies and lerps with emergence orifice) and of the psyllid (total number of *G. brimblecombei* nymphs).

Changes in parasitism by *P. bliteus* from 2004-2005 to 2008-2009 spring-summer seasons

Data obtained in this study in the same sampling sites as in 2004-2005 (SAG, 2006), allowed to compare the impact of de *P. bliteus* on *G. brimblecombei* in Santiago between both seasons. After importing, rearing and liberating *P. bliteus*, SAG began in 2004 to evaluate the biological control program in three sampling points in Santiago, Chile (MPS, Las Lilas Farm, and SSC), simultaneously with the monitoring corresponding to all that year. During 2005 the program continued being evaluated, adding as sampling sites in the Metropolitan Region, Lo Espejo and Rinconada de Maipú.

Determination of natural enemies

To know the possible existence of other natural enemies acting on the psyllid were collected at two sampling sites, MPS and SSC, using yellow sticky card traps 10 cm in diameter. The traps were placed in tree at 1.5 m of height. Ten eucalypt trees were selected at random to set each trap. All of them were taken every 15 days to the laboratory to determine predators collected.

Results

Level of parasitism by *P. bliteus*

The establishment of *P. bliteus* did not reach 35% in all sites evaluated from the last week of November through the second one of December 2008 (Fig. 1). However, parasitism during the second week of January 2009 varied between 52 and 72%, and the following samples had constant levels of parasitism greater than 60%. The higher levels were detected the second week of March 2009 (over 75%), and reached 100% in the MPS.

Parasitism of *P. bliteus* in the MPS increased progressively during the season (Fig. 2a), as the nymphs consumed grew from 6% at the beginning of the study to 98% at the end. With respect to nymphs being parasitized at the moment of the sampling, they were present always in the samples, ranging from 15-30% and during the first two months, and decreasing later to levels < 10%. The intact nymphs category declined continuously, from 74% at the beginning until they practically disappeared at the end of the season. Total

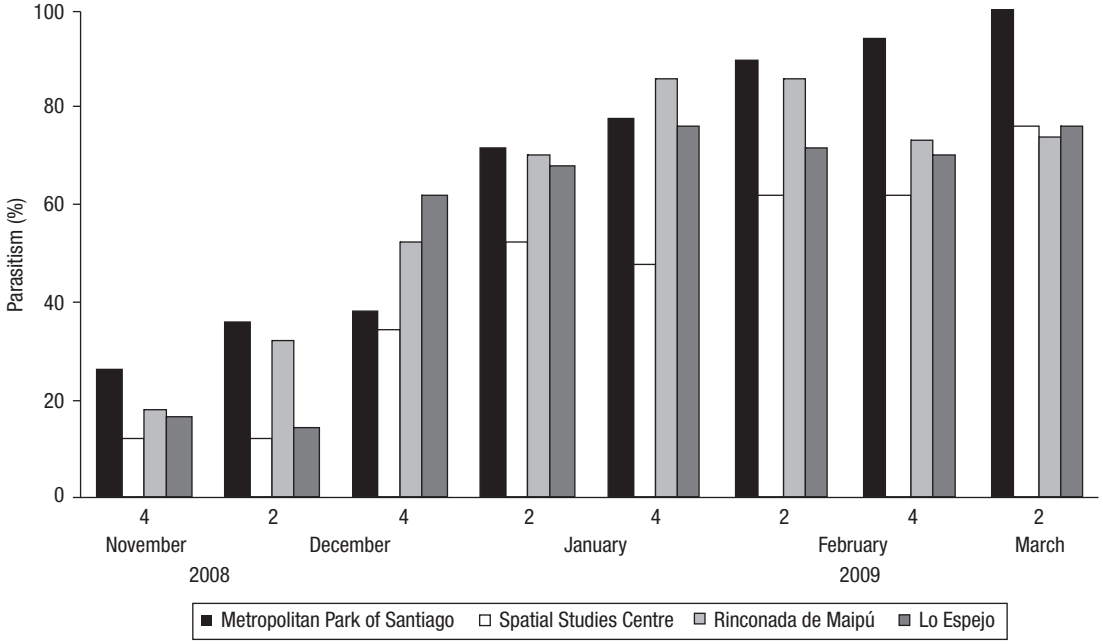

Figure 1. Parasitism (%) of *Glycaspis brimblecombei* by *Psyllaephagus bliteus* in the sampling sites in the 2nd and 4th weeks of November 2008 through March 2009 in Santiago, Chile.

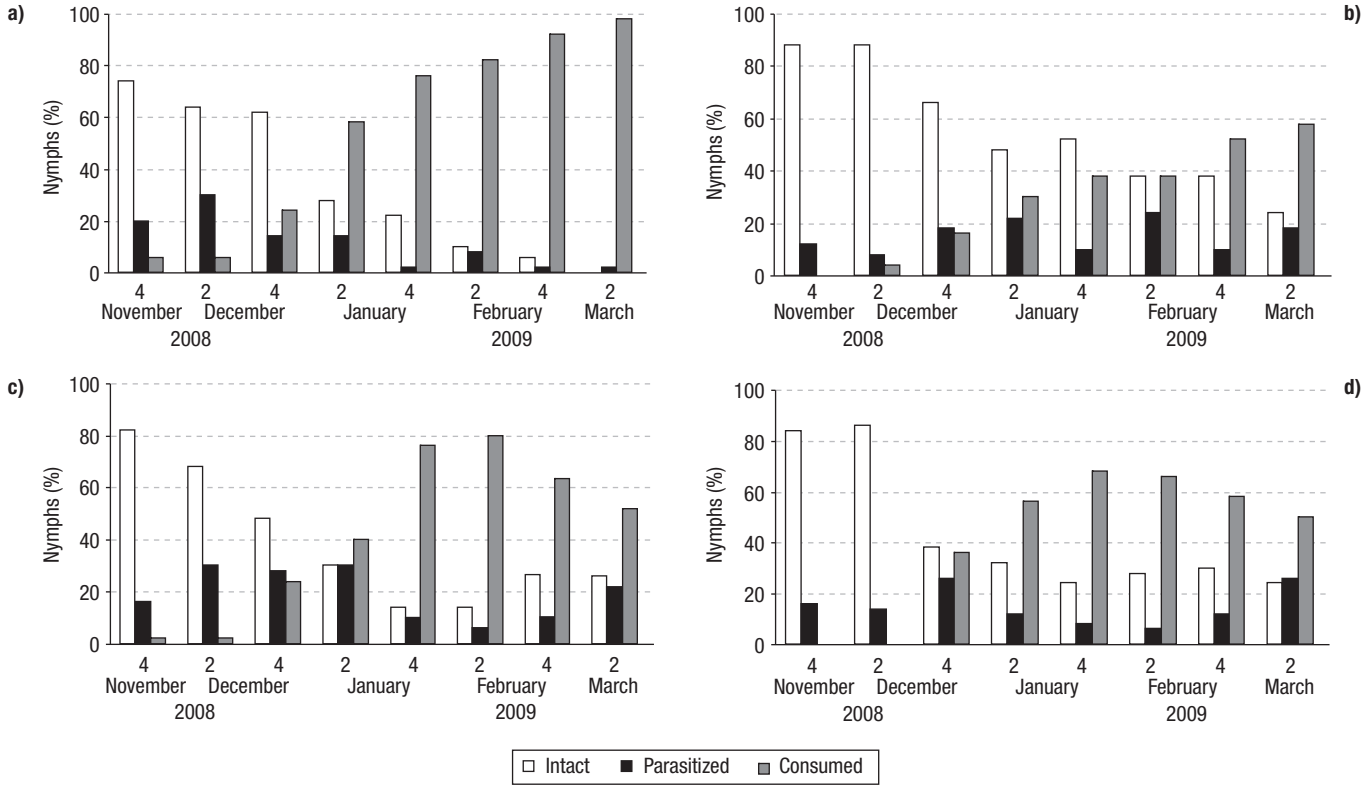

Figure 2. Seasonal development of parasitism by *Psyllaephagus bliteus* on *Glycaspis brimblecombei* measured as nymphs intact, parasitized and consumed in the sampling sites in the 2nd and 4th weeks of November 2008 through March 2009 in Santiago, Chile. a) Metropolitan Park of Santiago, b) Spatial Studies Centre, c) Rinconada de Maipú, and d) Lo Espejo.

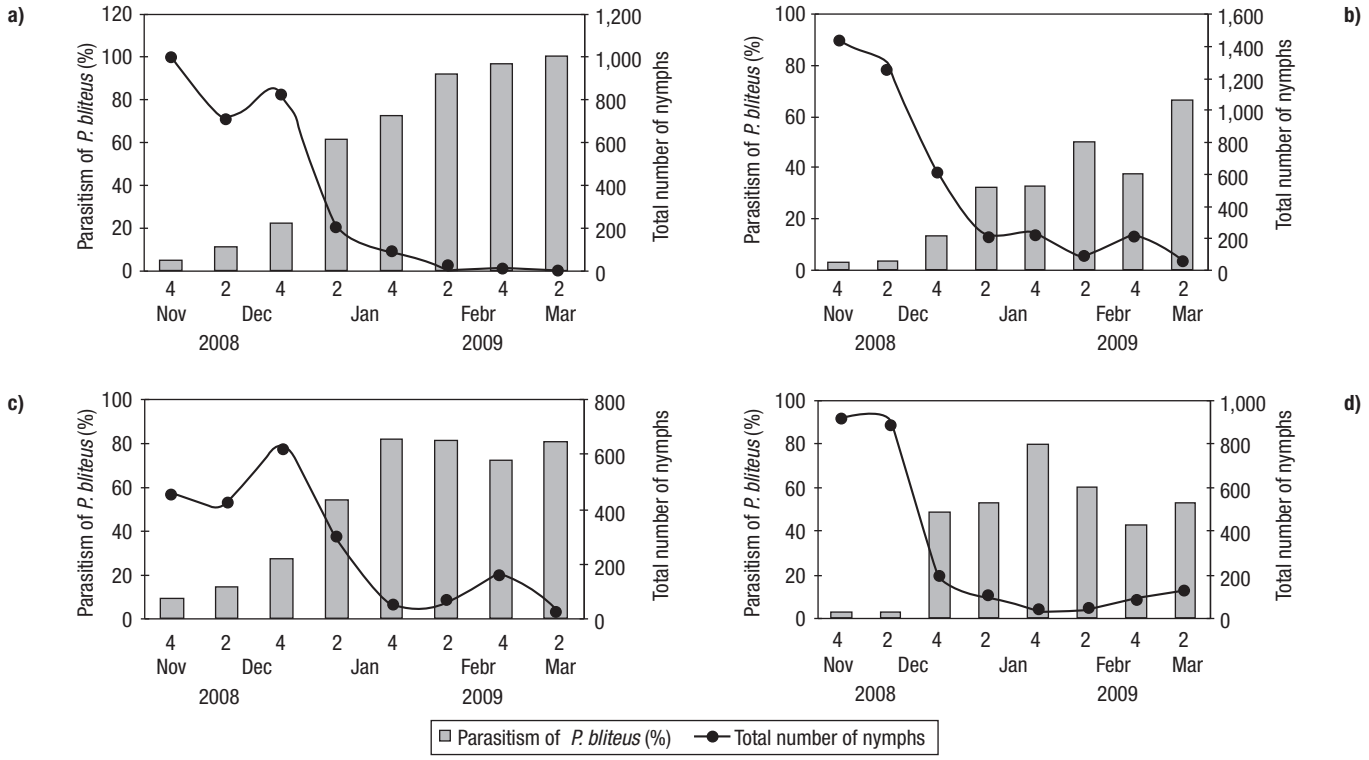

Figure 3. Total number of *Glycaspis brimblecombei* nymphs and nymphs parasitized by *Psyllaephagus bliteus* (%) in the 2nd and 4th weeks of November 2008 through March 2009 in Santiago, Chile. a) Metropolitan Park of Santiago, b) Spatial Studies Centre, c) Rinconada de Maipú, and d) Lo Espejo.

number of nymphs of all stages on the leaves collected and the proportion (%) of mummies and lerps with *P. bliteus* emergence orifice were determined (Fig. 3a). Over 800 nymphs of *G. brimblecombei* were obtained in the first sampling at MPS whereas *P. bliteus* had the least parasitism levels of the season (5%). From the last week of 2008, a sharp decrease of the nymph population occurred, simultaneously with the increasing of the parasitoid. This trend was confirmed the last three sampling dates, with > 92% parasitism by *P. bliteus*, and minimum intact nymphs.

Intact nymphs *G. brimblecombei* followed a similar pattern at the SSC (Fig. 2b) site but occurred at greater proportions during the whole season, scoring 88-66% during the last 3 sampling occasions and still around 50% by the end of January. Thus, *P. bliteus* had lower levels of parasitism in this site. The nymphs being parasitized varied between 8 and 12% early in the season, rose to its higher values (24%) at the end of February. There was an increasing seasonal trend in the number of consumed nymph, starting at none in the first sampling and reaching a 52% at the end of the season, far from the levels found in the MPS site. Density of *G. brimblecombei* nymphs in this second site was higher (Fig. 3b), over 1,250 individuals on each of the first two samplings. There was a steady decrease in the number of nymphs, being halved by the end of December, and gradually lowering to less than one hundred (56) late in the season. At the same time, *P. bliteus* occurred initially at lowest levels (3-4%), reached moderate levels (50%) by mid season and went up to highest parasitism in the last sampling (66%).

In Rinconada de Maipú, intact nymphs followed a steady decreasing trend from its maximun of 84% at the onset of the sampling to a minimum of 14% at the end of February, then nearing 30% by the end the season (Fig. 2c). From the last week of November to the second week of January 2009, there were between 16 and 30% of nymphs parasitized by *P. bliteus*, then proportion of nymphs under parasitism dropped to 6-22% during the second part of the season. *P. bliteus* impact in the consumed category increased from initial minimum levels (2%), steadily increasing during the following two months, to peak at 80% in the second week of February 2009, and then lowering to 52% by the last sampling. Number of nymphs of all stages was

not as high as in the two sites above sampling (Fig. 3c), starting at 420 individuals, and peaking with 620 nymphs by the end of December, when parasitism was only 27%. The second week of January 2009 the nymphs decreased almost by half (299 individuals), and *P. bliteus* increased significantly to 54%. Afterwards, samplings indicated relatively constant parasitism levels, around 80%, acting on a low number of nymphs, only 36, the least of the season, by the last sampling.

The intact nymphs class presented high levels (> 80%) in the first two samplings in Lo Espejo (Fig. 2d), but decreased considerably in the remaining samplings, staying quite stable at 28%. Parasitized nymphs occurred initially in 16% of the cases, and fluctuated through the sampling period from minimum 6% to a peak of 26% at the final sampling. The appearance of *P. bliteus* adults occurred later than in the other sites, as there were no consumed nymphs in the first two samplings. From the last week of December 2008 through the second one of March, the consumed nymphs category was always over 50%, being highest at the end January (68%). The population of nymphs was quite high (900) in the first two samplings, but later sharply decreased and established in levels between 200 and 100 individuals (Fig. 3d). In period, *P. bliteus* resulted very scarce initially (only 2% of the nymphs parasitized) by then rose considerably and set at a constantly high level for the rest of the season (over 50%). The lowest number of nymphs (36) occurred on January and coincided with the maximum proportion of the parasitoid (80%).

Changes in parasitism by *P. bliteus* from 2004-2005 to 2008-2009 spring-summer seasons

Parasitism levels of by *P. bliteus* at the MPS and SSC were compared from November through March existed from both sites and seasons. The levels in Rinconada de Maipú and Lo Espejo were available in both seasons only from January through March (Fig. 4). Parasitism was found higher in the 2008/9 season than observed four years before, for all sites in most sampling dates compared. There was no record of *P. bliteus* in November 2004 in the two first sampling sites; but it occurred at near 50% in both sites by December, and decreased in January and February and March. In the 2004-2005 season, the MPS averaged higher levels of parasitism (45%; Fig. 4a), than the SSC where parasitism occurred at levels below 25% in average (Fig. 4b). In contrast, in 2008-2009 parasitism increased steadily through season in both localities. In November, the parasitoid was found at levels near 20%, continuously rising to peak over 80% by end of season. In Rinconada de Maipú, parasitism of *P. bliteus* was constantly set at near 20% in 2005, whereas it occurred at 75% during all the 2009 season (Fig. 4c). In Lo Espejo, a similarly notorious difference was evident in March, the only

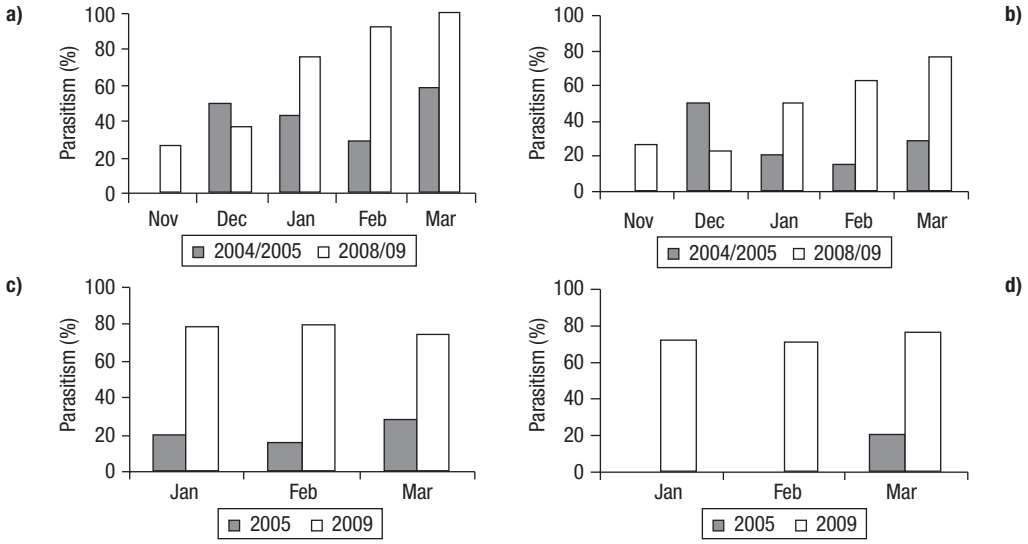

Figure 4. Monthly parasitism levels on *Glycaspis brimblecombei* by *Psyllaephagus bliteus* in the 2004-2005 and 2008-2009 spring-summer seasons at four sampling sites in Santiago, Chile. a) Metropolitan Park of Santiago, b) Spatial Studies Centre, c) Rinconada de Maipú, and d) Lo Espejo.

Table 1. Occurrence of predator species at two sampling sites in 2nd and 4th week of November 2008 through February 2009 in Santiago, Chile

Especies	Nov 2008	Dec 2008		Jan 2009		Febr 2009	
	4	2	4	2	4	2	4
Adalia bipunctata	♦ Δ	♦ Δ	♦	♦ Δ	♦ Δ		♦
Eriopsis connexa	♦ Δ	♦	♦	♦ Δ	♦ Δ		♦
Cycloneda sanguinea	♦ Δ	♦ Δ	♦	♦	♦ Δ		
Crysops sp.		♦		♦	♦ Δ		

♦: Metropolitan Park of Santiago (MPS). Δ: Spatial Studies Centre (SSC).

month with available record to compare both seasons (Fig. 4d).

Determination of predators

Several generalist predator insects were collected by sticker traps at the MPS and SSC sites, which co-existed in the eucalypt-psyllid system (Table 1): *Adalia bipunctata* L. (Coleoptera: Coccinellidae), *Cycloneda sanguinea* L. (Coleoptera: Coccinellidae), *Eriopis connexa* Germar (Coleoptera: Coccinellidae), and *Chrysops* sp. (Neuroptera: Chrysopidae). An unidentified hemerobid species (Neuroptera: Hemerobiidae) and predatory hemipterans (Lygaeidae) were also observed.

Discussion

Our results showed that *P. bliteus* exerted a good biological control on *G. brimblecombei* in Santiago, and this parasitoid could be considered completely established and in this area after six years from release. Similar results were obtained in Mexico, where Sánchez *et al*. (2005) pointed *P. bliteus* as the first successful case of biological control of a pest problem in that country. This biological control program obtained outstanding results in time for establishment and degree of dispersal by *P. bliteus*. It was verified that the wasp required only a year to get established successfully and that dispersed 500 km form its initial point of release, establishing ~80% control. Also, *P. bliteus* got well established in California (Dahlsten, 2005), but with differences between the coastal and inland areas. Studies in that country suggested that summer temperatures in the central valley (7°C higher than in coastal areas) may reduce the impact of the parasitoid, as they affect female lifespan and fecundity (Sime *et al*., 2004). However, it was concluded that, even though *P. bliteus* densities and parasitism level inland were low, its impact could continue growing, although at a slower pace than in coastal areas.

These conclusions may be extended to Chile, as in the 2004, study by SAG important differences occurred in levels of parasitism between the Metropolitan Region and adjacent Valparaiso, the later presenting always greater levels of parasitism than the former, reaching 80% in El Sauce (SAG, 2006). The high parasitism obtained in the four sampling sites (MPS, SSC, Rinconada de Maipú, and Lo Espejo) points out to a successfully development of this classical biological control program in Santiago, greatly due to the desirable attributes for an effective control of *P. bliteus*. This wasp is specific to *G. brimblecombei*, it is multivoltine, the female has a high reproductive rate and lifespan, it is a koinobiont, inserting the eggs into the host, and its life cycle is synchronized to host. All these features ensure strong relationships and maximum impact of a natural enemy on the host target (Hoddle, 2002).

From the point of view of the classical type of biological program established, the use of one specific parasitoid like *P. bliteus* may round up in the median term with this enemy being a permanent control agent that does not require more releases. The results of our study allow to conclude that establishment was successful, and that the required time period previously estimated by SAG were verified completely. The biological control program developed in Chile against the eucalypt pest *Ctenarytaina eucalipti* (Maskell) (Hemiptera: Psyllidae), using *Psyllaephagus pilosus* Noyes (Hymenoptera: Encyrtidae) as control agent also required a few releases for the wasp to become successfully established (SAG, 2002).

The high levels of parasitism by *P. bliteus* during the season suggest a permanent control action of this para-

sitoid on *G. brimblecombei*, as the former follows up naturally the development of the psyllid, affecting nymph density. In our study, maximum parasitism and minimum presence of nymphs of *G. brimblecombei* at any stage always coincided. It is necessary to proceed studying the impact of *P. bliteus* on the psyllid populations for each of the nymphal stages. According to Daane *et al*. (2005), egg laying may occur on all nymph stages, although *P. bliteus* has preference for the larger lerps, that is, the third, fourth, and fifth instar, and eggs are found on the third and fourth instars. Besides the effect of the parasitoid on the last stage nymphs, it is likely that *P. bliteus* adults represent an extra mortality factor on the smaller nymphs, as they may cause wounds when inserting the eggs, and also may feed on the host hemolymph (Rodríguez and Saiz, 2006).

On the other side, the comparison of the 2004-2005 and 2008-2009 seasons revealed a clear quantitative effect in the establishment of *P. bliteus* in Santiago. Despite the successful establishment of the parasitoid, Ide *et al.* (2006) showed that the severity and distribution of the pest is still high, with dense infestations of lerps on the foliage during the summer in this region. Population dynamics and survival of the psyllid, after Toro *et al.* (2003), depend on the weather, particularly the length of the dry season and prevalence of high temperatures. Silva *et al.* (2010) suggests that the maintenance of native vegetation around plantations is a promising managements practice to promote the natural biological of *G. brimblecombei*. Also, factors such as the production of fruits, high soil humidity, and host quality influence the abundance and survival of the psyllid, urban trees being most vulnerable to infestation (Halbert *et al*., 2001). Thus, trees under water stress are more prone to be infested by *G. brimblecombei* (Macías, 2001). On the other hand, Paine and Hanlon (2010), found that there were no differences in parasitism levels of the psyllids by *P. bliteus* under several treatment regimens of irrigation and nitrogen fertilization in field trials.

Several generalist predators were found in two of the sampling sites. Erbilgin *et al*. (2004), stated that understanding the consequences of the interaction between generalist predators and the parasitoid introduced was essential for the success of biological control of the psyllid in California. Their research showed that in the eucalypt-psyllid system, the occurrence of generalist predators, that is, species that do not have major restrictions related to the habitat, influenced the biological efficacy of the parasitoid, and that effective control of the psyllid could not occur in areas with a high population of predators. Bearing these conclusions in mind, it would be necessary to study the specific interactions between these generalists and *P. bliteus* in Chile, in order to promote a more effective biological control method.

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