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# Infestation level and natural enemies of *Prosopidopsylla* sp. (Hemiptera: Psyllidae) on *Neltuma alba* (Fabaceae) in the Chaco Seco ecoregion, Argentina

## Nivel de infestación y enemigos naturales de *Prosopidopsylla* sp. (Hemiptera: Psyllidae) sobre *Neltuma alba* (Fabaceae) en la ecoregión Chaco Seco, Argentina

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## SUMMARY

The native species *Neltuma alba* is promoted in the Chaco Seco ecoregion for its forestry, timber and ethnobotanical potential. *Prosopidopsylla* is a genus of insects that develops mainly on *Neltuma* spp., causing malformations in leaves and their subsequent fall. Due to the value of *N. alba,* the aims were to evaluate the incidence and severity of *Prosopidopsylla* sp. and record its main natural enemies. The study was carried out at the Fernández Experimental Station, Santiago del Estero, Argentina. According to the phenology of the trees, branches of cloned plants from four origins were collected. The abundance of psyllids and its ovipositions as well as the damage were recorded, to calculate the incidence (proportion of leaves on which they were present) and severity (number of specimens or associated damage per leaf). In addition, the abundance of predators, and parasitized immatures were recorded. The results demonstrate that there is a synchronization of the activity of the psyllids with the phenology of *N. alba*, with the bloom period being the most favourable for the development of these insects; there is a differential susceptibility among the different origins of the clones; the region has a variety of predators and parasitoids with the potential to regulate the *Prosopidopsylla* sp. population, the main ones being *Orius* sp., *Zelus renardii* and *Psyllaephagus* sp. The information presented in this work constitutes a basis for the management and selective improvement for *N. alba* resistance against *Prosopidopsylla* sp. and the promotion of its biological controllers in the Chaco Seco ecoregion.

*Keywords:* plant-sap sucker hemipteran, generalist predators, parasitoids, native species, algarrobo blanco.

## RESUMEN

La especie nativa *Neltuma alba* es promocionada en la ecorregión Chaco Seco por su potencial forestal, maderero y etnobotánico. *Prosopidopsylla* es un género que se desarrolla principalmente sobre *Neltuma* spp., ocasionando malformaciones en las hojas y su posterior caída. Debido al valor de *N. alba,* los objetivos fueron evaluar la incidencia y severidad de *Prosopidopsylla* sp. y registrar sus principales enemigos naturales. El estudio fue realizado en la Estación Experimental Fernández, Santiago del Estero, Argentina. Según la etapa fenológica de los árboles, se recolectaron ramas de plantas clonadas de cuatro orígenes geográficos. Se registró la abundancia de psílidos y sus oviposiciones, así como los daños, para calcular la incidencia (proporción de hojas en las que estuvieron presentes) y la severidad (número de ejemplares o daños asociados por hoja). Además, se registraron las abundancias de depredadores y ejemplares parasitoidizados. Los resultados demuestran que existe una sincronización de la actividad de los psílidos con la fenología de *N. alba,* siendo el período de floración el más favorable para el desarrollo de estos insectos; existe una susceptibilidad diferencial entre los diferentes orígenes de los clones; la región dispone de una variedad de depredadores y parasitoides con potencial para regular la población de *Prosopidopsylla* sp., siendo los principales *Orius* sp., *Zelus renardii* y *Psyllaephagus* sp. La información presentada en este trabajo constituye una base para el manejo y mejoramiento selectivo de la resistencia de *N. alba* frente a *Prosopidopsylla* sp. y la promoción de sus controladores biológicos en la ecorregión Chaco Seco.

*Palabras clave:* succionadores de savia, depredadores generalistas, parasitoides, especies nativas, algarrobo blanco.

## INTRODUCTION

Species of the genus *Neltuma* Raf. are considered as "multipurpose species" due to their ability to provide not only quality wood but also wood for fuel such as firewood and charcoal, fruits for human consumption, and fodder for livestock. Their flowers provide nectar and pollen for beekeeping activity (López Lauenstein et al., 2012, Fontana et al., 2020). In turn, it can be used as part of the tree component of silvopastoral and agroforestry systems, improving animal welfare and increasing productivity (López Lauenstein et al., 2012).

*Neltuma alba* (Hughes & Lewis), known as "algarrobo blanco" is a promoted native species in the Chaco Seco ecoregion due to its high forestry and timber potential, in addition to the cultural value it represents for local communities (López Lauenstein et al., 2012).

Currently, due to Argentine National Law 25,080 (renewed by Law 27,487 in 2018) on investments for cultivated forests and Law 26331 on minimum budgets for environmental protection of native forests, the deforestation rate has decreased, and the reforested area with native species has been increasing (Méndez & Martín, 2016). However, most of these plantations receive poor silvicultural management due to lack of knowledge of management techniques under different site conditions and their ecological potential, added to the false perception that they have a low growth rate (Marcó et al., 2014; Salto & Lupi, 2019). In this sense, it is essential to establish which are the main factors that influence the production of these species. These factors include environmental stresses during establishment and subsequent development (Niinemets, 2010), such as damage caused by herbivory (Lemoine et al., 2017).

At the moment, there are *N. alba* improvement programs that, among other things, have allowed the evaluation and improvement of various parameters of economic importance, such as growth traits and tolerance to environmental stresses (Carreras et al., 2016). The selection of material less susceptible or tolerant to herbivory is an effective tool for pest control since it is less expensive and does not have negative effects on natural enemies (Mojahed et al., 2012). In this sense, clonal orchards have been established from improved materials that were selected for their productive traits as well as their tolerance to phytophagous insects, fungi, and salinity (Carreras et al., 2016; Ewens et al., 2021); however, evaluations on the susceptibility to herbivory of these genetic materials are still incipient.

Herbivores associated with *N. alba* include a great diversity of insects, however, there are relatively few studies that evaluate the damage they cause to trees in Argentina (Carabajal de Belluomini & Fiorentino, 2006; Riquelme et al., 2016; Atanasio et al., 2024). Some studies mention that phytophagous insects are the most abundant during the nursery stage; however, they are rarely mentioned on field-established "algarrobo blanco" trees (Carabajal de Belluomini & Fiorentino, 2006; Riquelme et al., 2016).

The insects belonging to superfamily Psylloidea are small phytophagous hemipterans phloem- feeding characterized by being highly host specific (Burckhardt, 2008; Baliotte et al., 2023). There are only a few major pests, as *Diaphorina citri* and *Cacopsylla pyricola* on fruit orchards, and several other which are occasional pests to crop and ornamental plants, as *Platycorypha erythrinae* (Lizer) associated with *Erythrina cristagalli* and *Calophya schini* Tuthill on *Schinus areira* (Burckhardt, 2008). In heavy infestation these species cause yellowing leaves, distorted leaves and premature leaf drop (Burckhardt, 2008; Ruiz-Galván et al., 2015; Civolani et al., 2023).

The most harmful phytophagous that have been recorded on *Neltuma* spp. are psyllids of genera *Heteropsylla* Crawford and *Prosopidopsylla* Burckhardt, being *Heteropsylla texana* Crawford and *Prosopidopsylla flava* Burckhardt two species used in biological control programs where this tree is considered an invasive species (van Klinken et al., 2003; Mormontoy del Pino et al., 2020). *Prosopidopsylla* is a small genus with five described species and around ten undescribed species, distributed in Argentina, Bolivia, Brazil, and Chile, mainly developing on *Neltuma* trees (Burckhardt, 2008; Baliotte et al., 2024). On the other hand, predators and parasitoids are known as enemies of Psylloidea, corresponding to the insect orders Hemiptera, Coleoptera, Neuroptera, Diptera, and Hymenoptera (Hollis, 2004). However, there are no records of natural enemies to *Prosopidopsylla* in its native range.

The aim of this work is to determine the susceptibility of *N. alba* to *Prosopidopsylla* sp. infestation among phenological stages from different origins and to identify their potential natural enemies in the Chaco Seco ecoregion. The following hypotheses are addressed: 1 - the level of infestation and damage varies throughout the year according to the phenological stages of *N. alba*; 2 - susceptibility varies among clones of different origins; 3 - within the plantations, some natural enemies can regulate the psyllid populations.

## METHODS

*Study site.* The study was carried out at the Fernández Experimental Station (unit dependent on the Catholic University of Santiago del Estero within the framework of the agreement with the province of Santiago del Estero), located in the Chaco Seco ecoregion, Argentina (27.56°S 63.52°W). The area is characterized by an average temperature of 20 °C and annual rainfall that averages 600 mm with a marked seasonality towards summer (data from a 37-year series provided by the INTA-La María meteorological station, Santiago del Estero) (INTA 2018).

*Plant material.* The experimental plot was established in 2002 and consists of *N. alba* clones chosen after an initial selection based on plant height, pod production and pod sweetness (Ewens et al., 2021). The experimental design corresponds to that of the original plantation, in which the plants were placed following a randomized complete block design with four replications, covering a total area of 5,200 m$^2$. For this study, a total of 28 plants corresponding to four origins of *N. alba* were evaluated: Río Dulce (from the system irrigated by the Río Dulce river in Santiago del Estero province), Pinto (from the system irrigated by the Río Salado river in Santiago del Estero province), Ibarreta (from the Formosa province) and Castelli (from the Chaco province).

*Sampling.* Samplings were carried out between 2019 and 2022 according to phenology of the plants. The phenological stages that were considered are: dormancy (winter rest period), bloom (development of leaves and inflorescences), fructification (pod formation), pod maturation (development of seeds within pods), harvest (fully developed pods and seeds) and postharvest (podless period prior to dormancy). On each date, two branches of 20 cm length were randomly taken from the middle of the canopy of each plant. Samples were taken to the Laboratory of Agricultural Zoology of the National University of Luján and, using a Stemi 508 Zeiss® stereoscopic microscope, the damage and number of eggs, immatures and adults of psyllids were recorded. Damage is considered to be those malformations observed as a shortening of the rachis, which gives the leaves a compressed appearance. In addition, the abundance of predators was recorded and the parasitized psyllid immatures were reared under controlled conditions of temperature (25 ± 2 °C) until the emergence of parasitoid wasps. The material was preserved in 70% alcohol and sent for identification to the División Entomología, Museo de La Plata, Buenos Aires, Argentina.

*Data analysis.* For each plant origin and phenological stage, the following parameters were estimated:

$$Damage\ incidence = \frac{Damaged\ leaves}{Total\ number\ of\ leaves} \qquad [1]$$

$$Damage\ severity = \frac{Damaged\ leaflets}{Leaf} \qquad [2]$$

$$Prosopidopsylla\ sp.\ incidence = \frac{Infested\ leaves\ with\ Prosopidopsylla\ sp.}{Totalnumberofleaves} \qquad [3]$$

$$Prosopidopsylla\ sp.\ severity = \frac{Abundance\ of\ eggs\ or\ Prosopidopsylla\ sp.}{Leaf} \qquad [4]$$

$$Predators\ abundance = \frac{Abundance\ of\ each\ predator}{Leaf} \qquad [5]$$

$$Parasitoidism\ rate = \frac{Parasitized\ immatures}{Total\ number\ of\ immatures} \qquad [6]$$

To examine the effect of the clones origin and the phenological stage, data were analysed using generalized linear models assuming binomial and poisson distributions. When analyses were significant, the differences among treatments were determined by the Tukey's test. In the case of natural enemies, only phenology was taken into account for the analyses.

All the analyses were carried out using the RStudio software and a significance level of 5% was taken into account ($P < 0.05$).

*Material examined. Prosopidopsylla* sp. 200 ♂, 203 ♀, 106 immatures; Fernández, Santiago del Estero, Argentina; 8.x.2019; col. Gualterio Barrientos. Specimens obtained during this work were conditioned for its study and its correct identification following Burckhardt (1987) and Burckhardt (2008).

*Parasitoids. Psyllaephagus* sp. 2 ♀, 1 ♂; *Chartocerus* sp. 1 ♀; *Prochiloneurus* sp. 1 ♀; Fernández, Santiago del Estero, Argentina; 8.x.2019; col. Gualterio Barrientos. Specimens obtained during this work were conditioned for its study and its correct identification following Gibson (1997), Noyes (1980, 1994) and Woolley (1988).

*Predators. Zelus renardii* 22 ♀; *Orius* sp. 38 ♂, 25 ♀; Fernández, Santiago del Estero, Argentina; 8.x.2019; col. Gualterio Barrientos. Specimens obtained during this work were conditioned for its study and its correct identification following Herring (1966) and Zhang et al. (2016).

## RESULTS

When comparing the incidence, it was observed that in every phenological stage the Pinto and Ibarreta clones were the ones that generally had the highest incidence, while the Castelli clones were the ones with the lowest (Figure 1A). In turn, the incidence in the Pinto clones during the harvest period and in the Ibarreta clones during bloom, fructification and harvest periods was significantly higher than that found during the dormancy period, while in the remaining stages it was intermediate. On the other hand, the damage severity was significantly higher in Ibarreta clones than in Rio Dulce ones during the bloom and maturation stages (Figure 1B).

Regarding the oviposition records, the highest severity was observed during the fructification and maturation stages (Figure 2). The number of ovipositions per leaf was greater in the Río Dulce and Ibarreta clones than in Castelli and Pinto clones during fructification; however, during maturation the Ibarreta clones did not differ from Castelli clones, and the Pinto clones had intermediate values. Regardless of the phenological stage, the Castelli clones were generally the least preferred as oviposition site. In the case of Pinto clones, the severity during the dormancy period was significantly higher than the other origins and it was not statistically different from the values obtained during fructification and maturation periods (Figure 2).

On the other hand, the incidence (Figure 3) and severity (Figure 4) of immature and adult stages of *Prosopidopsylla* sp. were maximum during bloom stage, while in the rest of the phenological stages remained constant and relatively lower. Independent from clone and phenological stage, immatures were always more abundant than adults. Regarding the clones, Castelli stood out for being the ones with the lowest incidence and severity of psyllids in general.

During the sampling, predatory insects that could feed on *Prosopidopsylla* sp. were also captured. Figure 5 shows the abundances of each predator according to phenology, regardless of origin. The main predators recorded on site were *Zelus renardii* Kolenati (Hemiptera: Reduviidae) and *Orius* sp. Wolff (Hemiptera: Anthocoridae). The maximum abundance of these species occurred in different periods, not having overlapped, since *Orius* sp. was dominant during bloom, while in the other hand, *Z. renar-*

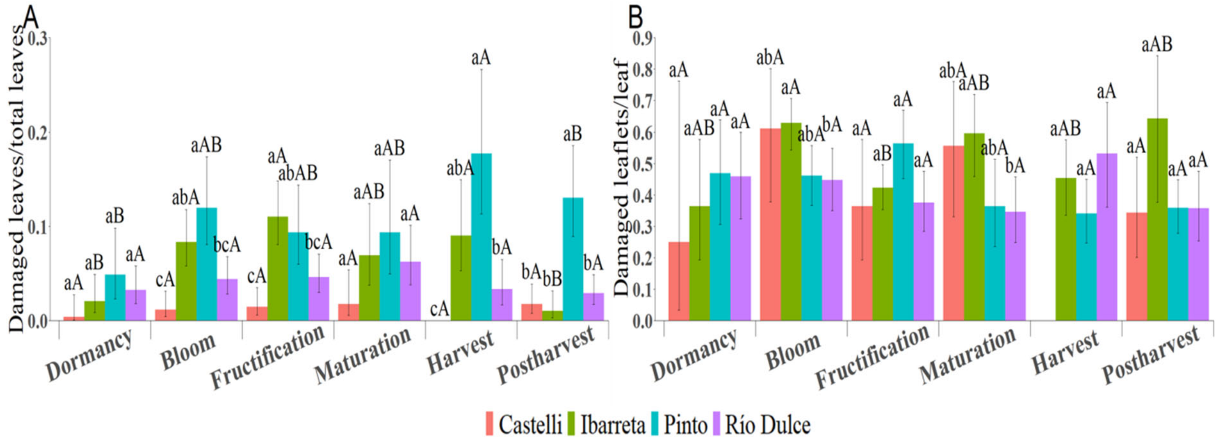

**Figure 1.** A- Damage incidence and B- damage severity (mean ± sd) of *Prosopidopsylla* sp. on *N. alba*. Different lowercase in the same phenological stage and uppercase letters in the same origin indicate significant differences by Tukey's test ($P < 0.05$) among origins and phenological stage, respectively.

A- Incidencia y B- severidad de los daños de *Prosopidopsylla* sp. sobre *N. alba.* Diferentes letras minúsculas en una misma etapa fenológica y letras mayúsculas en un mismo origen indican diferencias significativas según la prueba de Tukey ($P < 0,05$) entre orígenes y etapas fenológicas, respectivamente.

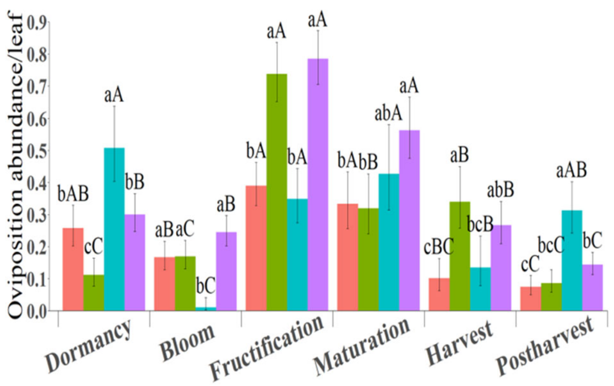

**Figure 2.** Oviposition severity of *Prosopidopsylla* sp. (mean ± sd). Different lowercase in the same phenological stage and uppercase letters in the same origin indicate significant differences by Tukey's test ($P < 0.05$) among origins and phenological stage, respectively.

Severidad de oviposición de *Prosopidopsylla* sp. Diferentes letras minúsculas en una misma etapa fenológica y letras mayúsculas en un mismo origen indican diferencias significativas según la prueba de Tukey ($P < 0,05$) entre orígenes y etapas fenológicas, respectivamente.

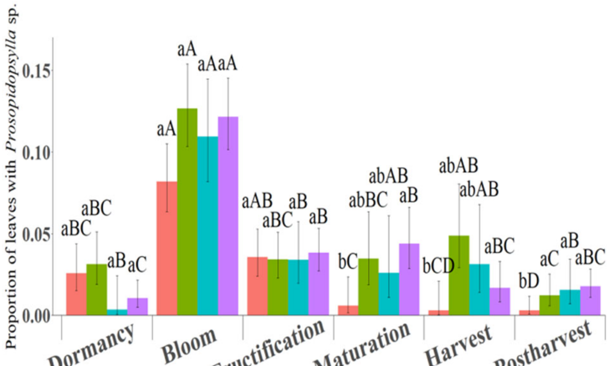

**Figure 3.** Incidence of adults and immatures of *Prosopidopsylla* sp. (mean ± sd). Different lowercase in the same phenological stage and uppercase letters in the same origin indicate significant differences by Tukey's test ($P < 0.05$) among origins and phenological stage, respectively.

Incidencia de adultos e inmaduros de *Prosopidopsylla* sp. Diferentes letras minúsculas en una misma etapa fenológica y letras mayúsculas en un mismo origen indican diferencias significativas según la prueba de Tukey ($P < 0,05$) entre orígenes y etapas fenológicas, respectivamente.

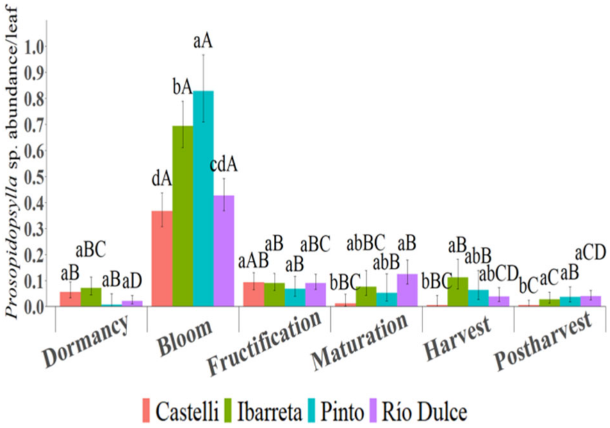

**Figure 4.** Severity of adults and immatures of *Prosopidopsylla* sp. (mean ± sd). Different lowercase in the same phenological stage and uppercase letters in the same origin indicate significant differences by Tukey's test ($P < 0.05$) among origins and phenological stage, respectively.

Severidad de adultos e inmaduros de *Prosopidopsylla* sp. Diferentes letras minúsculas en una misma etapa fenológica y letras mayúsculas en un mismo origen indican diferencias significativas según la prueba de Tukey ($P < 0,05$) entre orígenes y etapas fenológicas, respectivamente.

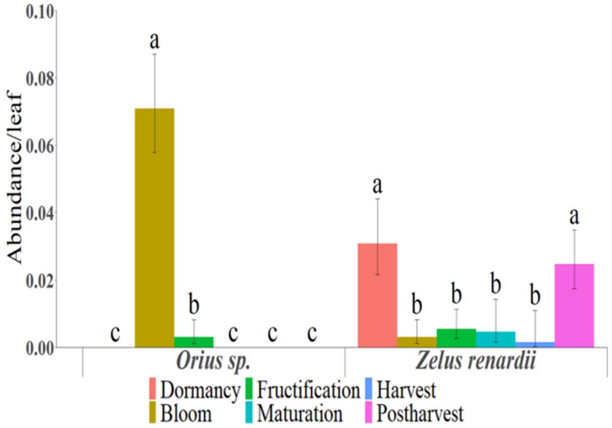

**Figure 5.** Predators abundance per leaf (mean ± sd). Different letters in the same species indicate significant differences by Tukey's test ($P < 0.05$) among phenological stages.

Abundancia de depredadores por hoja. Letras diferentes para la misma especie indican diferencias significativas según la prueba de Tukey ($P < 0,05$) entre etapas fenológicas, respectivamente.

*dii* was more abundant during dormancy and postharvest periods.

Regarding parasitoidism, the adults obtained from parasitoidized immatures belong to the genera *Psyllaephagus* Ashmead (Hymenoptera: Encyrtidae), *Chartocerus* Motschulsky (Hymenoptera: Signiphoridae) and *Prochiloneurus* Silvestri (Hymenoptera: Encyrtidae). The parasitoidism rate was 36.8%, being the fructification period the one with the highest rate.

## DISCUSSION

The results provided in this work demonstrate that there is a variation in the density of psyllids and their associated damage among phenological stages in clones from different origins. In general, it was observed that the Pinto and Ibarreta clones are the most susceptible and the Castelli clones are the most tolerant, in agreement with what was observed by Ewens and Felker (2010). As mentioned by other authors, the distribution of herbivores depends largely on the presence of their host plants in optimal conditions to sustain their growth and development (Hodkinson, 2009; Jia et al., 2023). It is important to highlight intraspecific susceptibility since for the same host species different degrees of susceptibility can occur depending on its place of origin, cultivar or variety, which could sometimes represent a great source of variability (Liu & Trumble, 2006; Jia et al., 2023). In the case of *Neltuma* trees, this differential susceptibility is enhanced by the great phenotypic variation within each species and the natural hybridization that occurs between them, resulting in plant families adapted to their place of origin (Fontana et al., 2020; Ewens et al., 2021). In this sense, susceptible hosts, such as the Pinto and Ibarreta clones, possibly present intrinsic characteristics that are favourable to *Prosopidopsylla* sp. Among these characteristics could be mentioned the tissue resistance to penetration, the colour of the leaves, the presence of attractive chemical compounds or the nutritional composition; which could influence variables such as initial attraction, oviposition rate, survival rate and development time among others (Liu & Trumble, 2006; Mauck et al., 2023).

In addition to the availability of hosts, to complete their development in seasonal environments it is essential that the herbivore cycle is synchronized with the phenology of their hosts (Hodkinson, 2009; Mauck et al., 2023). *Neltuma alba* is a seasonal species that is dormant during the winter and does not produce new tissues until the arrival of spring (Parizek et al., 2000). In the present study, during dormancy, the lowest incidence of damage was recorded, possibly due to a joint effect of the lower activity of psyllids in winter, natural defoliation and defoliation by herbivores prior to dormancy, as it was found by Mormontoy del Pino et al. (2020) in *Neltuma pallida* (Hughes & Lewis) in Peru for *Heteropsylla texana* (probably a misidentification of *H. obscura*). In turn, it is during this time that fewer immatures and adults were captured, while the number of ovipositions was not affected. This agrees with the general biology of Psylloidea, since the development of each generation is strongly influenced by the phenology of the host plant in which the immature will develop (Civolani et al., 2023; Mauck et al., 2023). Under the local environmental conditions there is a significant delay in development time during the winter as it was seen in *Prosopidopsylla flava* Burckhardt in Australia (van Klinken et al., 2003).

Blooming occurs during spring after the winter dormancy. During spring not only daily temperatures and day length are greater but also the availability of tender tissues increases, which are the main food resource of these insects (van Klinken et al., 2003; Hodkinson, 2009), resulting in maximum severity. Furthermore, most of the psyllid population was represented by the immature stage, which has been observed for this and other psyllid genera (van Klinken et al., 2003; Hodkinson, 2009; Mormontoy del Pino et al., 2020). In addition to being the most abundant, the immatures are responsible for most of the damage caused to new shoots, inflorescences and tender fruits because they are in continuous feeding activity (van Klinken et al., 2003; Mormontoy del Pino et al., 2020). This information turns out to be a valuable contribution since in pest management plans it is important to detect the optimal moment for the application of control measures. Psyllid pest control is usually carried out during the blooming season, when the most active individuals can be reached, increasing control efficiency (Ruiz-Galván et al., 2015; Civolani et al., 2023). Based on our results, the blooming period could be the appropriate moment to control *Prosopidopsylla* sp., since this is when there is the greatest population activity.

Oviposition was at a maximum during the fructification and maturation periods, possibly as consequence of the population peak produced during bloom, since females prefer to lay eggs on young and undeveloped leaves (van Klinken et al., 2003). However, a high number of ovipositions were recorded during dormancy in Pinto clones, which could be due to the fact that these trees, with a more voluminous crown, provide greater cover against environmental conditions (Ewens personal communication [August, 2024]). In this sense, some species of Psyllidae use woody species with a more complex canopy architecture as a refuge (Mauck et al., 2023).

Regarding damage, the harvest period had the highest incidence, while the lowest was during dormancy. This is probably due to the fact that the damage produced in the stages prior to harvest had accumulated, based on the fact that the highest severity of psyllids was recorded during bloom period and then decreases abruptly, however the damage incidence did not vary considerably, which is consistent with the fact that *N. alba* only produces new foliage in the bloom period (Parizek et al., 2000).

Also, this work has been useful to make a first approach to the natural enemies of *Prosopidopsylla* sp. that are available in the region. On the predator side, the main ones were the genera *Orius* and *Zelus*. Although these genera have already been cited in other works as predators of psyllids, in most of them they were always the least relevant, highlighting other arthropods such as spiders (Araneae) and coccinelids (Coleoptera) as the main biological regulators (Lyimo, 2016; Mormontoy del Pino et al., 2020). Regarding its population dynamics, *Orius* sp. has been found mainly in bloom period in coincidence with the maximum severity of psyllids, while *Z. renardii* was observed mainly in postharvest and dormancy. These events could be explained by the fact that both genera have generalist habits and can feed on different prey species, in addition to the fact that, due to the seasonal variation in the life cycle of psyllids, the supply of the prey may be scarce at certain periods and, consequently, predators could move to other plant species in search of new prey (Horton, 2024). It is important to mention that the recorded abundances of these predators were very low, possibly due to the sampling method, so for future evaluations it is recommended to make direct field observations.

This survey identified *Psyllaephagus, Chartocerus* and *Prochiloneurus* as the first record of parasitoids associated with *Prosopidopsylla* (Bistline-East & Hoddle, 2014; Lyimo, 2016; Triapitsyn et al., 2020; Marin et al., 2023). *Chartocerus* is mentioned as hyperparasitoid of Encyrtidae and Aphelinidae in different Sternorrhyncha including psyllids, and commonly parasitizing *Prochiloneurus* (Woolley, 1988; Löhr et al., 1990). However, based on the observations of this work, it was not possible to infer whether there was hyperparasitism by *Chartocerus* sp. and *Prochiloneurus* sp., which have been reported for several host species, which do not include *Psyllaephagus* sp. (Bistline-East & Hoddle, 2014; Triapitsyn et al., 2020).

The results of this work provide new information about the interaction of *Prosopidopsylla* sp. with *N. alba* and its natural enemies in the Chaco Seco, which contributes significantly to the knowledge about the pest associated to this native tree. The information presented here constitutes a basis for the management and selective improvement for *N. alba* resistance against *Prosopidopsylla* sp. and the promotion of its biological controllers in the Chaco Seco. Therefore, it is important to include Castelli clones in future breeding programs, and pay special attention to the bloom period for the detection and management of *Prosopidopsylla* sp.

## AUTHOR CONTRIBUTIONS

GB, AD, MS, ME and MRV were in charge of conceptualization, experimental design, methodology, sample collection, analysis and interpretation of results. CB, EM, FV, AA and DAA contributed to the methodology and discussion. EC and MRV were in charge of funding acquisition and project administration. All authors prepared the manuscript.

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