

Bionomics of *Neolasioptera aculeatae* (Diptera: Cecidomyiidae), a promising biological control candidate against *Parkinsonia aculeata* (Fabaceae)

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Bionomía de *Neolasioptera aculeatae* (Diptera: Cecidomyiidae), un agente promisorio para el control biológico de *Parkinsonia aculeata* (Fabaceae)

RESUMEN. Inspecciones de campo realizadas sobre *Parkinsonia aculeata* L. en el Norte-centro de Argentina entre 2008 y 2011 revelaron la presencia del mosquito agallícola *Neolasioptera aculeatae* Gagné (Diptera: Cecidomyiidae). La presencia de las agallas de *N. aculeatae* está restringida a la distribución norte de *P. aculeata*. La disección de agallas recolectadas a lo largo del año, reveló la presencia de larvas y/o pupas en distintos estados fenológicos de *P. aculeata*. La emergencia de adultos de *N. aculeatae* tuvo lugar 13 a 34 días desde la recolección en el campo y se extendió por un período promedio de 22 días. Entre once especies de leguminosas inspeccionadas en el campo, adultos de *N. aculeatae* emergieron únicamente de agallas recolectadas sobre *P. aculeata*. Los atributos biológicos y el restringido conjunto de plantas hospederas utilizadas en el campo, hacen de *N. aculeatae* un agente promisorio para el control biológico de *P. aculeata*.

PALABRAS CLAVE. Inspecciones de campo. Enemigos naturales. Control biológico de malezas. *Parkinsonia*. Cina-cina.

ABSTRACT. Field surveys conducted in North-central Argentina on *Parkinsonia aculeata* L. between 2008 and 2011 revealed the presence of the stem-galling midge *Neolasioptera aculeatae* Gagné (Diptera: Cecidomyiidae). Bionomical and field host range studies were conducted to determine the insect's suitability for biological control of *P. aculeata*. Presence of *N. aculeatae* galls was restricted to the northern distribution of *P. aculeata*. Larvae and/or pupae were found by dissecting galls collected throughout the year on *P. aculeata* plants at different phenological stages. Emergence of *N. aculeatae* adults occurred 13 to 34 days from field collection and over an average period of 22 days. Field host range surveys indicated that of the 11 legume species sampled, *N. aculeatae* adults only emerged from galls collected on *P. aculeata*. The biological attributes of *N. aculeatae* and its restricted field host range suggests that it will be a promising biological control agent for *P. aculeata*.

KEY WORDS. Field host range. Natural enemies. Weed biological control. *Parkinsonia*. Cina-cina.

INTRODUCTION

Parkinsonia aculeata L. ("cina-cina") (Leguminosae: Caesalpinioideae) is a thorny leguminous shrub native to the hot and dry regions of

North, Central and South America (Hawkins *et al.*, 2007). This species has a pan-tropical distribution following introduction as an ornamental, hedging, fodder and shade tree (Stewart *et al.*, 1992; Wagner *et al.*, 1999; PIER, 1999; Hawkins,

2001). In Australia, *P. aculeata* is recognized as one of the 20 worst weeds (Thorp & Lynch, 2000) and considered a weed of national significance due to its impacts on the environment and agricultural production (Heard & Bell, 2009). The detrimental effects include its propensity to form dense, thorny, impenetrable thickets along drainage lines, depressions, ephemeral wetlands and, to a lesser extent, uplands (van Klinken *et al.*, 2009).

Control techniques available to manage *P. aculeata* include the use of herbicides, machinery, fire, grazing, and classical biological control (Deveze, 2004; van Klinken *et al.*, 2009). Biological control efforts began in the 1980s and resulted in the release of three insect species: *Rhinacloa callicrates* Herring (Miridae: Hemiptera) (a sap-sucking mirid), *Mimosestes ulkei* (Horn) (Bruchidae: Coleoptera) from North America and *Penthobruchus germani* (Pic) (Bruchidae: Coleoptera) (seed-feeding bruchids) from Argentina (Julien & Griffiths, 1998; Flanagan *et al.*, 1996; Donnelly, 2000; Briano *et al.*, 2002; van Klinken *et al.*, 2008). Existing agents, do not appear to be having a significant impact, and new potential agents are unlikely to be found in the United States or northern Mexico (van Klinken *et al.*, 2009).

Recent genetic studies indicating very old dispersal events of *P. aculeata* in South America (Hawkins *et al.*, 2007), motivated the search for additional natural enemies in this area. Field surveys conducted recently in Argentina, revealed the presence of *Neolasioptera aculeatae* Gagné (Diptera: Cecidomyiidae), a gall midge responsible for a common and conspicuous stem gall that stunts the branches and often curbs further axillary growth of *P. aculeata* (Gagné *et al.*, 2011). It has been proposed as a biological control agent against *P. aculeata* (van Klinken & Heard, 2012).

Neolasioptera (Alycaulini) is a species-rich genus from the Americas, whose species are primary gall makers on a wide array of plant families and appear to be mostly host specific (Gagné, 1994, 2010). From the 65 described Neotropical *Neolasioptera*, only five species have been recorded from Argentina, with *N. aculeatae* being the only one associated with *P. aculeata* (Gagné, 2010; Gagné *et al.*, 2011). In this paper we present bionomical and field host range data on *N. aculeatae*, and discuss its potential as a

biological control agent against *P. aculeata*.

MATERIAL AND METHODS

The distribution of *N. aculeatae* was determined through extensive exploratory trips made along the main roads of North-central Argentina, between 2008 and 2011 (Fig. 1). In total, 20 surveys were conducted with 29 sites inspected, some of which were visited up to four times a year to account for seasonal variations. Surveys generally lasted seven days, and included two to three collectors. Time spent on-site rarely exceeded two hours. *P. aculeata* plants occurring mainly along the roadsides, rangelands and river beds were visually inspected for the presence of galls. Seasonal occurrence and field host range surveys were conducted at several sites along a longitudinal range of c. 500 km in northern Argentina (Chaco, Formosa and Salta provinces) (Fig. 1). The area has a tropical summer rainfall climate (world climate zone 2, as described by Walter *et al.*, 1975) with a mean annual temperature of 22 °C and annual rainfalls (500-1,000 mm), concentrated in the spring-summer months (October-March) (Servicio Meteorológico Nacional, 2000).

Seasonal occurrence

Plants of *P. aculeata* were sampled four times between March and November 2010 (Table I). For each site, the number of surveyed plants and the number of galls were recorded. Phenological stage of the plants was registered as flowering, fruiting or vegetative. Only fresh active galls with soft green tissue and developing insects were included in the study. Galls were collected by cutting *P. aculeata* branches with a heavy-duty pruner, kept in plastic containers and transported to the laboratory. A subsample of galls were dissected under a stereo microscope (Olympus SZ61; maximum magnification: 45X) to determine the life stage of specimens inside the galls. The rest of the galls were kept in controlled environmental chambers (25 ± 2 °C: 60-80% RH; 16:8 L:D) for subsequent emergence of adult specimens.

Field host range

During the summer 2009/10 and 2010/11, nine sites with populations of *P. aculeata* and ten co-occurring legume species were surveyed for

the presence of galls (Fig. 1). Plant species were selected for their phylogenetic proximity, ecological/distribution overlap and morphological similarity to *P. aculeata* (Table II). For each site, the number of surveyed plants and the number of collected galls were recorded. Samples were brought to the laboratory and kept in controlled environmental chambers (25 ± 2 °C; 60-80% RH; 16:8 L:D) for subsequent emergence of adults. Voucher specimens of the plants and insects collected were deposited in collections at the Fundación para el Estudio de Especies Invasivas (FuEDEI), Hurlingham, Buenos Aires.

Laboratory rearing

Preliminary rearing of *N. aculeatae* was attempted three times. On one occasion in September 2009, 10 adults were confined within a gauze-covered sleeve wrapped around the stem of a potted *P. aculeata* plant. A piece of cotton soaked in a water-sucrose solution was suspended from the upper access hole of the sleeve as a food source for the adults. A second attempt was conducted in 2011 when a collection of 800

galls rendered 400 adults (67 % female). A balanced number of male and female adults (9-51) were confined in 12 rearing aluminium frame cages lined with gauze and measuring 250 x 250 x 800 mm containing one *P. aculeata* potted plant. In 2012, a third rearing attempt was conducted using rearing cages upon a collection of 300 galls that rendered 150 adults.

RESULTS

Distribution. Presence of *N. aculeatae* galls was restricted to the northern distribution area of *P. aculeata* in Argentina (Fig. 1). Stem galls were collected from sites as far north as Embarcación, Salta (S 23.16°) and south to near Fortín Lavalle, Chaco (S 26.13°).

Galls. *N. aculeatae* stem galls were woody rounded/ovoid-shaped growths at the junction of the stem and the spines of *P. aculeata* plants (Fig. 2). Gall size ranged from 3-17 mm in length (9.3 ± 2.3) to 2-13 mm in width (6.6 ± 2.3) (mean \pm S.D.; n = 191). Galls were green-purple in colour, similar to *P. aculeata* branches and young

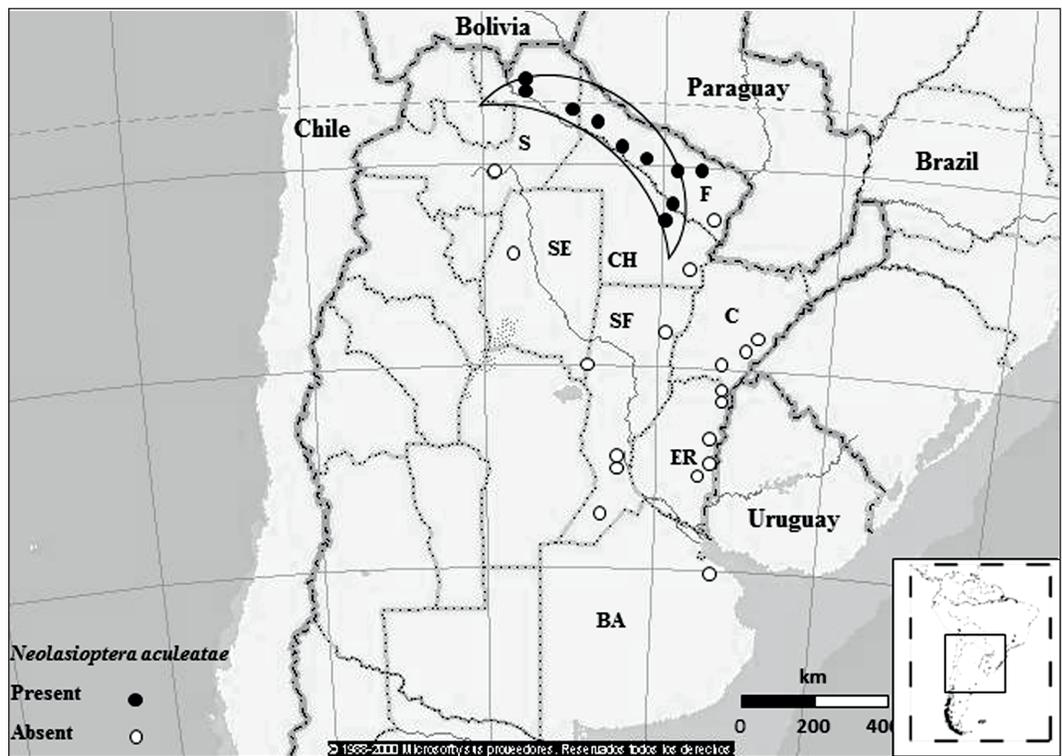


Fig. 1. Main collecting sites of *N. aculeatae* in Argentina. \frown : Area in which seasonal occurrence and field host range surveys were conducted for this study. BA, Buenos Aires; CA, Catamarca; CH, Chaco; C, Corrientes; ER, Entre Ríos; F, Formosa; S, Salta; SE, Santiago del Estero; SF, Santa Fe. This figure was created using Atlas Mundial Encarta 2001 (Microsoft).



Fig. 2. *Neolasioptera aculeatae* galls on *Parkinsonia aculeata*.

shoots. Inside each gall, 1-5 light orange larvae developed individually inside galleries into yellowish mature larvae which completely filled the cavity. Pupation occurred in the gall after which adults eclosed, leaving the pupal exuviae protruding from the gall.

Natural enemies. Hymenopteran parasitoids that emerged from *N. aculeatae* galls were identified as *Platygaster* sp. (Platygastridae) (M. Loíacono, Museo de La Plata, pers. comm.).

Seasonal occurrence

Fresh active galls were found on all four of the sampling dates during 2010 (Table I). The highest number of galls per surveyed plant was registered in June, which coincides with the cool-dry season, while the lowest number was registered in November, the beginning of the hot-wet season. In March, *N. aculeatae* galls were mostly recorded to be at an early developmental stage and growing near the shoot-tips of vegetative *P. aculeata* plants. At some sites, almost all the shoot-tips of plants had galls at high densities (2-3 galls per shoot-tip). In June, most plants were in a vegetative stage, bear-

ing medium- to large-sized galls. In September, small- to medium-sized galls were found near the shoot tips of flowering plants. In November, *P. aculeata* plants of vegetative/flowering stage bore very few medium-sized galls. Various larval instars were found when dissecting galls collected on each sampling date, while pupae were mostly found on galls collected in March. In addition, adults of *N. aculeatae* emerged from the remaining galls 13-34 days after collection and continued emerging for an average period of 22 (range: 15-28) days (Table I).

Field host range

From the nine sites visited, a total of 919 galls were collected from 416 *P. aculeata* plants. Adults of *N. aculeatae* emerged only from *P. aculeata* stem galls (Table II). *N. aculeatae* was not even found on the conspecific *Parkinsonia praecox*. However, different stem galls were found on *A. aroma* and *P. alba* species. Cecidomyiidae larvae dissected from galls on *Acacia aroma* were identified as "not *Neolasioptera*" (R. J. Gagné, USDA-ARS Systematic Entomology Laboratory pers. comm.).

Table I. Seasonal occurrence of *N. aculeatae* at selected sites in northern Argentina.

Sampling dates	N° of examined <i>P. aculeata</i> plants/sites	N° of collected/Dissected galls	N° of life stages found on dissected galls (Larvae/Pupae)	N° of adults emerged from remaining galls/Dates
18-23 March	159 (6)	279/62	130/20	137 (37♂; 100♀) 7-21 April
16-18 June	152 (5)	609/50	90/0	471 (200♂; 271♀) 19 July-9 Aug
25-26 September	228 (4)	496/61	100/0	247 (97♂; 150♀) 18 Oct-15 Nov
24-25 November	171 (4)	46/12	13/3	21 (14♂; 7♀) 6-29 Dec

Table II. Field host range of *N. aculeatae* in the native range of Argentina.

Plant species	N° of surveyed plants	N° of collected galls	Insect species emerged
<i>P. aculeata</i>	416	919	<i>N. aculeatae</i>
<i>P. praecox</i>	41	0	-
<i>A. aroma</i>	69	152	Unidentified Cecidomyiidae (not <i>Neolasioptera</i>)
<i>A. caven</i>	35	64	?
<i>N. pubescens</i>	10		-
<i>P. alba</i>	20	100	Unidentified Gracillariidae
<i>P. fiebrigii</i> or near	3	4	Unidentified Gracillariidae
<i>P. ruscifolia</i>	26	36	Unidentified Gracillariidae?
<i>S. morongii</i>	20	0	-
<i>S. obtusifolia</i>	10	0	-
<i>S. exasperate</i>	10	0	-

Gracillariidae moths emerging from *P. alba* and *P. fiebrigii* were identified as members of the Neurobathra group (unknown genus and species) (Don Davis, USDA-ARS Systematic Entomology Laboratory pers. comm.).

Laboratory rearing

Preliminary rearing of *N. aculeatae* using rearing sleeves produced two immature stem galls. However, neither full gall development nor adult emergence was recorded. Rearing attempts using cages proved unsuccessful.

DISCUSSION

Galls may act as physiological nutrient sinks on their host plants, (McCrea *et al.*, 1985; Larson & Whitham, 1991; Harris & Shorthouse, 1996; Goolsby *et al.*, 2000), and thus the impact of gall-forming insects on their host plants is not

necessarily centered on the attacked host plant organ or tissue (Hartnett & Abrahamson, 1979; Sacchi *et al.*, 1988; Fernandes *et al.*, 1992). Therefore, the formation of stem galls by *N. aculeatae*, may be acting as a metabolic sink, indirectly reducing the potential of *P. aculeata* plants to flower and set mature, viable seeds.

Biological traits of gall-forming insects that increase their potential as biocontrol agents have been described by several authors. Based on studies conducted on the hymenopteran gall-maker *Trichilogaster acaciaelongifoliae* on *A. longifolia* in South Africa, Dennill (1988) indicated different biological attributes that appear to increase the potential of gall-forming insects as biocontrol agents. Among these, the agent must live within the tissue that is galled, and the gall development must span the entire reproductive and/or growth phase of the plant. This was the case for *N. aculeatae*, where larvae and or

pupae were recorded in dissected *P. aculeata* galls throughout the year on plants at different phenological stages. This could be regarded as an attribute that would increase the potential effectiveness of *N. aculeatae* as biocontrol agent of *P. aculeata*.

Surveying closely related co-occurring species in the native range of the target contributes useful host-specificity data to prioritize agents for biocontrol (Witt, 2004; Goolsby *et al.*, 2006). Surveys of host plant use in northern Argentina indicated that *N. aculeatae* galls were found exclusively on *P. aculeata*, thus constituting its only known natural host. Although data could only be obtained from a small number of species, the fact that *N. aculeatae* galls were not found on the conspecific *P. praecox* constitutes strong evidence of a restricted host range. Given the potential of *N. aculeatae* as biocontrol agent, we consider that further investigations, especially to improve rearing methods of *N. aculeatae*, should be pursued.

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