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Biology, incidence and host susceptibility of *Pineus boerneri* (Hemiptera: Adelgidae) in Colombian pine plantations

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Adelgids (Hemiptera) in the genus *Pineus* have been reported as introduced insect pests causing serious losses to *Pinus* plantations worldwide. In 2008, *Pineus boerneri* was recorded for the first time in Colombia, with infestations noted on *Pinus kesiya*, *P. tecunumanii*, *P. maximinoi* and *P. oocarpa*. The lack of information on this insect in Colombia prompted investigations of its life cycle and infestation levels as well as host susceptibility of the main *Pinus* species planted in Colombia. In addition, the possibility of using a *Ceraeochrysa* species, an already established predator of adelgids in Colombia, for biological control was considered. Results showed that *Pineus boerneri* in Colombia has an anholocyclic life cycle comprised of four instars with a complete duration of between 49 and 97 d. Infestations were higher in the middle and upper part of trees. *Pinus kesiya* and *P. maximinoi* had the highest levels of susceptibility in field as well as in greenhouse trials. A survey of naturally infested trees showed *P. tecunumanii* to be moderately susceptible, whereas *P. patula* and *P. oocarpa* had low levels of susceptibility in a greenhouse trial but were not susceptible in the field. Investigations considering the impact of predation of *Ceraeochrysa* species showed a high predation rate of up to 140 *P. boerneri* consumed per day by a single *Ceraeochrysa* individual. Other predators of *P. boerneri* were recorded but were not sufficiently common to warrant detailed study.

Keywords: anholocyclic, biological control agent, pine woolly aphid, Pinus

Introduction

The superfamily Aphidoidea in the Hemiptera includes more than 170 species of pine sap-feeders distributed in the Adelgidae. Phylloxeridae and Aphididae (Blackman and Eastop 1994). The Adelgidae is a family known to feed exclusively on conifers (Scholtz and Holm 1985; Triplehorn and Johnson 2005) and has a complex multiple-generation life cycle where species are either holocyclic (alternates sexual reproduction with parthenogenetic reproduction) or anholocyclic (parthenogenetic reproduction) (Havill and Foottit 2007; Havill et al. 2007; Foottit et al. 2009). Some adelgids display a host-alternating behaviour (Dimond 1974) in which Picea is always the primary host associated with sexual reproduction, and other genera such as Abies, Larix, Pseudotsuga or Pinus are secondary hosts associated with parthenogenetic reproduction (Havill and Foottit 2007; Havill et al. 2007; Sano and Ozaki 2012).

Species in the adelgid genus *Pineus*, commonly referred to as woolly aphids, have emerged as some of the most serious pest in forests (Sano and Ozaki 2012). Since 1918, reports of *Pineus* infestations affecting both primary and secondary hosts throughout Europe were documented (Pierce 1918). *Pineus* species have subsequently spread throughout Africa, North America, South America, Australasia and Asia (Blackman and Eastop 1994; FAO 2007; Lazzari and Cardoso 2011) and species such as *P. pini* and *P. boerneri* have been reported as serious pests of *Pinus*, including *Pinus mugo* and *P. sylvestris* in Europe (Blackman and Eastop 1994; Soria et al. 1996), *Pinus patula* and *P. elliottii* in Tanzania (Petro and Madoffe 2011), and *Pinus kesiya* in Malawi (Chilima and Leather 2001). Symptoms of infestation include defoliation and, in extreme cases, dieback and tree death (Chilima and Leather 2001).

Despite their widespread occurrence, Pineus species had not been recorded in Colombia until 2008, when P. boerneri was detected in the Aguaclara Forestry Farm (Valle del Cauca) on 2.1-year-old Pinus kesiya (Table 1) (Rodas et al. 2014). Subsequent to this first report, infestations have been observed on Pinus kesiya, P. tecunumanii, P. maximinoi and P. oocarpa between the ages of 2.1 and 3.4 years (CAR unpublished data), in the departments of Valle del Cauca, Cauca, Caldas, Quindío and Risaralda (Figure 1). Characteristic symptoms of infestation were observed when populations of the pest were high. These symptoms included yellowing of needles, early senescence, tip dieback, growth reduction, shoot death and, in extreme cases, tree death. In addition, the presence of white cottony tufts (Figure 2a-c) can possibly reduce productivity of stands of Pinus species.

Location (farm) -	Coord	dinates	Altitude	Precipitation	Temperature	Planted	Plantation age	
	Latitude	Longitude	(m.a.s.l.)ª	(mm y⁻¹)	(°C)	species	(years)	
Aguaclara	clara 3°41'31" 76°32'48" 1 489 1		1 489	20.3	Pinus kesiya	2.1–3		
Laboratory	3°51′45″	76°29'49″	1 450	1 405	22.0	Pinus kesiya	0.6 (7 months)	
La Ponderosa	3°51′17″	76°29′39″	1 473	1 578	20.4	Pinus kesiya	2	
San Quin	3°34′40″	76°41′13″	1 524	1 439	21.1	Pinus kesiya	2	
Rancho Grande	3°51′43″	76°30′48″	1 479	1 418	21.0	Pinus tecunumanii	3.8	

Table 1: Location, climatic conditions and planted species for the different trial sites in the department of Valle del Cauca, Colombia

^a Metres above sea level

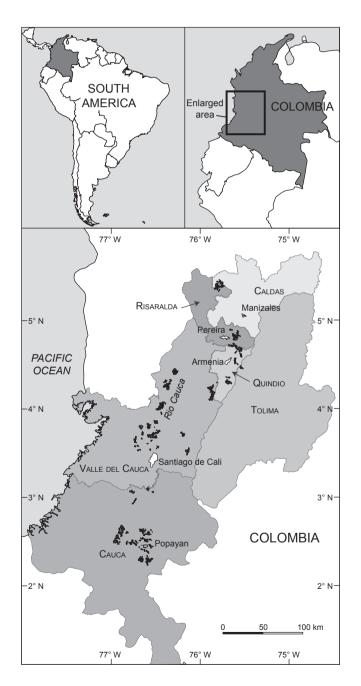


Figure 1: Map of Colombia showing the distribution of *Pineus* boerneri (areas shaded black)

The purpose of this study was to examine the life cycle of *Pineus boerneri* in *Pinus* plantations of Colombia. A further goal was to consider the susceptibility of different *Pinus* species planted in the country and to assess the impact of infestation. The possibility of using a species of *Ceraeochrysa* (Neuroptera: Chrysopidae), an already established predator of adelgids in Colombia, for biological control (Vargas et al. 1987) was also considered.

Materials and methods

Identification

A preliminary morphological identification of the *Pineus* species found in Colombia was made using the taxonomic characteristics documented by Blackman and Eastop (1994), namely the aspect of the cephaloprothoracic shield and the structure of wax pores. Specimens were also sent to Dr Nathan Havill (Northern Research Station, USDA Forest Service, USA) to confirm the preliminary identification using COI sequence data.

Life cycle

The life cycle of *Pineus boerneri* was investigated under controlled laboratory conditions. Insects were collected in the Aguaclara plantation (Valle del Cauca) in a three-year-old *Pinus kesiya* compartment (Table 1). The life-cycle studies were performed between September and December 2010 at the Smurfit Kappa Cartón de Colombia (SKCC) laboratory, located in Restrepo (Valle del Cauca) in Colombia using 100 seven-month-old *P. kesiya* trees.

Eggs were taken from field-collected white cottony tufts of *P. boerneri* using a scalpel and incubated in petri dishes on wet sterile filter paper, separated in groups of 10 eggs per dish. Ten emerged nymphs were transferred to trees. For each tree, a 20 ml syringe barrel was placed midway over the main branch to prevent escape (Figure 2d). Daily observations were made to assess aspects of the life cycle, including duration of the different life stages, percentage emergence from eggs, percentage nymph survival, percentage mortality and the number of eggs per female. The maximum length (from the eye to the tip of the abdomen) and width of the insect was measured for 10 individuals from each life stage.

Evaluation of the impact on Pinus plantations

To measure the impact of *Pineus boerneri*, evaluations were made monthly between November 2010 and September 2011 on two-year-old *Pinus kesiya* trees in plantations at two different sites, namely La Ponderosa

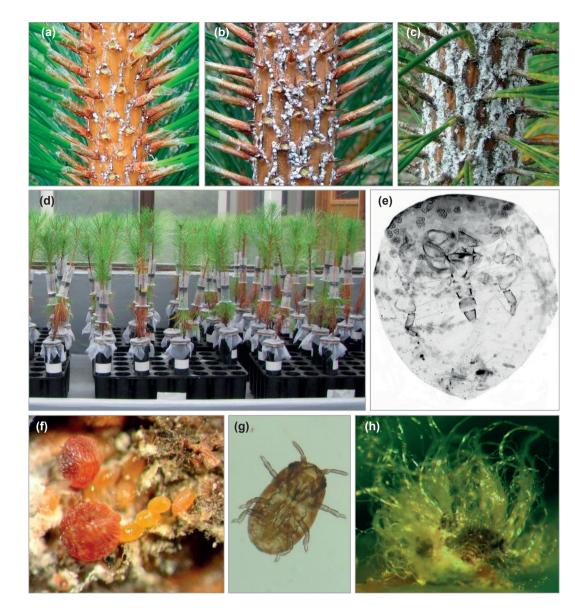


Figure 2: Low (a), moderate (b) and high (c) levels of infestation by *Pineus boerneri* on *Pinus kesiya*. (d) Syringe barrels (arrow) placed on trees for life-cycle determination. (e) Cephaloprothoracic shield and wax pores of *P. boerneri* (arrow). (f) *Pineus boerneri* adults and eggs. (g) *Pineus boerneri* first nymph instar. (h) *Pineus boerneri* nymph instar IV with the characteristic white cottony tufts

Farm and San Quin Farm in the Valle del Cauca department (Table 1). At each site there were four block-plots of 90 m² plot⁻¹, with 10 trees randomly selected per plot. The percentage infestation per plot was calculated based on the total number of trees infested.

To calculate the population density of *P. boerneri*, a tree was randomly selected within each plot and divided in three sections representing the bottom (lower section, lower branch 1, lower branch 2), middle (middle section, middle branch 1, middle branch 2) and top (higher section, higher branch 1, higher branch 2). Each section was divided into three parts, namely two lateral branches and the main stem. A circular bark sample (disc) of 2 cm² was cut from the middle of each of these parts. The samples were taken to the SKCC Laboratory (Restrepo, Valle del Cauca), where the total number of eggs, nymphs and adults per sample were counted over the 11-month period.

Susceptibility of Pinus species

To assess the susceptibility of Pinus species to infestation by P. boerneri, a survey was conducted using naturally infested trees as well as trees artificially exposed in the greenhouse. To assess natural infestation, five Pinus species were planted within a plantation of two-year-old Pinus kesiya at La Ponderosa Farm, where infestations of Pineus boerneri were observed. The five species were planted in randomly placed blocks of 20 trees per species, with the trees spaced 1 m \times 1 m apart. The species tested were Pinus oocarpa, P. patula, P. kesiya, P. maximinoi and P. tecunumanii High Elevation (HE) provenance. The trees were planted in May 2011 and monitored monthly until they were six months old (September 2011). In order to examine susceptibility where trees had not been planted in close proximity to an infested area, six-month-old trees of the same species were planted in plastic trays (29 trees

per species) and placed at a site in the Rancho Grande farm. Monthly evaluations of these trees were made from September 2010 to July 2011. In both cases, infested trees were counted to determine the percent infestation.

For the greenhouse trial, the same five *Pinus* species (20 trees per species) were used. The trees were nine months old and were planted in plastic trays. Individuals of *P. boerneri* were collected from Rancho Grande farm on 3.8-year-old *P. tecunumanii* (Table 1). Twenty individuals of nymphal stage I were placed on each tree. Monthly evaluations were made between September 2010 and January 2011. The number of infested trees was counted to determine the percentage infestation.

Impact of predation of Ceraeochrysa species and other predators

Observations in the field showed predation of *Pineus boerneri* by a *Ceraeochrysa* sp. (Neuroptera: Chrysopidae). This predator is commonly found in *Pinus* plantations in Colombia. The presence of other less common predators was also noted and these insects were identified.

Individuals of *Ceraeochrysa* were reared in the laboratory to assess their impact of predation on *P. boerneri*. Sixteen larvae of one-day-old *Ceraeochrysa* were placed singly in petri dishes. In each dish, 10 eggs, 10 nymphs and 10 adults of *P. boerneri* were provided initially for the newly emerged *Ceraeochrysa* sp. Thereafter, the number of *P. boerneri* individuals provided was increased daily according to the consumption rate of the developing *Ceraeochrysa* individuals. The total number of consumed insects was counted daily and the cumulative consumption rate for the different larvae instars of *Ceraeochrysa* sp. determined.

Results

Identification

Morphological examination based on the aspect of the cephaloprothoracic shield and the structure of the wax pores (Figure 2e) and COI sequence data (641 bp) confirmed that the insect was *Pineus boerneri*. The COI sequence was a match to an unnamed *Pineus* species from Footit et al. (2009), later confirmed to be *P. boerneri* (N Havill, USDA Forest Service, pers. comm., 2013; Genbank accession no. KM023326).

Life cycle

Infestations of *Pineus boerneri* in the field were observed only on *Pinus* species. The presence of male *P. boerneri* was not observed on any of the 100 plants used to investigate the life cycle, suggesting a possible parthenogenetic reproductive strategy. The complete life cycle ranged from 49 to 97 d (mean = 65.6 d, SD = 12.9, n = 714). The average egg length was 0.32 mm (0.30–0.35 mm, SD = 0.02) with an average width of 0.17 mm (0.16–0.18 mm, SD = 0.01). The egg eclosion time was on average 6.5 d (5–8 d, SD = 0.6, n = 100), and the percentage emergence was 90.5% from a total of 200 eggs. Eggs were a light yellow colour when newly laid, becoming red before hatching (Figure 2f).

Four instars of *P. boerneri* were observed. The total duration of the nymph stage was on average 24.2 d (22–29 d,

SD = 1.3, n = 527). Nymph stages of *P. boerneri* produced white woolly tufts. The nymphs showed small morphological differences when compared with adults. Colours varied from light yellow to reddish brown. Nymph I started to form white cottony tufts on the bark, had the longest duration (mean = 10.9 d, range = 10-12 d, SD = 0.3, n = 148), and an average body length and width of 0.34 mm (0.30-0.38 mm. SD = 0.03) and 0.21 mm (0.19-0.22 mm, SD = 0.01) (Figure 2g), respectively. Nymph II had an average duration of 5.2 d (5–6 d, SD = 0.4, n = 139), and an average body length and width of 0.39 mm (0.37-0.41 mm, SD = 0.01)and 0.28 mm (0.24–0.30 mm, SD = 0.02), respectively. Nymph III had a duration of 4.1 d (4-6 d, SD = 0.3, n = 126), and an average body length and width of 0.42 mm (0.40-0.46 mm, SD = 0.02) and 0.35 mm (0.31-0.39 mm, SD = 0.02)SD = 0.02), respectively. Nymph IV had a duration of 4 d (3–5 d, SD = 0.3, n = 114), and an average body length and width of 0.46 mm (0.40-0.48 mm, SD = 0.02) and 0.40 mm (0.38-0.41 mm, SD = 0.01) (Figure 2h), respectively. Nymphal survival rate for instars I, II, III and IV was 93.9% (n = 148), 90.7% (n = 139), 90.5% (n = 126) and 76.3% (n = 114). Adults had a lifespan of 34.9 d (22-60 d, SD = 11, n = 87), and an average body length and width of 0.53 mm (0.46-0.58 mm, SD = 0.04) and 0.44 mm (0.42-0.47 mm, SD = 0.02), respectively.

Examination of the duration of the adult life stage (n = 87) presented a pre-oviposition period of 1 d, an average oviposition period of 33 d (20–55 d, SD = 11.2) and an average post-oviposition period of 2.2 d (1–4 d, SD = 0.5). The average number of eggs oviposited per female was 65.6 (40–125 eggs female⁻¹, SD = 18.1). The number of eggs decreased across the duration of the adult stage, from an average of 2.6 eggs d⁻¹ (on day 2) to 0.2 eggs d⁻¹ (on day 58).

Impact on Pinus plantations

Per tree infestation rates of P. boerneri at La Ponderosa Farm were 85% at the time of the first observation (November 2010) and 92.5% when the last observation was made (September 2011). Infestation at San Quin was similar, with 87.5% infestation at the time of first observation and 95% when the last observation was made. The population of P. boerneri was highest in September 2011 (mean = 38.6) at La Ponderosa, and highest in March (mean = 37.7) at San Quin (Figure 3). The population density over the 11-month study period was higher at La Ponderosa Farm (total = 2 418 eggs, 1 848 nymphs and adults) than at San Quin Farm (total = 1 623 eggs, 1 316 nymphs and adults) distributed in the different tree sections. Pineus boerneri placed more eggs in the upper and middle tree sections at La Ponderosa Farm (mean = 36.5 and 36.4 per 2 cm², respectively) and in the upper and middle branch 1 at San Quin Farm (mean = 24.6 and 19.8 per 2 cm², respectively) (Figure 4). A greater number of nymphs and adults were registered in the higher tree sections at both farms over a period of 11 months (mean = 49.0 per 2 cm² for La Ponderosa, mean = $18.1 \text{ per } 2 \text{ cm}^2$ for San Quin) (Figure 4).

Susceptibility of different Pinus species

The most susceptible *Pinus* species in all trials were *P. kesiya* and *P. maximinoi* (Table 2). Infestation

percentages (number of trees infested) varied between the different assessments. Where natural infestation was assessed, mean infestation on *P. kesiya* was 88.3% at Rancho Grande and 23.4% at La Ponderosa, and mean infestation on *P. maximinoi* was 67.4% at Rancho Grande and 11.2% at La Ponderosa. In the greenhouse trial, mean infestation was higher on *P. kesiya* (93.8%) than on *P. maximinoi* (80.2%). *Pinus oocarpa* and *P. patula* had mean infestation levels in the greenhouse trial of 15% and 28%, respectively, but were not naturally infested at either of the two field sites (Table 2). *Pinus tecunumanii*

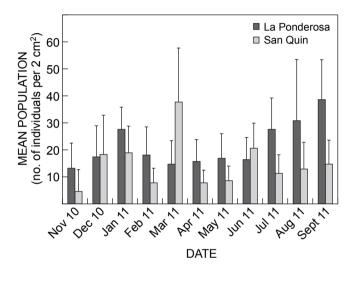


Figure 3: Mean population levels (eggs, nymphs and adults combined) of *Pineus boerneri* at two sites over an 11-month period (error bars indicate the SD)

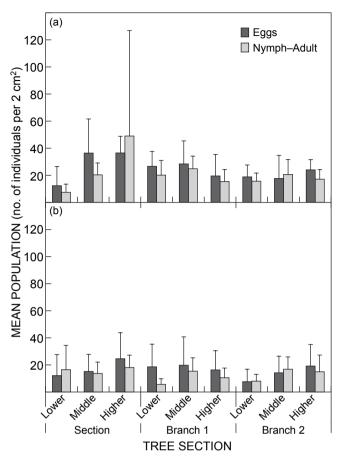


Figure 4: Mean population density of *Pineus boerneri* for the different tree parts sampled over an 11-month period (error bars indicate the SD) at (a) La Ponderosa Farm and (b) San Quin Farm

Table 2: Percent infestation of *P. boerneri* on different *Pinus* species in three different trials: natural infestation at Rancho Grande between September 2010 to July 2011; natural infestation at La Ponderosa between May and September 2011; and induced infestation in a greenhouse between September 2010 and January 2011

Species		2010			2011											
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	- Mean	SD	n
Natural infestation -	- Ranch	o Gran	nde													
P. kesiya	79	86	86	90	90	90	90	90	90	90	90	_	_	88.3	3.5	29
P. maximinoi	59	66	66	72	72	72	72	69	69	62	62	_	_	67.4	4.7	29
P. oocarpa	0	0	0	0	0	0	0	0	0	0	0	_	_	0	0	29
P. patula	0	0	0	0	0	0	0	0	0	0	0	_	_	0	0	29
P. tecunumanii HE	7	7	10	10	10	10	10	10	10	7	7	_	_	8.9	1.5	29
Natural infestation -	- La Por	nderos	a farm													
P. kesiya	_	_	_	_	_	_	_	_	0	5	5	25	82	23.4	34.1	20
P. maximinoi	_	_	_	_	_	_	_	_	0	0	5	18	33	11.2	14.2	20
P. oocarpa	_	_	_	_	_	_	_	_	0	0	0	0	0	0	0	20
P. patula	_	_	_	_	_	_	_	_	0	0	0	0	0	0	0	20
P. tecunumanii HE	_	-	_	_	_	_	_	-	0	0	0	0	0	0	0	20
Artificially exposed	– Greer	house	•													
P. kesiya	74	95	100	100	100	_	_	_	_	_	_	_	_	93.8	11.3	20
P. maximinoi	56	75	90	90	90	_	_	_	_	_	_	_	_	80.2	15	20
P. oocarpa	0	0	20	25	30	_	_	_	_	_	_	_	_	15	14.1	20
P. patula	25	25	30	30	30	_	_	_	_	_	_	_	_	28	2.7	20
P. tecunumanii HE	0	20	25	35	35	_	_	_	_	_	_	_	_	23	14.4	20

HE showed a mean infestation of 23% in the greenhouse trial. Natural infestation of this species was observed at Rancho Grande, with 8.9% infestation, but not at La Ponderosa, most likely because infestation levels were generally lower at that site.

Impact of predation of Ceraeochrysa species

The Ceraeochrysa sp. emerged from the eggs after an average of 6 d (n = 110). The duration of the larval instars was 7.2, 7.7 and 10.2 d for instar I (n = 109), instar II (n = 71) and instar III (n = 19), respectively. The average impact of predation of P. boerneri (eggs, nymphs and adults combined) per 24 h by the different Ceraeochrysa sp. instars was 15 (instar I), 33 (instar II) and 98 (instar III) individuals. The highest impact of predation per day was recorded for an individual of instar III, with a total of 142 P. boerneri (45 eggs, 47 nymphs and 50 adults) consumed per day. The average impact of predation of P. boerneri over the 33 d exposed to the 16 Ceraeochrysa sp. instars was 2 437 (817 eggs, 813 nymphs and 807 adults). The Ceraeochrysa sp. showed no obvious discrimination of food source in terms of the various P. boerneri instars. Other predators observed in this study were Harmonia axyridis (Coleoptera: Coccinellidae), Chrysoperla species (Neuroptera: Chrysopidae) and Cryptolaemus sp. (Coleoptera: Coccinellidae).

Discussion

Pineus boerneri was detected for the first time in Colombia in 2008 and is reported as a pest of *Pinus* (Rodas et al. 2014). This adelgid has also been reported in nearby Brazil and Chile (Zuñiga 1985; Blackman and Eastop 1994; Oliveira et al. 2008; Lazzari and Cardoso 2011), as well as other countries such as South Africa, New Zealand and the USA (McClure 1989; Blackman and Eastop 1994; Blackman et al. 1995), where it is considered a pest of various *Pinus* species. This study investigated the life cycle, impact and host susceptibility of this pest and also provided the first report of the impact of predation of *Ceraeochrysa* sp., a naturally occurring enemy of *P. boerneri* in Colombia. The findings presented will inform management decisions for this pest and potentially offset losses to plantation forestry in Colombia.

Results showed the apparent dominance of an anholocyclic life style for *P. boerneri* in Colombia. Adelgids can become anholocyclic when there is limited migration and the absence of an alternate host (Havill and Foottit 2007). These conditions were found in Colombia, where all *P. boerneri* individuals were apterous; no males were observed and no individuals were found on other nearby vegetation. Other reported anholocyclic *Pineus* species include *Pineus strobi* on *Pinus strobus* in North America (Blackman and Eastop 1994; Sano and Ozaki 2012), and *Pineus pini* on *Pinus sylvestris* in Europe (Blackman and Eastop 1994; Soria et al. 1996).

The duration of the life cycle has not been determined for an anholocyclic *Pineus* species, due to the complexity of the insect biology (Foottit et al. 2009). According to Sano and Ozaki (2012), the life cycle of adelgids that do not exhibit host-alternation or sexual reproduction could be completed within a year, but with a reduced number of generations. Generation time is closely related with climate, host condition and insect size, and can include between two and six generations per year (Arthur and Hain 1984; McClure 1989; Havill and Foottit 2007; Lazzari and Cardoso 2011). In Colombia, presence of *P. boerneri* throughout the year is a consequence of tropical environmental conditions, in which the number of generations per year was approximately 3.7.

Infestation levels were influenced by tree section. At both farms studied, younger tissues in the upper parts of trees harboured the greatest number of eggs, nymphs and adults. Nutritional quality as well as protection offered by branches is known to impact strongly on colonisation sites for *Pineus* species such as *P. boerneri* and *P. coloradensis* (McClure 1991). Losses due to infestation were not measured in this study, but McClure (1991) showed that *P. boerneri* can result in a 72% reduction of biomass in *Pinus* stands of less than one year old and a 53% reduction in *Pinus* stands of one to three years age for *P. resinosa*. In Colombia, *Pineus boerneri* was present throughout the year despite varying climatic conditions.

The most susceptible *Pinus* hosts examined in this study were *P. kesiya* and *P. maximinoi*. Although all five of the *Pinus* species tested showed susceptibility to *Pineus* boerneri when artificially exposed, *P. oocarpa* and *P. patula* showed no natural infestation, and *P. tecunumanii* HE showed low natural infestation at one site and no infestation at another site. A wide diversity of *Pinus* hosts has been documented for *P. boerneri*, including *P. oocarpa*, *P. patula*, *P. kesiya*, *P. taeda*, *P. caribaea*, *P. radiata* and *P. elliotti* (McClure 1991; Blackman and Eastop 1994; Chilima and Leather 2001; Oliveira et al. 2008; Lazzari and Cardoso 2011; Petro and Madoffe 2011). This study is the first to report on the susceptibility of *P. maximinoi* and *P. tecunumanii* to infestation by *Pineus boerneri*.

Ceraeochrysa sp. showed encouraging potential for the biological control of *P. boerneri* due to its high predation capacity on all life stages of the insect. Management strategies for forest insect pests can include the conservation, mass production and release of naturally occurring predators, as a form of biological control (Núñez 1988). The Neuroptera includes some of the most promising biological agents such as species of Chrysopidae, which are commonly known as green lacewings (New 1975; Tauber et al. 2009). For example, the genus *Chrysopa*, commonly found in tropical and subtropical regions of the Americas (Vargas et al. 1987), have been widely used as biocontrol agents (Klingen et al. 1996; Santa-Cecília et al. 1997; Cadena et al. 2007).

Conclusion

This study improves understanding of the life cycle of *P. boerneri* in Colombia and demonstrates the potential to use species selection and augmentation of natural predators to reduce impacts. The recent infestations of *P. boerneri* in Colombia have the potential to cause serious losses to commercial *Pinus* plantations. Continued monitoring of this insect in Colombia and rearing of natural enemies may contribute to the effective management of this pest.

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