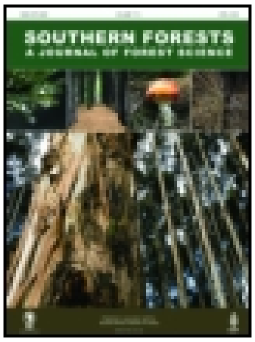

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Southern Forests: a Journal of Forest Science

ISSN: 2070-2620 (Print) 2070-2639 (Online) Journal homepage: http://www.tandfonline.com/loi/tsfs20

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Southern Forests 2016: 1–9
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This is the final version of the article that is
published ahead of the print and online issue

SOUTHERN FORESTS
ISSN 2070-2620 EISSN 2070-2639
http://dx.doi.org/10.2989/20702620.2016.1207132

Mega-trap-plots: a novel method of *Sirex* woodwasp management on *Pinus radiata* plantations in Chile

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***Sirex noctilio* is one of the most important invasive pests that affect *Pinus radiata* plantations in Chile. Its management is based on a biological control complex, the most important component of which is the nematode, *Deladenus siricidicola*. However, in some areas, *S. noctilio* populations attain epidemic levels and no effective control methods exist to reduce large populations in a short period. In this study, we evaluated a novel method called mega-trap-plots (MTPs), which consist of an area of 1 ha with trap trees, which were debilitated in four different months (from November to February) with the purpose of reducing the Sirex wasp population through harvesting of trees attacked. The main objective was to reduce the potential population of *S. noctilio*, by evaluating four periods or months of MTP installation to maximise the colonisation of trap trees by wood wasps. The results showed that the MTPs that were installed from November to January had the highest wood wasp infestation, which was coincident with the flight period of the insect. The trap trees were clearly attractive to *S. noctilio* females up to 90 d following their establishment. The MTPs that were established in November concentrated a potential population of 57 901 females of *S. noctilio*, which represents a population 5.4 times greater than that in the control, with 10 701 females. The population of *S. noctilio* attracted between November and January shows that the use of MTPs is an effective system for the management of wood wasps on plantations with a high level of infestation and thereby can reduce their spread and the attack of new trees within the same forest compartments.**

Keywords: mega-trap-plot, radiata pine plantations, *Sirex* control, Sirex wood wasp, trap tree

Introduction

The Sirex wood wasp, *Sirex noctilio* F. (Hymenoptera: Siricidae), is one of the most important invasive forest pests of *Pinus radiata* (D.Don) in the Southern Hemisphere (Hurley et al. 2007; Collett and Elms 2009; Carnegie and Bashford 2012). The wood wasp has caused significant damage in the countries where it has been introduced, due to tree mortality, degradation in wood quality, quarantine regulations and the cost of control (Madden 1975; Neumann et al. 1987; Bain et al. 2012; Hurley et al. 2012). Plantations with more than 5% of *S. noctilio* infestation are considered to represent an epidemic level, and are associated with outbreaks of the pest that can cause significant tree mortality (Villacide and Corley 2012), as was the case in Australia (Neumann et al. 1987; Madden 1988), South Africa (Hurley et al. 2007) and Argentina (Klasmer and Botto 2012).

Sirex noctilio was first reported in Chile on *Pinus radiata* trees during 2001 in two different localities: one in Guardia Vieja (32°51′43″ S, 70°24′45″ W) and the other in Ensenada (40°46′12″ S, 72°24′15″ W) (Beéche et al. 2012). The current distribution of *S. noctilio* is continuous between the El Maule region (34°48′16″ S, 72°01′33″ W) and the Los Lagos region (42°10′04″ S, 72°11′18″ W). *Sirex noctilio* is also present in the Metropolitan region (33°24′44″ S, 70°37′12″ W) and the Aysén region (47°04′23″ S, 71°57′46″ W) (SAG 2015). In these two regions, *P. sylvestris* stands have also been infested by *S. noctilio* and, to date, are the only non *P. radiata* plantations infected by the wood wasp (Rojas and Beéche 2010). Epidemic levels of attack in radiata pine plantations have been reported in the Biobío region, with about 50% of trees affected in different plantations of the Alto Biobío locality (37°47′20″ S, 71°45′16″ W) (Beéche 2012).

Pinus radiata is the most important forestry plantation species in Chile, comprising about 1.5 million ha of the approximately 2.3 million ha of commercial forest (INFOR 2014). Its geographical distribution lies between the regions of Valparaíso (32°02′ S) and Los Lagos (41°25′ S) (INFOR 2014). In this latitudinal range, *P. radiata* differs in productivity, according to variation in annual rainfall (Flores and Allen 2004). During the summer season, an increase in water stress occurs in plantations located in sandy soil areas (Biobío region, 37°14′ S) as well as in the central valley in the northern area of the *P. radiata* distribution in Chile, where the dry period extends for up to six or eight months (Huber and Trecaman 2004). This long period of water shortage partly coincides with the *S. noctilio* flight cycle, which occurs approximately between December and April (Rojas and Beéche 2010), and which increases the susceptibility of the plantations to attack by wood wasps (Madden 1977; Neumann et al. 1987).

Sirex noctilio normally causes tree wilting and death, due to a combination of the phytotoxic mucus (Talbot 1977) and the *Amylostereum areolatum* fungus (Chaillet) Boidin, both of which are deposited by the females during the oviposition process (Madden 1977; Spradbery and Kirk 1981; Neumann et al. 1987). For *Sirex* management, trap trees are used to attract *S. noctilio* females to stressed trees (Bordeaux and Dean 2012), via the artificial debilitation of trees by the injection of systemic herbicides into the tree stem (Neumann et al. 1982). As a response to herbicide action, organic compounds are emitted by the tree, which attract wood wasp females (Madden 1977; Crook et al. 2012). This method has been implemented in most of the countries where *S. noctilio* has been introduced (Hurley et al. 2007; Carnegie and Bashford 2012). Different methods exist to control the *S. noctilio* population, the most effective of which is the use of the parasitic nematode, *Deladenus siricidicola* Bedding (Nematoda: Neotylenchidae) (Bedding and Akhurst 1974; Corley et al. 2014), together with the parasitoid wasps *Ibalia leucospoides* Hochenwarth and *Megarhyssa nortoni* Cresson (Collett and Elms 2009; Fischbein and Corley 2014). Thinning with a phytosanitary purpose has also been performed in plantations infested by *S. noctilio* (Corley et al. 2007). Biological control agents require a long time to become successfully established (DeBach and Rosen 1991). The efficacy of *D. siricidicola* can decrease over time due to loss of aggressiveness, especially when the nematode is repeatedly reproduced under laboratory conditions (Bedding 2009). Moreover, its spread could be restricted, because the parasitised wasp is smaller and flies shorter distances than wasps unparasitised (Villacide and Corley 2008). Chemical control methods are not a feasible option, due to the low efficacy of insecticides on different stages of *Sirex*, as well as the environmental restrictions (FSC 2013). Ethological control using traps and lures have not been successful to date, due to the absence of an identified pheromone (Hurley et al. 2015) and the lack of knowledge concerning the behaviour of the population of the wasp (Böröczky et al. 2009; Crook et al. 2012), which might explain the low capture of *S. noctilio* adults (Zylstra et al. 2010; Bashford and Madden 2012; Sarvary et al. 2014) limiting the implementation of mass trapping as an useful control technique (El-Sayed et al. 2006).

The trap trees colonised by *S. noctilio* are used for the inoculation of *D. siricidicola* (Bedding 2009). The objective is to detect areas of spread of *S. noctilio* and to facilitate the inoculation of the biological control with the nematode (SAG 2008; Beéche et al. 2012). In addition, it is important to monitor efficacy of nematode and parasitoids using billet monitoring of trap trees (Carnegie et al. 2005). Given the presence of stands of *P. radiata* with epidemic infestation (>5%) of *S. noctilio* and the absence or low levels of biological control agents in the Biobío region, this study aimed to validate the use of trap-tree plots of 1 ha termed a mega-trap-plot (MTP), with trees being debilitated by herbicide injection during four consecutive periods. The hypothesis of this study is that the MTPs concentrate the attack of *S. noctilio* females, reducing the *Sirex* population, and thus reducing the level of attack of the remaining trees in the stand, through the harvest of attacked trees before the *Sirex* flight season. The aim of this study was to evaluate the infestation level and the *S. noctilio* population obtained using MTPs, as well as the effect of the time (months) since their establishment.

Materials and methods

Area of study and establishment of mega-trap-plots

The study was conducted between November 2010 and August 2011 in a thinned 19-year-old plantation of *P. radiata* that suffered attack by *S. noctilio* and was located to the east of Santa Bárbara town, in the Biobío region of Chile (between 37°43′13″ S, 71°44′16″ W and 37°46′12″ S, 71°43′31″ W) (Figure 1). The area near the foothills of Los Andes has a cold-to-temperate climate, with a daily temperature range from −5 to 37 °C, and contains a mixture of native vegetation, agricultural crops and forestry plantations (Aguayo et al. 2009; Table 1). *Sirex noctilio* was detected in the whole study area in September 2010, with levels of attack up to 60% of the trees at the stand level. Given these attack levels, the Servicio Agrícola y Ganadero (SAG) imposed quarantine regulations that required phytosanitary thinning, and restriction of the use of logs for the production of pulp only, to ensure the destruction of the immature *S. noctilio* stages within the logs.

Eleven MTPs were established in the first week of November 2010. Each MTP corresponded to 1 ha, and the trees were stressed by applying a sublethal dose of herbicide to use as a trap tree. Each hectare was divided into four equal quadrants of 0.25 ha (50 × 50 m) or treatments corresponding to four months of establishment. Each quadrant was randomly assigned one of the four treatments, established in the first week of each month (November 2010 to February 2011) (Figure 2). The total number of treated trees was 3 812 (347 trees per MTP).

The herbicide used to debilitate the trees was the systemic herbicide Tordon® 24K (a.i. picloram), which was injected into the tree stem following the standard procedure defined by SAG (2008), which means the use of 1 mL per 10 cm perimeter, injected with syringe on holes made in the trunk by a portable drill. To quantify the effect of the MTPs, 11 control plots without herbicide injection were established, located at a minimum distance of 200 m from each MTP. The total number of untreated trees was 3 607.

Emergence period of Sirex noctilio

The establishment of the MTPs started one month before the *S. noctilio* flight period (Zylstra et al. 2010). It was assumed that the flight period in the area would be similar to that observed in the south of Chile (Osorno province, 40°35′46″ S, 73°05′53″ W), which extends approximately from December to April (Rojas and Beéche 2010). This assumption was validated by records of the emergence of *S. noctilio* adults from December 2010 to April 2011, provided by the Consorcio de Plagas Forestales SA (CPF), and those obtained from their breeding cages, which were installed in the same area of this study to evaluate the presence of the *Ibalia leucospoides* parasitoid (C Goycoolea, CPF, pers. comm., 2014). Data for the relative number of emerged wasps were also obtained from the same cages. The emergence data from these containers were complemented by the daily temperatures recorded

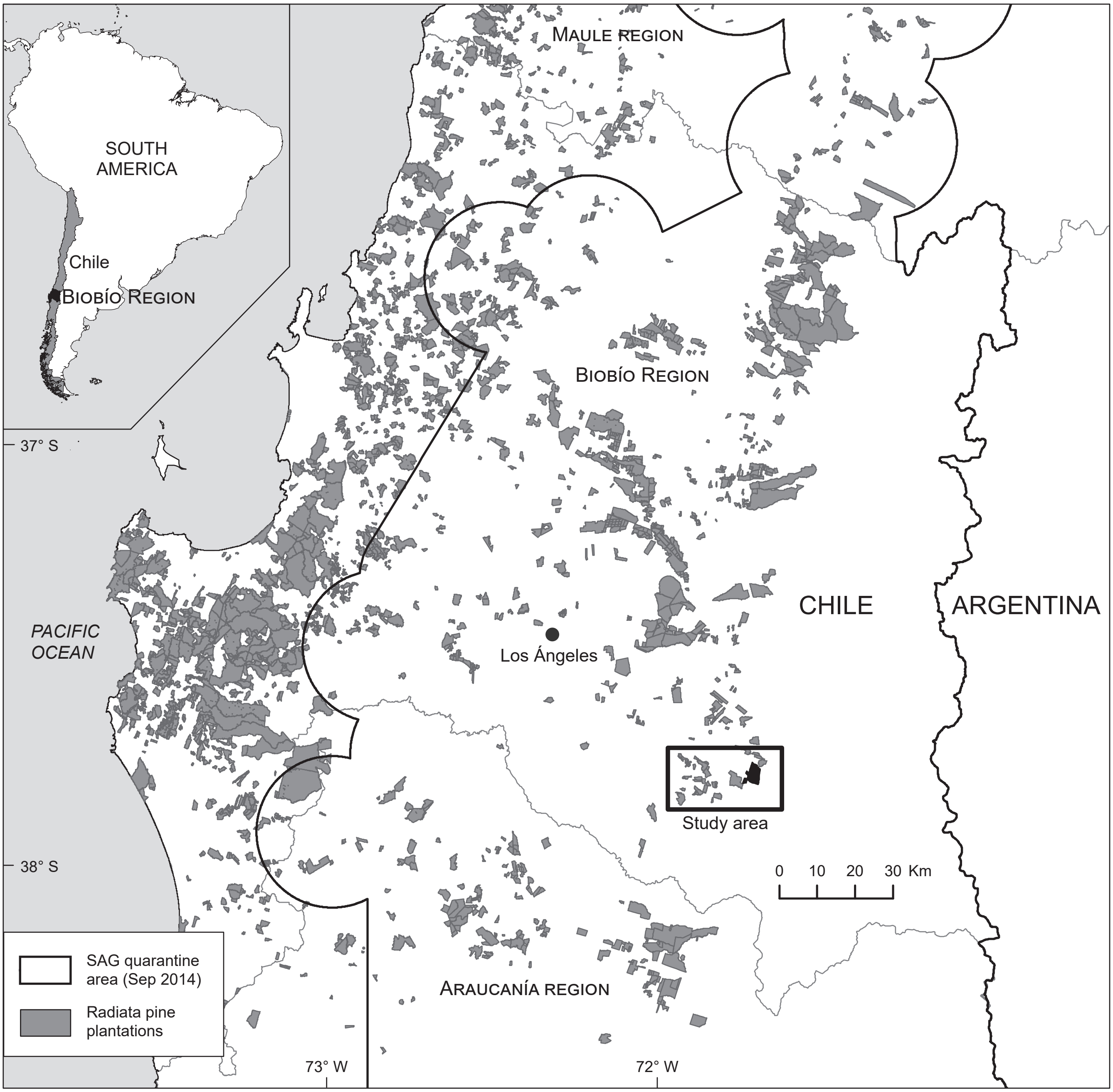

Figure 1: Study area located in the Biobío region, Chile. SAG = Servicio Agrícola y Ganadero

between 1 October 2010 and 1 August 2011 at the Human station, located near to Los Ángeles city (37°26′08″ S, 72°14′38.3″ W; 195 m above sea level), 53 km north-east of the study area.

Level of attack in the mega-trap-plots

The percentage attack level in each mega-trap was calculated using the number of trees that were attacked or showed symptoms of *S. noctilio* and the total number of trees in the plot. Attacked trees were identified through *S. noctilio* characteristic symptoms, such as the presence of resin beads and bleeds on the trunk, and staining caused by the fungus *A. areolatum* in the wood (Neumann et al. 1987; Dodds et al. 2010). Dead trees with the presence of emergence holes were considered to have been attacked by the insect in the previous season (Ismail et al. 2007) and were not included in the analysis. The level of attack was evaluated at the beginning of this study, in November 2010, and several subsequent evaluations were conducted at 30, 60, 90 and 240 d (July 2011), when the study ended. The number of healthy trees and those attacked by *S. noctilio* in each quadrant or treatment was recorded monthly and the trees affected by *S. noctilio* before the start of the treatment were not considered (Table 2).

Table 1: Climatic conditions, soil and site characteristics of the plantations where the trials were located

Climatic data[1]	
Average temperature (°C)	11.7
Average minimum temperature (°C)	5.7
Average maximum temperature (°C)	19.6
Rainfall (mm y^{-1})	1 500
Water stress index (mm y^{-1})	−82.8
Soil and site characteristics[2]	
Soil type	Sedimentary
Soil origin	Volcanic ash
Soil texture	Sandy loam
Altitude (m asl)	474.3
Slope (%)	14.1
***Pinus radiata* plantation**	
Plantation age (years)	19
Area (ha)	469.1
Site index (m)	32.2
Density (trees ha^{-1})	367
Average diameter at breast height (cm)	38.4

[1] Source: Quilaco weather station (37°40′08″ S, 71°59′60″ W), Dirección General de Aguas (DGA), Chile. Data: 1953–2013
[2] Data: Servicio Nacional de Geología y Minas (SERNAGEOMIN), Chile

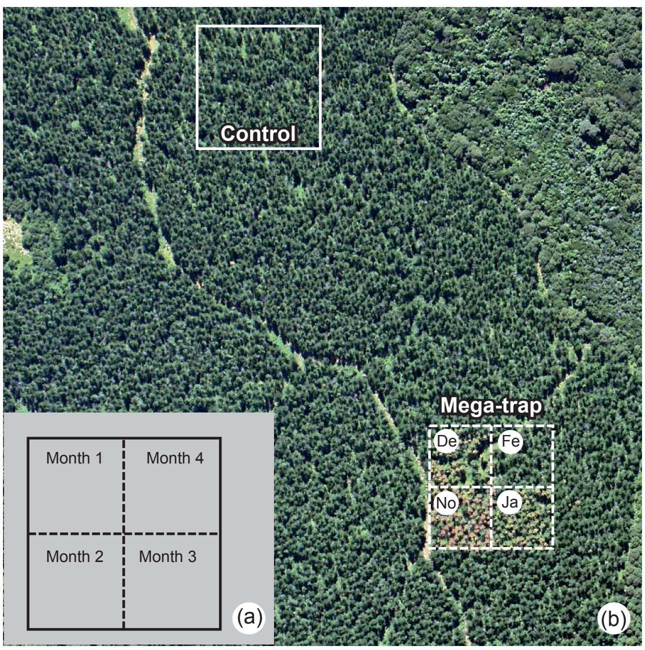

Figure 2: (a) Schematic representation of a mega-trap-plot four months after establishment. (b) A mega-trap-plot located in a *Pinus radiata* plantation near (290 m distant) to a control plot. Aerial view of a mega-trap-plot in February 2011

Infestation level in the mega-trap-plots

The level of infestation was determined by confirming the presence of larvae or internal galleries in symptomatic trees, and required the destruction of the sample. The infestation level was defined as the number of trees colonised by *S. noctilio* as a percentage of the number of symptomatic trees.

Infestation was evaluated in July 2011, prior to the harvest of the treatments, when the *S. noctilio* larvae and their galleries were easily recognisable.

The sampling of symptomatic trees from each MTP was completed in a set of four transects with five trees sampled per transect, totalling 20 trees per quadrant or treatment. Each transect was oriented towards the four cardinal points from the centre of the each quadrant. From this centre point, the sampling advanced following the first transect (north), until a symptomatic tree was found, which was then marked. This process was carried out until the next symptomatic tree was found and the same procedure was repeated for all the trees in each transect. Once the identification of symptomatic trees was completed, they were felled and were cut into sections 1 m in length, starting from the base of the tree up to a minimum diameter of 5 cm. For each cut section, the presence or absence of larvae and/or galleries (containing compacted sawdust) was monitored on the face of the logs. At the first confirmation of larvae or a gallery, cutting was suspended, and was continued on the next tree. In the quadrant established in February and in the control, the number of sampled trees was reduced to 12 (three per transect) and 16 (four per transect), respectively, due to the clear decrease in the number of symptomatic trees (Table 3).

Estimation of larval and adult populations of Sirex noctilio *in the mega-trap-plots*

The larval population of *S. noctilio* (z) was estimated from a sample of 20% of the trees found to be infested. The trees were marked in 1 m sections, from the base to a diameter of 5 cm, using an Artline® pen. All of the logs were cut, labelled and subdivided into three sections of 33 cm each. Each section was then carefully cut with an axe and the larvae were extracted using tweezers. The collected larvae of *S. noctilio* were counted, stored in 70% ethanol and were labelled with the number of the tree and the treatment for further analyses. The total larval population (w) was determined, and the larval population per hectare of *S. noctilio* (z) was estimated using the following function:

$$z \text{ (larvae ha}^{-1}) = y \times y_1 \times w \times \text{density}$$

where y = the percentage of trees scored positive for *Sirex* based on external characteristic, y_1 = the percentage of trees confirmed positive, w = number of larvae per tree, and density = number of trees per hectare. The potential population of adult females of *S. noctilio* (z_1) was estimated using the sex ratio of females to males obtained from the emergence in the breeding cages, assuming absence of mortality by parasitoids. The population of females per hectare (z_1) was calculated by multiplying the population of larvae per hectare (z) by the sex ratio.

Experimental design and statistical analysis

The level of attack (y) in the mega-traps was estimated with a completely randomised repeated measures design with a unifactorial structure of the treatments. The linear model of the response variable corresponded to:

$$y_{ijk} = u + t_i + r(t)_{ij} + p_k + tp_{ik} + \varepsilon_{ijk}$$

Table 2: Total number of trees attacked by *Sirex noctilio* in the mega-trap-plots during the entire period of evaluation for each treatment. Mean (±SE) attack level (%) of *S. noctilio* considers all trees in the mega-trap-plots and trees effectively weakened by herbicide. The statistical analysis shown is for each period of evaluation

Evaluation period (day)	Treatment	Tree condition[1]		*Sirex* attack on all trees (%)	Tree condition[2]		*Sirex* attack on mega-traps (%)[3]
		Healthy	Attacked		Healthy	Attacked	
Day 0	Control	3 607	0	0	3 607	0	0
	November	950	0	0	950	0	0
	December	926	0	0	926	0	0
	January	1 019	0	0	1 019	0	0
	February	917	0	0	917	0	0
Day 30	Control	3 499	108	2.99 ± 0.29	3 499	108	3.11 ± 0.29[a]
	November	918	32	3.37 ± 0.44	918	32	3.45 ± 0.44[a]
	December	902	24	2.59 ± 0.38	902	0	0
	January	979	40	3.93 ± 0.47	979	0	0
	February	894	23	2.51 ± 0.56	894	0	0
Day 60	Control	2 932	675	18.71 ± 3.10	2 932	675	19.53 ± 3.10[a]
	November	433	517	54.42 ± 3.59	433	517	53.84 ± 3.59[b]
	December	491	435	46.98 ± 4.12	491	411	44.50 ± 4.18[b]
	January	772	247	24.24 ± 2.08	772	0	0
	February	632	285	31.08 ± 4.90	632	0	0
Day 90	Control	2 715	892	24.73 ± 5.47	2 715	892	26.21 ± 5.47[a]
	November	396	554	58.32 ± 3.76	396	554	57.75 ± 3.76[b]
	December	340	586	63.28 ± 6.10	340	562	60.69 ± 6.23[b]
	January	355	664	65.16 ± 4.68	355	417	53.32 ± 6.70[b]
	February	567	350	38.17 ± 6.48	567	0	0
Day 240	Control	2 665	942	26.12 ± 5.65	2 665	942	27.57 ± 5.65[a]
	November	290	660	69.47 ± 4.97	290	660	68.87 ± 4.97[b]
	December	287	639	69.01 ± 6.00	287	615	66.54 ± 6.14[b]
	January	316	703	68.99 ± 4.98	316	456	57.08 ± 7.14[b]
	February	488	429	46.78 ± 7.39	488	79	21.98 ± 8.64[a]

[1] Includes all trees present in the mega-traps (with or without herbicide). Represents the sanitary condition (*Sirex* attack) of trees in the mega-trap-plot, for each evaluation period

[2] Excludes trees that have not been treated, with the exception of the control

[3] Mean values followed by the same superscript letter are not significantly different ($p < 0.05$), according to least squares means

Table 3: Number of symptomatic trees analysed and mean infestation by *Sirex noctilio* in the mega-trap-plots to the end of the evaluation period (July 2011)

Treatment	Symptomatic trees harvested		Mean infestation	SD	MC error[1]	Median	Credible interval	
	Total	Infested					2.5%	97.5%
Control	220	121	0.5494[a]	0.0333	2.977E−4	0.5500	0.4821	0.6129
November	220	201	0.9100[b]	0.0191	2.054E−4	0.9114	0.8692	0.9438
December	220	173	0.7838[b]	0.0280	2.757E−4	0.7847	0.7268	0.8355
January	176	138	0.7813[b]	0.0304	2.935E−4	0.7824	0.7187	0.8380
February	132	26	0.2011[c]	0.0345	2.409E−4	0.2001	0.1375	0.2725

[1] Monte Carlo (MC) error and the credible interval proportion (π) were calculated using Gibbs sampling. Mean values with the same letter are not significantly different according to the Bayesian credible interval

where y_{ijk} = the kth observation, u = the mean total, t_i = the fixed effect of the ith month of establishment (i = 1,2,…,5), $r(t)_{ij}$ = the random effect of the jth mega-trap (j = 1,2,3,…,11) in the ith month, p_k = the fixed effect of the kth evaluation period (k = 1,2,…,5), tp_{ik} = the fixed effect of the interaction month × period, and ε_{ijk} = the random effect of the residuals. The analysis was performed using the methodology of mixed models, with the MIXED procedure of SAS® 9.2 (SAS Institute, Cary, NC, USA). The normality assumption was verified with the Shapiro–Wilk statistical test and, when necessary, arc-sine transformation of the square-root ($\sin^{-1}\sqrt{y}$) was used to transform the percentage values and meet the assumption of normality required by the mixed model. The compound symmetry covariance structure, the unstructured covariance structure and the first-order autoregressive covariance structure were modelled using an approach of restricted maximum likelihood (Littell et al. 1998). The most parsimonious model was selected, using Schwarz's criteria for the Bayesian information. The classification of means obtained in each treatment for the periods of study was conducted using the GLIMMIX procedure of SAS® 9.2, by comparing the mean differences of minimal squares.

Bayesian statistics were used to analyse the infested trees during the final period, assuming a random binomial distribution for this variable. For the proportion of infested trees (y_1), a beta distribution was assigned *a priori*. Using the software WinBUGS® (Bayesian Inference Using Gibbs Sampling), the Bayesian credibility interval was calculated for this proportion (π). The Gibbs algorithm was run for 11 000 iterations, using the first 1 000 iterations as burn-in.

The larval population of *S. noctilio* present in the infested trees (w) and the estimated potential population of adult females (z_1) was adjusted to the variance model:

$$w_{ij} = u + t_i + e_{ij}$$

where w_{ij} represents the mean value of the population at the *i*th month in the *j*th mega-trap, u is the total mean, and e_{ij} is the experimental error. The data were analysed using the GLM procedure of SAS® 9.2. The transformation of the square-root $\sqrt[2]{z}$ was applied, to comply with the normality assumption according to the Kolmogorov–Smirnov test. The significance of differences between means was determined using the Tukey test.

Results

Emergence period of Sirex noctilio

The emergence of *S. noctilio* adults between November 2010 and April 2011 showed that emergence started in December and extended until April (Figure 3). The peak emergence was observed in January, which was 75.7% of the total, whereas February and March showed emergence that was 16.8% and 6.3% of the total, respectively. The sex ratio was 1:0.56 males to females.

Level of attack in the mega-trap-plots

A total of 3 812 trees in the mega-traps and 3 607 trees in the control plots were evaluated during each period. The establishment of the MTPs in each month showed significant statistical differences in the level of wood wasp attack ($F_{4,44.7} = 2.02$; $p < 0.0001$), the effect of the evaluation period ($F_{4,174} = 216.4$; $p < 0.0001$) and the interaction between both effects ($F_{16,174} = 42.14$; $p < 0.0001$). The month of mega-trap establishment interacted significantly with the period of evaluation (Table 2). The evaluation conducted at 240 d after the establishment of the MTPs showed that the highest level of attack was observed by those that were established in November, with 68.8%, followed by December with 66.5% and January with 57.1%, with no significant differences among them observed. The level of attack in the treatment established in February was 21.9%, and was significantly different from the control, which showed an attack level of 27.5% (Table 2).

Infestation level in the mega-trap-plots

A total of 748 symptomatic trees were harvested in the MTPs during the entire period of evaluation. Of these trees, 538 trees (71.9%) were infested or colonised by *Sirex* (Table 3). In the control, 121 (55%) trees out of a total of 220 were infested. The Bayesian interval of credibility showed a level of infestation of 91% in November, 78.3% in December and 78.1% in January, although no significant statistical differences were observed among the months. However, significant differences were observed between the control, with 54.9% of infestation, and the MTP installed in February with 20.1% infestation, and the other treatments (Table 3).

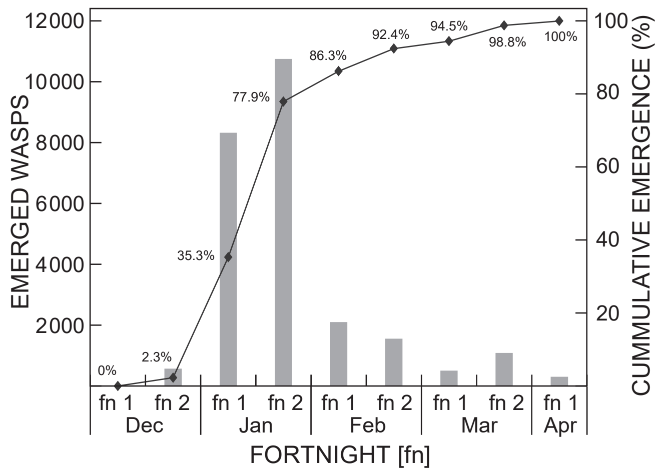

Figure 3: Sirex wasp emergence fortnightly from December 2010 to April 2011 in the study area and cumulative emergence (%)

Estimation of the larval and adult population of Sirex noctilio *in the mega-trap-plots*

In the MTPs the effect of month on the larval populations of *S. noctilio* was significant ($F_{4,50} = 32.65$; $p < 0.001$). The ranges obtained were from 101 938 larvae ha^{-1} in November to 64 858 larvae ha^{-1} in January. The control and February MTPs contained populations of 18 841 and 2 224 larvae ha^{-1}, respectively, which were significantly different (Tukey test; $p < 0.05$) to those in the other treatments (Table 4).

Similarly to larvae populations, the estimated total population of *S. noctilio* females showed significant differences between months ($F_{4,50} = 32.65$; $p < 0.001$). The highest number of adults was achieved in the mega-traps installed in November, with 57 901 females ha^{-1}, followed by December and January with 44 158 and 36 839 females ha^{-1}, respectively, with no statistical differences between them observed. However, the mega-trap of February, which possessed a potential number of 1 263 adult females ha^{-1}, differed significantly from the population in all other treatments. In the control mega-traps, the population of 10 701 females ha^{-1} also differed significantly from that in all other treatments (Table 4, Figure 4), exhibiting a potential population of females 5.4-fold lower than that obtained in the mega-trap installed in November and 4.1-fold lower that those installed in December.

Discussion

The efficiency of the use of MTPs in removing significant numbers of *S. noctilio* by harvesting and destroying the logs (pulp, sawn timber or any action to prevent the emergence of wasps) when the population was at an epidemic level was evaluated for the first time in Chile. At the final period of evaluation, the degree of attack by female *S. noctilio* was significantly higher in the quadrants corresponding to the first three months of MTP establishment, fluctuating

Table 4: Mean ± SE *Sirex noctilio* larval population per tree and per hectare for each treatment. Estimation of the potential population of *S. noctilio* females (Mean ± SE), according to attack level, infestation and larvae population

Treatment	*Sirex* attack (%)	*Sirex* infestation (%)	Larvae tree^{-1}*	Larvae ha^{-1}*	*Sirex* females ha^{-1}*†
Control	27.6	54.9	334 ± 40[a]	18 841 ± 5 132[a]	10 702 ± 2 915[a]
November	68.9	91.0	435 ± 34[b]	101 939 ± 12 043[b]	57 901 ± 6 841[b]
December	66.5	78.4	415 ± 50[b]	77 744 ± 9 673[b]	44 159 ± 5 494[b]
January	57.1	78.1	384 ± 46[b]	64 859 ± 14 834[b]	36 840 ± 8 426[b]
February	22.0	20.1	136 ± 55[c]	2 224 ± 1 030[c]	1 263 ± 585[c]

* Means within a column followed by the same superscript letter are not significantly different ($p < 0.05$), according to the Tukey test
† The sex ratio used was 1:0.56 males to females. Absence of mortality was assumed

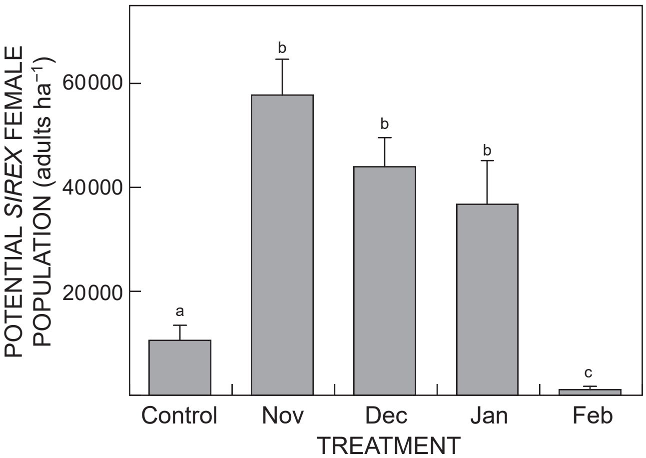

Figure 4: Potential population of *Sirex noctilio* females (adults ha^{-1}) for each of the treatments evaluated. Treatments with the same letter are not significantly different (Tukey test; $p < 0.05$). Error bars represent the SE

between 69% in November to 57% in January, compared with 28% in the controls and 22% in the treatment establishment in February. These results could be explained by the degree of synchrony between the months of establishment of the MTP with the flight period of *S. noctilio* (Zylstra et al. 2010) and by the effect of the aggregation of the females of *S. noctilio* when they emerge and start to attack the trees (Corley et al. 2007).

The MTPs spatially concentrated trees that were weakened by herbicide in different months of establishment, and then they effectively attracted females of *S. noctilio* by emitting volatiles into the environment (Neumann et al. 1987; Böröczky et al. 2009). To overcome the resistance barriers of the tree, the females make repeated injections of phytotoxic mucus and inoculations of *A. aerolatum* spores to successfully oviposit (Bordeaux and Dean 2012; Ryan and Hurley 2012). In the MTP, these resistance mechanisms are inhibited by herbicide action and the degree of attack was greater in the months where the trees were exposed, from three to five months depending on the month of installation, to the females that were found flying within the area (Neumann et al. 1982; Zylstra et al. 2010). Consequently, the *Sirex* attack process in the MTP's control was slower than in the treated plot, due to the fact that they were not stressed by herbicide application.

The establishment period of the MTPs (November to February) affected the attack, infestation and the captured population of *S. noctilio*. In this study, a greater level of attack was observed by females that were concentrated between November and January, and this seasonality was related to the flight period of the insect (Iede et al. 2012). The flight period in the study area occurred from December to April, which coincides with other records from Chile (Rojas and Beéche 2010), and resulted in the largest emergence percentage between January (75.7%) and February (16.8%). Mega-trap-plots were installed in November, December, January and February and were evaluated for attack by *S. noctilio* in December (30 d), January (60 d), February (90 d), March (120 d) and June (240 d). All these periods were relative to the November treatment. In December, only 2.3% of the total population had emerged and the attack on the traps tree in November was only 3.1%. In January, the degree of attack on the mega-traps established in November and December was 53.8% and 44.6%, respectively.

The total attack on these traps increased by 3.9% and 16.1% between January and February. In contrast, the plots installed in January showed a 52.3% level of attack after 30 d. These values confirm that the traps tree installed one month before or at the beginning of the flight period exhibited the highest percentage of attack, similar to observations in the Northern Hemisphere (Zylstra et al. 2010). The MTPs established in February only reached a 22% level of attack by June (240 d), although the population of female *S. noctilio* was still 20.3% of the maximum observed in February and March. From the obtained results, it follows that the MTPs are attractive to *S. noctilio* for at least 90 d from their establishment based on the results of attack obtained from November and December. The duration of this period might be related to various factors, such as tree species, the type of herbicide, its mechanism of action, the number of doses per tree and the environmental conditions. In Chile, the herbicide used for the MTP installation (picloram) resulted in the gradual wilting of trees and not the rapid dehydration of trees (SAG 2008; MAP and RA unpublished data), which then caused the tree to die, thereby reducing the attraction of *S. noctilio* (Hurley et al. 2007). In the present study an average of 12 mL tree^{-1} of Tordon® 24K (3 g picloram) was used. For up to 90 d (the start of February), the presence of dead trees with chlorotic (reddish brown) foliage was not observed during the visual observations of the MTPs, which is a characteristic symptom of drying and tree death (Ismail and Mutanga 2010). In contrast, the creation of trap trees of *Pinus sylvestris* and *P. ponderosa* in the north-eastern USA, using 0.96 mL dicamba tree^{-1} as a herbicide, showed chlorosis and death between the third and fourth week after treatment (Zylstra et al. 2010). In Australia, the application of dicamba to *P. radiata* trap trees resulted in mortality from the fourth month (Neumann et al. 1982), using an equivalent dose to that applied in Chile, before the herbicide was prohibited by the Forest Stewardship Council.

The degree of infestation and population of *S. noctilio* in the MTPs was closely related to the level of attack observed. The largest infestation occurred in the MTPs installed between November and January, which were also the periods when the greatest attack was observed. This can be explained by the highest exposure to females during their flight period within this period, and to the greatest susceptibility of trees that had already been weakened by the repeated inoculations of phytotoxic mucus and *A. aerolatum* spores by the wasps (Zylstra et al. 2010; Bordeaux and Dean 2012). Repeated inoculations possibly represent one of the most important factors in the attack of *S. noctilio*. In a dynamic context of spatial aggregation, the probability of a large number of inoculations on the trees is increased, which affects the physiology of the tree and creates conditions for the optimal development of the *A. aerolatum* fungus (Corley et al. 2007; Slippers et al. 2015).

In the present study, the largest populations of larvae collected per hectare were observed in the MTPs installed in November, when 101 938 specimens were collected. These trap trees were also exposed to attack by females for the longest period of time. In contrast, the treatment installed in February yielded the lowest population, with only 2 224 larvae ha^{-1} recovered at the end of the evaluation period. This decrease in the wasp population in the MTPs of February might be because the trap trees were not sufficiently debilitated by herbicide for a successful attack by the female population to occur. In the control plots, the attack by *S. noctilio* was observed during the entire evaluation period, but was possibly influenced by the mechanisms of resistance of the trees, which inhibited the successful colonisation of the wasps (Madden 1977; Taylor 1981). The population of larvae in the control trees, which was 5.4-times lower than that in the MTPs in November, allows an estimate for that month of a potential population of 57 901 females, whereas that in the control only reached 10 701.

Conclusions

This research has been the first to evaluate a method of *Sirex* management population, using mega-trap-plots (MTPs) as a mass trapping system that increase the *Sirex* populations in a single large area then remove the future populations of wood wasp females via harvesting of the trap trees for use as pulpwood, before all of the wasps emerge. This process is a classical method of ethological control and demonstrates the efficacy of the MTPs as a tool of *Sirex* management in stands of radiata pine plantations with epidemic level attack by the pest.

Mega-trap-plots can be installed over a three-month period (November to January) to synchronise with the peak *S. noctilio* flight period without affecting the potential of attraction of the females populations.

Mega-trap-plots could be used in an intensive biological control program as a massive inoculation system for the nematode *D. siricidicola.* The efficacy of this method could be improved, installing MTPs that combine the best two months to attract the main population of *Sirex* females (November–December).

Further research is needed to assess the optimum size of the MTPs, in terms of the number of trap trees to install monthly, and their optimum spatial distribution. The overall results of this study suggest that MTPs could be used as a novel method of *Sirex* wood wasp management in *P. radiata* plantations.

Acknowledgements — We thank Bioforest SA and Forestal Arauco for financial and technical support. We also thank various colleagues including Mr R Gómez, Mr M Oliva and Mr A Durán for support and useful discussions that helped the completion of this study. Mr P Salas from Universidad de Concepción and Mr R Saavedra are thanked for assistance with statistical analysis and GIS work, respectively.

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Received 4 January 2016, revised 24 March 2016, accepted 27 March 2016