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Long-term quantification of leaf-cutting ant damage in willow forestations in the lower delta of the Paraná River, Argentina

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Abstract

1. Leaf-cutting ants are major pests of Neotropics forest plantations. The lower delta of the Paraná River contains the main Argentine Salicaceae production, strongly attacked by *Acromyrmex lundii* and *Acromyrmex ambiguus*. Nevertheless, there is no damage quantification in willow plantations attributed to leaf-cutting ant species.
2. In an area without leaf-cutting ant control, we installed 15 blocks with eight willow stakes each: four clones × two treatments; with and without leaf-cutting ant exclusion. We used two traditional (Americano, Nigra 4) and two new (Géminis, Yaguareté) commercial clones. During 2014–2018, we measured the damaged foliage, height and diameter of each tree.
3. Foliage was damaged intensely during the first 2 years. After 1537 days, the loss in height and diameter was greater in Americano (70%), followed by Géminis (50%–60%), Yaguareté (40%–50%) and Nigra 4 (45%–40%). Stake survival with exclusion was greater (>80%) than stakes without exclusion (<50%). Total loss of wood volume was 93% for Americano, followed by Géminis (77%), Yaguareté (66%) and Nigra 4 (51%).
4. Although the new clones were heavily attacked, they produced two to three times more wood volume than Americano; replacing Americano with the new clones would help to reduce leaf-cutting ants impact on plantations and pesticides released into the environment.

KEYWORDS
Acromyrmex, forest plantation, Neotropical region, *Salix*, wood losses

INTRODUCTION

Leaf-cutting ants (LCA) are one of the most important herbivores in the Neotropical region, even surpassing mammals and other insects, such as Homoptera and Lepidoptera (Hölldobler & Wilson, 1990). By damaging or consuming different plant structures, LCA impact the life cycle of plants, reducing its growth and reproduction and thus increasing its mortality. The damage can also favour the entry of fungi and pathogens in the attacked vegetal species, which reduces its survival (Pérez et al., 2011; Prins & Verkaar, 1992). Plant responses to herbivory involve different mechanisms such as physiological changes in photosynthesis rates, hormones and redistribution of resources, and morphological changes in apical domination or type of ramification (Prins & Verkaar, 1992).

LCA are considered pests because they defoliate a large number of crops of economic importance, such as coffee, cocoa, citrus, sorghum, sunflower, sugar cane, vineyards and managed forest (Anglada et al., 2013; Cantarelli et al., 2008; Cantarelli et al., 2019; Montoya-Lerma et al., 2012; Pérez et al., 2011; Sánchez-Restrepo et al., 2019). In forestry systems, *Eucalyptus*, *Pinus*, *Populus* and *Salix* plantations are the most affected by LCA (Jiménez et al., 2021; Montoya-Lerma et al., 2012; Sánchez-Restrepo et al., 2019). Damage is intense in the first years of planting, when their control can represent between 30% and 75% of the total budget dedicated to pest management (Della

Lucia et al., 2014; Vilela, 1986). Later on, the management intensity can be reduced (Cantarelli et al., 2008; Cantarelli et al., 2019; Lewis & Norton, 1973; Nickele et al., 2012; Vasconcelos & Cherrett, 1995). Consequently, a proper estimation of LCA damage in the early stages of plantation growth is crucial to optimize control strategies.

Previous studies showed that LCA are responsible for high levels of defoliation that negatively affect plant growth in several types of plantations. In Brazilian eucalyptus plantations (*Eucalyptus* spp.), 75% defoliation was followed by a loss of 16%–42% in volume of wood production (Zanuncio et al., 1999). Losses of 12% in height and 19% in diameter were observed by Matrangolo et al. (2010). Della Lucia (1993) reported that LCA attacks can be higher on young trees (<6 months old) in *Eucalyptus grandis* Hill ex Maiden forestations, causing reductions of up to 32% in height, 25% in circumference and 60% in wood production. Mortality in *E. grandis* occurred after three consecutive defoliations and can reach 30% (Mendes Filho, 1979). Other strongly affected plantations are pines (Araújo et al., 1997; Hernández & Jaffé, 1995; Montoya-Lerma et al., 2012; Nickele et al., 2012; Nickele et al., 2020). Defoliation levels caused by LCA varied between 14% and 50% in coniferous seedlings in Brazil and Venezuela (Montoya-Lerma et al., 2012). Hernández and Jaffé (1995) quantified the damage in under 10-year-old *Pinus caribaea* Morelet plantations in Venezuela, where nests of *Atta laevigata* Smith had a negative effect on the timber production; the harvested wood volume was reduced by half. Meanwhile, an intense defoliation caused by *Acromyrmex crassispinus* Forel in *Pinus taeda* L. plantations in Brazil caused a reduction of 13% in height, 20% in diameter and a mortality between 4% and 8% per hectare in the first 30 days of planting (Nickele et al., 2012). In a recent study, Nickele et al. (2020) found that a complete defoliation (including the apical meristem) during the first 30 days could result in a loss of 22% in diameter and 43% in volume, measured 10 years after planting. All these studies provide consistent evidence that LCA have significant negative effects on forest plantations, both due to a reduction in wood production and an increase in the cost of LCA management and control. As a consequence of the high levels of damage reported in the Neotropical region, excessive doses of synthetic insecticides are used to control LCA, which results in a high environmental cost due to its toxicity and lack of specificity (Della Lucia et al., 2014; Isenring & Neumeister, 2010; Lemes et al., 2017; Montoya-Lerma et al., 2012; Zanetti et al., 2014; Zanuncio et al., 2016). As an alternative strategy to the use of insecticides, studies in the region are also being oriented towards the genetic improvement of forest species (e.g., pines, eucalyptus, willows and poplars). By crossing different species of the same forest genus, individuals with a better performance than their predecessors can be selected (e.g., wood production, health, adaptation to different environments and suitability of the wood). These selected individuals can then be cloned from stakes and finally released on the market, offering improved implantation material to forest producers (INTA, 2022).

In Argentina, the largest extensions of native and cultivated forests are located in the Mesopotamian (subtropical-temperate) region with great emphasis on the production of pines, eucalyptus and Salicaceaes (poplars and willows) (Borodowski, 2011; Cantarelli et al., 2008; Cantarelli et al., 2019; Jiménez et al., 2021; Sánchez-Restrepo et al., 2019). The highest LCA species richness is also found in this region (12 to 3 species along a north–south latitudinal gradient), although only two to three species are locally problematic (Farji-Brener & Ruggiero, 1994; Sánchez-Restrepo et al., 2019). LCA has been considered a pest in Argentina after 1907, in particular *Acromyrmex* species (Bonetto, 1959). Research on *Pinus* spp. in Corrientes province revealed that *Acromyrmex* species can cause a reduction of 17% in diameter, 12% in height and a loss of 21% of seedlings during the first 65 days (Cantarelli et al., 2008); the attack remained high for the first 2 years, and then decreased in the third. Other studies mention LCA as potential pests because of their high defoliation rates in pine plantations due to *Acromyrmex ambiguus* Emery in the Mesopotamian province of Entre Ríos (Elizalde et al., 2015) and to *Acromyrmex lobicornis* Emery southernmost in northern Patagonia (Pérez et al., 2011). The willow forest plantations (*Salix* spp.) in the lower delta of the Paraná River are highly affected by two usually sympatric LCA species: *Acromyrmex lundii* Guérin-Méneville and *A. ambiguus* (Sánchez-Restrepo et al., 2019). A third LCA species, *Acromyrmex heyeri* Forel, was reported in the delta region, but not associated to Salicaceae plantations (Sánchez-Restrepo et al., 2019). A recent study related mortality of a 1-year-old *Salix nigra* Marshall plantation and lost wood volume to *A. lundii* nest density in Entre Ríos province (Jiménez et al., 2021). Knowing the pest LCA species and their true level of damage in Salicaceae forest plantations helps to promote more rational LCA control methods.

The aim of this study was to quantify the damage produced by *A. lundii* and *A. ambiguus* in an experimental willow plantation according to (i) the forest species or clone planted, and (ii) the age of the plantation, (1) to establish which are the species or clones least attacked (least defoliated) by the LCA and the species or clones most tolerant to LCA damage (those whose growth and survival were least affected by foliage damage); and (2) to determine up to what age of the plantation LCA control is necessary.

MATERIALS AND METHODS

Experimental design

In August 2014 (winter in the Southern Hemisphere), an experimental willow plantation was established in the Experimental Agricultural Station Delta of the Paraná River (−34.171440S, −58.860867W), which belongs to the National Institute of Agricultural Technology (EEA INTA Delta, acronym in Spanish) in Campana, Buenos Aires province, Argentina. Four clones of willow (*Salix* spp.) were used; two clones are widely used in the region: Soveny Americano (*Salix babylonica* L. var. *sacramenta*) and Alonzo Nigra 4 INTA (*S. nigra*), and two new clones recently released on the market: Géminis INTA CIEF NZ694 (*Salix matsudana* Koidz) and Yaguareté INTA CIEF SI64-004 (*Salix alba* L.) from authorship of Teresa Cerrillo in EEA INTA Delta (INTA, 2020).

A total of 120 stakes (30 per clone), 150-cm-high (buried 50 cm) were planted in a sector of 1 ha without LCA control and with livestock exclusion. The most common form of reproduction of forestry species is by stakes (Casaubón, 2013). Each clone or species is planted in a plot or staker from which stakes are cut each year according to the use that they will be given. The stakes of the four clones (Americano, Géminis, Nigra 4 and Yaguareté) were planted in 15 blocks (~20 m apart from each other). Each block contained two stakes of each clone randomly assigned to one of two treatments: LCA exclusion (stake with trunk physical barrier to avoid ants) and control (stake with no trunk physical barrier) treatments. Stakes were randomly located within each block, 2 m away from each other and 2 m from the other clones (2 × 4 = 8 stakes per block, Figure 1). The exclusion consisted of a sliding external plastic barrier with foam rubber on the inside; wrapped round the stake (trunk) 50–60 cm from the ground that impedes LCA access to the leaves. The success of this exclusion method was previously tested (Casaubón et al., 2018; Montoya-Lerma et al., 2012; Moressi et al., 2007; Pérez et al., 2011). Each block was surrounded by a matrix of spontaneous native (e.g., *Solidago chilensis* Meyen, *Erythrina crista-galli* L., *Prosopis nigra* (Griseb.) Hieron, *Acacia caven* (Molina) Molina, *Panicum grumosum* Nees, *Ludwigia elegans* (Cambess.) Hara) and exotic (e.g., *Phytolacca*

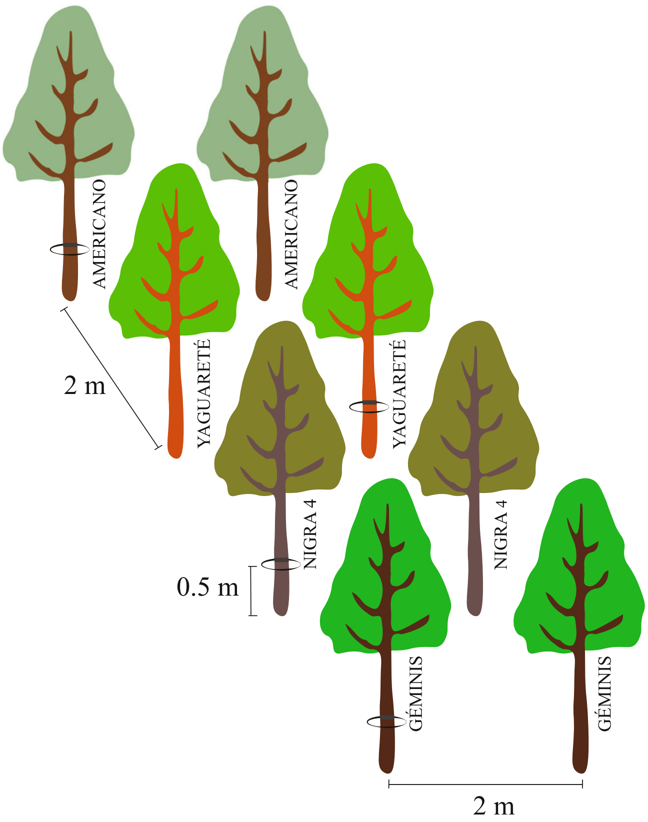

FIGURE 1 Diagram of an experimental block with eight stakes, two per *Salix* clone, one with a leaf-cutting ant exclusion (physical barrier, represented with a black circle around the tree) and another without the exclusion (without a barrier). The order of the clone inside the block and the stake with the exclusion were randomized.

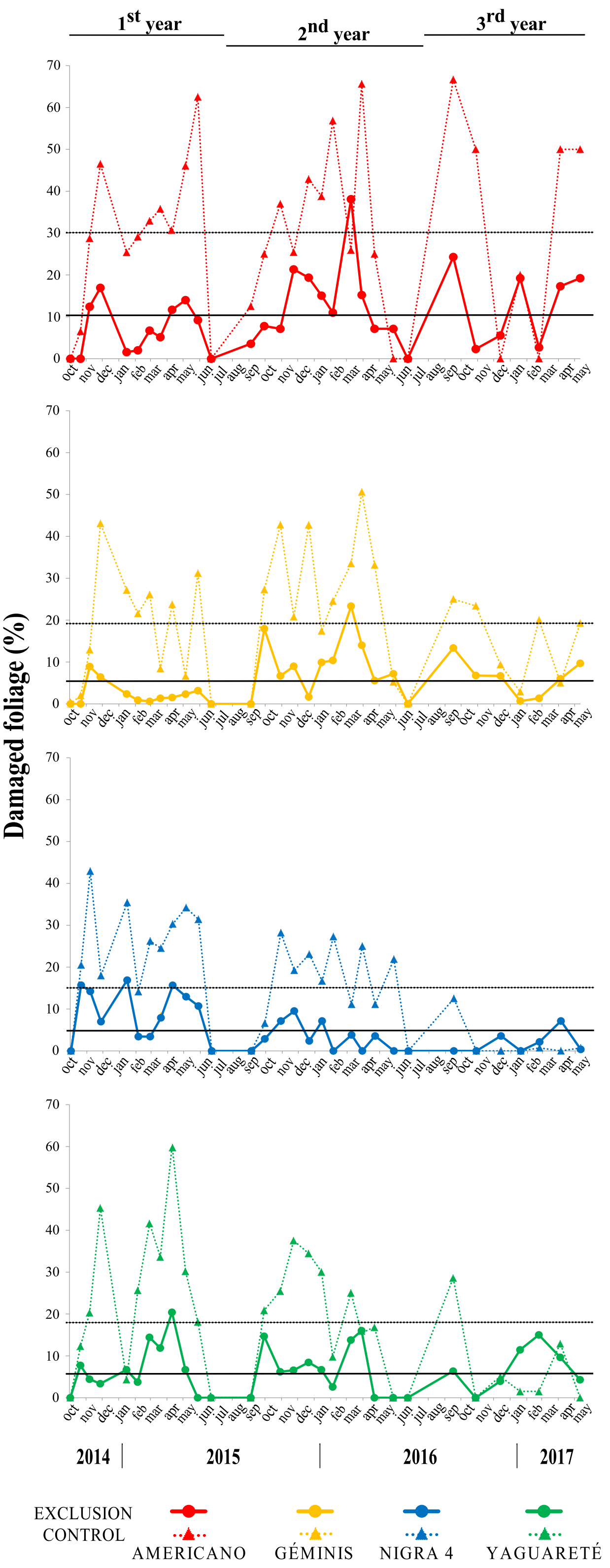

FIGURE 2 Percentage of damaged foliage (sprouts and branches) of stakes/trees of four *Salix* clones with and without leaf-cutting ant (LCA) exclusion, from implantation in august 2014 until the third year (mid-2017). The average damage percentages of each clone with (solid black lines) and without (dashed black lines) LCA exclusion over the first 3 years are shown in each frame. CONTROL = with LCA access (no physical barrier), EXCLUSION = with LCA exclusion (physical barrier to exclude ant access)

TABLE 1 Statistical values (*F* and *p* values) of generalized linear mixed models (GLMMs) of height and diameter in a field experiment in blocks with four *Salix* clones under two treatments, one with barriers on the trunks to prevent the leaf-cutting ants climbing stakes/trees to attack foliage (exclusion) and the other without the barriers (control)

Variable	Year	Interaction	*F* value	*p* value
Height	1st (2014–2015)	clone × exclusion × time	$F_{33,1344} = 0.61$	0.959
		clone × exclusion	**$F_{3,1344} = 4.96$**	**0.002**
		clone × time	**$F_{33,1344} = 19.16$**	**<0.0001**
		exclusion × time	**$F_{11,1344} = 18.35$**	**<0.0001**
	2nd (2015–2016)	clone × exclusion × time	$F_{33,1344} = 0.15$	1.000
		clone × exclusion	**$F_{3,1344} = 4.43$**	**0.004**
		clone × time	**$F_{33,1344} = 3.12$**	**<0.0001**
		exclusion × time	**$F_{11,1344} = 10.72$**	**<0.0001**
	3rd (2016–2017)	clone × exclusion × time	$F_{12,560} = 0.10$	1.000
		clone × exclusion	**$F_{3,560} = 3.70$**	**0.012**
		clone × time	$F_{12,560} = 1.01$	0.442
		exclusion × time	**$F_{4,560} = 3.22$**	**0.013**
	5th (2018–2019)	clone × exclusion	$F_{3,112} = 0.74$	0.534
		clone	**$F_{3,112} = 23.50$**	**<0.0001**
		exclusion	**$F_{1,112} = 91.37$**	**<0.0001**
Diameter	1st (2014–2015)	clone × exclusion × time	$F_{12,560} = 0.10$	1.000
		clone × exclusion	$F_{3,560} = 1.48$	0.220
		clone × time	$F_{12,560} = 1.62$	0.081
		exclusion × time	**$F_{4,560} = 10.80$**	**<0.0001**
	2nd (2015–2016)	clone × exclusion × time	$F_{12,560} = 0.14$	0.999
		clone × exclusion	$F_{3,560} = 1.19$	0.314
		clone × time	**$F_{12,560} = 1.84$**	**0.039**
		exclusion × time	**$F_{4,560} = 11.84$**	**<0.0001**
	3rd (2016–2017)	clone × exclusion × time	$F_{9,448} = 0.16$	0.988
		clone × exclusion	$F_{3,448} = 1.09$	0.353
		clone × time	$F_{9,448} = 0.89$	0.538
		exclusion × time	**$F_{3,448} = 6.32$**	**0.0003**
	4th (2017–2018)	clone × exclusion	$F_{3,112} = 0.56$	0.642
		clone	**$F_{3,112} = 3.32$**	**0.020**
		exclusion	**$F_{1,112} = 22.80$**	**<0.0001**
	5th (2018–2019)	clone × exclusion	$F_{3,112} = 0.78$	0.510
		clone	**$F_{3,112} = 7.82$**	**0.0001**
		exclusion	**$F_{1,112} = 78.21$**	**<0.0001**

Note: Significant values are in bold.

americana L., *Lonicera japonica* Thunb., *Gleditsia triacanthos* L., *Fumaria capreolata* L., *Rubus fruticosus* L., *Ligustrim sinense* Lour., *Sonchus oleraceus* L., *Carduus acanthoides* L., *Cirsium vulgare* (Savi) Ten., *Iris pseudacorus* L.) vegetation, characteristic of the lower delta of the Paraná River (Kandus et al., 2006; Perri et al., 2020). Inside each block, vegetation was periodically cut to avoid LCA using it as a bridge to overcome barriers and access to foliage.

Four variables were measured: (1) percentage of foliage damaged (sprouts and branches with damaged leaves) on stakes—later trees—by LCA (*A. lundii* and *A. ambiguus*; the only two LCA species found in the EEA INTA Delta) to characterize direct damage on plants; (2) plant height; (3) stem diameter (measured on trunk at ground level); and (4) survival of stakes and trees to determine how the direct LCA damage on the foliage affected their growth and survival indirectly (Cantarelli et al., 2008; Pérez et al., 2011). The percentage of damaged foliage was estimated by counting shoots and branches of each stake with and without damaged leaves during the first 2 years. In the third year, the quantitative estimation of damaged foliage was changed into a visual qualitative estimation, because the size of the trees made it difficult to count branches with and without damaged leaves. The crown of each tree was visually divided in quarters (25% each), and depending on defoliation in each quarter, a percentage of damage was assigned. The percentage could later on be adjusted depending on the intensity of the damage obtaining damage percentage values in increments of 5 (e.g., 10%, 25%, 60%, 85%). During the first 2 years of the plantation (2014–2015 and 2015–2016), the percentage of damaged foliage and the height of the stakes were measured every 20–30 days, whereas the diameter was measured every 60 days. In the third year (2016–2017), the percentage of damaged foliage and height were measured every 60 days and the diameter was measured every 60–90 days. Sampling was then spaced out, considering previous studies in which only the first 2–3 years of a plantation of several forestry species were the most affected by LCA (Cantarelli et al., 2008; Cantarelli et al., 2019; Nickele et al., 2012). In the fourth year (2017–2018), only the diameters were measured once. In the fifth year (December 2018), the height and diameter of the trees were measured for the last time.

Stake and tree mortality attributable to LCA damage (plants that had been heavily defoliated by LCA, easily identified by their semi-circular leaf-cutting patterns, usually starting at the apex of the stake/tree) and other factors (drought or injury by animals such as deer or cows) were recorded. A stake was considered dead, if after a total defoliation by LCA, it did not recover in the two following samplings. When mortality was due to other causes, the sampling date of when the damage was discovered was recorded as the death date.

Finally, trunk volumes were estimated by considering heights and diameters of each individual, control and LCA exclusion, in a cone area to estimate the lost wood of each clone. Tree volume estimations are generally performed using the Smalian formula considering the height of the main axis from the base to the smallest usable diameter (generally 5 cm, Manuel García Cortés, EEA INTA Delta, pers. comm.). Nonetheless, trees were still standing in the experiment when the data were analysed and this height could not be measured. The experiment was followed for 1537 days (4 years and 4 months, August 2014–December 2018) and involved 40 sampling instances.

Data analyses

For all the variables measured, the damage attributed to LCA resulted from the difference between defoliation values observed in control stakes (and later trees) without exclusion (damage caused by LCA plus other causes) and injury values observed in stakes/trees with LCA exclusion (damage to be expected only by other causes). Analyses of percentage of damaged foliage, height and diameter were modelled by using generalized linear mixed models (GLMMs), using clone (four levels: four clones), exclusion (two levels: control and LCA exclusion) and time (number of levels depended on the variable and the year analysed) as fixed factors and the block identity as a random factor. All analyses were performed with the R software (version 3.1.5; R Development Core Team, 2012) through the RStudio inter-phase (version 1.1.453; RStudio, 2012). The percentage of damaged foliage was analysed year by year and modelled using a binomial distribution (number of sprouts and branches damaged, number of sprouts and branches not damaged) for the first 2 years with the *glmer* function of the *lme4* package. The third year was analysed in a similar way but using the visual percentage of damaged foliage obtained as a proportion of the total foliage, as an independent variable. Months of June,

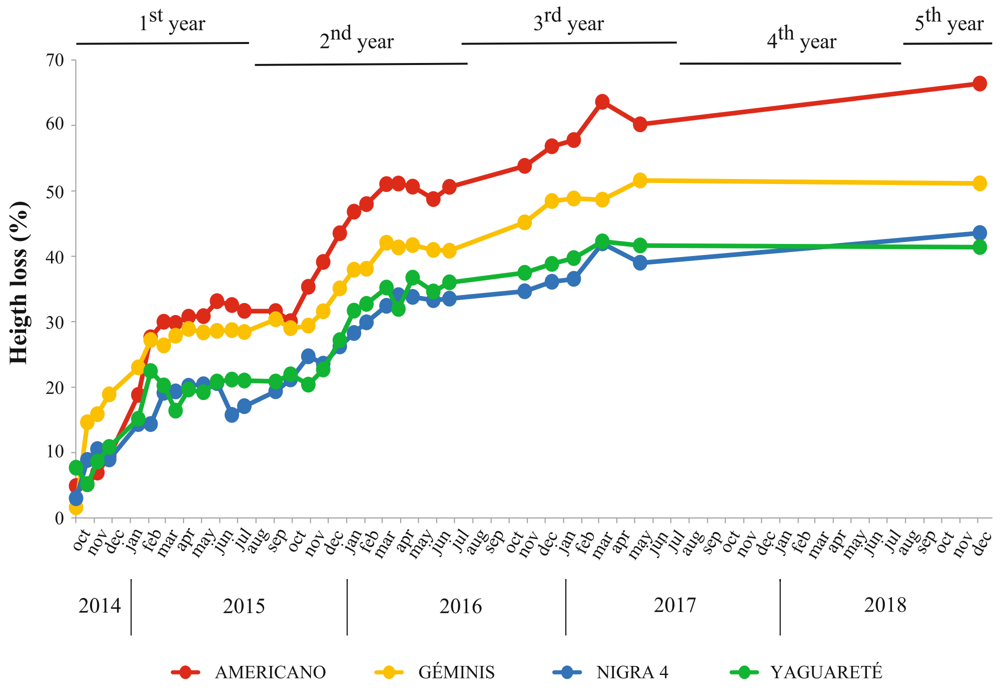

FIGURE 3 Height loss of stakes/trees of four *Salix* clones over 4 years and 4 months in an experimental plantation. Values correspond to the percentage of difference in height recorded for each clone between individuals with and without leaf-cutting ant exclusion

July and August (winter in the Southern Hemisphere), were not included in the statistical analyses because there are no leaves on willow trees in winter, so no data were recorded.

Height and diameter were analysed year by year for the first 3 years, with a normal distribution using the *gls* function of the *nlme* package. All possible models were run from null model to full model, the most complex (Bolker et al., 2009; Zuur et al., 2009) (e.g., models with only one factor, with all factors, with all the possible combinations, with and without interaction between the factors) also modelling the correlation matrix with the *corCompSymm* function and the heterocasticity through *varIdent* function. In the fourth year, only one diameter sampling instance was analysed and in the fifth year, height and diameter were analysed using the same function with only one measurement for each variable. For all measured variables, the most suitable minimum model was chosen according to the Akaike information criterion (AIC, the one with the lowest value) and the parsimony principle. Pairwise comparisons were performed controlling the global error with the *emmeans* function of the homonym package. For height and diameter, the percentages of loss per sampling instance was the difference between the mean of each variable per clone with LCA exclusion (considered as the maximum possible to be obtained, 100%) and the percentage that represents the mean of each variable per clone with control treatment.

Clone survival was modelled with a Kaplan–Meier analysis through *survfit* function of the *survival* package. This is a nonparametric statistic used to estimate the survival function from a lifetime dataset through a step function in a graphical representation that allows comparing populations using their survival curves (Kaplan & Meier, 1958). Kaplan–Meier also contemplates censoring, that is when the actual date of death of an observed individual is only partially known.

Lost volume estimations at the end of each year of the plantation were the difference between the mean of volume per clone with LCA exclusion (considered as the maximum possible to be obtained, 100%) and the percentage that represents the mean of volume per clone with control treatment. The total wood volume of the trees estimated at the end of the experiment resulted from the sum of the volumes of each tree in each treatment (clone × exclusion).

RESULTS

Damaged foliage

The model that best explained the evolution of LCA damage in each of the first 3 years was one that considered only exclusion and block identity. This model indicated a higher damage in the control treatment than in the LCA exclusion treatment in the first ($\chi^2_1 = 22.61$, $p < 0.0001$) and the second year ($\chi^2_1 = 5.77$, $p = 0.016$), regardless of the clone. No differences were found in the third year between treatments ($\chi^2_1 = 0.12$, $p = 0.729$). During each of the first 2 years, the damage pattern was highly variable for all clones, with samplings with great damage and others with low damage, showing a strong "peaks and valleys" pattern (Figure 2). During the third year, damage decreased in all clones, except in the Americano control treatment (without barriers) that continued to be characterized by a high level of damage (Figure 2). The average percentage of damaged foliage over the first 3 years for the control and the LCA exclusion treatments respectively was: for Americano 30.2 and 10.4%, Géminis 19.5 and 5.7%, Nigra 15.5 and 5.1% and for Yaguareté 17.9 and 6.6% (Figure 2). The average percentage of damaged foliage grouping all clones was 21% for the control treatment and only 7% for the LCA exclusion treatment. Thus, the difference in the average percentage of damaged foliage by defoliation attributed to the two LCA species in the experiment was 14%.

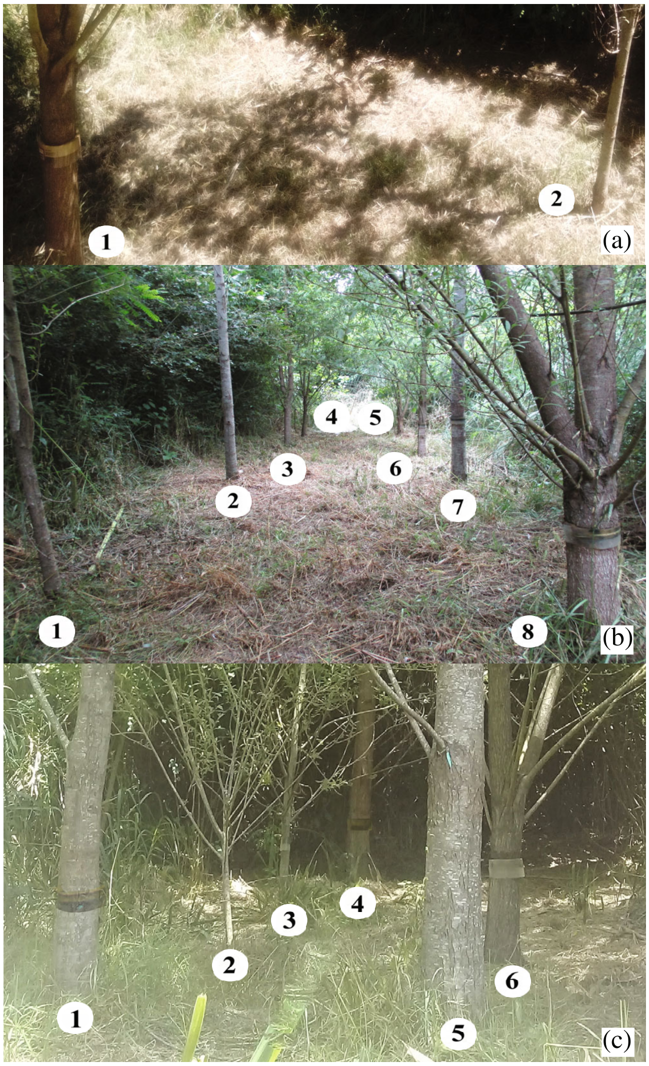

FIGURE 4 Comparison in diameter of (a) Yaguareté = 1: EXCLUSION and 2: CONTROL (December 2016). (b) CONTROL = 1: Yaguareté, 2: Nigra 4, 3: Géminis and 4: Americano; EXCLUSION = 5: Americano, 6: Géminis, 7: Nigra 4 and 8: Yaguareté (November 2017). (c) EXCLUSION = 1: Nigra 4, 3: Americano, 4: Géminis and 6: Yaguareté; CONTROL = 2: Yaguareté and 5: Nigra 4 (December 2018). CONTROL = with leaf-cutting ant access (no physical barrier), EXCLUSION = with leaf-cutting ant exclusion (physical barrier to exclude ant access)

Height

Over time, the height of stakes/trees differed between clones with and without the LCA exclusion. The model with a better fit to explain the variation in height in each of the first 3 years, considered clone, exclusion, time and block identity, modelling the correlation matrix and the heterocasticity. In the fifth year, the best model considered clone, exclusion and block identity, modelling the correlation matrix.

In each of the first 3 years of the plantation, the simple interactions clone × exclusion and exclusion × time were significant (Table 1). Pairwise comparisons for the clone × exclusion interaction showed higher stakes within LCA exclusion treatment than in the controls, with this difference more noticeable for Americano and Géminis, than for Nigra 4 and Yaguareté. The exclusion × time interaction showed that stakes, both with and without exclusion, were similar in height at the beginning of the plantation. Stakes in the LCA exclusion treatment were higher than in the control treatment in late spring of each year (November–December). In addition, during the third year, heights of trees of all clones with LCA exclusion did not differ in two consecutive measurements taken, but they did differ with the next measurements. The clone × time interaction was only significant for the first 2 years (Table 1). The clones did not show differences in height during the initial sprouting stage of each cycle (September–November), demonstrating their homogeneity. However, Americano grew less in height than the other three clones starting in late December.

During the fifth year, the effects of each fixed factor (clone and exclusion) were significant (Table 1). Pairwise comparisons showed that the height of Americano trees was lower than the other three clones, although the height of Géminis and Yaguareté trees did not differ, but they did differ from Nigra 4 trees. All trees within LCA exclusion treatment were significantly higher than the controls.

At the beginning of the fifth year (December 2018), all clones had at least one specimen that was over 9 m high. Trees of Nigra 4, Géminis and Yaguareté reached around 14–15 m in height, whereas Americano only reached approximately 10 m. On average, Nigra 4 was the tallest clone, followed by Géminis and Yaguareté, and finally Americano (Figures S1 and S2). At the end of the experiment, Americano was the clone that was most affected by LCA defoliation with a loss of almost 70% in height, followed by Géminis (50%), Nigra 4 (45%) and Yaguareté (40%) (Figure 3).

Diameter

The main difference in diameter, as in height, was observed between stakes/trees with and without LCA exclusion, with the maximum width difference in Americano trees at the end of the experiment. The modelling for each one of the first 3 years showed the best fit to the same model that explained height patterns over time (clone, exclusion, time as fixed factor and block identity as random factor, modelling the correlation matrix and the heterocasticity). The best model for the fourth and fifth years, considered clone, exclusion and block identity with the modelling of the correlation matrix.

In each one of the first 3 years, the exclusion × time interaction was significant (Table 1). Pairwise comparisons showed no difference in diameter of stakes with and without LCA exclusion at the beginning of the experiment, again showing their homogeneity. As from the first summer (February 2015), stakes within the LCA exclusion treatment showed a wider diameter than in the control treatment, regardless of

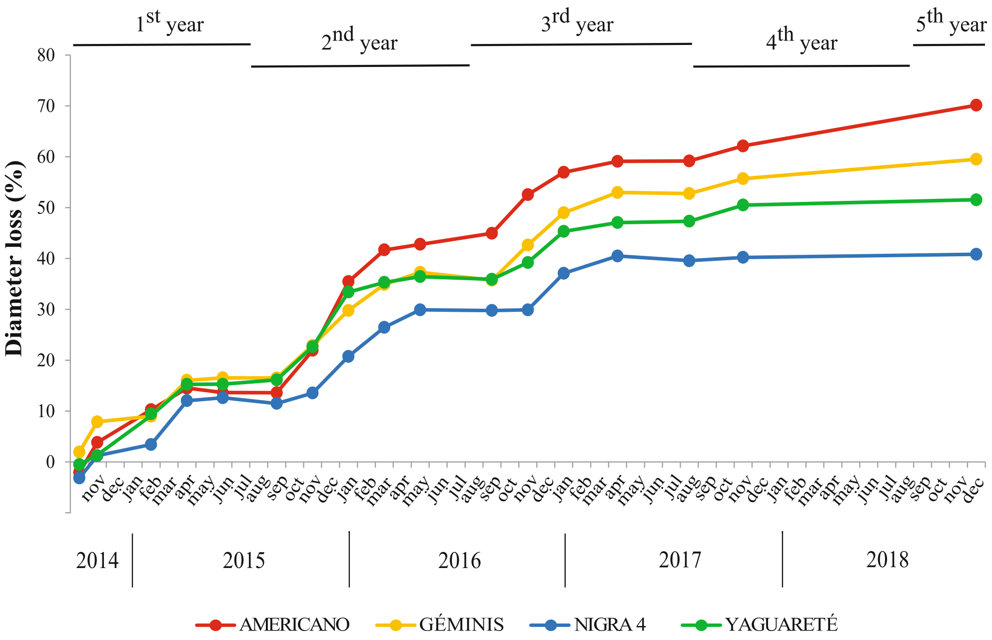

FIGURE 5 Diameter loss of stakes/trees of four *Salix* clones over 4 years and 4 months in an experimental plantation. Values correspond to the percentage of difference in diameter recorded for each clone between individuals with and without leaf-cutting ant exclusion

the clone. In the second year, there were no differences in stake diameters among control treatments between September 2015 and June 2016, indicating a low growth rate likely to be a consequence of LCA attacks. However, there was a significant and progressive increase in diameter during the same period of time in trees within the LCA exclusion treatment compared with the control treatment. In the third year, trees within the LCA exclusion treatment had a significantly larger diameter than in the control treatment from January to April (summer–early autumn) than from September to November (the first months of growth cycle) (Figures 4 and 5). In addition, the second year had a significant difference in the clone × time interaction (Table 1). Pairwise comparisons did not show differences in diameter until January 2016 (479 days after the start of the experiment), but during the rest of the second year, trees of Americano (the control and the LCA exclusion treatments) had smaller diameters than the other three clones (Figures 4 and S3).

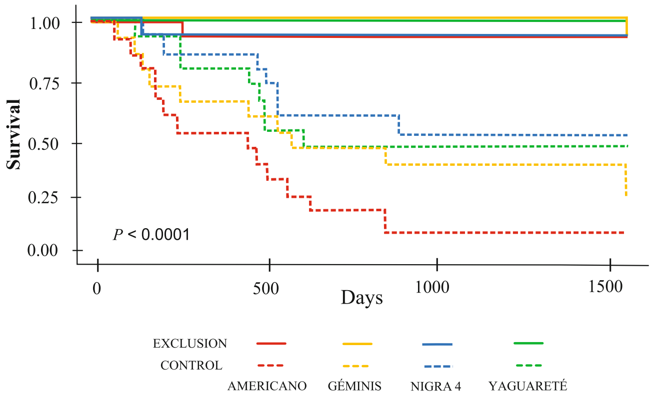

FIGURE 6 Survival of the stakes/trees of four *Salix* clones between August 2014 and December 2018. CONTROL = with leaf-cutting ant access (no physical barrier), EXCLUSION = with leaf-cutting ant exclusion (physical barrier to exclude ant access)

In the fourth and fifth years, the effects of clone and exclusion were significant (Table 1). Pairwise comparisons showed Americano had a smaller diameter than the other three clones, whereas there were no differences among Géminis, Nigra 4 and Yaguareté. As observed with height, trees within the LCA exclusion treatment had larger diameters than the control treatments (Figures 4 and S3). This shows that the LCA exclusion treatment had a significant effect on the growth in diameter of stakes/trees.

Géminis, Nigra 4 and Yaguareté stakes/trees had the largest diameters at the end of the experiment (December 2018, Figure S3) with trees exceeding 20 cm in diameter. Surprisingly, an Americano tree within the LCA exclusion treatment had the largest diameter, reaching 29 cm. The greatest percentage of loss in diameter at the end of the experiment was again observed in Americano by almost 70%, followed by Géminis (60%), Yaguareté (50%) and Nigra 4 with 40% (Figure 5).

Survival

The survival of willows differed between each treatment ($p < 0.0001$). Stakes and trees under the LCA exclusion treatment were always greater than 80% (Figure 6). Conversely, control stakes survival curves started to decrease after 100 days to a constant rate with a similar pattern for all clones, but with a higher slope for Americano and Géminis clones. At 600 days approximately, only 50% of the control stakes survived, and less than 30% survived at the end of the experiment in Americano and Géminis control stakes (Figure 6). One extreme case was observed in Block 6, where only the four trees with LCA exclusion survived.

The main cause of mortality was attributed to the LCA attacks (63.6%), although other causes of death, such as drought, damage while mowing the internal vegetation of blocks and stakes knocked over by livestock were also recorded (Table 2). In December 2018,

TABLE 2 Number of dead stakes/trees of each *Salix* clone with and without leaf-cutting ant (LCA) exclusion after 4 years and 4 months of experiment. Each treatment (control and LCA exclusion) started with 15 stakes (120 in total)

	No. dead stakes/year (no LCA damage causes)					
Clon	1°	2°	3°	4°	5°	Total
Control						
Americano	7 (4)	5 (1)	1	0	0	13 (5)
Géminis	4	3	1	0	2 (2)	10 (2)
Yaguareté	3	5 (1)	0	0	0	8 (1)
Nigra 4	2	5 (3)	1 (1)	0	0	8 (4)
LCA exclusion						
Americano	1 (1)	0	1 (1)	0	0	2 (2)
Géminis	0	0	0	0	1 (1)	1 (1)
Yaguareté	0	1 (1)	0	0	0	1 (1)
Nigra 4	1	0	0	0	0	1
Total	18 (5)	19 (6)	4 (2)	0	3 (3)	44 (16)

Note: Numbers in parentheses correspond to dead stakes/trees whose death cannot be attributed to leaf-cutting ant damage.

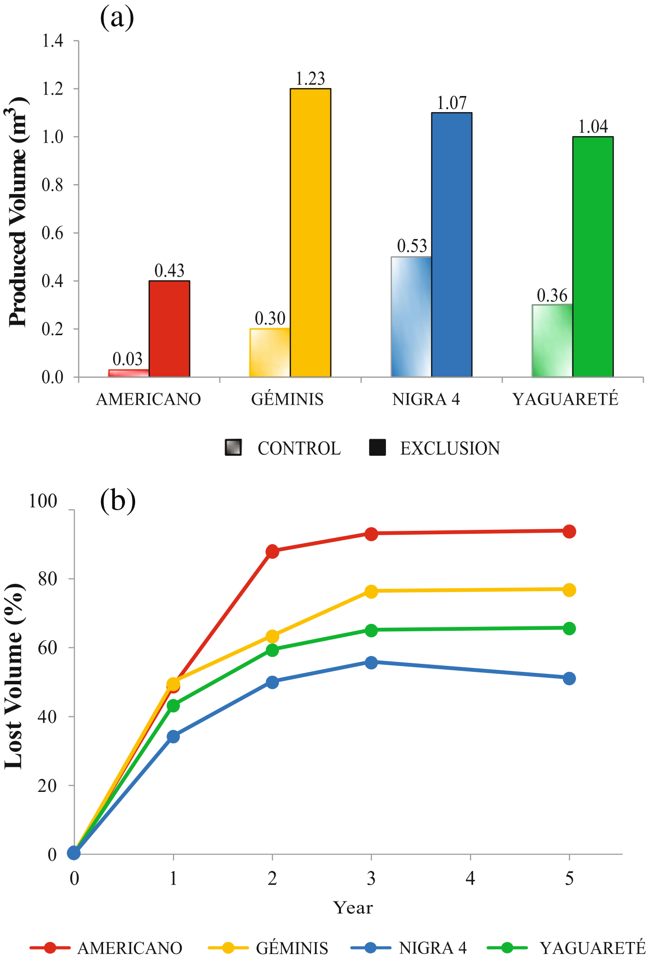

FIGURE 7 (a) Total obtained volume at the end of the experiment (1537 days) for each treatment (clone × exclusion) and (b) the percentage of loss volume of four *Salix* clones year per year. CONTROL = with leaf-cutting ant access (no physical barrier), EXCLUSION = with leaf-cutting ant exclusion (physical barrier to exclude ant access)

only one block of the original 15 remained complete with its eight trees alive. The highest mortality attributed to LCA damage (between 75%–80%) was observed in Americano and Géminis trees in the control treatment (Figure 6).

Volume

The total wood volume of the trees estimated at the end of the experiment differed among clones, in both the control and the LCA exclusion treatments. The highest volume in both treatments was reached by Nigra 4, followed by Géminis, Yaguareté and Americano (Figure 7a). The greatest volume loss was in Americano (93%), followed by Géminis with 77% (Figure 7b). However, Géminis reached a final volume three times greater than Americano when LCA were excluded. Nigra 4 and Yaguareté reached a similar final volume in the LCA exclusion treatment, but a reduction of 51% and 66%, respectively, in the control treatment (Figure 7). Regarding the evolution through the years, percentage of loss in volume at the end of the first year was approximately 50% for Americano and Géminis (Figure 7b). In the second year, volume loss of Americano was greater than the remaining clones. All clones increased their loss in volume between the first and the third year of the experiment, stabilizing into the beginning of the fifth year (Figure 7b).

DISCUSSION

Foliage damaged by *A. lundii* and *A. ambiguus* was relatively similar in the four willow clones tested throughout this experiment. Thus, injury to the leaves was equally high in the two clones traditionally planted (Americano and Nigra 4) in the lower delta of the Paraná River and in the two clones recently released to the market (Géminis and Yaguareté). However, damaged foliage had a differential indirect effect on the biomass produced by each of the four clones, mainly due to differences in their growth rates. This difference was clearly manifested in the greater loss in height and diameter (consequently in volume) and in the higher mortality of Americano, the clone with the slowest growth, and thus, the most affected by these LCA at the study site.

Damaged foliage

Damaged foliage varied through time in the different clones, but in general it was very intense during the first 2 years, stabilized in the third year and later decreased. The same result was observed during the first 3 years in a *P. taeda* plantation in Argentina (Cantarelli et al., 2008; Cantarelli et al., 2019). Except Jiménez et al. (2021), who studied *A. lundii* damage to a Nigra 4 plantation in Entre Ríos province, no other study had previously quantified LCA damage in Salicaceae plantations, especially over such a long period.

Progression of damage throughout the first 2 years only showed differences between stakes/trees with and without exclusion for LCA, but not among the four studied clones. Unfortunately, it was impossible to discriminate between the damage caused by *A. lundii* and *A. ambiguus* as both species cut leaves in a similar way. These LCA species made a semi-circular cut (Garcia et al., 2020; Jiménez, 2019) in all clones and usually began on the top of the stakes or crown of trees and then gradually descended, leaving leafless branches.

Though it was not possible to determine the preference of each LCA species for a particular willow clone in this field experiment, it seems they do not have a predilection for any one of these four clones, according to paired choice tests conducted in the laboratory (Jiménez, 2019). Likewise, damaged foliage could not be related to nest density for each LCA species in the field. However, the density of LCA nests in nearby areas in the lower delta of the Paraná River can vary between 0.6 and 18.7 nests/ha depending on if the estimation was carried out in open or in closed areas, and if both LCA species (*A. lundii* and *A. ambiguus*) are in sympatry or not (Casaubón et al., 2018; Jiménez, 2019; Jiménez et al., 2021). For example, in a neighbouring plot to our experiment, the *A. ambiguus* density was 5.4 nests/ha (Casaubón et al., 2018). Thus, it could be supposed that in our adjacent plots the density of this LCA species was similar. In addition, in a nearby poplar plantation in the EEA INTA Delta, the densities of both LCA species seem to be similar (authors pers. obs.). However, *A. ambiguus* nests were easier to find due to their more conspicuous nests, with an aerial mound covered with dry leaves and branches, whereas *A. lundii* nests are mainly subterranean with only one or more holes at ground level (Jiménez, 2019). Moreover, a mean density of 18.7 nests/ha of *A. lundii* damaged more than 60% of 70-cm-high stakes of Nigra 4 clone, 50 days after their implantation in six experimental plots in the south of Entre Ríos province (Jiménez et al., 2021).

Even though physical barriers were not fully effective in avoiding damage to the foliage by LCA, there was, however, a reduction of about 67% in damage in stakes with the LCA exclusion treatment. This could be due to LCA having managed to overcome the sliding surface of the barrier (some were damaged), or they might have used spontaneous vegetation growing next to the trunks as a bridge (authors pers. obs.). Garcia et al. (2020) also found differences, yet no so marked, in the amount of foliage damaged by LCA when using physical barriers to prevent the attacks in various pioneer and non-pioneer tree species.

Height and diameter

Losses recorded in this experiment as a consequence of the defoliation by *A. lundii* and *A. ambiguus* during the first months were similar in height and lower in diameter, than those reported by Nickele et al. (2012) in *P. taeda* plantations attacked by *A. crassispinus* in Brazil. The same relation was found by Reis Filho et al. (2011) in 1-year-old seedlings of *P. taeda* and *E. grandis* subjected in their first month to artificial damage simulating LCA herbivory. Instead, if we compared our results at the end of the experiment (4 years and 4 months), height and diameter losses were higher than those obtained by Nickele et al. (2020) in the tenth year (10% and 23%, respectively), when they simulated 100% of artificial LCA damage, including the apical meristem, in a *P. taeda* plantation.

The highest mean height was reached by Nigra 4, the clone with the lowest percentage of height loss and less affected by LCA herbivory. In the field, it was observed that Nigra 4 trees were only just being attacked by LCA, when the rest of the clones already had high levels of damage. This could be interpreted as a lower preference of LCA for this clone in the field; however, this difference was not statistically detected in the model that explained the progress of damaged foliage over time. The next tallest trees were Yaguareté and Géminis, but the latter had a greater percentage of loss in height, surpassed only by Americano. This might indicate that although Americano was more affected than Géminis by LCA, it also had a much lower rate of growth in height. This has been previously corroborated by field observations (Teresa Cerrillo from the EEA INTA Delta, pers. comm.). In our experiment, Géminis and Americano were frequently attacked by LCA, even in some circumstances in stakes/trees with barriers, which could also be an indication of higher preference.

Despite some isolated cases, effectiveness of barriers was proven in the four clones with a higher height in the trees within the LCA exclusion treatment with respect to the control treatment, increasing the difference as the experiment progressed. The percentage of loss in diameter was the same as for the loss in height (Americano > Géminis > Yaguareté > Nigra 4), but not exactly with the same magnitude in each clone. The diameter probably has a lower growth rate than the height because during the first years, the plant uses its first resources to generate photosynthetic mass growing in leaves, branches and height to reach the light. As from the third year, trees already established in the LCA exclusion treatment began to grow faster in diameter than those in the control treatment that were more negatively affected by LCA damage.

Survival

The survival of the stakes/trees with LCA exclusion was seven times greater than the survival of stakes with the control treatment. This demonstrates both the great impact of the herbivory by these two LCA species on willow clones and the effectiveness of the physical barriers to prevent this type of damage. A huge difference observed in the field was the tolerance of Géminis. Although the stakes/trees of Americano found it difficult to recover after attacks to their foliage, Géminis stakes/trees recovered faster from similar damage. This implies a duality for Géminis, because although the clone is frequently attacked by LCA, it has a remarkable ability to recover, probably due to its high growth rate. Respect to Nigra 4, mortality in a first year of plantation in this experiment (10%) was six times lower than mortality obtained by Jiménez et al. (2021) in a 1-year-old experimental plantation, conducted with this same clone in Entre Ríos province, but using shorter stakes (70 cm), no spontaneous vegetation at the beginning of the plantation, and without other clones in the experiment. However, stake mortality at the end of the first year of our experiment was similar to those obtained in other forestry species, such as *P. taeda* (15%) and *E. grandis* (10%), in the same period of time (Nickele et al., 2012; Reis Filho et al., 2011).

Volume

The volume of lost wood at the end of the experiment (4 years and 4 months) ranged between 51 and 93%, depending on the clone. These values were overall higher than those obtained in older pine forestations (6-to-10-year-old plantations) of *P. caribaea* (50%) attacked by *A. laevigata* in Venezuela (Hernández & Jaffé, 1995) or a 10-year-old *P. taeda* plantation (43%) with artificial defoliation simulating attacks of LCA species in Brazil (Nickele et al., 2020). This could indicate that willows are more susceptible because, in a shorter period of time, they exhibit higher volume losses in younger plants compared with the losses observed in older pine plantations. Nevertheless, the same percentage of volume loss implies a higher loss of wood product in a large tree than in a smaller tree.

As for survival, Americano and Géminis had the greater loss of volume mainly during the first 2–3 years that stabilized into the fourth-fifth years. It is important to note that though Americano is the commercial clone that has been more widely used in the lower delta of the Paraná River, its volume at the beginning of the fifth year was two to three times lower than the volume of the other clones. Americano also had the lowest growth rate in height and diameter during the first years, which could be due, in part, to its lower yield in the higher elevation areas (less flood prone) of the plantation site (Teresa Cerrillo, EEA INTA Delta, pers. comm.). In fact, the Americano tree, with the best performance during the 5 years, was in a block located in a more flood prone area. By contrast, Yaguareté and Nigra 4 had lower losses and greater stability over the years. For instance, Nigra 4 exhibited a third of volume losses (34%) during its first year compared with another experiment (90%) conducted with shorter stakes, no other clones and no spontaneous vegetation in the proximity (Jiménez et al., 2021).

Implications for LCA management

Variables measured showed a similar pattern over the 5 years of the experiment in the four clones. Based on all the information obtained about the direct damaged foliage caused by *A. lundii* and *A. ambiguus* in the studied clones and its effect on height, diameter, volume and survival, we can conclude that Nigra 4 was the clone that was least affected by LCA. This traditional clone, probably less preferred by the ants, showed the highest growth rate and the lowest damage by LCA, which resulted in the lowest loss of wood volume. Besides, this clone with apical dominance had the lowest mortality. However, owing to the dark colour of its wood it is not suitable for paper production and tends to break easily in the apex (Teresa Cerrillo, EEA INTA Delta, pers. comm.). Meanwhile Géminis, despite being heavily attacked by LCA, showed a faster growth rate and yielded a good wood volume production. The other new clone, Yaguareté, could also be a good candidate due to its high growth rate and low LCA damage. It is important to mention that this clone has a great ramification, whereby in commercial plantations pruning is usually done "to guide" the trees to develop a single trunk or main axis. No clone was pruned in this experiment, as it was considered a possible artificial damage, difficult to handle equally in all clones. However, if they had been pruned during the experiment, Yaguareté trees might have increased their height. Therefore, Yaguareté and Géminis could be two excellent candidate clones to replace Americano in the lower delta of the Paraná River.

One important fact to note is the effectiveness of physical barriers used to prevent the attacks of LCA. These barriers successfully prevented 67% of damage caused by LCA during the first 3 years in all the studied clones. The use of physical barriers to control *A. laevigata* defoliation had a 90% success in small native species reforestation areas in Brazil (Moressi et al., 2007) and reduced up to eight times the damage of *A. lobicornis* in *Pinus contorta* Dougl. ex Loud var. *murrayana* in northern Patagonia in Argentina (Pérez et al., 2011). Thus, it would be interesting to assess the feasibility of using physical barriers against LCA at a larger scale. Although it may have a relatively high cost, and although it is not 100% effective, perhaps it can be applied on several rows in the outer perimeter of a plantation to prevent the access of LCA present in the spontaneous vegetation matrix during the first 2 years in which their impact is higher. Nevertheless, barriers cannot be used effectively on 70-cm stakes, the most commonly used to start a plantation. In this system, stakes are buried 40 cm, leaving only 30 cm above the soil surface in which to place the barrier (Jiménez et al., 2021). This is not high enough to avoid LCA using the spontaneous vegetation, usually taller than 30 cm, to climb to the leaves. Instead, physical barriers could be used in 150-cm stakes like those used in this experiment, or taller ones, such as guides of 2–3 m used in another experiment in EEA INTA Delta, in which damage was low and there was no mortality by LCA (Casaubón et al., 2018).

Tall stakes and guides are commonly used in the silvopastoral system (forest grazing), usually combined with apiculture, where livestock already in the second year of the plantation can graze the spontaneous vegetation growing freely around the guides/trees (Araújo et al., 2003; Casaubón et al., 2018; Montoya-Lerma et al., 2012; Pérez, 2009; Zanetti et al., 2000). This can provide a greater plant offer for the livestock like *Amorpha fructicosa* L., *L. japonica* and *Monteiroa glomerata* (Hook. & Arn.) Krapov., also preferred by LCA and bees; and others like *I. pseudacorus* consumed by LCA, but not palatable for livestock (Perri et al., 2020). In addition, allowing native and exotic spontaneous vegetation to grow between planting lines would help to provide an alternative substrate for the fungus of LCA, as well as food and cover for various herbivorous species, and some natural enemies (Araújo et al., 2003; Vasconcelos & Cherrett, 1995; Zanetti et al., 2000).

This is, to our knowledge, the first study that quantifies long-term LCA damage in Salicaceae plantations. Our results show that the foliage damage caused by both, *A. lundii* and *A.ambiguus*, could be very high in a willow forest plantation and particularly intense during the first 2 years. Without control measures against LCA during this critical time period, levels of damage by LCA reported in this experiment could show that the lower delta of the Paraná River is not suitable for forestry. Thus, the use of barriers on tall stakes/guides could be considered in management programs to reduce both, damage caused by LCA and insecticide doses applied in the region, one of the most important wetlands of Argentina (Ramsar, 2008). It is important to mention that the National Service of Agri-Food Health and Quality (SENASA, acronym in Spanish) recently completely prohibited (August 2021) the use, importation and fractioning of insecticides with Chlorpyrifos as the active agent (Resolution 414/2021; SENASA, 2021a). In August 2021, SENASA also restricted the use, importation and commercialisation of products based on Fipronil (Resolution 425/2021; SENASA, 2021b), which can now only be used for treatments of seeds and baits, such as those used for LCA control, but not for concentrated suspension or as dispersible granules (SENASA, 2021b).

Although there was no clear laboratory experimental evidence supporting LCA preferences for the willow clones implanted in the EEA INTA Delta (Jiménez, 2019), direct damage caused by LCA in foliage affected growth differently in height, diameter,and consequently in volume and survival of the clones. The new recently released willow clones, Yaguareté and Géminis, could be seriously considered to replace Americano, due to their higher tolerance to LCA attack, likely because of their higher growth rates, which result in two to three times more wood volume than the Americano clone. Although Nigra 4 has a similarly high growth rate and the least LCA attack rate, this clone could also be replaced because the two new clones have a better quality cellulose fibre for producing paper.

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AUTHOR CONTRIBUTIONS

All authors conceived the idea and designed the methodology, interpreted the results and wrote the manuscript, contributed critically to the drafts and approved the manuscript for its publication. Nadia Lis Jiménez and Luis Alberto Calcaterra installed the experiment. Nadia Lis Jiménez collected, digitized and analysed the data.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request and are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Figure S1. Comparison in height of (a) Yaguareté (February 2015), (b) Nigra 4 (September 2015), (c) Géminis (September 2015), (d) Americano (December 2016), (e) Nigra 4 (November 2017) and (f) Yaguareté (December 2018). CONTROL (dashed line) = with leaf-cutting ant access (no physical barrier), EXCLUSION (solid line) = with leaf-cutting ant exclusion (physical barrier to exclude ant access).

Figure S2. Mean height of stakes/trees of four *Salix* spp. clones over 4 years and 4 months in an experimental plantation. CONTROL = with leaf-cutting ant access (no physical barrier) and EXCLUSION = with leaf-cutting ant exclusion (physical barrier to exclude ant access).

Figure S3. Mean diameter of stakes/trees of four *Salix* spp. clones over 4 years and 4 months in an experimental plantation. CONTROL = with leaf-cutting ant access (no physical barrier) and EXCLUSION = with leaf-cutting ant exclusion (physical barrier to exclude ant access).

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