Austral
ECOLOGY A Journal of ecology in the Southern Hemisphere

Ecological Society of Australia

Austral Ecology (2016) ●●, ●●–●●

The role of leaf cellulose content in determining host plant preferences of three defoliating insects present in the Andean-Patagonian forest

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Abstract Phytophagous insects choose their feeding resources according to their own requirements in addition to properties of the host plants, such as biomechanical defences. The feeding preferences of the native folivorous insects of the Andean-Patagonian forest (Argentina) have rarely been studied. These environments present a wide diversity and abundance of insects associated with trees of the *Nothofagus* and *Lophozonia* (Nothofagaceae) genera, which represent the main tree species of the forests of the southern hemisphere. In particular, *Lophozonia alpina* and *Lophozonia obliqua* are of great interest because they have a wide distribution, a high capacity for hybridization and exhibit great phenotypic plasticity. This versatility causes substantial variation in the biomechanical properties of leaves, affecting the feeding preferences of insects. The purpose of this work was to study the food selection behaviour of three leaf-chewing insects (*Polydrusus nothofagii*, *Polydrusus roseaus* (Coleoptera: Curculionidae) and *Perzelia arda* (Lepidoptera: Oecophoridae)) associated with *L. alpina* and *L. obliqua* as host plants. Based on their choices, our aim was to determine a preference scale for each insect species and the variables on which these preferences were based. Therefore, we selected trees of *L. alpina* and *L. obliqua*, measured several properties such as cellulose content and recorded which leaves were eaten. As a result, we determined that the three species of insects feed on both host plants but prefer the leaves of *L. obliqua*, with cellulose content being the main determining factor for their decisions. However, in the case of *P. arda*, there was a positive relationship between cellulose and host plant preference, whereas there was an opposite relationship for the weevils. We conclude that during feeding selection, there are some properties of the leaves that have a more important role than others and that the same property does not exert the same behavioural response in all folivorous insects.

Key words: Bayesian analysis, biomechanical properties, feeding preferences, native forest, phytophagous, Thurstone scale.

INTRODUCTION

During the process of searching for and selecting a host plant, folivorous insects may exhibit some degree of selectivity among their feeding resources. This selection process involves not only choosing the right species of plant but also selecting an individual plant within that species (Bernays & Chapman 1994; Mayhew 1997; Price *et al.* 2011). Several biomechanical characteristics of a leaf regulate insect behaviour, affecting the preferences and patterns of feeding (Hanson 1983; Schoonhoven *et al.* 2005; Hong *et al.* 2012). According to several authors, the toughness of the leaves (determined by cell wall components: cellulose, hemi-cellulose and lignin) is the main property that influences the feeding behaviour and food preferences of an insect (Schoonhoven *et al.* 2005; Peeters *et al.* 2007). This behaviour may also vary depending on the biology, habits and developmental stage of each particular species of chewing insects in the following two different ways: either by preventing the initiation of feeding by early larval instars by creating a physical obstacle and/or by causing selective feeding in the smaller chewers that attempt to avoid the toughest tissues (Ohmart *et al.* 1987; Choong 1996; Wheeler *et al.* 1998).

The western area of Patagonia, a geographical region located at the southern end of South America that includes the territories of Argentina and Chile, is characterized by the presence of the Andean forest. These environments have a temperate-cold climate and are dominated by trees of the Nothofagaceae (Fagales) family. Further, there is a wide diversity and abundance of folivorous insects, many of which have not been identified or studied in terms of any

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Accepted for publication September 2016.

doi:10.1111/aec.12460

aspect of their behaviour (Gentili & Gentili 1988; McQuillan 1993). This family comprises approximately 35 living species present in various temperate forests of New Zealand, Australia, New Caledonia, New Guinea and South America (Ward 1965). In Argentina, the following two genera are represented: *Nothofagus* Blume and *Lophozonia* Heenan & Smissen (2013). In particular, *Lophozonia alpina* 'raulí' and *Lophozonia obliqua* 'roble pellín' (previously named *Nothofagus nervosa* and *Nothofagus obliqua* respectively) are species of great importance due to their important ecological value, excellent robustness and timber quality (Sabatier *et al.* 2011); it is for these reasons that both species are included in domestication and conservation plans (Gallo *et al.* 2006). Furthermore, they have the ability to grow in a wide range of environments, including different altitudes and latitudes (Ramirez 1987; Donoso *et al.* 2004). This varied distribution reflects their high phenotypic plasticity and hybridization ability, qualities that often hinder their taxonomic identification (Premoli 1996). These factors, in turn, are the cause of large variation in their biomechanical properties, including the toughness of leaves and possibly the complex of associated insects (Donoso 1987; Donoso *et al.* 1990; Premoli & Mathiasen 2011; Premoli *et al.* 2012; Martínez Pastur *et al.* 2013). Therefore, phytophagous insects may show an unclear pattern of selectivity for a particular species, given that their preferences could be more linked to the biomechanical properties of the leaves of a particular individual.

Several studies have focused on the interactions between Patagonian Nothofagaceae and their associated native insects based on the damage caused by these insects using either a descriptive or quantitative approach (Russell *et al.* 2000; Mazía *et al.* 2004, 2009, 2012; Rousseaux *et al.* 2004; Garibaldi *et al.* 2010, 2011a,b; Paritsis & Veblen 2010; Altmann 2011; Chorbadjian & Francino 2013; Chaneton *et al.* 2014). However, few studies have evaluated such interactions in terms of insect behaviour (Russell *et al.* 2004; Pietrantuono *et al.* 2014). Such studies (common in biological control trials) contribute to understanding in a variety of different ways by providing knowledge regarding the ecology and natural history of native phytophagous insects and information that can be used for the development of conservation plans and even for the silvicultural management of different species of Nothofagaceae.

The purpose of this work was to study the food selection behaviour of three species of chewing insects associated with *L. alpina* and *L. obliqua* as host plants. Based on their choices, our aim was to determine the preference scale for each insect species and if its feeding preferences were related to the biomechanical properties that determine the toughness of the leaves.

METHODS

Study system

The study system is composed of three species of phytophagous insects and their host plants, which are present in the Andean-Patagonian forest, Argentina. *Polydrusus nothofagii* Kuschel, *P. roseaus* Blanchard (Coleoptera: Curculionidae) and *Perzelia arda* Clarke (Lepidoptera: Oecophoridae) are native insects that are commonly found feeding on the foliage of *L. alpina* and *L. obliqua* (Kuschel 1952; Giganti & Dapoto 1990; Grandón 1996; Morrone 1999; Vergara & Jerez 2010). The Curculionidae larvae are root-feeders, whereas the adults are skeletonizing defoliators that are mainly found during the months of January and February (Gentili & Gentili 1988; Elgueta & Marvaldi 2006). In the case of *P. arda*, the larvae cause leaf-tying and defoliating damage. They are commonly found from December to March (Cerda *et al.* 1982; Grandón 1996).

Selection of host plants and experimental insects

Several individuals of *L. alpina* ($n = 8$) and *L. obliqua* ($n = 8$) were selected from a plantation in a nursery at the INTA EEA Bariloche (Instituto Nacional de Tecnología Agropecuaria. Estación Experimental Agropecuaria Bariloche, Río Negro, Argentina 41°7′22.36″S, 71°15′4.94″W) (National Institute of Agricultural Technology – Bariloche Research Lab). The selected trees were used as a source of seeds for the production of seedlings to be used for conservation, restoration or timber production. At the nursery, all individuals were in a healthy state, were never exposed to insecticides, and experienced the same humidity, light, substrate and temperature conditions. Leaves were harvested from these individuals for the analysis of foliar traits and were offered as stimuli in the experiment, ensuring that the leaves were mature, of similar size, had not been chewed and did not harbour fungi. At the same site, non-host plants (*Populus nigra* Linneo, $n = 1$ and *Salix nigra* Marshall, $n = 1$ (Malpighiales: Salicaceae)) were selected to be used as controls in the experiment.

Regarding the insects, adult *P. nothofagii* ($n = 107$) and *P. roseaus* ($n = 51$) and the last larval instar of *P. arda* ($n = 68$) were collected from the same nursery.

Analysis of foliar traits

For each host plant, several biomechanical properties related to leaf toughness and digestibility were measured (e.g. dry matter, neutral detergent fibre, neutral detergent insoluble protein, lignin, ash, total nitrogen and cellulose, Appendix S1). To determine the amount of dry and organic matter, one gram of each sample, composed of dried and ground leaves, was analysed. The dry matter was estimated by drying the samples in an oven at 105°C until a constant weight was obtained, whereas the amount of organic matter was obtained by the calcination of the samples in a muffle furnace at 550°C for eight hours.

doi:10.1111/aec.12460

Afterwards, the residue was weighed as ash (organic matter % = 100% − % ash). To determine the neutral detergent fibre (NDF or structural carbohydrates), cellulose, hemicellulose and lignin contents, a similar sample was used, and the detergent method of Goering and Van Soest (1970) was followed. For the protein content (or NDIP (neutral detergent insoluble protein)), a 0.2 g sample was analysed using the Kjeldahl method. The total amount of nitrogen was measured, which was then multiplied by 6.25 to estimate the amount of protein in each sample (Ruiz & Ruiz 1990; Sikorski 2007).

The concentration of non-structural carbohydrates (NC) was estimated using the formula provided by Cornell University (Sniffen *et al.* 1992), where NC = 100 − (NDF − NDIP) − (ash + ether extract + crude protein). The determination of ether extract was performed by the method described in AOAC (Association of Official Analytical Chemists, 1990). Finally, apparent digestibility (AD) was obtained from the implementation of the Accumulative Goering and Van Soest Equation (1970) to estimate the dry matter digestibility.

Experimental design

To evaluate host plant selection, a series of paired comparisons was performed. One insect was placed in the centre of a rectangular plastic container (5 × 8×14 cm) and stimuli (i.e. leaves from different trees) were placed in the opposite corners (45 combinations including host and non-host plants) (David 1988; DeShazo & Fermo 2002; Raffa *et al.* 2002; Bruzzone & Corley 2011). For the assays with *P. nothofagii* and *P. roseaus*, we used a single leaf as each stimulus, whereas for *P. arda*, we used two overlapping leaves because of its leaf-tying habits and to simulate plant architecture (Marquis *et al.* 2002; Marquis & Lill 2010). After 24 h, we recorded which of the two stimuli was eaten by the insect (response variable). All experiments were performed in the laboratory under semi-controlled temperature and humidity conditions (20.4 ± 0.1°C; 39.5 ± 0.1% RH).

Data analysis

The data were analysed using a Bayesian approach that proposes a series of explanatory models, choosing one of them using a model selection procedure. By using the deviance information criterion (DIC), the best model in terms of balancing fit and complexity was selected from a set of plausible candidate models. Consequently, the analysis of the data proceeded using a two-step methodology for the generation and selection of models. First, the decisions made by the insects were analysed to establish a scale for host plant preferences (i.e. simple model). This scale, based on the Thurstone model of comparative judgments case V (Thurstone 1927/1994; Bradley & Terry 1952; Tsukida & Gupta 2011), provides a continuous unidimensional dimensionless variable for preference. This linear scale expresses the degree of attraction/preference that an experimental subject shows for one of the options. Higher values indicate an increasing preference, and lower/negative values indicate rejection. Because the Thurstone scale is an 'interval scale' (Stevens 1946), we used one of the host plant individuals (*L. alpina* number 9 or La9) as the reference value or origin ordinate of the scale system. This individual was arbitrarily chosen as the reference value and corresponds to zero and, therefore, has no estimation error. In addition, the units of this scale were expressed in terms of the standard deviations of the discriminal dispersion of the reference host plant. Thus, the preference for each tree within the scale was positioned relative to this value, and its values were expressed in terms of the discriminal dispersion.

To determine specifically which parameter best explains the scale of preferences, several models were developed (based on the simple model including only preference). These were developed using regressions between the Thurstone scale and the explanatory variables (i.e. continuous variables: properties of each host plant). Linear models for each variable (i.e. linear functions for cellulose, lignin, etc.) were generated, followed by quadratic and cubic functions. Several models that included two or more parameters with or without interactions were created. The generation of these models continued until the complexity of the models exceeded the improvement in the likelihood function.

According to Bayesian methods, it is mandatory to choose and justify an *a priori* distribution to express one's beliefs about a particular variable before the data are taken into account. In this case, because we did not have *a priori* information, we used a wide distribution so as to have the least possible influence on the results. Therefore, an uninformative *a priori* distribution was selected for all of the parameters (i.e. a normal distribution with a mean of 0 and a deviance of 10 and a normal likelihood to measure the fit of the function) (Gelman 2003). For each model, 110 000 iterations were performed, from which ten thousand were discarded as a burn-in. From the remainder, one in one hundred was chosen to avoid autocorrelation. To test convergence, Geweke plots were used, and a visual inspection of the variable traces was carried out (Geweke 1992). The analyses were performed using the PyMC library for Bayesian estimation (Patil *et al.* 2010) in the Python programming language. Finally, the lowest value of the DIC was used to select the model (Gelman *et al.* 2004).

Using a Bayesian approach allows the two steps of the analysis (creation of the preference scale and the regression analysis for preference and leaf properties) to be conducted simultaneously and more easily, thereby increasing the sensitivity and precision of the analyses. It provides a more straightforward way to fit and select among a great number of models, unlike frequentist statistical methods, and it is widely used by ecologists in animal behaviour studies (Ellison 2004; McNamara *et al.* 2006; Valone 2006; Pereira *et al.* 2013; Pietrantuono *et al.* 2014, 2015).

RESULTS

The three insect species fed on the leaf tissue from the two species of *Lophozonia* used in the experiment. The leaf biomechanical properties varied significantly among host plant species and individuals (Table 1, Appendix S1). The preference scale for

Table 1. Biomechanical and nutritional properties of both host plant used in the preference test

	Lophozonia alpina			*Lophozonia obliqua*		
	Mean	Range	SE	Mean	Range	SE
Lignin (%)	12.55	10.9–13.5	0.37	10.63	9.4–12.5	0.35
Hemi-cellulose (%)	22.85	20.8–26.7	1.43	21.25	16.6–27.4	0.67
Cellulose (%)	20.18	17.3–22.6	0.45	16.10	14.4–18.0	0.67
Crude protein (%)	13.95	11.9–16.2	0.75	14.45	11.3–17.4	0.59
Dry matter (%)	94.2	93.1–95.1	0.25	93.78	92.6–94.4	0.26
Organic matter (%)	93.06	89.8–96	0.86	91.4	87.3–95.2	0.92
Nonstructural carbohydrates (NC) (%)	44.48	38.2–49.1	1.72	52.06	43.9–56.0	1.45
Apparent digestibility (AD)	46.13	41.9–48.9	1.08	51.04	46.2-54.3	0.92

each insect species was established based on the decisions they made. The variation in host plant preferences at the intra- and inter-specific scales is shown in Figures 1 and 2 (i.e. not all host plants and even not all individuals of the same species are equally attractive). According to the preference scale, *L. obliqua* was the preferred host plant because it ranked first for all three species of insects. However, it should be noted that not all insect species chose the same individual and that in the following positions of the ranking, this preference was maintained only for *P. nothofagii*. These results suggest that the preferences for a host plant are not established by the species according to their taxonomic classification; if so, we would have observed a section of the scale that was well-differentiated by the greater preference of a particular species and another section that was less preferred.

The control or non-host trees were rejected as host plants by the three species of insects.

In the process of model selection, 45 models were generated for each insect species, and these were used to analyse the relationships between preferences and the explanatory variables. One model was selected for each insect species according to the lowest DIC value (Table 2). In all three cases, cellulose was the variable that best explains the choices made by the insects, both individually and in association with the other variables. However, the relationship between cellulose content and preference was not the same for all of the insect species. For *P. arda*, there was a positive relationship between the amount of cellulose and host plant preference (Fig. 3), whereas, for *P. nothofagii* and *P. roseaus*, there was an inverse relationship (Fig. 4).

DISCUSSION

Here, we present the first work evaluating the relationship between the feeding preferences of three species of folivorous insects from the Andean-Patagonian forest and the biomechanical properties of their host plant. The host plants evaluated had different nutritional and digestibility properties, and the three species of insects were able to distinguish among them, allowing us to establish a preference scale for each species. These scales showed a gradient of selectivity for the different hosts; each insect species has its own preference scale, which was determined by the individual characteristics of each host plant, regardless of their taxonomic classification.

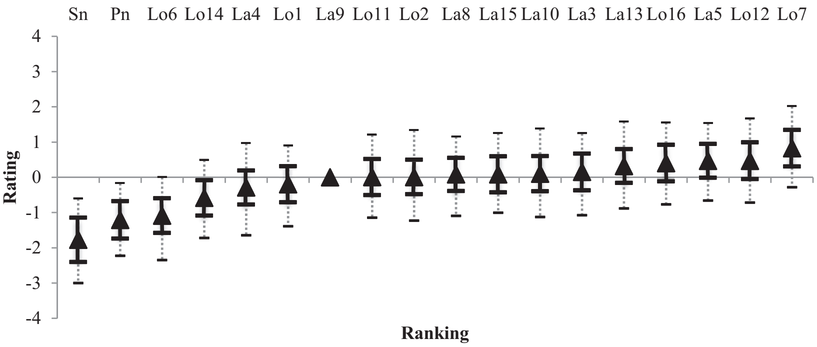

Fig. 1. Ranking of the feeding preferences of *Perzelia arda*, for which *Lophozonia obliqua* (Lo) was the preferred species. The black lines indicate the error estimates for the mean value for each point on the Thurstone scale. The grey dotted lines indicate the discriminal dispersion according to case V of the Thurstone model (Thurstone 1927/1994).

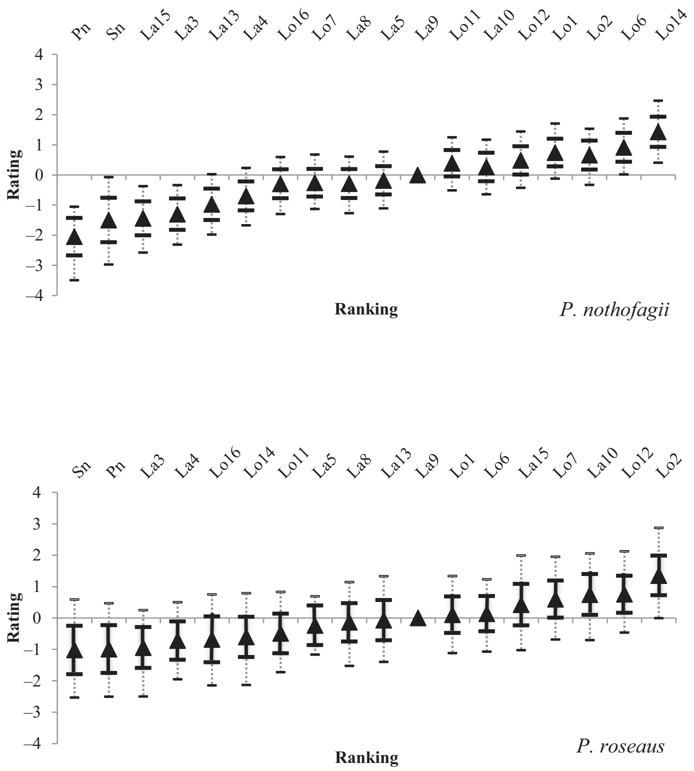

Fig. 2. Ranking of the feeding preferences of *Polydrusus nothofagii* and *P. roseaus*, for which *Lophozonia obliqua* was the preferred species. The black lines indicate the error estimates of the mean value for each point on the Thurstone scale. The grey dotted lines indicate the discriminal dispersion according to case V of the Thurstone model (Thurstone 1927/1994).

Table 2. Values of deviance information criterion (DIC) for the models proposed to measure the preferences related to the nutritional properties of host plants

Insect	Model	Function	DIC
Perzelia arda	0-Simple		113.44
	1-Cellulose*	Lineal	110.80
	2-NC	Lineal	110.92
	3-AD	Lineal	111.65
Polydrusus nothofagii	0-Simple		181.60
	1-Cellulose*	Lineal	153.64
	2-Cellulose-NC	Bi-Lineal NI	153.47
	3-Cellulose-AD	Bi-Lineal NI	153.48
P. roseaus	0-Simple		81.79
	1-Cellulose*	Lineal	74.96
	2-Cellulose-NC	Bi-Lineal NI	76.40
	3-Cellulose-Dry matter	Bi-Lineal NI	76.42

The asterisk (*) indicates the selected model for each insect. AD, apparent digestibility; NC, nonstructural carbohydrates; NI, no interaction.

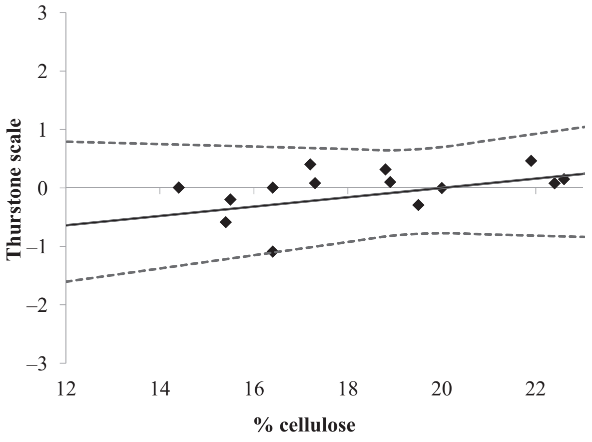

Fig. 3. The scale of preferences according to the percentage of cellulose for *Perzelia arda* (equation of the solid line: $y = 0.08x - 1.6$). The dotted lines indicate a credibility interval of 95%.

According to these results, *L. obliqua* was the preferred species in all of the cases, agreeing with Grandón's field observations, which suggested that this species experiences a higher rate of herbivory (Grandón 1996). However, *P. arda* and *P. roseaus* do not show a strong preference for *L. obliqua*, given that there were also individuals of *L. alpina* within the top five of the ranking. This result may be explained if we consider that *L. alpina* and *L. obliqua* are able to produce natural hybrids, combining their

doi:10.1111/aec.12460

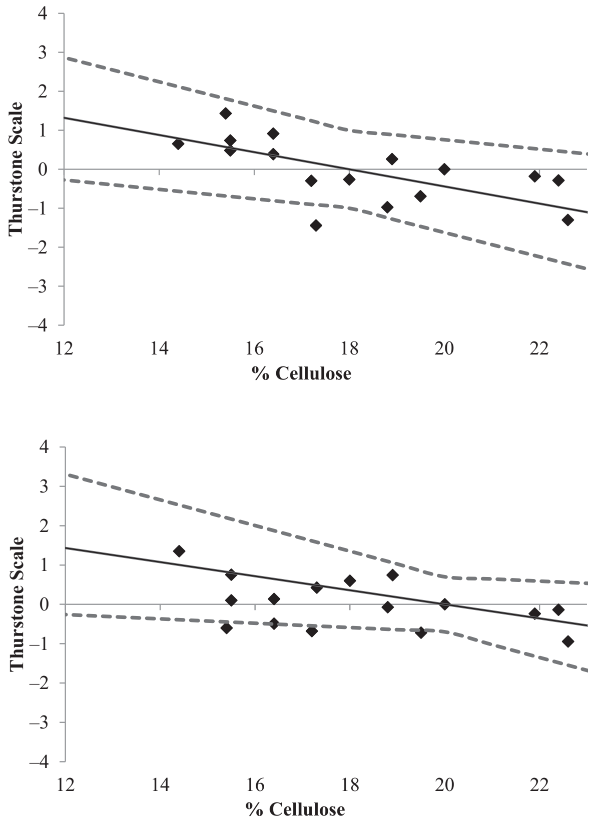

Fig. 4. The scale of preferences according to the percentage of cellulose for *Polydrusus nothofagii* (equation of the solid line: $y = -0.22x + 3.7$) and *P. roseaus* (equation of the solid line: $y = -0.179x + 3.58$). The dotted lines indicate a credibility interval of 95%.

phenotypic properties and are, therefore, perceived as similar species (Donoso *et al.* 1990).

According to the selected models, the concentration of cellulose in the leaves influenced host plant selection for the three insect species (it should be noted that high cellulose content results in increased toughness; Niklas 1992). For *P. nothofagii* and *P. roseaus*, preference decreased together with the cellulose content. This correlation would explain the skeletonization damage pattern caused by these insects, because the vascular tissue of leaves tends to be a tougher tissue (Malishev & Sanson 2015). Similarly, Barker (1989) found that the feeding preferences of *Listronotus bonariensis* (Curculionidae) were negatively correlated with the fibre content of the foliage (cellulose + hemicellulose + lignin), indicating a possible effect of strength due to the toughness of the leaves. Bogacheva (1994) found that *Polydrusus ruficornis* showed a clear preference for small and young leaves. In that paper, he argued that this preference might be related to the amount of cellulose and/or leaf toughness. In addition, Brunt *et al.* (2006) showed that the toughness of the leaves of *Nothofagus moorei* is an effective anti-herbivore defence. According to Raupp (1985), tough leaves erode the cutting surface of beetle jaws such as those of *Plagiodera versicolora* (Coleoptera: Chrysomelidae), indicating that leaf toughness may act as an effective defence strategy, affecting the morphology, feeding behaviour and ultimately the spatial and temporal patterns of herbivores. Peeters *et al.* (2007), found similar results and suggested that the negative relationship with cellulose may indicate that Curculionidae require leaf tissue of higher quality compared to that required by other external chewers.

In the case of *P. arda* larvae, the cellulose content also influenced the host plant preferences but showed a positive relationship. This result suggests that an increase in cellulose does not represent a barrier against insect herbivory. Indeed, there are cases in which leaf-tying insects have been shown to have the ability to reduce the anti-herbivore defences of their host plants (Sagers 1992; Fukui *et al.* 2002), thus allowing them to consume leaves with high cellulose content or a higher toughness. According to the literature, the main food source for *P. arda* is *L. alpina* seeds (Cerda *et al.* 1982; Carrillo & Cerda 1987). Therefore, we can infer that perhaps their mouthparts and digestive capacity are stronger than those of other chewing insects, allowing them to exploit leaves with higher toughness (Bernays *et al.* 1991). For future work, it would be interesting to integrate a comparative analysis of the mandibular morphology of these species into our research.

From the analysis of the results of this study, it could be inferred that the role played by cellulose is different for each insect species and that different feeding guilds may respond differentially to this variable (Peeters *et al.* 2007). In fact, the role of cellulose is more important than that of other nutritional properties of the leaves, such as the amount of protein, which has also been considered to be a main nutritional factor (Haukioja *et al.* 1991; Schowalter 2006). Mazía *et al.* (2012) demonstrated that in forests of *Nothofagus pumilio* located in Patagonia, leaf toughness increases during the summer season, with a peak in January. In the same month, there is also a peak in foliar damage caused by leaf-tying insects. Because the mature leaves of all three species are tougher than younger leaves, this indicates that there is no apparent competition for food resources and that perhaps a sequence of foliage use occurs (Lowman & Box 1983; Hochuli 2001).

Understanding the relationship between host plant preference and the intraspecific variation the biomechanical properties of host plant leaves provides valuable information to plant breeders, especially in the case of *L. alpina* and *L. obliqua*, as these species are included in forestry management and conservation plans (Laclau 1997; Schlichter & Laclau 1998; Gallo *et al.* 2006). Determining the feeding preferences of phytophagous insects also allows us to infer which resources they can or will exploit, thereby allowing for the identification of potential host plants and preventing the development of forest pests (Peeters 2002; Rossetti *et al.* 2014). Therefore, in addition to developing a theoretical framework for the understanding of the insects inhabiting the native forest, this information also provides a starting point for phytosanitary care and the conservation of systems containing native species (Schowalter 2006; Altmann 2011; Pietrantuono *et al.* 2014).

ACKNOWLEDGEMENTS

This work was financed by the following projects: PICT-2010-2508 (Agencia Nacional de Promoción Científica y Tecnológica), PIP No. 11220100100266 (Consejo Nacional de Investigaciones Científicas y Técnicas) and PE-PNFOR44321 (Instituto Nacional de Tecnología Agropecuaria). We want to thank Tec. Luis Ernesto Cohen from the Laboratory of Textile Fibers (Instituto Nacional de Tecnología Agropecuaria) for his collaboration in the foliar analysis and Dr. Fernando Guerrieri for useful comments on previous versions of this manuscript. We are also thankful to the Associate Editor and to the anonymous reviewers who provided useful suggestions for improving the style of the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Detailed biomechanical and nutritional properties of each host plant.

doi:10.1111/aec.12460