Feeding preferences of the willow sawfly *Nematus oligospilus* (Hymenoptera: Tenthredinidae) for commercial *Salix* clones

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Preferencias alimentarias de la avispa sierra de los sauces *Nematus oligospilus* (Hymenoptera: Tenthredinidae) por clones de *Salix* comerciales

■ **RESUMEN.** *Nematus oligospilus* Förster (Hymenoptera: Tenthredinidae), o la avispa sierra de los sauces, es nativa del Hemisferio Norte y se ha convertido en un serio defoliador en plantaciones de sauces (*Salix* spp.) del Hemisferio Sur, después de su introducción a principios de 1980. Los estudios sobre las preferencias de hospedador aportan información útil para el desarrollo de estrategias, donde la avispa sierra puede producir daño a los árboles y pérdidas económicas. Se evaluó la preferencia alimentaria de las larvas de *N. oligospilus*, mediante ensayos de laboratorio; se ofrecieron en forma simultánea hojas de cuatro clones de sauce, usados comúnmente en plantaciones comerciales en Argentina (*Salix babylonica* var *sacramenta* Hortus, *Salix nigra* Marsch., *S. babylonica* L. x *Salix alba* L. 131-27 and *Salix matsudana* Koidz. x *S. alba* L. 13-44). Las larvas de *N. oligospilus* se alimentaron de las hojas de los cuatro clones. Sin embargo, consumieron una proporción significativamente mayor de las hojas de *S. babylonica* var *sacramenta*. Estos resultados indican que todos los clones utilizados en los ensayos fueron palatables para el insecto y que *S. babylonica* var *sacramenta* es el hospedador preferido para la herbivoría de las larvas.

PALABRAS CLAVE. Defoliación. Herbivoría. Salicaceae. Avispa sierra.

■ **ABSTRACT.** *Nematus oligospilus* Förster (Hymenoptera: Tenthredinidae) is a willow sawfly native to the Northern Hemisphere which became a serious defoliator in willow plantations (*Salix* spp.) of the Southern Hemisphere after being introduced in the early 1980´s. Studies on host preferences provide useful information for the development of pest management strategies where the willow sawfly may produce tree damage and economic loss. Feeding preferences of *N. oligospilus* larvae were evaluated in laboratory trials by simultaneously offering leaves from four willow tree clones commonly used in commercial plantations in Argentina (*Salix babylonica* var *sacramenta* Hortus, *Salix nigra* Marsch., *S. babylonica* L. x *Salix alba* L. 131-27 and *Salix matsudana* Koidz. x *S. alba* L. 13-44). Larvae of *N. oligospilus* fed on leaves from the four clones. However, insects consumed a significantly higher proportion of *S. babylonica* var *sacramenta* leaves than of leaves

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from the other clones. Results indicate that all clones used in the trials were palatable to the insect, and that *S. babylonica* var *sacramenta* is the preferred host for larval herbivory.

KEY WORDS. Defoliation. Herbivory. Salicaceae. Willow sawfly.

INTRODUCTION

Nematus oligospilus is a willow sawfly native to the Northern Hemisphere, and it was introduced into South America in the early 1980s (Koch & Smith, 2000). It was first detected in Argentina in 1981 (Smith, 1983) and then spread into Chile where it was reported in 1986 (González *et al.*, 1986). Later, it was found in South Africa (Urban & Earldley, 1995), New Zealand (Berry, 1997) and more recently in Australia (Bruzzese & McFadyen, 2006). The larva of *Nematus oligospilus* Förster (Hymenoptera: Tenthredinidae) feeds on leaves of several species of the Salicaceae family, especially on willows (*Salix* spp.) used for commercial plantations.

Nematus oligospilus is a common willow-feeding sawfly in the Holarctic Region (Koch & Smith, 2000). It has low impact on commercial plantations within its original distributional range, where it occurs in relatively low population density (Carr *et al.*, 1998). In the Southern Hemisphere, however, outbreaks of *N. oligospilus* can completely defoliate (and even kill) trees (Dapoto & Giganti, 1994; Urban & Earldley, 1995; Ede, 2009). The high reproductive and dispersal capability of this species has been partially attributed to thelytokous parthenogenetic reproduction, favorable environmental conditions, and the occurrence of extensive monoculture plantations of exotic Salicaceae (Koch & Smith, 2000).

Poplars (*Populus* spp.) and willows are important resources for pulp production and non-wood products such as fodder and fuel. Furthermore, they are used for environmental purposes such as soil and water protection, windbreaks, riparian buffers, and phytoremediation (e.g. Dimitriou & Aronsson, 2005; Marchand & Masse, 2007). The global area of planted Salicaceae is approximately 7 million hectares, and China, India, France, Turkey, Italy and Argentine hold the most extensive areas of planted forests (Ball *et al.*, 2005). In Argentina, most willow and poplar plantations are located in the wetlands of the Lower Delta of the Paraná River (FAO 2001), covering approximately 58000 ha (Bodorowski, 2006). Approximately 90% of the planted willows are used for paper and chipboards. *Nematus oligospilus* was first detected in the region in the mid-1980's (Toscani *et al.,* 1992) where it caused up to 60% loss in timber production (Cabarcos, 1995), and it is currently considered a forest pest accounting for significant economic loss (Giménez, 2006; Dapoto *et al.*, 2011). The reproductive strategy (Koch & Smith, 2000), wide host range, favorable environmental conditions and the presence of extensive monocultures of Salicaceae, likely influence the success of *N. oligospilus* populations in the Lower Delta of the Paraná River.

Salicaceae species may show different susceptibility to *N. oligospilus* infestation. Previous reports indicate that poplars were less infested than willows (Dapoto & Giganti, 1994; Urban & Earldley, 1995; Ede, 2009), and that willow shrubs were less infested than willow trees (Charles *et al.*, 1998: Finlay & Adair, 2006). Although some studies have pointed out the relevance of determining *N. oligospilus* preference for oviposition (e.g. Charles *et al.*, 1998) or feeding (e.g. Urban & Eardley, 1995), no study has explored experimentally feeding preferences by willow species or hybrids. Such information may contribute to a better understanding of plant-insect interaction, given that the existence of food preference could provide information on the sawfly´s ability to cause damage to commercial willow plantations, and help to decide the most appropriate

clone to grow. Thus, using *N. oligospilus* larvae we conducted food preference trials by simultaneously offering leaves from four willow tree clones commonly used in commercial plantations in Argentina.

MATERIAL AND METHODS

We conducted food preference trials involving *N. oligospilus* larvae and leaves from four willow clones. Larvae and leaves used in the trial were collected from commercial willow plantations, located in the Lower Delta of the Paraná River, Buenos Aires province, Argentina (33° 55'S – 58° 59'W). The area has temperate and subhumid climate; mean annual temperature range 16.7 C – 18 C and annual precipitation is 1000 mm (Malvárez, 1999). The Lower Delta of the Paraná River is a freshwater wetland showing high environmental heterogeneity and biological diversity (Kandus *et al*., 2006); part of the region is a UNESCO Biosphere Reserve since 2000. All willows and poplars species, varieties and hybrids cultivated in commercial plantations are reproduced asexually (clonally) by ramets.

To collect larvae for the experiment, branches from infested trees were clipped, placed in glass jars containing a piece of wet cotton, and brought to the laboratory. Third- and fourth-stage larvae of *N. oligospilus* showing adequate activity after six hours of starvation were selected for the trial. Larval activity was considered adequate when it responded immediately to stimulation with a fine brush. Leaves for the experiment were also collected by branch clipping in commercial plantations from four of the most commonly used clones in the study area: *Salix babylonica* var *sacramenta* Hortus, *Salix nigra* Marsch., *S. babylonica* L. x *Salix alba* L. 131-27 and *Salix matsudana* Koidz. x *S. alba* L. 13-44 (Cerrillo, 2009). Branches from each clone were labeled and wrapped in plastic bags with wet cotton to avoid dehydration. Leaves selected for the trial were of similar size, with intact leaf laminae and without visual signs of dehydration.

Traditional cafeteria trials were conducted in the laboratory under ambient temperature and photoperiod (approximately 24 ± 4 °C and 14L: 10D). A trial consisted of simultaneously offering one leaf of each clone to six larvae over a 48-hour period. To conduct the trial, leaves and larvae were placed in a 250 cc-glass jar (6 cm in diameter and 13 cm in height) containing a piece of wet cotton to avoid dehydration. Thus, the jar contained four leaves of similar size, one from each clone and six larvae of *N. oligospilus.* Leaves were arranged equidistant to each other, and each larva was used for a single trial and then discarded. The location of leaves in each trial was recorded in each jar. The trial was replicated 12 times.

Leaf margins were drawn prior to the trial and 48 hours after the trial started, and leaf areas were estimated using Image-Pro Plus 4.5. The percentage of leaf area consumed by larvae after 48 hours was calculated by subtracting the final to the initial leaf area. Typically, food preferences are quantified by comparing food consumption and availability using a variety of possible indices (see Krebs, 1999). In our study, we offered a similar amount of each food type by using similar initial leaf area for all willow clones. Thus, we estimated preferences by comparing the mean percentages of leaf area consumed using a one-way ANOVA with willow clone as the factor, followed by Tukey's post-hoc pair comparisons. By using ANOVA, we tested for significant differences in the consumption of willow clones. Prior to statistical analysis, percentage data were arcsine-square-root transformed to meet the ANOVA assumptions.

RESULTS

Prior to the trials, the leaf area offered to larvae was similar among willow clones ($F_{3, 44} = 1.44$, $p = 0.24$). During the trials, larvae of *N. oligospilus* fed on leaves from the four clones used (Fig. 1), indicating that all clones used here were palatable to the insect. However, mean percentage leaf area consumed during the trial differed among clones indicating food preference ($F_{3, 44}$ =

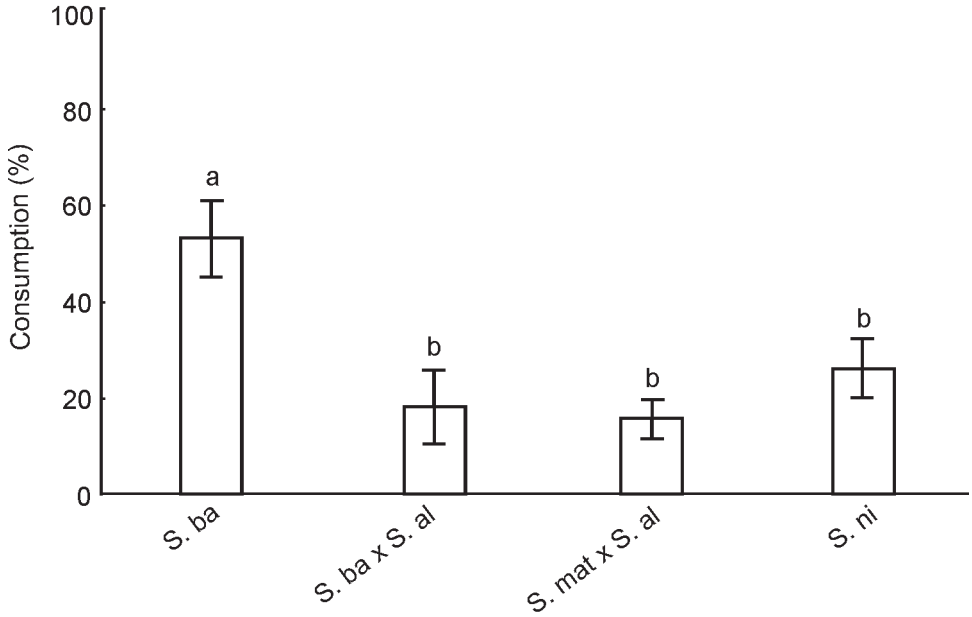

Fig. 1. Mean percentage leaf area (± ES) consumed by larvae of *Nematus oligospilus* of the four willow clones included in the food preference trial: S. ba = *Salix babylonica* var *sacramenta,* S. ba x S. al = *S. babylonica* x *Salix alba* 131-27, S. mat x S. al = *Salix matsudana* x *S. alba* 13-44, and S. ni = *Salix nigra*. Different letters indicate significant differences (Tukey's test, P < 0.05).

5.92, p < 0.05). Post-hoc pair comparisons showed significantly higher consumption of *S. babylonica* var *sacramenta* leaves than of leaves from the other clones (p < 0.05). Larvae fed on similar proportions of *Salix nigra* Marsch., *S. babylonica* L. x *Salix alba* L. 131-27 and *Salix matsudana* Koidz. x *S. alba* L. 13-44.

DISCUSSION

Our results are consistent with previous studies examining *N. oligospilus* feeding habits and preferences in other regions where the species has been introduced. In our trials, *N. oligospilus* showed a flexible use of host willows. Similar results were found in other laboratory studies where the hatched larvae successfully developed to adult by feeding on a variety of willow species such as *Salix lasiolepis* in USA (Carr *et al.*, 1998), *S. babylonica*, *S. fragilis* and S. *mucronata* in South Africa (Urban & Earldley, 1995), and on 22 willow species/hybrids and two poplar species (Charles & Allan, 2000; Charles *et al.*, 2005) commonly planted in New Zealand. Thus, diet of *N. oligospilus* could be categorized as specialist in *Salix*, but including a wide range of willow and some poplar species.

Although *N. oligospilus* feeds on a variety of willow species, it did preferred *S. babylonica* var *sacramenta*. Similar to our findings, Roininen and Tahvanainen (1989) conducted choice experiments using larvae of two willow sawflies (*Nematus pavidus* Serville and *Nematus salicis* (Linnaeus)) common in Finland, and found that *N. pavidus* fed on all willow species offered but preferred *Salix cv. aquatica* and *S. viminalis*, whereas *N. salicis* preferred *S. fragilis* and scarcely fed on the other willow species; the authors speculated that host flexibility would lead *N. pavidus* to a more effective use of those food resources that are only marginally exploited, potentially favoring the inclusion of new hosts. According to Charles *et al.* (2005), although some host tree species were more favorable (e.g. *S. alba, S. glaucophylloides,*

S. nigra and *S. viminalis*) than others (e.g. *S. babylonica, S. exigua* and *S. lasiolepis*), all hosts allowed larvae to complete their development to the adult stage. Similarly, different levels of damage in a wide variety of willow species were observed in plantations after severe defoliations caused by willow sawfly outbreaks (Dapoto & Giganti, 1994; Ede, 2009). In the Argentine Patagonia (Río Negro and Neuquén provinces), leaves of *S. babylonica* L. emerge early in the growing season and are the first to be consumed by *N. oligospilus*; however, other willow species may become infested as the growing season progresses, i.e., the sawfly can even attack poplars when willow foliage is scarce (Dapoto & Giganti, 1994). Therefore, *N. oligospilus* has a flexible use of host, and the Salicaceae species attacked seemed to be related to the availability of willow species in the region and throughout the season.

CONCLUSION

The results of this study showed that larvae of *N. oligospilus* preferred feeding on *S. babylonica var sacramenta* leaves over *S. babylonica* x *Salix alba* 131-27, *Salix matsudana* x *S. alba* 13-44 and *Salix nigra*. This information should be considered in the development of pest management strategies in willow plantations, through the development of resistant *Salix* phenotypes or genotypes where the willow sawfly may produce tree damage and economic loss, in the context of both tree production and environmental purposes.

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