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SCIENTIFIC NOTE

Oviposition of *Naupactus cervinus* (Boheman) and *Naupactus xanthographus* (Germar) (Coleoptera: Curculionidae) under laboratory conditions on orange fruit

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Fuller's rose weevil, *Naupactus cervinus* (= *Asynonychus* = *Pantomorus cervinus*) (Boheman), is a cosmopolitan pest that currently represents a serious quarantine problem for the Chilean citrus industry due to its similarity at the egg stage with the quarantine pest *Naupactus xanthographus* (Germar). The objective of this research was to study the biological aspects associated with *N. cervinus* and *N. xanthographus* oviposition habits under laboratory conditions on orange (*Citrus sinensis* [L.] Osbeck) fruit. Adult *N. cervinus* and *N. xanthographus* were collected from the field to carry out the research. Results show that *N. cervinus* fecundity was higher than *N. xanthographus* by more than 70%, but egg viability was lower. It was recorded that 71.90% *N. cervinus* ovipositions were under the calyx. Likewise, 52.46% *N. xanthographus* ovipositions occurred on leaves and 40.98% on the surface of the plastic cage. In the present study, only *N. cervinus* lays egg masses under the calyx of citrus fruit.

Key words: *Citrus sinensis*, egg masses, Fuller's rose weevil, oviposition, *Naupactus*.

INTRODUCTION

The Naupactini (Curculionidae: Entiminae) tribe consists of approximately 65 genera (Alonso-Zarazaga and Lyal, 1999) with over 500 species mainly distributed in Central and South America (Wibmer and O'Brien, 1986); it is one of the most diverse tribes and is highly important for phytosanitary reasons (Lanteri, 1994; Lanteri et al., 2002a). Within this tribe, the main curculionidae pests found in Chilean citrus trees are *Naupactus* (= *Asynonychus* = *Pantomorus cervinus*) (Boheman) and *Naupactus xanthographus* (Germar) (Ripa and Luppichini, 2008; Olivares et al., 2014).

Naupactus cervinus was first described in 1840 by Boheman, but its taxonomic history is mixed because it has had several changes in its generic designation and species. Among those changes are the following: *Asynonychus godmani* (Crotch), 1867; *Aramigus fulleri* (Horn), 1876; *Naupactus simplex* (Pascoe), 1881; *Pantomorus olindae* (Perkins), 1900; *P. cervinus* (Boheman), *P. godmanni* (Crotch), *Asynonychus cervinus* (Boheman), Hustache 1947; *Pantomorus cervinus* (Boheman), and Kuschel 1949 (Chadwick, 1965; Woodruff and Bullock, 1979; Alonso-Zarazaga and Lyal, 1999). It subsequently went back to its first taxonomic designation (Alonso-Zarazaga and Lyal, 1999). The taxonomic history of *N. xanthographus* is simpler. It was described by Germar in 1824, who had previously classified it as *Leptocerus xanthographus* (Elgueta and Marvaldi, 2006).

Both *Naupactus cervinus* and *N. xanthographus* are polyphagous species. Adult *N. cervinus* feed mainly on roses and citrus fruit in addition to a great variety of plants such as lucerne, raspberry (*Rubus idaeus* L.), strawberry, blueberry (*Vaccinium corymbosum* L.), peach tree (*Prunus persica* [L.] Batsch), apple tree, and avocado tree (*Persea americana* Mill.) (Elgueta, 1993; Ripa and Rodríguez, 1999; Del Rio et al., 2010). It damages *Citrus* spp. (Rutaceae) in Brazil (Lanteri et al., 2002b) and ornamental plants in the USA (Lanteri et al., 2002a). In Chile, *N. xanthographus* is largely associated with grapevine (*Vitis vinifera* L.), custard apple (*Annona cherimola* Mill.), plum (*Prunus domestica* L.), peach, cherry (*Prunus cerasus* L.), walnut (*Juglans regia* L.), pear (*Pyrus communis* L.), kiwifruit (*Actinidia deliciosa* [A. Chev.] C.F. Liang & A.R. Ferguson), orange (*Citrus sinensis* [L.] Osbeck), lemon (*Citrus limon* [L.] Burm. f.), avocado, blueberry, persimmon (*Diospyros kaki* Thunb.), and raspberry, as well as other vegetable crops such as beetroot (*Beta vulgaris* L.), potatoes (*Solanum*

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tuberosum L.), and bean (*Phaseolus vulgaris* L.) (Ripa and Luppichini, 2010). Moreover, it is part of the lucerne curculionidae complex in Argentina and it was frequently found in soybean crops in recent years (Lanteri, 1994; Lanteri et al., 2002a).

Although the Naupactini tribe has many morphofunctional similarities, the species group has distinct biological characteristics, especially in their type of oviposition (Lanteri and Del Rio, 2008). Lanteri et al. (2002b) classified the Naupactini tribe into two categories: insects that lay their eggs in masses attached to the crevices and folds of leaves and insects that lay their eggs on the soil.

The Naupactini tribe prefers narrow grooves that are wide enough for females to introduce their distended ovipositor and not too far so as to expose the eggs to desiccation or natural enemies. It is also mentioned that oviposition in fruit is related to insect size where those that are larger than 2 cm tend not to lay their eggs on the fruit (Guedes and Parra, 2004).

In Chile, more than 80% of pest-related rejections in citrus production –mainly oranges–are due to the presence of curculionidae egg masses under the calyx (SAG, 2013). However, there is no evidence indicating that *N. xanthographus* lays eggs under the orange calyx; this is done only by the female species of *N. cervinus*.

Therefore, the objective of this research was to study the biological aspects associated with the oviposition habits of the *N. xanthographus* and *N. cervinus* species under laboratory conditions on orange fruit.

MATERIALS AND METHODS

Biological material

Adult *N. cervinus* and *N. xanthographus* were collected from the field in January 2013. Collections were conducted in citrus orchards located in the two municipalities of Ovalle (30°35.370' S, 71°22.423' W) and Pan de Azúcar (30°06.753' S, 71°12.997' W). January is the month when adult emergence of *N. cervinus* (Olivares et al., 2014) and *N. xanthographus* begins (Ripa, 1992).

Biological material was collected by the Instituto de Investigaciones Agropecuarias (INIA) staff. Adults of both species were collected by the branch banging technique (Ripa and Luppichini, 2008; Olivares et al., 2014).

Fecundity and fertility of *N. cervinus* and *N. xanthographus*

Trials were carried out in the laboratory at 20 ± 4 °C, 57 ± 10% RH, and 14:10 h photoperiod. Each experimental unit was a plastic cage (25 × 15 × 11 cm) adapted for adult insect rearing. A female *N. cervinus* was placed inside this cage with one orange fruit (*Citrus sinensis* [L.] Osbeck) 'Navel Late' (Guedes and Parra, 2004). For the *N. xanthographus*, a female and a male with one fruit were placed in each experimental unit. Three orange leaves were added as substrate feed in each experimental unit. For each weevil species, 15 replicates were done with newly emerged females.

Each experimental unit was evaluated daily. The presence of egg masses on different substrates was recorded. Eggs were removed and counted under a stereomicroscope (Carl Zeiss, Oberkochen, Germany). The oviposition date and number of eggs were recorded. Eggs were then placed in plastic 5-cm diameter containers on moistened filter paper to determine the mean number of eggs per female per day. These containers were kept in the laboratory and monitored to determine the number of hatched larvae.

The number of eggs laid by every female in its lifetime was added to calculate total fecundity. Fertility was calculated by adding the number of hatched larvae in relation to the total number of eggs laid in its lifetime.

Oviposition substrate preference of *N. cervinus* and *N. xanthographus*

Each experimental unit of *N. cervinus* corresponded to a cage (25 × 15 × 11 cm) adapted for adult insect rearing with one female and containing one fruit and three orange 'Navel Late' leaves as oviposition substrate. For *N. xanthographus*, a couple was placed in the oviposition substrates described above. Vine leaves were used as food for this species (*V. vinifera*) (Guedes and Parra, 2004).

Experimental units were checked on a daily basis to record the presence or absence of *N. cervinus* and *N. xanthographus* eggs masses under the calyx, on the fruit surface, leaves, and rearing cages. When an egg mass was found on a fruit, it was replaced with another. If eggs were not detected, fruit were replaced every 2 d to maintain them hydrated and turgid. Leaves were replaced daily and sprayed with water to keep them hydrated. For each *Naupactus* species, 15 replicates were done.

Statistical analysis

The number of ovipositions per female, number of eggs per mass, fecundity, fertility, and oviposition substrates were analyzed by ANOVA. Means were separated by the least significant difference (SAS Institute, 2009). Data that did not show a normal distribution were transformed by $\sqrt{(x+0.5)}$ (Little and Hills, 1976).

RESULTS AND DISCUSSION

Fecundity and fertility of *N. cervinus* and *N. xanthographus*

Naupactus cervinus produced a mean of 25.35 eggs per mass with 9.26 masses per female (Table 1). For *N. xanthographus*, the mean of eggs per mass was 44.71 eggs with 4.13 masses per female. Coats and McCoy (1990) indicate that the mean of eggs per mass of *N. cervinus* is 38.5 and the mean of ovipositions is 1.8 for a 2-wk period.

Table 1. Oviposition parameters of *Naupactus cervinus* and *Naupactus xanthographus*.

Species	Masses per female	Eggs per mass*	Fecundity (eggs per female)*	Fertility (% hatched larvae)
N. cervinus	9.26 ± 2.89a	25.35 ± 3.29b	235.47 ± 82.85a	33.92 ± 10.42c
N. xanthographus	4.13 ± 1.72b	44.71 ± 9.12a	180.0 ± 64.54ab	53.92 ± 19.03b

Different letters indicate a significant difference between treatments according to the LSD test ($p \leq 0.05$). Data are presented as mean ± standard deviation.
*Eggs were collected daily from each experimental unit.

McCoy (1994) shows that the number of *N. cervinus* eggs per mass varies between 70 and 100 eggs and with a total of 300. Guedes et al. (2007) reported that *N. cervinus* lays 120.9 eggs per female with a mean of 4.2 ovipositions and a mean of 28.7 eggs per mass.

Additionally, Guedes et al. (2007) indicate that *N. cervinus* compared with *N. versatilis* exhibits a smaller body size, which would explain in part its reduced oviposition ability.

The highest fertility corresponded to *N. xanthographus* (53.92%) and then *N. cervinus* (33.92% hatched larvae, viable eggs). Guedes and Parra (2007) obtained viability at the egg stage at different temperatures and registered a mean of 59.4% for *N. cervinus*. As temperature increased, egg viability was higher, which led to 79.6% eggs hatching at 32 °C. The same authors studied the fertility of *N. versalis*, another species of the Naupactini tribe, and recorded mean fertility of 56.1%.

The same study indicates that death can occur during oviposition due to the presence of fungus (4%) and dehydration (3%). Gallego et al. (2012) studied feeding and oviposition behavior in *Compsus viridivittatus* and registered 94% viability, whereas it was only 10% in *C. latifolia*; this shows that there is a significant host effect.

Total fertility was 235.4 eggs for each *N. cervinus* female and 180 eggs for the *N. xanthographus* female. These results differ from those reported by Ripa (1983), who indicates that each *N. xanthographus* female can oviposit 600 eggs on the average under laboratory conditions. East (1977) reported a variation in the mean number of eggs per female in *N. leucoloma* and associated fertility with the host.

Lanteri (2010) indicates that the species of parthenogenetic Curculionidae would double their fertility with respect to those that produce balanced sex offspring. From these results, it can be observed that *N. cervinus* species fecundity was higher than for *N. xanthographus* by more than 70%, but egg viability was lower.

Oviposition site preference of *N. cervinus* and *N. xanthographus*

It was observed that *N. cervinus* oviposition was 71.94% under the calyx, 13.66% on leaves, and 14.38% on the rearing cage surface. In *N. xanthographus*, results show that 51.6% of oviposition occurs on leaves, 6.44% on the fruit surface, and 40.1% on the cage surface (Table 2). Then it was observed that *N. cervinus* oviposition was protected under the fruit calyx; *N. xanthographus* egg masses were laid on an unprotected area of the fruit surface.

Results of oviposition preference in this study are similar to those reported by Barattini (2008), who states that the first oviposition preference of *N. cervinus* is under the calyx of lemon fruit (88%) and dry leaves (12%) under field conditions in a lemon orchard. In addition, authors that collected egg masses in the field from citrus orchards observed that 100% of the eggs under fruit orange sepals corresponded to *N. cervinus*.

Guedes and Parra (2004) determined that *N. cervinus* and *Parapantomorus fluctuosus* (Boheman) oviposition occurred only on the fruit by using free choice substrates that provided leaves, plastic, and fruit. Moreover, oviposition was observed on the plastic, but not on leaves when there was no choice of substrate.

Coats and McCoy (1990) note that 83.2% *N. cervinus* eggs are laid on the fruit, 16% on leaves, and 0.8% on branches. This preference may be influenced by the population density of this pest because 60% of the eggs were found at lower densities on the fruit, whereas the percentage rises to 90% at higher densities. McCoy (1994) indicates that females lay 80% of their eggs under the citrus fruit calyx. Maher and Logan (2004) note that 45% oviposition occurred on the experimental container nets, 41% on blocks with polyethylene bands, 6.3% on foliage, and the remaining 8% is divided on different sites. The results of the present study verify that *N. cervinus* lays egg masses mostly under the citrus fruit calyx.

Table 2. Percentage oviposition of *Naupactus cervinus* and *Naupactus xanthographus* egg masses on different substrates.

		Oviposition substrates		
Species	Calyx	Leaves	Fruit surface	Plastic surface
N. cervinus	71.94 ± 1.65aA	13.67 ± 0.71bB	0aC	14.38 ± 0.79bB
N. xanthographus	0cB	52.46 ± 1.73aA	6.56 ± 0.75bB	40.98 ± 1.82aA

Different vertical lowercase letters indicate a significant difference between species and horizontal uppercase letters indicate a significant difference between substrates according to the LSD test ($p \leq 0.05$). Data are presented as the percentage of egg masses ± standard deviation.

CONCLUSIONS

Under laboratory conditions, oviposition habits of the *Naupactus xanthographus* and *N. cervinus* species were different. The most important oviposition substrate preference of *N. cervinus* corresponded to the calyx of the citrus fruit. Female *N. xanthographus* did not lay egg masses under the calyx. The results of this study add to the knowledge of oviposition behavior in the *N. xanthographus* species. This information will prevent the rejection of Chilean citrus fruit at export control points due to the similarity of *N. xanthographus* and *N. cervinus* eggs.

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