



RESEARCH NOTE

A method for rearing Fuller's rose weevil, *Naupactus cervinus* (Coleoptera: Curculionidae) on an artificial diet

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(Original submission received 22 September 2025; accepted in revised form 2 May 2026)

Summary

Fuller's rose weevil, *Naupactus cervinus* (Boheman) (Coleoptera, Curculionidae) adults are only available in the field for a short period of time each year. As field-collected insects are not of a known standardised quality or age, they are not suitable for experimental work. Our goal was to develop a laboratory rearing technique to enable access to all life stages of this insect year-round. This paper reports on life-cycle data for two generations of *Naupactus cervinus* reared on an artificial diet. Key life cycle parameters were determined, and a laboratory colony was successfully established. The life cycle parameters at 20°C and an 18-h photoperiod were as follows: the egg development period was approximately 30 days, the neonate-to-adult period was about 21 weeks, and the average generation time (egg to egg) was approximately 27 weeks. The diet and rearing technique enabled the year-round, laboratory rearing of standardised adult insects of known quality and age, with larvae fed artificial diet and adults fed mature citrus leaves for two successive generations.

Keywords Weevil, diet, rearing, fecundity

INTRODUCTION

Fuller's rose weevil *Naupactus cervinus* (Boheman) (Coleoptera: Curculionidae), sometimes known as the Fuller's rose beetle, is a flightless, parthenogenetic weevil that is distributed throughout fruit-growing areas of the world. In the USA, it is a pest on many horticultural crops, such as apples, apricots, and citrus (Chadwick 1965). In California, *N. cervinus* adults lay their eggs under the calyx of navel oranges (*Citrus sinensis*) and therefore, pose a quarantine issue for yearly exports to Korea (Blake 2013). In South Africa, Australia, and Chile, *N. cervinus* is a quarantine pest of citrus and is an issue for some export markets, such as China and North Korea (Baker 2013, Olivares et al. 2014, Akter et al. 2023). *Naupactus cervinus* has been present in New Zealand since the late 1930s and is likely to have been introduced from Australia (Gourlay 1940). Adults may be a threat to young avocado trees in New Zealand due to damage caused by foliage feeding (Siebert 2016). *Naupactus cervinus* on pipfruit are a quarantine pest in the export markets of China, Thailand, New Caledonia and Mexico (Shaw & Wallis 2016).

Until 2006, *N. cervinus* was a quarantine pest of citrus and kiwifruit (*Actinidia chinensis*) for the Japanese export market. (Mander et al. 2003, Anonymous 2006, Logan et al. 2008). The Japanese ultimately updated their quarantine policy for *N. cervinus* on kiwifruit because *N. cervinus* was already present in Japan (Steven 2005, Anonymous 2006). However, *N. cervinus* remains a market access problem for exports to China, Taiwan and Korea.

Larvae of *N. cervinus* are root-feeding and soil-dwelling for 11 months of the year (May 1979), making them challenging to study. Masaki and Takahashi (1999) developed a successful rearing method for *N. cervinus* larvae using potted potatoes, strawberry and citrus plants in soil. The development of an artificial diet and rearing method would likely improve the study of these insects. In the family of Curculionidae, many artificial diets have been developed for rearing weevils in the laboratory (Beavers 1983, Mau & Lai 1988, Blossey et al. 2000; Olo & Omoloye 2012, El-Shafie et al. 2013), but only one diet has been published for *N. cervinus* (Morse & Lindegren 1996), which produced

only 12 adults (1.2% survival) and some viable eggs.

Research to prevent this pest from causing major market access problems is dependent on having access to all life stages of *N. cervinus* of a known age. Field-collected insects are not usually of a standardised quality and known age and therefore are not always suitable for repeatable experimental work. The purpose of this study was to develop a rearing technique and collect life cycle developmental data based on an artificial diet. This information was used to establish a laboratory colony of *N. cervinus* and supply insects of a known age for research on its biology, dispersal patterns, host plant preferences (Maher & Logan 2004) and pre-harvest control options.

MATERIALS AND METHODS

All rearing was carried out in controlled temperature rooms maintained at $20 \pm 1^\circ\text{C}$, 55–65% relative humidity (RH), and an 18:6 light: dark (L:D) photoperiod. To initiate the laboratory colony, approximately 200 *N. cervinus* adults were collected around December 2000 from kiwifruit vines, located at Te Puke ($37^\circ 82' \text{S}$, $176^\circ 32' \text{E}$), New Zealand.

Adult rearing

Adults (Figure 1A) were group-reared ($n = 20\text{--}25$) in plastic containers (190 mm diameter $\times$ 190 mm high) (Figure 1B), covered with a lid containing two gauze-covered holes (10 mm diameter). They were provided with a food source of mature leaves of the navel orange (*Citrus sinensis* (L.) Osb.), stored and held in water, which were changed once a week. Four strips of Mono® scale wax paper (Clorox New Zealand Ltd) (each 200 $\times$ 250 mm, fan-folded into 20-mm wide folds and stapled along their length) were used as an egg laying substrate and placed vertically, against the inside wall of each adult rearing container.

Experimental oviposition trials were carried out using the first filial generation (F1) and F2 generation adults that emerged from larvae reared on artificial diets. The oviposition rate per week and total fecundity were determined from 18 (F1) and 19 (F2) adults for each generation. Each experimental unit consisted of a single diet reared adult placed in a clear plastic container (680 mL volume) with a clip-on lid, ventilated with about 10 holes. The adults were provided with mature citrus leaves and a strip of fan-folded wax paper placed around the inside wall of each container as an egg-laying substrate (Figure 1C). Containers were checked weekly, and eggs were collected. The F1 generation adults were fed mature leaves, field collected from mid-June (winter), and F2 generation adults were fed mature leaves, field collected from mid-November (summer). Adult mortality was recorded, and egg collection ceased when egg laying had been zero for three weeks.

Eggs

After collection, eggs were left on the wax paper (Figure 1C), cleaned of all insect frass and debris using a fine camel-hair (size 00) brush and stored dry at 20°C in round plastic containers (680 mL volume) with an RH of 55–65% for the complete duration of the development. Egg hatch (for eggs 30 days or older) was initiated by sterilising eggs in aqueous

sodium hypochlorite solution (0.1% v/v/) for 10 minutes, followed by rinsing them in water for another 10 minutes, then removing any excess water.

Egg-development duration and percentage hatch trials were conducted for F1 generation only. These trials consisted of four replicates ($n = 753$ eggs), each consisting of 10 randomly chosen egg batches (Figure 1D) from a mixture of adults per replicate. A few days before the first expected hatch, eggs were sterilised and each egg batch was placed in a separate Petri dish. The dishes were placed in a plastic container, and the number of larvae hatching from each egg batch was recorded daily.

Eggs required to maintain a laboratory colony could be accumulated by storing them at a low temperature (15°C) and sterilising them before initiation of hatch.

Larval rearing

The larval diet was modified from Beavers (1983), and the percentage composition of ingredients is presented in Table S1. The diet was prepared in 3-kg batches, where the dry ingredients were slowly added to water and thoroughly mixed. The mixture was sterilised in an autoclave at 121 kPa pressure for 20 minutes. The hot diet mixture was extremely thick, and extra care was taken during mixing until it had cooled to about 60°C . The vitamin/mould inhibitor mixture was then added slowly and mixed thoroughly. Due to the thickness of the finished diet, it was added to plastic (non-vented) Petri dishes (12 mm high $\times$ 85 mm diameter) in the amount of 65 g per dish using a metal spatula. At this stage, the Petri dishes of diet were either packed up and stored at 4°C or set up for diet pre-conditioning. It was essential that the diet was appropriately pre-conditioned before use. This was accomplished by removing the lids from the Petri dishes of diet and placing them in a sterile cabinet (3–4 days) or a laminar flow unit (6 h) to allow for drying of the diet to achieve a 16% loss of moisture (percentage moisture loss was measured by recording loss in actual weight of artificial diet). After this period, the diet had shrunk away from the sides and base of the dishes. To facilitate larval entry into the diet, the top and then the undersurface of the dried diet were heavily scored by using sterile fine stainless-steel forceps. The diet was carefully flipped over using a sterile spatula to achieve the scoring on both sides. A 10-mm diameter hole was made in the centre of the conditioned diet with a cork borer. Ten neonate larvae were carefully inoculated onto the diet at the bottom of the hole in each Petri dish. The lids of the Petri dishes were then taped closed (top to bottom, with four pieces of Sellotape®), (Figure 1E), and larvae were left to feed and develop on the diet (Figures 1F, 1G and 1H) until adult emergence. Determination of the development period from neonate larva to adult was carried out for F1 generation only. This consisted of three replicates of 18 Petri dishes per replicate (inoculated with 10 larvae per dish). Adults were removed daily from Petri dishes as they emerged.

Statistical analysis

A mixed-effects model was used to estimate coefficients for egg counts over time from F1 and F2 generations using the R package glmmTMB (Brooks et al. 2017, R Development Core Team 2022). The number of weeks since experiment

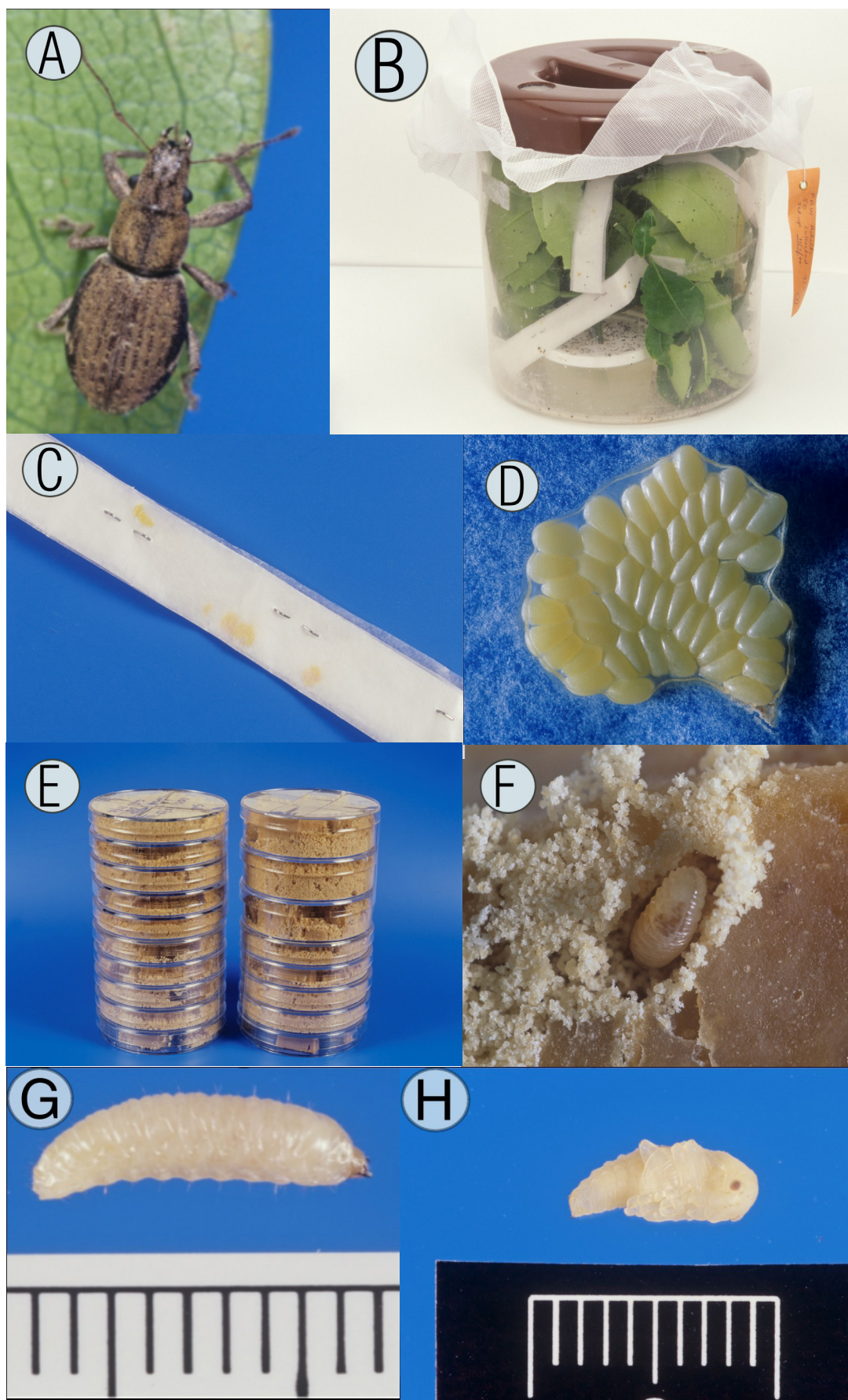


Figure 1. Fuller's rose weevil *Naupactus cervinus*: diet-reared adult (A); adult oviposition container (B); fan-folding wax paper with eggs laid between folds (C); egg batch (D); Stack of larval rearing containers (E); mature larvae in diet (F); mature diet-reared larvae (G) and mature diet-reared pupa (H). Scale 1 division = 1 mm.

inception and a factor of generation were considered fixed effects. Container identity was included as a random effect term to reduce over-dispersion of residuals and to allow for repeated measurements over time. Residual diagnostics were undertaken using the R package DHARMA (Hartig 2022). The best candidate model used a Gaussian error term, an interaction of week and generation with a quadratic term for week. Candidate models were selected based on residual diagnostics, AIC values and goodness of fit assessed using R^2 (Nakagawa et al 2017).

RESULTS

Eclosion of first instar larvae from eggs began as early as 27 days after egg laying, with a mean egg development period of 29.4 days (Table 1). Eggs were laid in batches, range 2–69 (F1, n=388) and 3-100 (F2, n=1936) eggs/batch. When zero extra moisture was added to the egg storage containers, the egg hatch was only 28%. After sterilisation with hypochlorite solution, egg fertility was 84% for F1 and 76% for F2 even though the relative humidity was the same in both cases.

Neonate larvae established initially on the bottom surface of the diet (between diet and Petri dish), burrowing into the diet, forming tunnels and pupating in the diet. Determination of the actual date of pupation (Figure 1H) was difficult without disturbing the diet and so was not attempted. The average neonate larva to adult period was just under five months (146.2 days), with a range of 4.5 to 5.5 months (135–163 days) (Table 1). The mean survival rate on the diet from neonate larva to adult was 36%.

Approximately four adults (range 1–8) were obtained per Petri dish with an average adult weight of 29 mg (Table 1). Diet-reared adults, upon emerging, fed and survived on the larval diet but did not lay any eggs unless fed mature

citrus leaves. For citrus leaf-fed adults, the pre-oviposition period was ≈ 15 days for F1 adults and ≈ 11 days for F2 adults (Table 1) and were significantly different. Adult oviposition, for both generations, had a general increase to a peak and then a long, gradual decline (Figure 2). F1 generation adults reached their peak egg laying of ≈ 30 eggs per week ≈ 10 weeks after eclosion, whereas F2 generation adults reached their peak egg laying of ≈ 105 eggs per week ≈ 13 weeks after eclosion. For F1 generation, 56% and 89% of adults stopped laying eggs after 20 and 25 weeks after eclosion, respectively. The F2 generation fed adults (the summer emergence) were superior to F1 generation fed adults (winter emergence) in most aspects of oviposition data collected (Table 1). The pre-oviposition period of F2 generation fed adults was four days quicker; oviposition period was 1.7 times greater; fecundity was five times greater; egg fertility was about the same at around 80%. At 30 weeks after eclosion, only 10% of adults had stopped laying. When egg counting ceased, most adults were still alive. The adult mortality was low, with only three adults dying (14.3%) in F1 generation and only one adult dying (5%) in F2 generation (up to 34 weeks). Laboratory-reared insects completed a full generation (from egg to egg) in an average of 192 days.

DISCUSSION

Our aim was to develop an artificial diet and method for rearing *N. cervinus*. When trying to develop a larval artificial diet for *N. cervinus*, Morse and Lindegren (1996) stated that larval mortality was especially high for early larval instars and that diet moisture content may be important. Therefore, the method presented here involves a pre-conditioning period designed to remove excess surface moisture.

Table 1. Life cycle data for Fuller’s rose weevil, *Naupactus cervinus*, reared on a larval artificial diet at 20±1°C, 55-65% RH, and 18 h photoperiod. (F1 generation adults - fed mature citrus leaves from mid-June [winter] and F2 generation adults -fed mature citrus leaves from mid-November [summer]). Eggs sterilised in sodium hypochlorite.

Parameter	Generation	n	Mean ± SE	Range	P. value
Egg development period (days)	F1	753	29.4 ± 0.9	27 – 35	
Neonate to adult period (days)	F1	196	146.2 ± 0.4	135 – 163	
Adult weight (mg)	F1	122	29.22 ± 0.3	20.8 – 36.1	
	F2	30	29.1 ± 0.5	24.6 – 33.6	P=0.869
Pre-oviposition period (days)	F1	21	15.5 ± 0.8	11 – 25	
	F2	20	11.5 ± 0.4	10 – 18	P<0.001
Oviposition period (weeks)	F1	18	18.9 ± 1.2	12 – 34	
	F2	19	31.4 ± 0.6	25 – 34	P<0.001
Fecundity	F1	18	479.5 ± 109	109 – 903	
	F2	19	2465.1 ± 81.3	1876 – 3318	P<0.001
Egg batches/adult	F1	18	19.4 ± 1.4	8 – 35	
	F2	19	101.3 ± 3.4	76 – 133	P<0.001
Egg fertility/adult when fed citrus leaves (%)	F1	18	84.2 ± 1.29	74.5 – 92.9	
	F2	19	75.9 ± 1.26	60.2 – 83.9	P<0.001

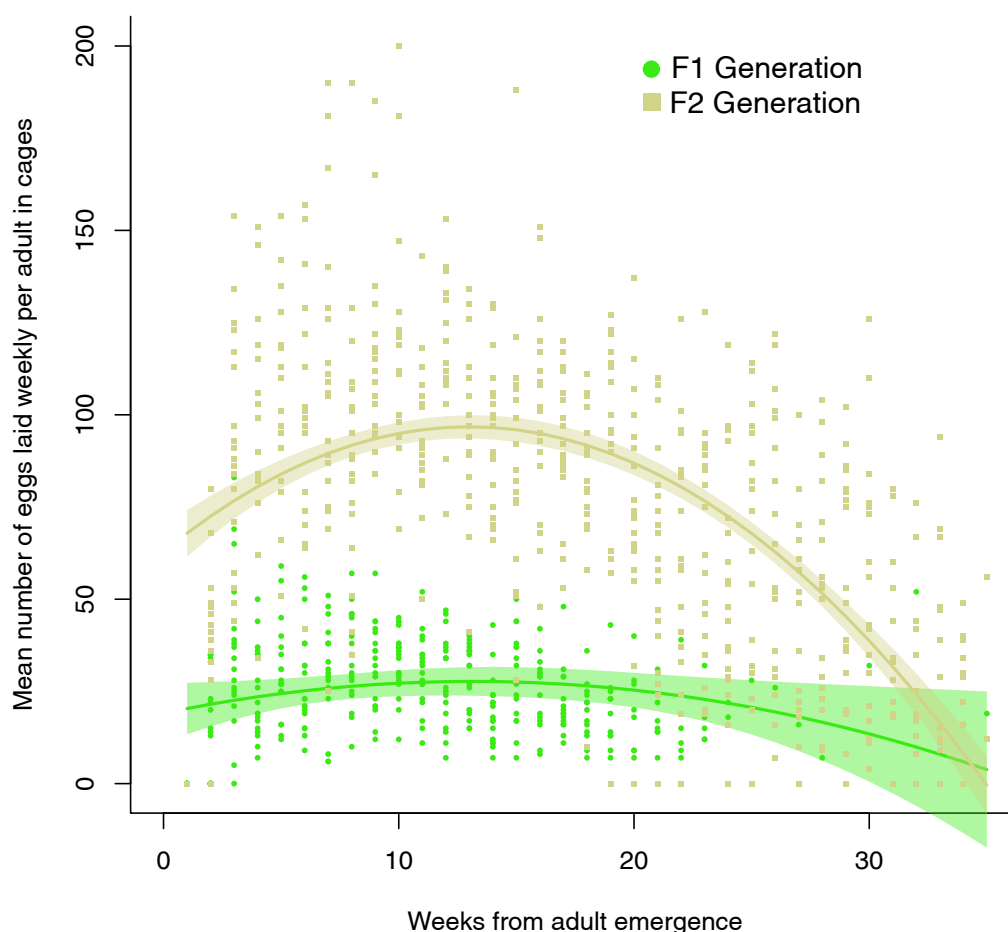


Figure 2. Egg counts per week for consecutive generations of diet-reared Fuller's rose weevil, *Naupactus cervinus*. Adults were maintained at 20°C, 55–65% RH, 18h LD photoperiod. Lines are predicted fits from a mixed effects model and the shaded areas represent estimated 95% confidence intervals. F1-generation adults were fed mature citrus leaves from mid-June [winter] and F2-generation adults were fed mature citrus leaves from mid-November [summer].

Adults emerging from the artificial diet and fed only on the artificial diet did not lay eggs, while adults emerging from the artificial diet and fed mature citrus leaves did lay eggs. This raised two important points. Firstly, some important ingredient(s) for adult maturation may be present in mature citrus leaves but lacking in the artificial diet. The development of an adult artificial diet may therefore be possible by the addition of freeze-dried host plant powder to the diet. Secondly, it shows that, in *N. cervinus*, adult maturation feeding determines fecundity, not the larval diet. This effect was also shown by Masaki and Kadoi (1997), who carried out extensive host plant feeding trials on Fuller's rose weevil (as *Pantomorus cervinus*), and concluded that adult fecundity was influenced by adult host plant, during adult maturation feeding, rather than by the larval host plant.

There was a 500% increase in the fecundity of F2 generation compared to F1 generation. This could have been caused by either differences in leaf nutrition or a generation effect. The first possibility is the nutritional quality of the adult host plant during adult maturation feeding. Seasonal changes in the mineral nutrient status of citrus leaves are well established and have been shown to change from

summer to winter (Jones and Parker 1951, Labanauskas et al. 1960, Edwards et al. 1997, Ayoub et al. 2014). It is well established that nutrition can have a major impact on insect development and oviposition (House 1961, Dadd 1973, Thompson 1999). Adults in the field begin emerging naturally in December and January (summer) each year and eat citrus leaves. Laboratory-reared adults of F1 generation (emerged in winter) were thus fed mature leaves from mid-winter onwards. This could have influenced their fecundity in the same way as adults in F2 generation (emerging in summer), who were fed mature leaves. Thus, the superior oviposition results obtained from F2 generation are probably nutrition-based. In an ovipositional study in Florida, USA, using field-collected *N. cervinus*, a seasonal variation was found in the number of eggs laid by adults throughout the year, with more eggs being laid in summer, and only a weak correlation with temperature (Coats & McCoy 1990). The variation could not be explained. However, seasonal changes in leaf nutrition could have been a factor.

The second possibility for the difference in fecundity between F1 and F2 generations is the effect of laboratory colonisation of the insects. Placing insects on nutrient-rich artificial diets and maintaining colonies at constant

temperatures and photoperiods can affect an insect's performance in many ways. During this process, changes can occur in phenotypes such as reproductive parameters (Mason et al. 1987).

The mean egg development period in this study (29.4 days) compares closely with the degree-day model predictions for *N. cervinus* egg development at 20°C (Lakin & Morse 1989, Tarrant & McCoy 1989, Masaki et al. 1996) of 30.2, 28.6 and 27.2 days, respectively.

Lakin & Morse (1989) found that a relative humidity (RH) greater than 75% was necessary for a high percentage (82–96%) egg hatch. In the current study, egg hatch was only 28% when zero extra moisture was added to the egg-storage containers, which had an RH of 55–65% for the complete duration of their development. the presence of moisture alone has been found by Haley (1948) to stimulate the hatching of *N. cervinus* eggs that had completed their development but had not hatched. sodium hypochlorite used for egg sterilisation is a strong oxidizing agent and is well known to partially dechlorinate insect eggs (Soares 1992) and thus may aid egg hatch.

CONCLUSIONS

In summary, all life stages of *N. cervinus* were completed and a laboratory colony successfully established using the larval artificial diet and rearing method presented here. Two successive generations of *N. cervinus* were reared in the laboratory, with larvae fed artificial diet and adults fed mature citrus leaves, producing over 2000 diet reared adults. The diet and rearing method enabled the year-round rearing of standardised insects of a known age and quality in the laboratory and the collection of life cycle data greatly facilitated the study of these insects. Further research is needed to develop an adult artificial diet.

ACKNOWLEDGEMENTS

We would like to thank the reviewers, David Logan, Manoharie Sandanayaka, Samuel Brown, Peter Lo, and Asha Chhagan, for their helpful critique of the manuscript. We would also like to thank Dave Rogers, who initiated the project, Vicky Davis for technical assistance and Patrick Connolly for initial statistical analysis. This research was funded by the New Zealand Foundation for Research, Science and Technology (No. C06X0006).

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SUPPLEMENTAL INFORMATION

Table S1. Ingredients of Fuller's rose weevil, *Naupactus cervinus*, larval artificial diet as percentages [(a) = water added to dry mix; (b) = water added to vitamin mix/mould inhibitor solution].

Ingredient	%
Dry mix	
Cellulose powder	8.52
Potato starch/powder	6.94
Fine soybean powder	2.87
Sucrose	1.98
Casein	1.98
Wheatgerm (finely ground)	1.69
Fine cornmeal	1.19
Wesson salt mix	0.44
Agar	2.97
Water (a)	66.40
Vitamin mix/mould inhibitor solution	
Vanderzant vitamin mix	0.89
Methyl <i>-p</i> -hydroxybenzoate	0.10
Sorbic acid	0.07
Water (b)	3.96
Total	100.00