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Thunb. (Dipsacales: Caprifoliaceae) in Aotearoa / New Zealand

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Abstract

Aotearoa / New Zealand harbours more naturalised non-native plant species than almost any other island group in the world, some of which are serious threats to indigenous biodiversity. Japanese honeysuckle (Lonicera japonica), a non-native climbing vine, is widespread across the country, and is considered a serious threat due to the nature of its growth habits and smothering effects upon indigenous flora. In 2014, the Honshu white admiral butterfly (Limenitis glorifica) was released in Aotearoa / New Zealand as a biological control agent, as this species has been shown to be narrowly oligophagous to Japanese honeysuckle. It is believed that the larva of this butterfly could cause substantial feeding damage; sufficient to reduce the plants' fitness. However, the amount of foliage that could be consumed by Honshu white admiral larvae is unknown. This study monitored 30 larvae from egg to pupation, and found that a single larva could consume 2.13g of Japanese honeysuckle foliage before pupating. This equates to approximately 5.6 leaves (based on the mean weight of a mature honeysuckle leaf). These findings give an insight into the defoliation potential this biocontrol agent could have upon Japanese honeysuckle, although further research is needed to determine the physiological response of the invasive plant based on this level of herbivory.

Keywords

Biocontrol, invasive plant, Aotearoa / New Zealand, biodiversity, Lepidoptera, Nymphalidae, Dipsacales, Caprifoliaceae, Lonicera japonica, Limenitis glorifica

Introduction

Aotearoa / New Zealand's ecosystems are being impacted by over 2800 species of naturalised plant, of which over 300 are considered to be actual or potential invasive weeds (Froude 2002; Ogle et al. 2020; Schönberger et al. 2021). This estimate of invasive plant species is but a subset of the more than 19,000 non-native freshwater and terrestrial species currently in Aotearoa / New Zealand, thousands of which have naturalised since the late 19th century (Froude 2002). The consequences that these introductions pose are severe, with many highly invasive weeds having the potential to transform natural landscapes, community composition and ecological dynamics (Effah et al. 2020). As it is, approximately 50% of all uncultivated vascular plant species present in Aotearoa / New Zealand are non-indigenous, resulting in many coastal habitats, lowland forests, shrublands, wetlands and tussock grasslands being invaded and subsequently dominated (Hulme 2020; Aikio et al. 2012). This includes hundreds of thousands of hectares of conservation land and critically endangered ecosystems, where plant invasions threaten up to one-third of all this country's nationally threatened plant species (Hulme 2020).

Most flora introduced into Aotearoa / New Zealand lack the natural enemies that are present in their native range, and without these coevolved controls to limit their vigour and density, their growth and spread can become excessive (Poland et al. 2021). Japanese honeysuckle (Lonicera japonica Thunb., Caprifoliaceae) is native to Japan, Korea and eastern China. It was first recorded as being naturalised in this country in 1926, and today is abundant throughout the North Island, upper regions of the South Island, and a growing number of offshore islands, including the Chatham Islands (Webb et al.



Figure 1. Lonicera japonica (Caprifoliaceae) in flower, Split Apple Bay (western Tasman Bay), Te Wai Pounamu / South Island, 26 November 2019. Photo: Peter J. de Lange.

1988). The Department of Conservation (DOC) has listed Japanese honeysuckle as one of the 18 "A-category" invasive weeds, prevalent in Protected Natural Areas in 70% of DOC conservancies (Williams & Timmins 1998).

Japanese honeysuckle is a perennial vine that spreads by rhizomes, above-ground runners, and occasionally by seed. In Aotearoa / New Zealand it can be seen in bloom (Figure 1) from September through to May, flowers are sweetly fragrant white-yellow, and the fruit is a many-seeded black berry that matures in the autumn months (Waipara et al. 2007). Leaves are ovate or oblong in shape (young leaves are often lobed), 4–8 cm long, sparsely pubescent, with a short petiole (Nuzzo 1997). The leaf phenology of Japanese honeysuckle varies depending on its locality, deciduous in the colder parts of its range in Aotearoa / New Zealand, to evergreen in warmer regions (Schierenbeck 2004).

Once introduced to a site, Japanese honeysuckle can quickly build up a mass of vegetation, using surrounding vegetation and its own stems for support to form dense, tangled curtains. It competes with native flora and restricts seedlings from establishing beneath its canopy, leading to a simplified vegetation structure with lower biodiversity (Williams & Timmins, 1998). Herbicides are most commonly used to control Japanese honeysuckle, which are either sprayed directly onto standing foliage, or applied directly onto cut stem stumps and regrowth (Standish 2002). Although effective, conventional control is regarded as impractical at most



Figure 2. Honshu white admiral *Limenitis glorifica* Fruhstorfer, 1909 (Lepidoptera: Nymphalidae) imago. Photo: Adam Parkinson.

sites as herbicide application can cause significant collateral damage to the non-target species (Peterson et al. 2020), which Japanese honeysuckle is often found smothering. This approach is also costly. DOC has estimated \$791,621 per year over a 10-year period to eradicate Japanese honeysuckle at Kopuatai Peat Dome (-37.41653, 175.55092) in the North Island of Aotearoa / New Zealand, an area of 2111 ha. DOC emphasises the considerable risk of failure this approach may have (Paynter et al. 2017).

In 2012, the Greater Wellington Regional Council, in collaboration with Manaaki Whenua Landcare Research, made an application to the Environmental Protection Authority (EPA) to introduce the Honshu white admiral butterfly (*Limenitis glorifica* Fruhstorfer, 1909) as a biological control agent for Japanese honeysuckle. Although this species has not been used as a biocontrol agent anywhere in the world before, permission was granted by the EPA in 2013 and the butterfly was released in spring of 2014 (Paynter et al. 2017). The purpose of introducing the Honshu white admiral butterfly was to establish self-sustaining populations that would contribute to the suppression of Japanese honeysuckle (Environmental Protection Authority 2019).

The Honshu white admiral is endemic to the island of Honshu, Japan, and is mainly found in open areas or light shrubland in dry warm-temperate habitats (Tanaka 1978). It is widely distributed from the western lowlands of Yamaguchi Prefecture to Shimokita Peninsula in the



Figure 3. Honshu white admiral, *Limenitis glorifica* Fruhstorfer, 1909 (Lepidoptera: Nymphalidae) larvae final instar. December 2021. Photo: Adam Parkinson.

north, and has an altitudinal limit of 1450 metres but is most abundant in lowland areas (Tanaka 1978). Honshu white admiral imago (Figure 2) are black with have a distinctive white band across the fore and hindwings, with reddish-brown, white and black markings underneath (wingspan 50-70 mm). Females will deposit 150-200 eggs individually on the upper and lower surfaces of Japanese honeysuckle leaves over a 2-4-week period. Fertile eggs take c.7 days to hatch in optimal conditions (Paynter et al. 2017). As the larvae (Figure 3) feed on Japanese honeysuckle foliage, they develop and grow through five instars. Initially hatching as a minute (4-5 mm) brown larva, when fully grown (25 mm) has a double row of branched reddish spines along its darkgreen body. Development from egg to imago can take place within c.8 weeks if temperatures are warm and favourable. Within its natural range of Japan, this species will produce 1–4 generations annually; however, it is still uncertain how many generations will be produced in this country (Paynter et al. 2017).

The EPA reported that each larva could destroy several leaves during its development (Hill 2019). However, the quantity of Japanese honeysuckle leaves a single larva can consume has not been tested. Despite being a common butterfly in Japan, the Honshu white admiral is little studied, other than the species' distribution and host plant preferences. This study aims to collect preliminary data on the consumption quantities of Honshu white admiral larvae on Japanese honeysuckle under controlled conditions.

Methods

Japanese honeysuckle was sourced from naturalised plants growing on the Unitec, Te Pūkenga campus, Tāmaki Makaurau / Auckland. These were grown on from cuttings in the Unitec butterfly enclosure (36°52'47.5"S 174°42'21.0"E), under naturalistic conditions, in 13 m plots in organic potting mix (Living Earth). No additional fertilisers were used. Mature leaves were harvested for feeding trials six months after initial planting. Consumption tests were conducted between 27 November and 21 December 2021.

Fertile eggs of the Honshu white admiral were obtained from an established captive population, donated by Manaaki Whenua Landcare Research, Lincoln. To ensure that the larvae were in similar stages of development, those selected for consumption measurements had emerged from the egg within a 3-hour window of one another. Between consumption tests the larvae were kept in 33 cm x 33 cm x 61 cm mesh enclosures in the Unitec butterfly enclosure, with access to the host plant.

Undamaged mature leaves that had reached their full dimensions were cut from the main stem at the midpoint of the petiole. The weight (g) of each leaf was recorded using a Sartorius (type 1702) precision scale prior to each consumption test, and these amounts were combined to give a mean value. The ends of the petioles were then wrapped in moistened cotton to prevent desiccation (3 ml of water added to the cotton by pipette). Larvae were placed individually on each single leaf of weighed honeysuckle. Each leaf and larva were enclosed in a paper cup (10 cm height, 7 cm diameter), covered with monofilament polyethylene bio-mesh, securing the larvae, protecting them from predation and allowing ventilation (Figure 4). After 24 hours, the larvae, frass and silk were removed, and remaining leaf material was weighed. This trial was repeated every 4 days until the larvae pupated. Growth rates of the larvae were not monitored; however, observations on instar stages were noted throughout the experiment.

A single control leaf was monitored during each consumption test to calculate moisture loss caused by evaporation. These control leaves were exposed to the same conditions as the experimental group (petiole submerged in cotton wool with 3 ml of water added), and weighed before and after the 24-hour test period.

The larvae were managed as a cohort, and their consumption amounts were combined. Individual larva consumption quantities were not recorded. The mean

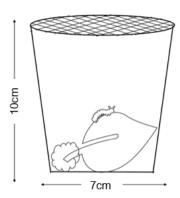


Figure 4. Honshu white admiral larva, and Japanese honeysuckle leaf with moistened cotton around petiole to prevent desiccation in containment over 24-hour test period. Secured with monofilament polyethylene bio-mesh.

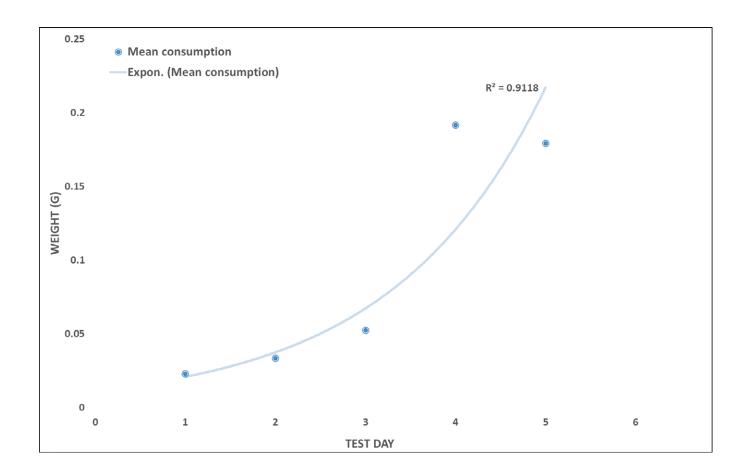


Figure 5. Exponential curve fitted to the collected data points (blue circles) of the combined mean consumption values (n = 30) for each test day, and predicted consumption weight.

Date	Test	Leaf consumption (g) after 24 hours (Mean)	Larval instar	Mean leaf weight (g)	Single control leaf moisture loss (g)	Temperature (°C)	Humidity (%)
27–28 Nov 21	1	0.02291	1	0.26567	0.0203	High 23.9 – Low 13.4	71.5
3-4 Dec 21	2	0.03336	2	0.483253	0.0060	High 24.5 – Low 18	59
9–10 Dec 21	3	0.05249	3	0.317543	0	High 26 – Low 18	64
15-16 Dec 21	4	0.19152	4	0.43985	0.1012	High 23.9 – Low 17	70
21–22 Dec 21	5	0.17930	5	0.407787	0.1012	High 23 – Low 17	52

Table 1. The combined mean larva consumption weight (n = 30) of each test day, and observed stage of larval development. Combined mean weight of honeysuckle leaf (n = 30) prior to consumption test, singular control leaf moisture-loss quantities, and ambient environment variables during test period.

consumption per larva was estimated by subtracting the weight of remaining leaf tissue post-consumption from the initial weight of leaf tissue. Thirty replicates (larvae) (n=30) were used for each consumption, and were tested until feeding ceased in the pre-pupal stage or pupation. To determine the quantity of foliage consumed, a regression model was used and an exponential curve was fitted to the mean value of the data. The goodness of fit (r2) was calculated to determine how well the data fitted within the regression curve. The fitted curve was used to predict the mean consumption of the larvae between consumption tests, and to give an approximate quantity a single larva could consume. Leaf moisture-loss was not deducted from the combined mean consumption values.

Results

By using exponential model analysis (exponential regression curve; $y = 0.0189e^{0.0977x}$), Figure 5 presents predicted consumption weight between the plotted data points. This study shows mean consumption per larva was 2.13g of Japanese honeysuckle foliage, which equates to 5.6 leaves. While a gradual increase in consumption was observed through test dates 1–3, leaf consumption during the first three instars was very

low, with a combined mean total of 0.10867 g (Table 1). There was significant increase in Test 4 (instar 4), with larvae consuming almost four times the amount of foliage to that of Test 3 (instar 3). A slight decrease in consumption was observed between Tests 4 and 5. The developmental time from hatch to pupal stage spanned 25–27 days (Table 1).

Discussion

This study presents preliminary data on the potential quantity of Japanese honeysuckle the Honshu white admiral larvae can consume, a mean of 2.13 g or 5.6 leaves per larva. These findings support Hill (2019), who states that each larva can destroy several leaves during its development, further maintaining confidence in the biocontrol. The consumption tests aligned with larval instar development, and the consumption increase over the course of development was gradual, with peak consumption quantities occurring on Tests 4 and 5 (instars 4 and 5). The exponential model was statistically significant ($R^2 = 0.9118$), indicating confidence in the overall consumption-quantity prediction. A slight decrease in consumption was observed between Tests 4 and 5, which can largely be accounted for by five of the larvae entering pupation during Test 5. These larvae did

not consume any proportion of the honeysuckle leaf; as such the combined mean consumption amount for Test 5 incorporates the 25 remaining larvae. It is plausible that these five early-stage larvae consumed foliage at a faster rate, which accelerated development and earlier morphological change, which is not uncommon amongst Lepidoptera (Mukerji & Guppy 1970). As the larva consumption rates were not tracked individually, this assumption cannot be verified. Due to limited resources and processing constraints, moisture loss of the leaves used in this study were not deducted from the mean consumption values.

To estimate the overall defoliation success the Honshu white admiral would have on Japanese honeysuckle in the wild, other variables would need to be taken into consideration. The high availability of Japanese honeysuckle for the Honshu white admiral could increase the population densities across sites where the butterfly has successfully established, as previous studies have found a strong relationship between population density and the abundance of associated host plant, particularly species of butterfly that are dietary specialists (Curtis et al. 2015). However, the biology and ecology of the Honshu white admiral is little understood, and population densities maybe mediated by species traits to not exceed the carrying capacity of their host plants, further limiting the impact they could have on Japanese honeysuckle. There is sufficient evidence to suggest that tolerance to herbivory is common, and that plant tissues removed by herbivores might not in fact reduce plant fitness (Agrawal 2000). Schierenbeck (2004) found that under combined herbivory from mammals and insects, and a comparison of growth and biomass allocation patterns across three herbivory treatments, Japanese honeysuckle has a compensatory response to herbivory and greater biomass allocation to leaves than in nonherbivory treatments. Schierenbeck (2004) went on to conclude that this response to herbivory plays a key role in the spread and persistence of the species. This study may not be directly comparable, yet it does outline the significance of gaining a greater understanding of the relationship between the Honshu white admiral and Japanese honeysuckle.

Conclusion

While this study does demonstrate that Honshu white admiral larvae can indeed consume multiple leaves, it does not suggest that the quantity of leaves consumed would reduce the fitness of the plant, and therefore no conclusions can be drawn as to whether this level of herbivory would have any effect upon reducing Japanese honeysuckle in Aotearoa / New Zealand. Further investigation into the response of defoliation, and how this may disrupt the normal physiological processes, is needed to determine the effectiveness of the Honshu white admiral as a biocontrol agent.

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