Response of two *Chrysolina* species to different *Hypericum* hosts

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Summary *Chrysolina hyperici* and *C. quadrigemina* (Coleoptera: Chrysomelidae) were introduced to New Zealand for biological control of St John's wort (SJW), *Hypericum perforatum*, following successful biological control in Australia. In other parts of the invaded range of SJW worldwide *C. quadrigemina* is generally accepted as the more significant contributor to SJW successful biocontrol. Their ability to feed and develop on indigenous *Hypericum* species was not tested. *Chrysolina hyperici* established well while *C. quadrigemina* was initially thought to have failed to establish in New Zealand, although it is now widespread. Thus, identifying differences between *Chrysolina* species in host preference and performance would have important implications for assessing the potential risks to indigenous *Hypericum* species. We compared the performance in the lab of the two *Chrysolina* species on SJW to that on four other *Hypericum* hosts. More *C. hyperici* larvae successfully completed development on the indigenous *H. gramineum* than on *H. perforatum*, but fewer eggs were deposited on the former by *C. hyperici* females. In contrast, *C. quadrigemina* females deposited more eggs on *H. gramineum* than on *H. perforatum* and larvae of this species developed similarly on both hosts. There was no difference in the two species' response to the other three hosts.

Keywords Classical weed biocontrol, *Chrysolina hyperici*, *C. quadrigemina*, *Hypericum perforatum*, *H. gramineum*, *H. pusillum*, *H. rubicundulum*, *H. androsaemum*.

INTRODUCTION

St John's wort, *Hypericum perforatum* (L.), is a perennial herb of European, west Asian and north African origin which, by the early 1940s had become a serious weed in New Zealand (Miller 1944). Following the successful control of St John's wort in Australia, New Zealand imported biological control agents from its neighbour. The lesser St John's wort beetle, *Chrysolina hyperici* (Forst.) (Coleoptera: Chrysomelidae), which was introduced in 1943, established immediately and was distributed widely throughout the country (Hancox *et al.* 1986). The greater St John's wort beetle, *C. quadrigemina* (Suffrian), which is generally considered the more successful of the two, was introduced in 1963 but, for many years was thought to have failed to establish (reviewed by Hancox *et al.* 1986). *Chrysolina quadrigemina* was rediscovered in the late 1980s (Fraser and Emberson 1987). It is now abundant in mixed populations with *C. hyperici* (R. Groenteman personal observations).

St John's wort beetles are not strictly restricted to *H. perforatum*, and are known to be able to develop on other *Hypericum* species (some examples are reviewed by Harris 1988). New Zealand hosts 10 naturalised (Healy 1972) and four indigenous (Heenan 2008) *Hypericum* species. Little is known about the suitability and impacts of either *Chrysolina* species on these *Hypericum* species.

We examined the difference in response by the two *Chrysolina* species to various *Hypericum* hosts. Larval feeding and adult female oviposition response to St Johns wort were compared to the response to three indigenous *Hypericum* species, as well as to the exotic *H. androsaemum* (tutsan), which is a weed in New Zealand and in parts of Australia.

MATERIALS AND METHODS

Insects and plants Gravid *Chrysolina* species females were collected in autumn (March–May) 2009 from a mixed population in North Canterbury (42°51'S, 172°46'E).

Hypericum perforatum, *H. androsaemum* and *H. gramineum* plants were grown from field-collected seeds, and *H. pusillum*, *H. rubicundulum* and some *H. gramineum* were grown from cuttings.

***Chrysolina* species identification** Females were held individually and identified to species morphologically (by size, following Fraser and Emberson 1987), and confirmed with DNA bar-coding of eggs using partial sequence from COI gene with primers LCO1490 [5'-GGTCA ACAAATCATAAAGA TATTGG] and HCO2198 [5'-TAAACTTCAGGGT GACCAAAAAATCA] (Folmar *et al.* 1994). PCR conditions were: 95°C for 4 min (×1); 94°C for 45 sec, 50°C for 45 sec, 72°C for 1 min (×38); 72°C for 10 min (×1); 10°C). Eggs deposited by the individual females were collected and held separately so newly hatched larvae could be identified.

Larval development Twenty-five newly hatched larvae were placed five per dish in five Petri dishes (90 mm^2), lined with moist filter paper and containing a small branch (a leaf in the case of the much larger *H. androsaemum*) of one of the five host species, and left at 12:12 h light:dark and 20:10°C. The experiment was replicated five times on different dates for each *Chrysolina* species to a total of 250 larvae (25 larvae per host species × five host species × two *Chrysolina* species). Survival was recorded initially once every 24 h and later at intervals of up to 72 h. Food was replenished and filter paper moistened and replaced as necessary. When surviving larvae reached full size (indicated by change of colour and feeding cessation) they were considered to have successfully completed development. Effects of host species, *Chrysolina* spp. and their interaction on duration of larval development were tested in a mixed effects model in R (R Development Core Team 2008) with a Poisson distribution. The chance of a larva to complete development was tested in a mixed effects model with the same explanatory variables but with a binomial distribution (development either completed or not).

Female oviposition choice Gravid field-collected females were introduced individually into one of five ventilated Perspex cages (50:75:50 cm width:length:height), each containing five potted plants – one of each host species. Orientation of the plants within each cage was randomised. The females were left for 24 h under 10:14 h light:dark and 20:10°C, after which the host they were found on was recorded, eggs were counted, and each female was introduced into a new cage. This process was repeated at least once more for each female to a total of 15 females per *Chrysolina* species and 117 'female nights'.

The effects of host species, *Chrysolina* sp. and their interaction on the proportion of instances in which each host was selected for oviposition were tested in a generalised linear model with a binomial distribution (the proportion of instances each host was selected for oviposition). In addition, the effects of host species, *Chrysolina* sp. and their interaction on mean number of eggs per plant per 'female night' was analysed in a mixed effects model with a Poisson distribution; female and cage were the random grouping factors.

RESULTS

***Chrysolina* species identification** DNA bar-coding confirmed that morphological identification was correct in all cases but one.

Larval development Larvae of both *Chrysolina* species were able to complete development on *H. perforatum*, *H. gramineum* and *H. pusillum* (Figure 1). None survived on *H. rubicundulum* and *H. androsaemum*. There was no difference between *Chrysolina* species in the time it took to complete development on the different hosts. It took both species significantly longer to develop on *H. pusillum* (Figure 1; $z_{1,62}$ = 4.26, P <0.001).

There was a significant interaction between *Chrysolina* species and host species in the probability of individuals completing development (Figure 1b):

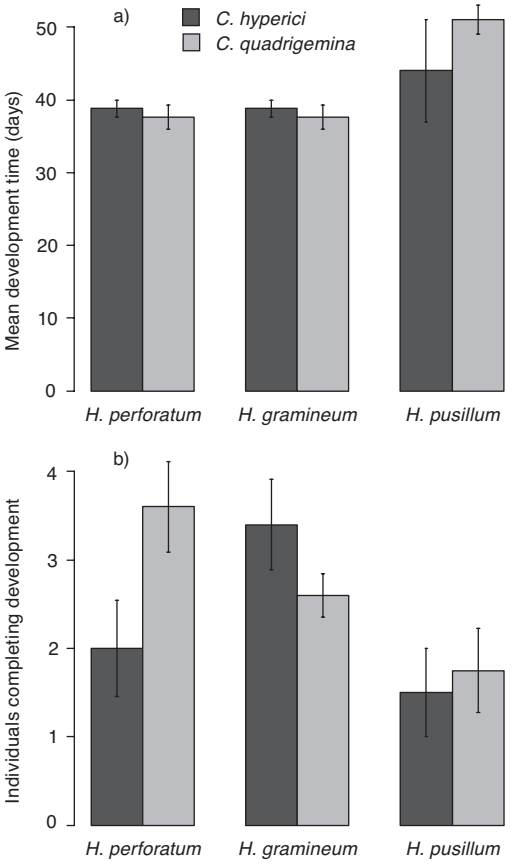

Figure 1. (a) Duration (days ±SEM) of larval development to completion of life cycle and (b) the mean number (±SEM) of *C. hyperici* (dark bars) and *C. quadrigemina* (light bars) larvae per replicate that successfully completed development on three *Hypericum* hosts. No successful development occurred on *H. androsaemum* and *H. rubicundulum.*

C. quadrigemina larvae had a higher chance than *C. hyperici* larvae to complete development on *H. perforatum* ($z_{1,238} = 2.23$, P = 0.026), but *C. hyperici* larvae had a higher chance than *C. quadrigemina* larvae to complete development on *H. gramineum* ($z_{1,238} = -2.40$, P = 0.016). There was no difference between the two *Chrysolina* species in likelihood of completing development on *H. pusillum* ($z_{1,238} = -0.31$, P = 0.76). *Chrysolina hyperici* individuals had a higher chance of developing successfully on *H. gramineum* than on *H. perforatum* ($z_{1,238} = -1.96$, P = 0.050) or on *H. pusillum* ($z_{1,238} = -2.15$, P = 0.032). *Chrysolina quadrigemina* larvae had a similar chance of completing development on *H. perforatum* and on *H. gramineum* but a lower chance to complete development on *H. pusillum* ($z_{1,238} = -2.99$, P = 0.003).

Female oviposition choice There was no significant interaction between *Chrysolina* species and host species and there was no significant difference between *Chrysolina* species in the proportion of instances each host was selected (Table 1; Figure 2). Oviposition was avoided altogether in 24% of instances by *C. hyperici* and in 33% of instances by *C. quadrigemina*.

A comparison of egg numbers deposited per plant shows *C. quadrigemina* females deposited significantly more than *C. hyperici* on *H. gramineum* ($z_{1,102} = 2.94$, P = 0.003) and on *H. pusillum* ($z_{1,102} = 1.95$, P = 0.051; Figure 3). *Chrysolina quadrigemina* deposited the least eggs per plant on *H. androsaemum* ($z_{1,102} = -3.75$, P <0.001). On the other three hosts, *C. quadrigemina* deposited more eggs per plant compared to *H. perforatum* (*H. rubicundulum*: $z_{1,102} = 3.62$, P <0.001; *H. pusillum*: $z_{1,102} = 2.28$, P = 0.02; *H. gramineum*: $z_{1,102} = 1.81$, P = 0.07), whereas *C. hyperici* deposited similarly on *H. perforatum*, *H. pusillum* and *H. rubicundulum* but fewer eggs per *H. gramineum* ($z_{1,102} = -2.874$, P = 0.004) and *H. androsaemum* ($z_{1,102} = -4.80$, P <0.001) plants.

DISCUSSION

The two *Chrysolina* species differed slightly in their response to the main host, *H. perforatum*, and to *H. gramineum*. *Hypericum*

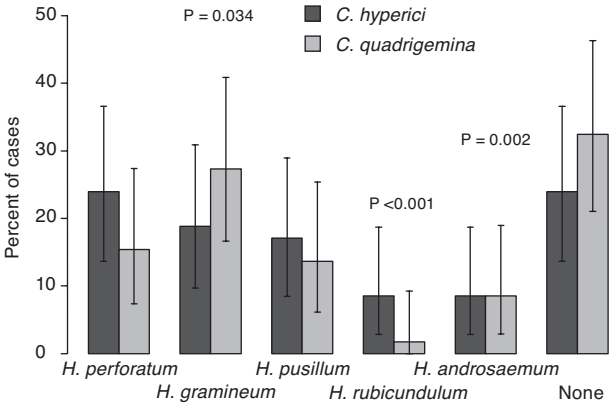

Figure 2. Percentage (±95% CI) of instances each host species was selected for oviposition in choice arenas by *C. hyperici* (dark bars) and *C. quadrigemina* (light bars). P values represent percentages of preference significantly different than would be expected at random. The category 'none' represents instances where no host was selected.

Table 1. ANOVA table for the generalised linear model testing the proportion of instances each host was selected for oviposition.

	df	Deviance	Residual deviance	P(>|Chi|)
NULL			38.33	
Chrysolina sp.	1	0.01	6.36	0.922
Host	5	31.96	6.37	<0.001
Interaction	5	6.36	0.00	0.272

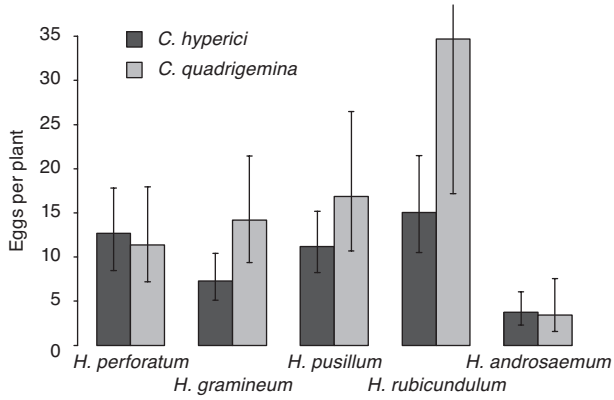

Figure 3. Mean (back transformed ±95% CI from generalised linear mixed effect models) eggs deposited per plant by individual *C. hyperici* (dark bars) and *C. quadrigemina* (light bars) females given choice between five hosts.

gramineum emerged as a more suitable host than *H. perforatum* for *C. hyperici*, with more larvae completing development on the former, although females deposited fewer eggs per plant on *H. gramineum* than on *H. perforatum*. *Chrysolina quadrigemina* larvae did similarly well on *H. perforatum* and *H. gramineum*, although females deposited more eggs on the latter.

Larvae of both *Chrysolina* species developed well on *H. pusillum*, and for larvae that failed to complete development, persistence on this host was no shorter than persistence on *H. perforatum* and *H. gramineum* (data not shown). Substantial plant material was consumed during this time.

Both *H. androsaemum* and *H. rubicundulum* were similarly unsuitable for larval development of both *Chrysolina* species. Larvae of neither *Chrysolina* species could complete development on these hosts, and only persisted on them for a very short time, never developing past the second instar. It is interesting to note that in the late 1940s several explicit attempts were made to release *C. hyperici* as a biocontrol agent against *H. androsaemum* in New Zealand, but the beetles failed to establish on this host (Miller 1970). Poor larval development on *H. androsaemum* documented in our study confirms the unsuitability of *H. androsaemum* as a host for both *Chrysolina* species and explain these past failures. Little preference for oviposition (Figure 2) and negligible egg deposition (Figure 3) provide further explanation.

Although in the laboratory *C. hyperici* larvae seemed to benefit more from feeding on a host other than *H. perforatum* than did *C. quadrigemina* larvae, records from North America and Australia indicate that in the field, it is *C. quadrigemina* that can be found feeding on other *Hypericum* species, both exotic and indigenous (Harris 1988, Willis *et al.* 2003). From our study, we may predict that *C. hyperici* will feed on the indigenous *H. gramineum* disproportionately frequently, that *H. pusillum* will be fed on less frequently by both *Chrysolina* species, and that *H. rubicundulum* will largely be avoided.

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