Evaluation of the host range of *Lathronympha strigana* (L.) (Tortricidae), and *Chrysolina abchasica* (Weise) (Chrysomelidae), potential biological control agents for tutsan, *Hypericum androsaemum L.*

Summary

- *Lathronympha strigana* only laid eggs on *Hypericum* spp.
- *H. androsaemum* was strongly preferred over other *Hypericum* species, and only a few eggs were laid on native New Zealand *Hypericum spp.*
- *Lathronympha strigana* larvae survived only on *H. androsaemum* and *H. perforatum*. There is therefore no significant risk that damaging populations could develop on other *Hypericum* species.
- There is no significant risk of non-target attack on species outside the family Hypericaceae or the genus *Hypericum*.
- There is a slight risk of low level non-target attack on the *H. perforatum*, and on exotic Hypericum species that were not tested.
- *Lathronympha strigana* is not expected to have significant impact on populations of native or valued *Hypericum* species if released in New Zealand.

- *Chrysolina abchasica* did not lay eggs on *Hypericum involutum, Melicytus ramiflorus, Viola lyallii, Passiflora tetrandra, Linum monogynum* and *Euphorbia glauca* and no larval survival occurred on these species during the first no-choice starvation test. These species are clearly not hosts.
- *H. calycinum; H. perforatum*, and the native species *H. pusillum* and *H. rubicundulum* supported completed development in the laboratory and can be considered fundamental hosts.

- Eggs were laid on *H. pusillum* and *H. rubicundulum* in the choice test, but significantly fewer eggs than on the *H. androsaemum* controls
- Significantly fewer larvae survived to adult on these species. Many died quickly on completing development while those reared on tutsan survived.
- Combined Risk Scores for these species ranged from 0.01 to 0.06 for *H. pusillum* and *H. rubicundulum* - much lower than the predicted threshold for non-target attack in field conditions.

- Combined risk scores were much lower than for the two related control agents already released against St John's wort (0.307 - 1.135) which have trivial impacts on *H. pusillum* and *H. involutum* in New Zealand.
- The combined risk scores for *H. perforatum* (0.17 – 0.21) were higher, but still below the threshold for host-use in the field.
- *Chrysolina abchasica* is not expected to have a significant impact on populations of native or any valued *Hypericum* species identified in New Zealand.

Test plant selection

Hypericum androsaemum L. belongs to the family *Hypericaceae* Jussieu. *Hypericum* is the only genus of this family present in New Zealand (http://www.nzflora.info/factsheet/Taxon/Hypericaceae.html). There are 19 *Hypericum* species in New Zealand. Four of these are native. Of the 15 exotic species, 12 have naturalised in New Zealand, including another serious weed, *H. perforatum* (St John's wort). Some species are common garden plants.

The family Hypericaceae belongs to the Order *Malpighiales* Martius (Fig. 1). There are 9 other families in this order present in New Zealand. Three of these have no native species and were not considered for testing (Ochnaceae, Putranijvaceae, Salicaceae). Five families are represented by only one or two native species (Elatinaceae, Euphorbiaceae, Linaceae, Passifloraceae, Phyllanthaceae). The family Elatinaceae has one native species, but this is an aquatic plant. The family Violaceae has 14 native species, 11 of which are endemic.

No families present in New Zealand are closely related to the Hypericaceae. The closest is the family Elatinaceae which has only one species in New Zealand; an aquatic plant. The next closest is the Violaceae, but this is found in a distant clade from Hypericaceae. Other families are even less closely related to the Hypericaceae (Fig. 1).

Three of the four native species of the genus *Hypericum* were selected for testing. Plants of *H. minutiflorum* were unobtainable. This species could be represented in tests by *H. rubicundulum* which is similar in form and habit, and is considered to be a sister species phylogenetically (Heenan 2008). Two naturalised *Hypericum* species were selected to represent the exotic species, some of which are valued as ornamentals.

The species selected for testing are listed in Table 1. One or two representative species were selected from those families of the order represented in the native flora. The exceptions were the Elatinaceae, which only has one aquatic species in New Zealand (and is therefore not at risk from the proposed control agents) and the Phyllanthaceae. Specimens of the two *Poranthera* species native to this family were unobtainable. Other test plants provided representation of families outside the Hypericaceae so that testing these remotely related native species was not considered necessary.

The legume *Lotus corniculatus* (Fabaceae) has been recorded as a host of *L. strigana* in Europe. This is likely to be a misidentification, but this species was included in one test.

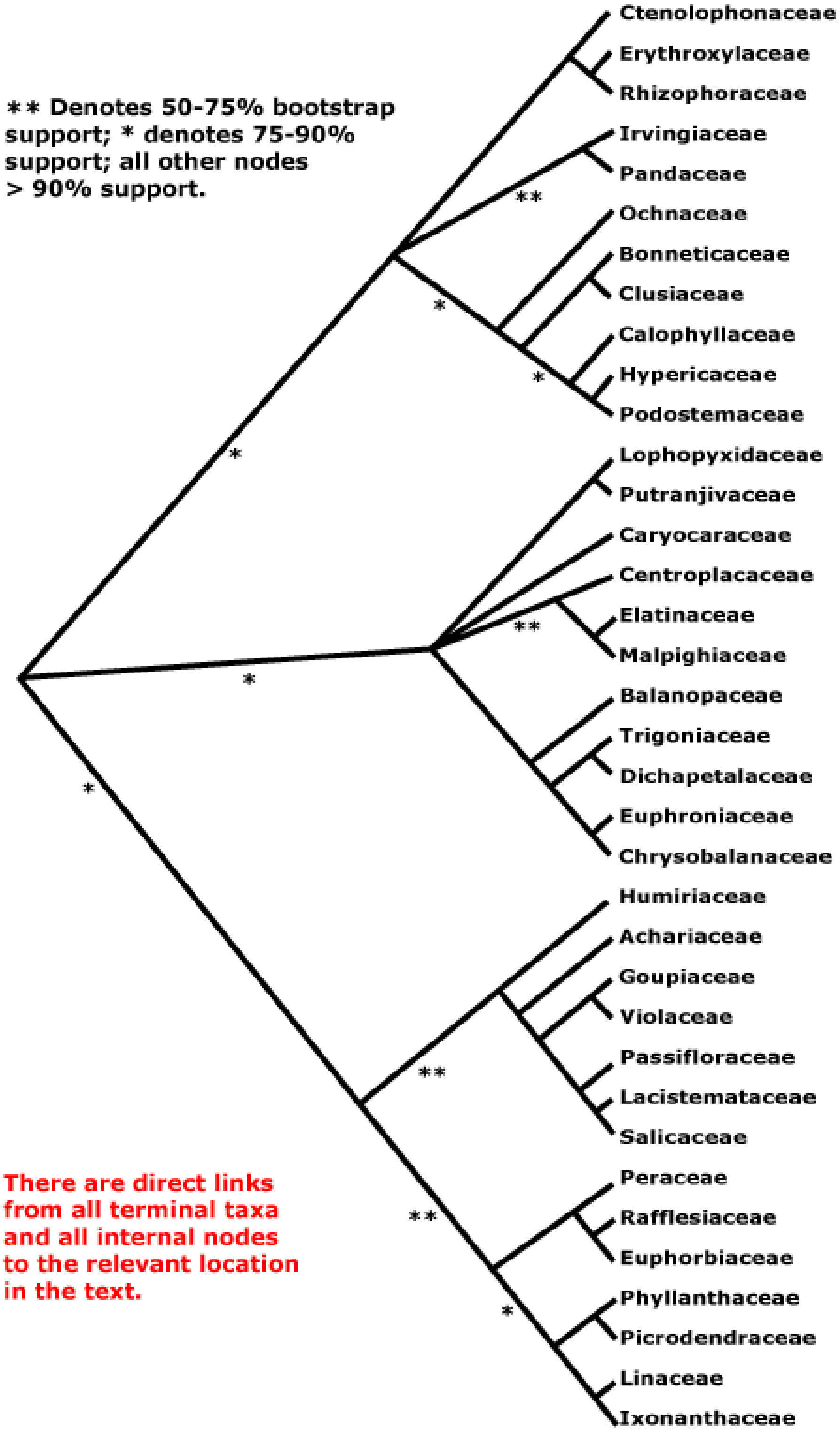

Figure 1. Phylogenetic relationships between families of the order Malpighiales (From Angiosperm Phylogeny Website of the Missouri Botanic Gardens, http://www.mobot.org/MOBOT/research/APweb/).

Family, in descending relatedness	**Species recorded in NZ**	**Native species**	**Test species selected**	**Notes**
Hypericaceae	19	4	*Hypericum androsaemum*	Target weed
			H. involutum	Indigenous
			H. rubicundulum	Indigenous, endemic
			H. pusillum	Indigenous, endemic
			H. calycinum	Naturalised
			H. perforatum	Weed, St John's wort
Violaceae	23	14	*Melicytus ramiflorus*	Indigenous, endemic
			Viola lyallii	Indigenous, endemic
Passifloraceae	10	1	*Passiflora tetrandra*	Indigenous, endemic
Euphorbiaceae	34	2	*Euphorbia glauca*	Indigenous, endemic
Phyllanthaceae	3	2	nil	Not tested
Linaceae	6	1	*Linum monogynum*	Indigenous

Table 1. Plants of the order Malpighiales selected for testing against control agents for tutsan. Data from eFlora of New Zealand (http://www.nzflora.info/factsheet/Taxon/Malpighiales.html)

Testing the host range of *Lathronympha strigana*

Choice oviposition tests

Three male and three female adult *Lathronympha strigana* were released into a Perspex box with potted plants: *Hypericum androsaemum, H. calycinum, H. perforatum, H. pusillum, H. rubicundulum, H. involutum, Melicytus ramiflorus, Viola lyallii, Passiflora tetrandra, Linum monogynum, Euphorbia glauca, Lotus corniculatus* and left for 4 days after which the number of eggs laid on each plant was counted. Five replicates were performed.

The tests were repeated, but in the absence of tutsan to confirm the pattern of oviposition between potential hosts.

An average of 97.4 eggs were laid on each *H. androsaemum* plant in choice tests where tutsan was present. A small numbers of eggs were laid on the exotic *H. calycinum and H. perforatum.* There was no oviposition on *Hypericum rubicundulum* but one or two eggs were laid on the native *H. involutum* and *H. pusillum* (Fig. 2). No eggs were laid on *Melicytus ramiflorus, Viola lyallii, Passiflora tetrandra, Linum monogynum* or *Euphorbia glauca.*

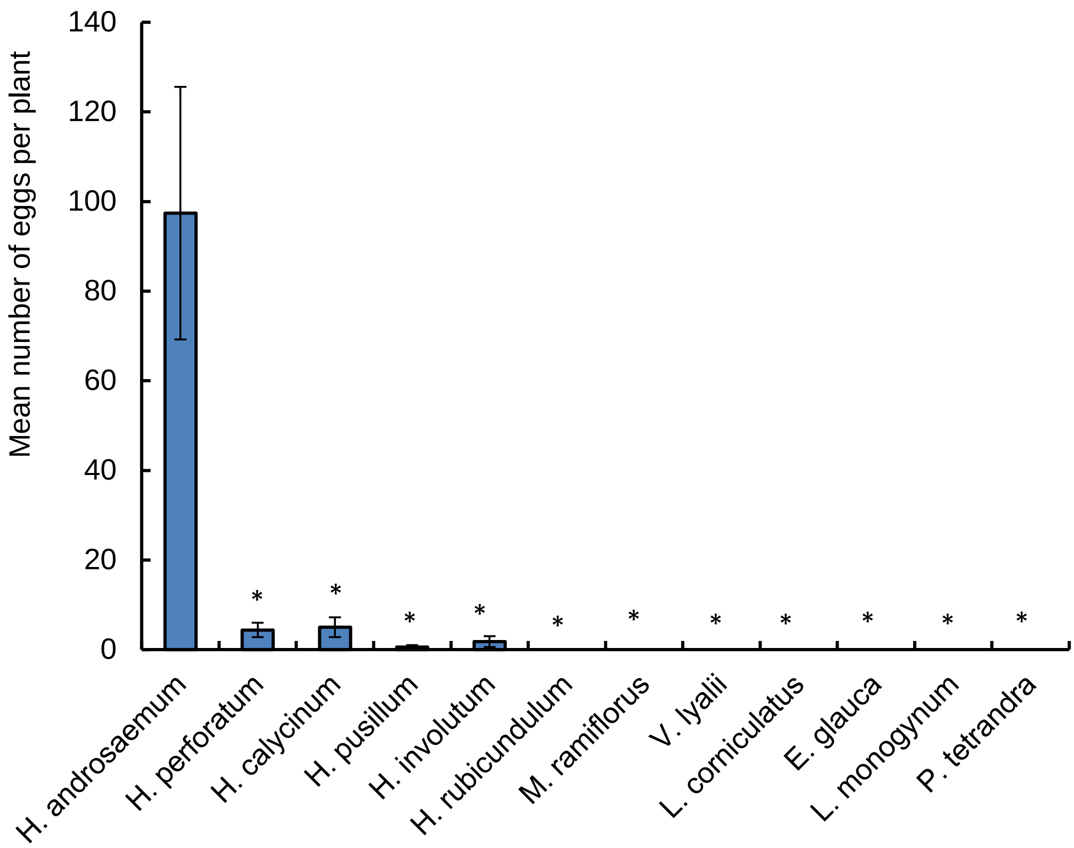

Fig. 2. Number of eggs laid by *Lathronympha strigana* adults on Tutsan *Hypericum androsaemum,* five other *Hypericum* spp., and six other test plant species (an asterisk indicates a significant difference in survival between a test plant and *H. androsaemum;* Pairwise comparisons using Wilcoxon rank sum test; $P < 0.05$).

Analyses were performed using the R statistical package (R Core Team and contributors worldwide). Preliminary investigation of the data indicated that the data were not normal and lacked homogeneity of variances between treatments. Non-parametric statistics were therefore used to analyse the data.

To test whether *Lathronympha strigana* demonstrated a preference between plant species that for oviposition, a Friedman rank sum test rank sum test was performed where the number of eggs laid was the response variable and plant species and replicate (= block) were grouping factors.

Treatment was highly significant (Friedman chi-squared chi-squared = 41.1585, df = 11, p-value = 2.263e-05) indicating that oviposition preference varied significantly between plant species.

Pairwise comparisons were performed using Wilcoxon rank sum test, which indicated that the number of eggs laid on *H. androsaemum* was significantly greater than on *H. calycinum, H. perforatum, H. involutum* and *H. pusillum* (Fig. 2).

A similar pattern of oviposition was observed when test plants were exposed to moths in the absence of tutsan (Table 2), but in this case, a mean of 2.2 eggs per replicate were laid on *H. rubicundulum.*

	Mean no. eggs/replicate	
Plant	**Choice**	**Choice minus host**
H. androsaemum	97.4	-
H. perforatum	4.4	1.2
H. calycinum	5	4.6
H. pusillum	0.6	0.4
H. involutum	1.8	3.2
H. rubicundulum	0	2.2
M. ramiflorus	0	0
V. lyallii	0	0
P. tetrandra	0	0
E. glauca	0	0
L. monogynum	0	0
L. corniculatus	0	0

Table 2. Comparison of egg deposition by *L. strigana* on test plants in the presence and the absence of tutsan.

Larval starvation tests

Three tests investigated whether *L. strigana* larvae could survive and complete development on non-target host plants.

Survival on transfer to whole plants - Ten newly hatched *Lathronympha strigana* larvae were transferred to each test and control plant. There were five replicates of each plant species. *Hypericum androsaemum, H. calycinum, H. perforatum, H. pusillum, H. rubicundulum, H. involutum, Melicytus ramiflorus, Viola lyallii, Passiflora tetrandra, Linum monogynum, Euphorbia glauca*) were tested. Larval survival was recorded after 25 days.

After 25 days, 58% of larvae placed on *H. androsaemum* were alive, as were 36% of those placed on *H. perforatum, and* 10% of those placed on *H. calycinum.* All larvae placed on other plants were dead.

At 30 days the larvae were recovered from deteriorating plants and fed in Petri dishes until completion of development or death. No larvae completed development on *H. calycinum.* Ten percent of larvae completed development on *H. perforatum* but 26% of larvae completed development on tutsan controls.

Development of naturally deposited eggs – The development of eggs deposited on plants of five *Hypericum* species during oviposition tests were monitored. After 30 days the larvae were removed from deteriorating plants and fed on various plant structures in Petri dishes until completion of development or death.

Larvae were only ever found in the flowers of *H. calycinum* and none completed development (Table 3). Larvae colonised leaves and stems of *H. perforatum* and *H. androsaemum* and completed development on both hosts

Species	Number of plants	Total eggs monitored	Larvae present at 30 days	Larvae completing development
H. androsaemum	5	140	15%	yes
H. perforatum	5	36	17%	yes
H calycinum	5	43	14%	no
H. pusillum	3	5	0%	-
H. involutum	4	25	0%	-

Table 3. Development success of larvae hatching from *L. strigana* eggs laid on five *Hypericum* species.

Host selection of wandering larvae - Later stage larvae of *L. strigana* are mobile and could theoretically wander from defoliated tutsan plants in search of a new host plant. This test was performed to investigate the potential for wandering larvae to attack three non-target native *Hypericum* species. Five third-instar larvae (that had been reared on tutsan) were placed on whole plants of *Hypericum androsaemum, H. pusillum, H. rubicundulum, H. involutum*). This was repeated in five replicates.

No larvae completed development on the native *Hypericum* species. Eighteen of the 25 large larvae placed on *Hypericum androsaemum* control plants were alive at day 30 (72%) and all went on to complete development. Twelve percent of larvae placed on *H. pusillum* survived at 30 days but were dead by day 35.

Discussion
The oviposition tests indicated that *Lathronympha strigana* only laid eggs on *Hypericum* spp. Moreover, there was a very strong preference for oviposition on *H. androsaemum* over other *Hypericum* species, and only a few eggs were laid on native NZ *Hypericum spp.* (*H. pusillum, H. involutum,* but not *H. rubicundulum*). Furthermore, larval survival was confined to *H. androsaemum* and *H. perforatum*. There is no significant risk that damaging populations could develop on native *Hypericum* species. There is a slight risk of non-target attack on *H. perforatum* (but this species is considered a weed in NZ). There is no significant risk of non-target attack on species outside the family Hypericaceae or the genus *Hypericum*.

Testing the host range of *Chrysolina abchasica*

Choice oviposition test with target present
Ten adult *Chrysolina abchasica* were released into a Perspex box with potted plants. Five replicates were performed where one each of the following plant species were included in the Perspex box: *Hypericum androsaemum, H. calycinum, H. perforatum, H. pusillum, H. rubicundulum, H. involutum, Melicytus ramiflorus, Viola lyallii, Passiflora tetrandra, Linum monogynum, Euphorbia glauca* and left for 5 days after which the number of eggs laid on each plant was counted. Five further replicates were performed where the Perspex box included only *Hypericum androsaemum, H. calycinum, H. perforatum, H. pusillum, H.*

rubicundulum, H. involutum (*i.e.* omitting *Melicytus ramiflorus, Viola lyallii, Passiflora tetrandra, Linum monogynum, Euphorbia glauca*).

No oviposition occurred on *Hypericum involutum, Melicytus ramiflorus, Viola lyallii, Passiflora tetrandra, Linum monogynum* and *Euphorbia glauca.*

Analyses were performed using the R statistical package (R Core Team and contributors worldwide). Preliminary investigation of the data indicated that the data were not normal and lacked homogeneity of variances between treatments. Non-parametric statistics were therefore used to analyse the data.

To test whether *Chrysolina abchasica* preferred any plant species, a Friedman rank sum test was performed where the number of eggs laid was the response variable and plant species and replicate (=block) were grouping factors. Plant species on which no eggs were laid were omitted from the analysis (Fig. 3).

Treatment was highly significant (Friedman chi-squared chi-squared = 15.9358, df = 4, p-value = 0.003106) indicating that oviposition preference varied significantly between plant species. Pairwise comparisons were performed using Wilcoxon rank sum test. Compared to *H. androsaemum*, oviposition was significantly lower on *H. calycinum, H. pusillum* and *H. rubicundulum*. Oviposition was also lower on *H. perforatum*, but this difference was not statistically significant.

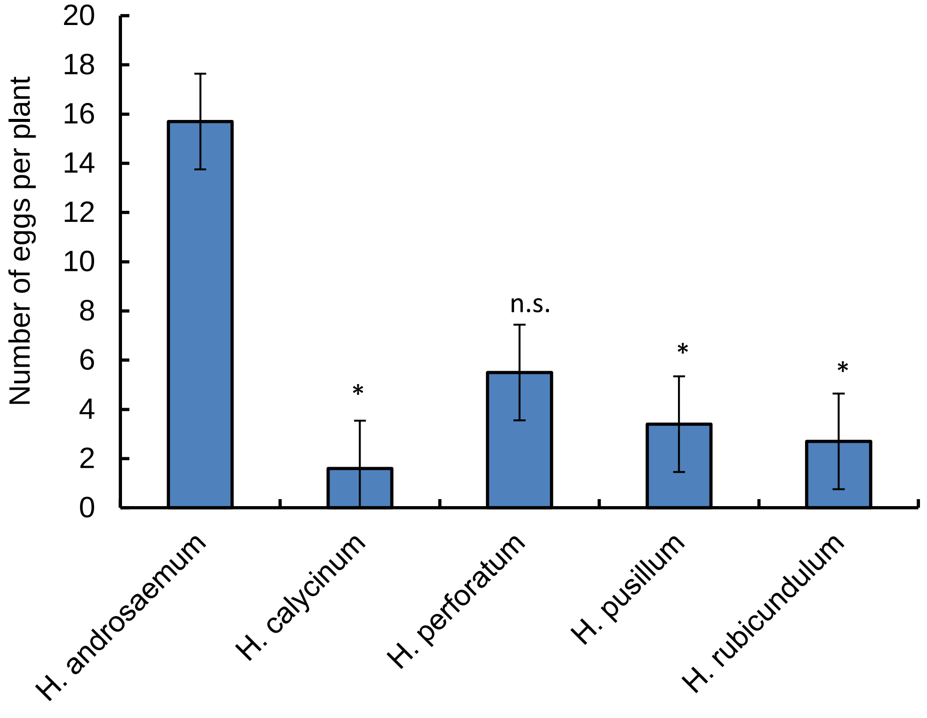

Fig. 3. Number of eggs laid by *Chrysolina abchasica* adults on Tutsan *Hypericum androsaemum* and four other *Hypericum* spp. (an asterisk indicates a significant difference in survival between a test plant and *H. androsaemum;* Pairwise comparisons using Wilcoxon rank sum test; $P < 0.05$).

No-choice larval starvation test 1

Chrysolina abchasica larvae were transferred to test and control plants (five larvae per plant). Larvae were not neonates and had fed on *H. androsaemum* for up to a week when used in the tests. Five replicates of each plant species (*Hypericum androsaemum, H. calycinum, H. perforatum, H. pusillum, H. rubicundulum, H. involutum, Melicytus ramiflorus, Viola lyallii, Passiflora tetrandra, Linum monogynum, Euphorbia glauca*) were performed.

Survival was high on the host plant *Hypericum androsaemum*, moderate on *H. perforatum* and low on *H. calycinum*, *H. pusillum* and *H. rubicundulum* (Fig. 4). The following plants did not support the survival of larvae to adult: *Hypericum involutum, Melicytus ramiflorus, Viola lyallii, Passiflora tetrandra, Linum monogynum* and *Euphorbia glauca.*

Analyses were done using the Genstat statistical package (VSN International Ltd). A one-way ANOVA was performed to test the effect of treatment (plant species) on the proportions of larvae (i.e. out of five) surviving on each plant. Proportion data were arcsine square root transformed, prior to analysis. Plant species which supported no larval survival were considered not to be hosts and excluded from the analysis.

The proportion of larvae that survived to adulthood varied significantly according to treatment ($F_{4,24} = 14.55$, $P < 0.001$). Survival on *H. calycinum, H. perforatum, H. pusillum* and *H. rubicundulum* plants was significantly lower, compared to *H. androsaemum* controls.

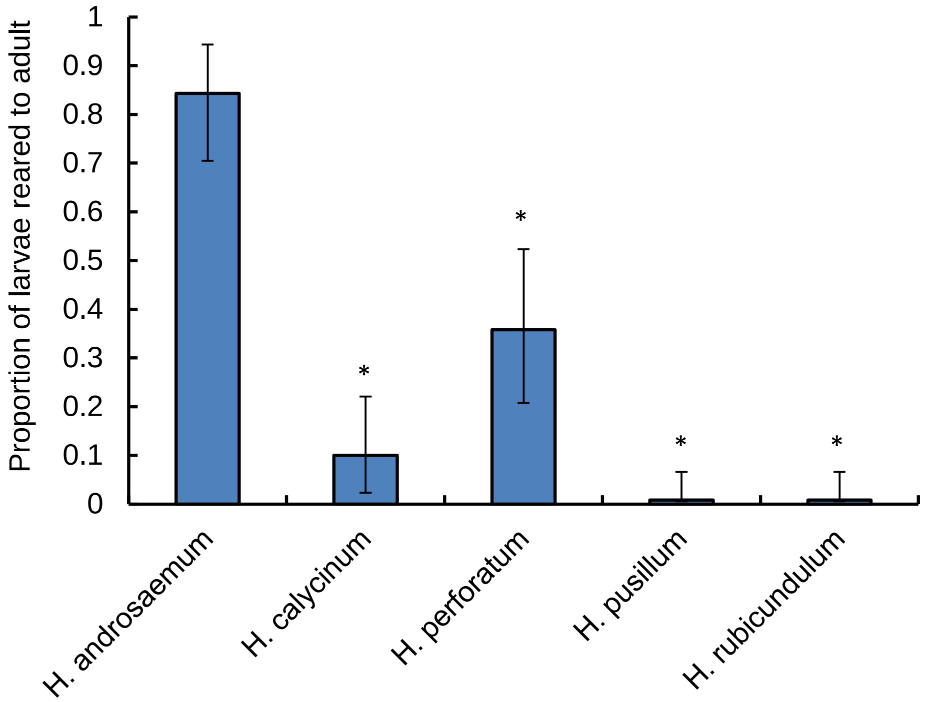

Fig. 4. Proportion of *Chrysolina abchasica* larvae that survived to adult on Tutsan *Hypericum androsaemum* and four other species of *Hypericum* (test 1; back transformed data; an asterisk indicates a significant difference in survival between a test plant and *H. androsaemum; LSD P* < 0.05).

C. *abchasica* no-choice larval starvation test 2

Because the first starvation test did not use neonate larvae, a second test was performed that monitored the eggs laid on *H. androsaemum* and test plants during the oviposition test. Larvae were allowed to emerge naturally and feed. The number of larvae per plant varied according to the number of eggs laid, so the proportion of larvae that survived to adulthood was used as the response variable (Fig.5).

Analyses were done using Genstat. A one-way ANOVA was performed to test the effect of treatment (plant species) on the proportions of larvae surviving on each plant. Proportion data were arcsine square root transformed, prior to analysis.

As in the first starvation test, the proportion of larvae that survived to adulthood varied significantly according to treatment ($F_{4,23} = 6.26$, $P < 0.001$).

Survival on the key test plants *H. pusillum* and *H. rubicundulum* was higher than in the first no-choice test, but was still low compared to survival on *H. androsaemum*. Furthermore, it was noted that many of the adults which reared through on these plants died soon after emergence. When we counted only adults that survived long enough to enter diapause, the difference between treatments was more marked ($F_{4,23} = 28.81$, $P < 0.001$; Fig. 6).

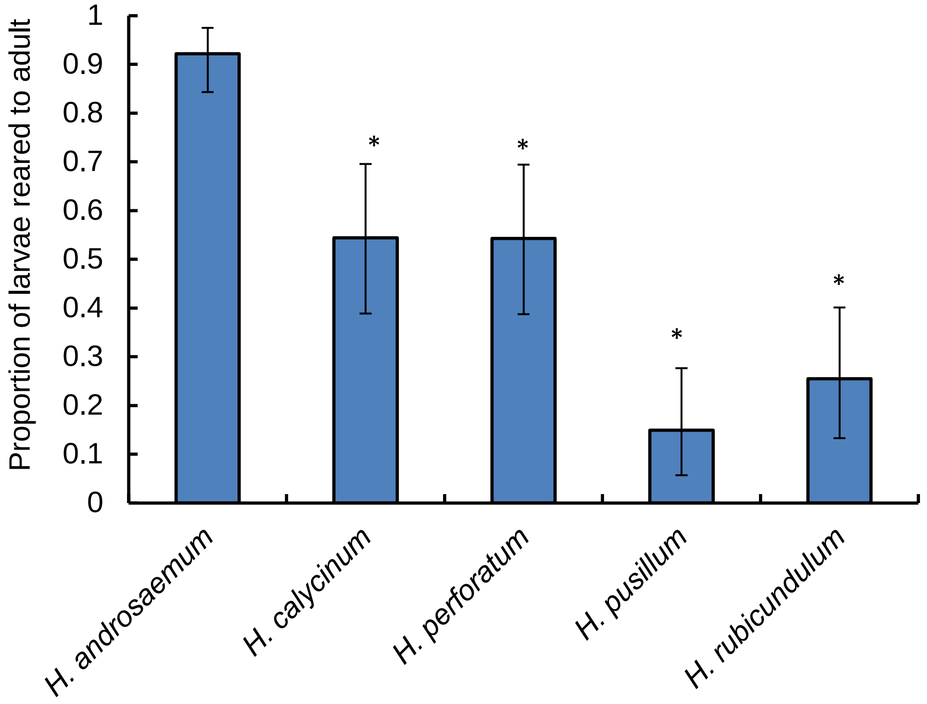

Fig. 5. Proportion of *Chrysolina abchasica* larvae that survived to adult on Tutsan *Hypericum androsaemum* and four other species of *Hypericum* in the second no-choice test (back transformed data; an asterisk indicates a significant difference in survival between a test plant and *H. androsaemum; LSD P* < 0.05).

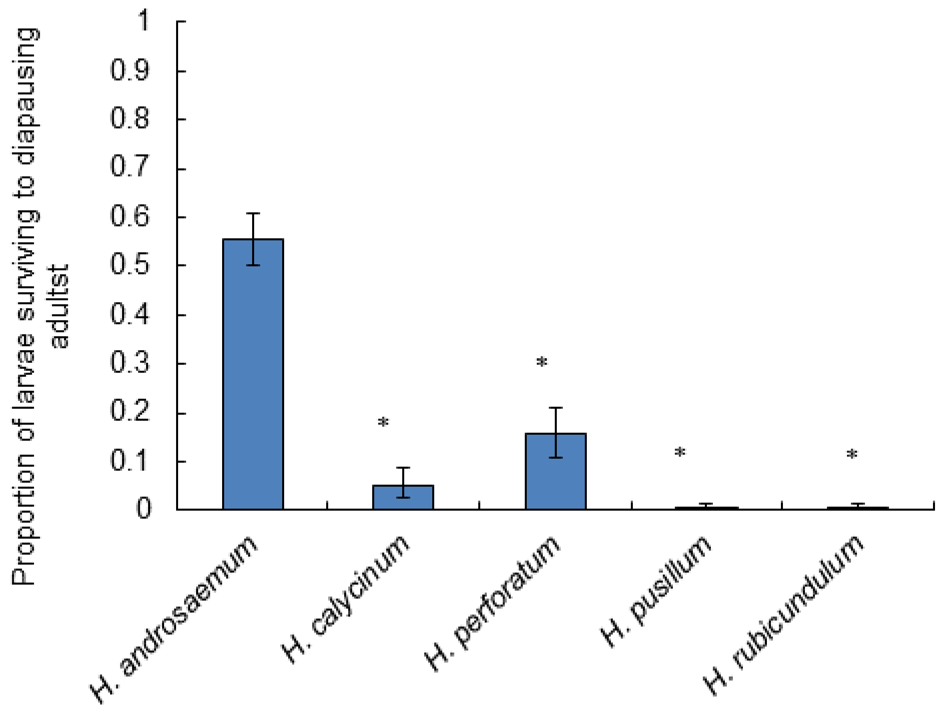

Fig. 6. Proportion of *Chrysolina abchasica* larvae that survived to diapausing adults on Tutsan *Hypericum androsaemum* and four other species of *Hypericum* in the second no-choice test (back transformed data; an asterisk indicates a significant difference in survival between a test plant and *H. androsaemum; LSD P* < 0.05).

Predicted risk

Risk scores were calculated according to Paynter *et al.* (2015), using raw data (rather than the back-transformed data presented in the figures) and are presented in Table 4.

Table 4. relative risk scores calculated according to Paynter *et al.* (2015). R_o = Relative oviposition score; R_{s1} = Relative survival (first test); R_{s2}= Relative survival (second test); CRS_1 Combined risk score = R_o x $R_{s1;}$ CRS_2 Combined risk score = R_o x R_{s2}

Plant species	Oviposition	R_o	Proportion surviving (raw data test 1)	R_{s1}	Proportion surviving (raw data test 2)	R_{s2}	CRS_1	CRS_2
H. androsaemum	15.7	1.00	0.76	1.00	0.89	1.00	1.00	1.00
H. perforatum	5.5	0.35	0.36	0.47	0.54	0.61	0.17	0.21
H. calycinum	1.6	0.10	0.16	0.21	0.56	0.63	0.02	0.06
H. pusillum	3.4	0.22	0.04	0.05	0.23	0.26	0.01	0.06
H. rubicundulum	2.7	0.17	0.04	0.05	0.31	0.34	0.01	0.06

Depending on which data were used for larval survival (i.e. starvation test 1 or 2), the combined risk scores varied between 0.17-0.21 for *H. perforatum*, and between 0.01-0.06 for the two native species that supported *C. abchasica* development (*H. pusillum* and *H. rubicundulum*).

Conclusions

No oviposition occurred on *Hypericum involutum, Melicytus ramiflorus, Viola lyallii, Passiflora tetrandra, Linum monogynum* and *Euphorbia glauca* and no larval survival occurred on these species during the first no-choice starvation test. These species are clearly not hosts.

The remaining *Hypericum* species (namely *H. calycinum; H. perforatum, H. pusillum* and *H. rubicundulum*) were fundamental hosts. Both native species (*H. pusillum* and *H. rubicundulum*) were oviposited upon in the choice test, but significantly fewer eggs were laid on these species, compared to the *H. androsaemum* controls, moreover larval survival to adult on these species was significantly lower than on *H. androsaemum* controls: only 4% of larvae survived to adult in the first test. Survival to adult was higher in the second test, although there is a possibility that this result is due to eggs being under recorded in the oviposition test. Moreover, it was noted that most adults that reared through on *H. pusillum* and *H. rubicundulum* died soon after emergence, unlike adults that were reared on *H. androsaemum* controls.

Combined Risk Scores for these species calculated using larval survival from the first or second test (CRS_1 and CRS_2, respectively) ranged from 0.01-0.06 for *H. pusillum* and *H. rubicundulum* - much lower than the predicted threshold for non-target attack in field conditions. For example, the threshold scores for the combined risk score for choice oviposition/no-choice starvation tests were 0.307 and 0.56 for all non-target attack, and for full utilization alone, respectively (Paynter *et al.* 2015).

It is noteworthy that the equivalent combined risk scores for host-range tests performed on *Chrysolina hyperici* and *C. quadrigemina*, which have trivial impacts on *H. pusillum* and *H. involutum* in NZ, were much higher than for *C. abchasica* (Table 5).

Table 5. Combined risk scores and field use of non-target plants by *Chrysolina hyperici* and *Chrysolina quadrigemina* (from Paynter *et al.* 2015), compared to combined risk scores for *Chrysolina abchasica*. Field use of *Hypericum involutum* and *H. pusillum* by *Chrysolina hyperici* and *Chrysolina quadrigemina* in NZ is low to absent (Groenteman, Fowler & Sullivan 2011).

Test plant	Beetle species	Combined risk score
Hypericum involutum	*Chrysolina quadrigemina*	1.135
Hypericum involutum	*Chrysolina hyperici*	0.631
Hypericum involutum	*Chrysolina abchasica*	0
Hypericum pusillum	*Chrysolina quadrigemina*	0.537
Hypericum pusillum	*Chrysolina hyperici*	0.307
Hypericum pusillum	*Chrysolina abchasica*	0.01-0.06

The ecology of *Chrysolina hyperici* and *C. quadrigemina* is very similar to that of *C. abchasica*. We conclude that the risk of *C. abchasica* causing serious non-target attack to native NZ *Hypericum* spp. is very low.

The combined risk scores for *H. perforatum* (0.17 – 0.21 using the larval survival data from the first test and second test, respectively) were higher, but still below the threshold for host-use in the field. Moreover, this species is an introduced noxious weed so any no-target feeding is not considered to be a problem.

References

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Heenan, PB (2008). Three newly recognised species of *Hypericum* (Clusiaceae) in New Zealand New Zealand Journal of Botany 46: 547-558.

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