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Temperature effects on the alligator weed flea-beetle, *Agasicles hygrophila* (Coleoptera: Chrysomelidae): implications for biological control in New Zealand

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Abstract. *Agasicles hygrophila*, a beetle of South American origin, was introduced into New Zealand in 1982 for the biological control of alligator weed, *Alternanthera philoxeroides*. Substantial damage to alligator weed has not been widely observed in New Zealand. Investigations into possible reasons for this indicated that low field-temperatures may be partially responsible. Laboratory experiments have shown that *A. hygrophila* has a low temperature threshold for development of 13.3°C. The highest rates of development occurred at 27 and 30°C, temperatures of 23 and 25°C were optimum for survival. In oviposition experiments, 25°C was optimal for egg-laying and the mean viability of eggs was significantly higher at 25°C than at 20°C. Experiments demonstrated that significant adult mortality occurred during exposure to low temperatures. Mortality was 92% for adults exposed for four weeks to 10°C and 70% for adults exposed for 12 weeks to 15°C . These observations suggest that the low spring- and summer-temperatures experienced in New Zealand may inhibit population build-up and low winter temperatures may cause severe over-wintering mortality. Field temperatures experienced by *A. hygrophila* in New Zealand may be unsuitable for it to be able to control alligator weed. Therefore, additional biological control agents and integrated control strategies are required against this weed in New Zealand.

Introduction

Alligator weed, *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae), a plant of South American origin, has become a problem in waterways and pastures in many parts of the world, including the southern United States of America, Puerto Rico, Burma, Thailand, Indonesia, India, China, Australia and New Zealand (Julien 1980; Julien *et al.* 1992). It grows primarily as an emergent, aquatic plant, rooted in the substrate below shallow water but also grows as a terrestrial plant in a range of ecosystems from almost arid conditions to swampy areas (Julien and Broadbent 1980). In aquatic habitats, alligator weed forms large floating mats of interwoven hollow stems which extend over the surface of the water. Mats can become sufficiently thick to support the weight of a person walking on the weed, and also allow normally-terrestrial plants to establish on the weed mat. This can accelerate succession and turn enclosed water bodies into swamps.

Alligator weed was first recorded in New Zealand in 1906 at Aratapu near the Northern Wairoa River (Cheeseman 1906). It is currently distributed from North Cape to the Waikato River in the North Island and appears to be spreading southwards (C. Stewart unpublished data).

Biological control agents were selected for alligator weed control from searches made in South America by the United States Department of Agriculture from 1960 to 1974 (Coulson 1977). Two of these agents, *Agasicles hygrophila* Selman & Vogt (Coleoptera: Chrysomelidae), and *Vogtia malloi* Pastrana (Lepidoptera: Pyralidae) were imported into Australia from the USA in 1976. An additional species, *Disonycha argentinensis* Jacoby (Coleoptera: Chrysomelidae), was imported into Australia from Brazil in 1979. Between 1981 and 1988, *A. hygrophila*, *V. malloi* and *D. argentinensis* were introduced into New Zealand from Australia (Roberts and Sutherland 1989), but *D. argentinensis* did not establish. Although *V. malloi* established in Northland, no assessment of its effectiveness has been made.

Agasicles hygrophila was introduced into

Northland and Auckland in 1982 by the Entomology Division of DSIR, (Roberts and Sutherland 1989). The beetle established and spread rapidly throughout Northland (Philip *et al.* 1988) but there is limited quantitative information on its biology and effectiveness as a biological control agent.

Temperature is one of the major factors influencing insect development. In this study we investigated the rates of development from egg to adult, egg production and viability at a range of temperatures, and responses of adult beetles to chilling.

Methods

A colony of beetles for use in experiments was collected from a field population in Waitoki (Rodney District, Northland) and was maintained on fresh, field-gathered alligator weed in a greenhouse.

Rates of development

Development time from egg to adult was measured at eight constant temperatures between 10 and 30 ±1°C with a 16:8 L:D photoperiod. Newly-laid egg batches (25-35 eggs per batch on a single leaf) were incubated at each temperature. When each egg-batch hatched, 10 first-instar larvae (comprising one replicate) were randomly selected and reared individually in opaque, plastic containers. Damp filter paper was placed at the bottom of each 500 ml cylindrical container. Two shoots of plant material, with their roots sealed in 30 ml vials of water, were placed in each container and the top was covered with nylon gauze held in place by a rubber-band. At each temperature, 10 replicates were prepared. The insects were checked daily for life-stage changes and mortality until they reached adulthood. Difficulty was experienced in obtaining full replicates of 10 larvae from single egg-batches at 15°C. Nevertheless, the individuals that hatched were used and the sizes of the replicates were uneven at this temperature.

Egg production and viability

Female longevity, total life-time egg production, and egg viability were measured at four constant temperatures (15, 20, 25 and 30 ±1°C). Adult females were taken from the 'rates of development' experiments and paired with males from the greenhouse colony. Each pair of beetles was confined in a 500 ml container, as described above. The intention was to use the first females which eclosed at each temperature, for the oviposition experiments, but at 15, 20 and 30°C only two, 19 and eight adult females, respectively, became available from the original 100 larvae. Each day, at each temperature, egg batches were removed, incubated and viability was recorded. Males were replaced after they died.

Chilling experiments

Three- to four-day-old adults were chilled at 10 and 15 ±1°C for one, two, four and 12 weeks in a 2x4 factorial design with 20 replicates, each replicate included 10 adult beetles confined in a 500 ml container. Beetles were checked weekly and mortality was recorded. Alligator weed shoots were replaced fortnightly to provide adequate food. After the chilling period ended, containers were placed at progressively higher temperatures to acclimatize the beetles to 26°C. Beetles from 10°C were placed for a day at each of the following temperatures: 15, 20, 23 and 26°C. Beetles from 15°C were placed for a day at each of the following temperatures: 20, 23 and 26°C. Twenty pairs of males and females from each treatment, were maintained at 26°C and the viability of eggs from the first 12 egg-batches laid per female was recorded.

Three- to four-day-old adults were also exposed to three low temperatures (2, -4 and -8°C) for 13 h. Each replicate contained 10 adult beetles in a single container and there were 20 replicates. Following the cold period, the containers were placed at 26°C for one hour, after which the mortality of adults was measured. Twenty pairs of males and females from each treatment were retained and the viability of eggs from the first 12 egg-batches laid per female was recorded.

Data analysis

All data were analysed using the SYSTAT statistical package (Wilkinson 1990). Regression analyses were used to calculate the threshold for development from egg to adult and confidence intervals were calculated using the 'Prediction of X from Y: Linear calibration' described by Snedecor and Cochran (1980). The datum point from the 30°C development study was excluded from the regression analysis because this point was clearly not on the linear section of the development curve. Mortalities were analysed using Chi-squared analysis and standard deviations were calculated for the mortality data. Longevities, numbers of eggs laid and percentage viabilities were analysed using an ANOVA in a log $(X+1)$ transformation to stabilize variance, and

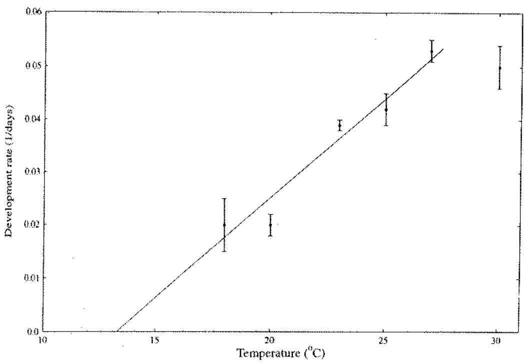

Fig. 1. Regression of development rate on temperature of *A. hygrophila* from egg to adult (bars represent standard deviations). Equation of line: $Y = 0.00376X - 0.05$, $R^2 = 90.4$. The 30°C datum point was not included in the regression calculation.

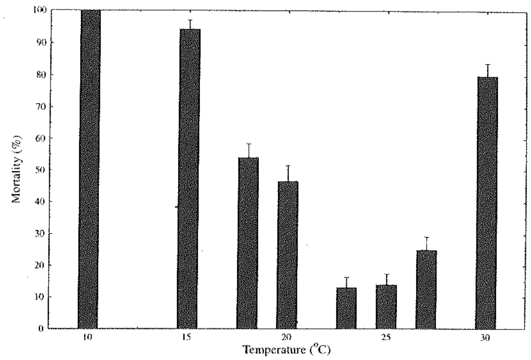

Fig. 2. Mortality of *A. hygrophila* during development (first instar to adult) at constant temperatures (bars represent standard deviations).

confidence intervals (95%) were estimated for the means. Survival curves from chilling treatments were compared using the log rank test and are graphed as Kaplan-Meier estimates (Kaplan and Meier 1958).

Results

Rates of development

Figure 1 shows the mean development rates (egg to adult) for *A. hygrophila* at seven temperatures from 15-30°C. No egg hatch occurred at 10°C. The highest rate of development occurred at 27°C and the low temperature threshold for development was 13.3°C (95% confidence interval: 11.2-15.5°C).

Mortality was greatly influenced by temperature (Fig. 2). Survival was highest at 23 and 25°C. At 15 and 30°C, mortality was >80%.

Egg production and viability

The effect of four temperatures on adult longevity, total life-time egg production per female and egg viability is shown in Table 1. Longevity was generally inversely proportional to temperature within the range tested. There was no significant difference (P>0.05) between the number of eggs laid by females at 20 and 25°C, but egg viability at 20°C was significantly lower (P<0.01) than at 25°C. At 15°C no viable eggs were produced and at 30°C only one of the eight females produced viable eggs.

Chilling experiments

The effects of low temperatures on adult survival are shown in Fig. 3. At 15°C, adult survival decreased steadily with duration of chilling; 30% of the adults were still alive after 12 weeks. The data fitted the linear regression equation: $Y = 0.991 - 0.061X$ ($R^2 = 98\%$). From this, the last beetle was predicted to die after an exposure of 16.2 weeks. At 10°C the effect was greater; only 8% of beetles still alive after four weeks and none were alive after seven weeks. The duration of the chilling-exposure affected the viability of eggs that were subsequently laid by females (Fig. 4). After four weeks at 15°C, or two weeks at 10°C, egg viability was less than 10%.

Most beetles survived exposure to 13 h at 2 or -4°C (Table 2). There was a significant difference (P<0.01)

Table 1. Female longevity, eggs laid per female and viability at constant temperatures (±95% confidence intervals). The data for 15°C are based on two adults only. Geometric means are given for 20, 25 and 30°C.

Temperature	15°C	20°C	25°C	30°C
Longevity (days)	154	79 (60.6-101.8)	48 (33.1-69.8)	11 (7.0-17.0)
Eggs laid per female	118	833 (575-1205)	769 (341-1730)	49 (8-265)
Eggs hatched per female	0	108 (30.6-373.8)	410 (151.6-1104.8)	0.6 (0-4.4)
Viability (%)	0	16 (6.9-36.5)	51 (31.6-81.9)	0.4 (0-1.9)

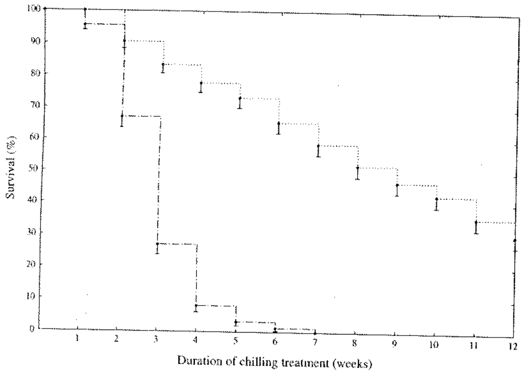

Fig. 3. Survival (%) of adult *A. hygrophila* chilled at 10°C (broken line) and 15°C (dotted line) (standard errors are shown).

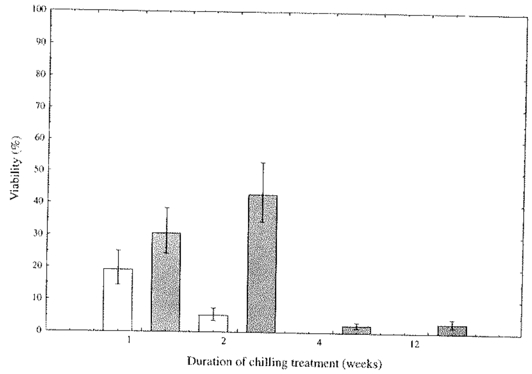

Fig. 4. Viability (%) of eggs laid after chilling adults of *A. hygrophila* at 10°C (unshaded histogram bars) and 15°C (stippled bars) for 1-12 weeks (95% confidence intervals are shown). At 10°C no pairs survived the four-week treatment.

between the viability of eggs laid following exposure to 2 or -4°C, but overall viability was low. Exposure to -8°C for 13 h killed all the beetles that were tested.

Table 2. Effect of 13 h exposure to cold temperatures on adult survival and subsequent egg viability of *A. hygrophila*.

Temperature	Survival of adults (%)	Egg viability (%) (±95% CI)
-8°C	0	-
-4°C	90.5	4 (2.7-5.9)
2°C	99.5	27 (20.1-34.8)

Discussion

Compared to some widely-distributed New Zealand insects, *A. hygrophila* has a high optimal temperature range. Optimal temperatures for development and survival occur between 23°C and 27°C (Figs 1 and 2). By comparison *Ctenopseustis obliquana* (Walker), a native pest of tree crops that occurs throughout New Zealand, has an optimum temperature for development of 20°C (Clare and Singh 1990). The lower temperature threshold for development of *A. hygrophila* (13.3°C) is also high, compared to that

Table 3. Lower temperature development thresholds of widely-distributed insects studied in New Zealand.

	Lower development threshold	References
Coleoptera		
Coccinella undecimpunctata L.	10°C	French (1966)
Listronotus bonariensis (Kuschel)	10°C	Goldson *et al.* (1982)
Longitarsus jacobaeae Waterhouse	4.3°C, egg 9°C, larva 8°C, all other stages	Delpachitra (1991)
Sitona discoideus Gyllenhal	15°C, prepupa	Goldson *et al.* (1988)
Hemiptera		
Acyrthosiphon kondoi Shinji	2.6°C	Rohitha (1979)
Lepidoptera		
Ctenopseustis obliquana (Walker)	7.8°C, egg 5.2°C, larva 7.2°C, pupa	Clare and Singh (1990)
Tyria jacobaeae (L.)	11°C, egg, 1st, 2nd, 4th instar 12°C, 3rd instar 6°C, 5th instar	Harman *et al.* (1989)
Neuroptera		
Micromus tasmaniae (Walker)	5.8°C	Syrett and Penman 1981)

Table 4. Mean number of days of air-frost per month, from May to September (M-S), for selected North Island, New Zealand meteorological sites (Anonymous undated). Readings between October and April are all zero.

	M	J	J	A	S	Total per year
Kaitaia	0	0.1	0.1	0	0.1	0.3
Whangarei	0	0.2	0.4	0	0	0.6
Dargaville	0.3	1.0	2.3	1.2	0.2	5.0
Owairaka	0	0.5	0.7	0.3	0	1.5
Thames	0	1.0	2.0	0.3	0	3.3
Te Kauwhata	0.5	1.9	2.2	0.6	0.1	5.3

of some insect species with wide distributions in New Zealand (Table 3).

The data from the chilling experiments indicate that over-wintering adults of *A. hygrophila* may suffer high mortality and reduced egg viability as a consequence of exposure to low field temperatures. The laboratory experiments showed that adult mortality was 92% after four weeks exposure to 10°C. Beetles exposed to 20°C and 25°C suffered 0% and 21% mortality, respectively, over the same time period (C. Stewart unpublished data). Exposure of *A. hygrophila* adults to periods (2-4 weeks) of chilling (at 10°C and 15°C, respectively) significantly reduced the viability of the eggs that they laid (Fig. 4). Egg viability after adult chilling was less than 10% compared to 51% for adults that were not exposed to chilling (Table 1). After exposing adults to short periods of severe chilling (2°C and -4°C for 13 h) egg viability was reduced (27% and 4%, respectively) compared to the viability of eggs reared from adults not exposed to chilling (51%).

It is likely that *A. hygrophila* will experience low temperatures under field conditions in New Zealand similar to those investigated in the laboratory. An air-frost occurs when the air temperature falls below zero and, even in northern New Zealand, most places have several air-frosts per year (Table 4). Given the susceptibility of *A. hygrophila* to low temperatures, the population growth-rate of *A. hygrophila* is likely to be limited in New Zealand.

Using the climate matching program, CLIMEX, Stewart *et al.* (1995) predicted *A. hygrophila* could control alligator weed from Auckland to Northland and in several coastal areas of the North Island. Our data indicate that this species may be more limited than the modelling study suggested. Optimum temperatures for development and survival (23-27°C) are only sustained for a short period of time and then only in the very north of New Zealand (Table 5). Mean daily maximum temperatures are above 23°C for four months per year in Kaitaia but for only two months per year in Te Kauwhata (Table 5).

Conclusions

On the basis of the data presented here, we believe that the present strain of *A. hygrophila* is not suitable for widespread control of alligator weed in New Zealand. The next step in this biological control programme

Table 5. Mean daily maximum and minimum temperatures for each month of the year (J-D) for selected North Island, New Zealand meteorological sites (Anonymous undated).

	J	F	M	A	M	J	J	A	S	O	N	D
Kaitaia	25.2	25.6	24.7	21.8	19.3	17.1	16.2	16.8	17.7	19.0	21.3	23.1
35.07S 173.16E	14.2	14.6	14.8	12.5	9.8	8.7	7.2	8.1	8.8	10.2	11.7	13.0
Whangarei	24.7	24.7	23.4	20.8	18.3	16.2	15.3	16.0	17.1	18.9	21.0	22.9
35.44S 174.18E	14.6	14.9	14.4	12.1	9.5	8.0	6.7	7.5	8.6	10.0	11.6	12.9
Dargaville	23.4	24.2	23.0	20.4	17.8	15.7	14.9	15.3	16.3	17.8	19.6	21.4
35.57S 173.50E	13.7	13.9	13.1	11.3	9.3	7.8	6.5	7.1	8.2	9.8	10.9	12.3
Owairaka	23.0	23.5	22.2	19.8	17.1	15.0	14.1	14.8	16.1	17.8	19.6	21.4
36.54S 174.44E	14.9	15.5	14.4	12.0	9.6	7.6	6.4	7.1	8.5	10.3	11.8	13.5
Thames	23.8	24.2	22.7	20.1	17.2	14.8	14.3	15.0	16.5	18.3	20.2	22.1
37.08S 175.32E	15.0	15.3	14.2	11.7	9.1	7.0	6.3	7.0	8.6	10.7	12.2	13.9
Te Kauwhata	23.9	24.2	22.8	19.9	16.7	14.1	13.5	14.6	16.1	18.1	20.0	22.1
37.25S 175.08E	13.9	14.1	13.1	10.6	8.1	6.1	5.2	6.3	7.9	9.6	10.7	12.5

should be to assess the impact of *V. malloi*. If results show that this species is also limited in its ability to control alligator weed in New Zealand, then new cool-adapted agents should be sought in South America. This will be especially important if alligator weed continues to spread further south.

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