Bulletin of Entomological Research (2006) **96**, 145–152 DOI: 10.1079/BER2005408

Population regulation of a classical biological control agent: larval density dependence in *Neochetina eichhorniae* (Coleoptera: Curculionidae), a biological control agent of water hyacinth *Eichhornia crassipes*

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Abstract

The release of classical biological control agents has reduced the economic, environmental and social problems caused by water hyacinth, *Eichhornia crassipes*; however, additional control measures are needed in some locations. Water hyacinth plants were treated with different densities of eggs of the weevil *Neochetina eichhorniae* Warner, one of the main control agents, under different nutrient regimes in a controlled experiment. Plants were destructively sampled and the development of *N. eichhorniae* was assessed. The survival of first and second instars declined as larval density increased. Plant nutrient status did not directly affect the mortality rate of larvae, but at higher nutrient concentrations larvae developed faster and were larger at a given developmental stage. It is argued that the density dependence operating in *N. eichhorniae* occurs through an interaction between young larvae and leaf longevity. Consequently, events which disrupt water hyacinth leaf dynamics, e.g. frost or foliar herbicides, will have a disproportionately large effect on the control agents and may reduce the level of control of the host.

Keywords: integrated pest management, density dependence, classical biological control, weeds, *Neochetina eichhorniae*, *Eichhornia crassipes*, water hyacinth

Introduction

The use of classical biological control has reduced the problems caused by many invasive weeds (McFadyen, 1998) and has been particularly successful in controlling aquatic plants (Room *et al*., 1981; Ajuonu & Neuenschwander, 2003; McConnachie *et al*., 2004). However, many control agents do not provide adequate levels of control. Where agents provide partial control, different methods of managing weeds need

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to be integrated. This requires a detailed knowledge of the ecology of the biological control agent (Murdoch & Briggs, 1996; Paynter & Flanagan, 2004). Control may also be expected to depend on host plant quality. Leaf nitrogen concentration, in particular, has been shown to influence the efficacy of many biological control agents (Room *et al*., 1989; Wheeler *et al*., 1998; Wheeler, 2001).

Water hyacinth *Eichhornia crassipes* Solms-Laubach (Pontederiaceae) is an invasive aquatic plant that has caused serious social, economic and environment problems in its introduced range (Gopal, 1987; DeGroote *et al*., 2003). The classical biological control agents *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) and *N. bruchi* Hustache have reduced some infestations by 95% (Deloach & Cordo, 1983; Haag & Center, 1988; Jayanth, 1988) but, even after decades of classical biological control, water hyacinth is still being actively managed in other locations (Center *et al*., 1999a). To assess the impact of *Neochetina* spp., information is required on: (i) what controls the dynamics of the insect; (ii) the rate at which insects damage the plant; and (iii) how these relationships vary with environmental conditions.

Density-dependent mortality in insects has been reported more frequently from larval stages than from adult, egg or pupal stages (Sinclair, 1989). Previous work on *Neochetina* spp. suggests that weevil population size is also regulated during the larval stage (Chikwenhere, 2000; Heard & Winterton, 2000).

Several studies have looked at the effect of the weevils on water hyacinth plants (Forno, 1981; Center *et al*., 1982, 1999b; Bashir & Bennett, 1984; Bashir *et al*., 1984; Center & Van, 1989), where a fixed number of adult weevils was added to each plant. Larvae were observed to cause more damage to plants than adults. However, in these experiments the number of adults may not be tightly correlated to the number of larvae, particularly as the oviposition rate can vary markedly between females (range 0 to 15 per day (DeLoach & Cordo, 1976b; Abjar & Bashir, 1984; Shih *et al*., 1994)). Even if the total adult lifetime fecundity is similar, differences in the timing of oviposition will mean adult numbers are not a good predictor of herbivore pressure or larval density dependence.

Nutrients, in particular nitrogen, limit the growth rate of both the weed and the control agent (Heard & Winterton, 2000; Wilson *et al*., 2005). Heard & Winterton (2000), showed that nitrogen has a large effect on the water hyacinth/weevil interaction, concluding that higher nitrogen levels lead to faster weevil population growth and increased damage. However, to predict the long-term effect of nutrient conditions, the effect of nutrients on the population regulation of the control agent must be understood.

In the present experiment, the effect of *N. eichhorniae* larval density on larval development is explored under high and low nutrients.

Materials and methods

Work was conducted at the International Institute of Tropical Agriculture (IITA) biological control station in the Republic of Benin, West Africa from June to October 2001. Daily temperatures were between 19–23°C and 27–31°C; relative humidity was 50–90% to 90–100%; and solar radiation averaged 325 Gm-cal cm^{-2} day^{-1} (range 140–480).

Plants were collected several kilometres north of the nearest population of *Neochetina* spp. (2° 33′ E, 9° 13′ N). Adult *N. eichhorniae* were collected from a field population on 18 July 2001 (2° 00′ E, 7° 55′ N). No *N. bruchi* have been found at this site (Ajuonu *et al*., 2003). *Neochetina eichhorniae* was used as it is the most commonly released water hyacinth biological control agent (Julien *et al*., 1999), and is the most abundant agent in Benin (Ajuonu *et al*., 2003).

Plants were grown in a series of eight paddling pools ($1.65 \times 2.5 \times 0.5$ m) in water 0.3–0.35 m deep. The pools were kept under wooden shelters to stop rainfall from disrupting water nutrient levels, and to prevent direct sunlight from damaging the plants. In preliminary experiments plants grown in unshaded pools turned brown and died, in part because of a 'clothes-line' effect. The experimental pools were relatively small and not set into the ground, and so the micro-climate was less buffered than it would have been if the plants were growing in a pond. This effect has lead to gross over-estimates of the rate of evapo-transpiration caused by invasive aquatic plants (Allen *et al*., 1997). The shelters did not appear to reduce the growth rate of the plants relative to rates recorded in other studies (cf. fig. 1 and (Wilson *et al*., 2005)).

Within each pool, experimental replicates were separated by grouping plants into four plastic buckets. To allow water to circulate freely within each pool, the bottom of each bucket was cut off and the buckets were suspended on a frame. The top of each bucket was 0.54 m in diameter, and the hole in the bottom ~0.32 m, with ~0.2 m of the bucket above the water-line and ~0.1 m below.

The experiment was a split-plot design. Treatments were water nutrient level, time to sampling, and egg load per plant. Pools were kept at either a low or high water nitrogen concentration. Plants were sampled when the larvae were 25–26 or 45–46 days old. There were 2, 4, 8 or 12 eggs inserted per plant. Each replicate consisted of nine plants, two plants at each egg density and one plant as a control. At the start of the experiment plants had 7–10 leaves and weighed 156 ± 43 g (wet weight ± 1 standard deviation, $n = 288$). Any offshoots that had developed were removed.

Nutrient conditions

Pools were initially filled with tap water (0.3 mg l^{-1} phosphate, < 0.0 mg l^{-1} nitrate-nitrogen, ~0.02 mg l^{-1} nitrite-nitrogen, < 0.00 mg l^{-1} ammonia-nitrogen). A water-soluble mineral nutrient fertilizer (Plantafert® 9-Hydro 15N : 7P : 22K, 4 nitrate-nitrogen : 1 ammonium-nitrogen, with trace elements) was then added to maintain the nitrate-nitrogen concentration at either 4 mg l^{-1} or 0.4 mg l^{-1}. These levels are representative of nitrate concentrations observed in tropical lakes (ILEC & UNEP, 2001). Moreover, the rate of growth of nitrogen-limited water hyacinth plants roughly doubles over this change in nitrate concentration (Wilson *et al*., 2005). To avoid iron deficiency, chelated iron was also added at the start of the experiment (Murphy™ Sequestrene® granules). Water nutrient concentrations were measured using hand-held ion-specific meters (Hanna instruments).

Plants were cultured for 30 days before the start of the experiment. Eggs were therefore inserted into plant material that had been produced under controlled nutrient conditions.

Insect treatments

The weevils were given fresh high nutrient water hyacinth leaves to oviposit on. These leaves were removed daily and stored under damp paper. Four days later, by which time any eggs laid would have hardened, the eggs

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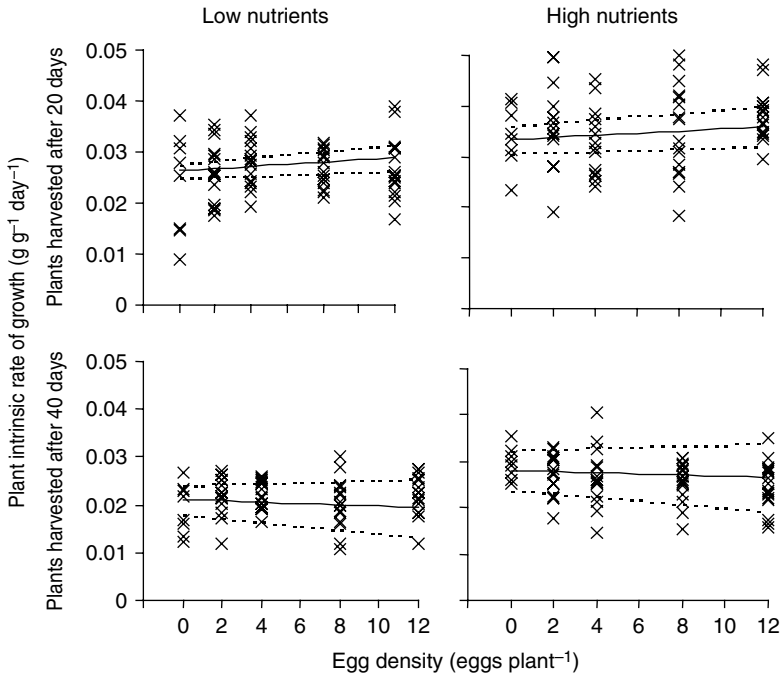

Fig. 1. Effect of *Neochetina eichhorniae* egg density and water nutrient concentration on water hyacinth growth rate. Each point is the growth rate of a plant measured between the start of the experiment and sampling. The panels are different combinations of nutrient treatment and time to harvest. The solid and dotted lines show the fitted relationship ± 1 SE.

were dissected out of the leaves. The next day the eggs were inserted into the plants. *Neochetina eichhorniae* prefer to oviposit in the youngest three leaves (Center, 1987). Taking into account a leaf turnover of about 0.2 leaves per day, the 5–6-day-old eggs were therefore placed in the third and fourth youngest leaves. Two slits of 5–10 mm were made in the bottom third of each petiole and eggs were evenly distributed between the cuts. Although *N. eichhorniae* oviposit in many plant plants, they frequently lay in the bottom third of a petiole (DeLoach & Cordo, 1976a; Shih *et al*., 1994), and this was the most practical option. The plants were left for 20 or 40 days before being destructively sampled, by which time the weevils were 25–26 or 45–46 days old.

To estimate eclosion probability, two pools were set up as for the rest of the experiment, but plants were harvested after 5 days. The percentage eclosion was also measured for eggs stored in Petri dishes.

Monitoring

The depth and nutrient content of each pool were measured every five days (nitrate, phosphate, ammonium and nitrite). Any water lost from the pools was replaced and fertilizer was added to bring each pool back to a depth of 0.35 m and a concentration of 4.0 or 0.4 mg l^{-1} of nitrate-nitrogen. To check the rate of leaf production, new leaves were tagged.

At the end of the experiment each plant and its offshoots were drip dried for at least 30 min before being weighed. In many tropical aquatic plants wet weight is highly correlated to dry weight (Little & Henson, 1967) and, moreover, in water hyacinth this ratio is not affected by insect damage (Little & Henson, 1967; Gopal, 1987).

Water hyacinth roots were examined for pupal cases. All the petioles, leaves, and rootstock of every plant were checked for eggs and larvae, and larvae were carefully removed from the petioles and rootstock. The head capsule width of each larva was then measured to determine its instar. In some cases it was possible to follow larval tunnels and find the fate of living larvae. While the tunnels of older larvae frequently went into the crown and into other petioles (making very visible exit and entry holes in the surface of the petiole), no exit holes were found after 24–25 days, and the tunnels of the younger larvae were not found to go into the harder material at the base of the petiole. Dead larvae and eggs could not be counted accurately due to decomposition and rotting of damaged leaves.

To determine the nutrient status of the water hyacinth plants, the third leaf was removed, dried at 80°C, and its percentage nitrogen content measured using the Kjeldahl method. This leaf is the first mature leaf and has a nutrient status similar to most older leaves (Center & Wright, 1991).

Data analysis

The statistics program R v.1.4.1 was used for all analyses (copyright 2002, The R Development Core Team). Due to the split plot design and temporal repetition in monitoring, data were analysed using mixed effect models. Mixed effects models allow the estimation of the correlation structure that exists in grouped data (Pinheiro & Bates, 2000). In this study, data are grouped by plant and by pool (repeated measures on the same plant over time and repeated measures on the same spatial scale of a pool). To allow for temporal correlation, pool, bucket, and plant identity were included in the model as random effects.

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The minimal adequate model was arrived at by step-wise deletion of the fixed effects from the full model. The depleted model was then compared with the full model using an F-test of the likelihood ratios ($LR_{x,y}$). The final model was fitted using restricted maximum likelihood to reduce the bias in the estimation of the variance components (Venables & Ripley, 1997). The subscript x is the difference in degrees of freedom between the larger and smaller models, and the subscript y is the degrees of freedom of the smaller model.

Plant growth rate was calculated as the intrinsic rate of growth: $\ln(B_{final}/B_{initial})/t$, where B is the biomass of the plant and t the duration of the experiment in days. This calculation gives a single value, r, which is the proportional change in plant biomass per day.

Larval survival rates were analysed using generalized linear models with binomial error structures within the mixed effect models (using the glmmPQL function in R (Venables & Ripley, 1997)). The number of larvae that died was assumed to be the number of eggs inserted minus the number of larvae and pupal cases found. However, by 45–46 days, a small number of larvae had migrated onto control plants (6 larvae out of 312). Assuming the larvae moved randomly between plants and the rate of movement was not affected by plant size or nutrients,

$$\#\text{ larvae observed} = \#\text{ before movement} \cdot (1 - \text{p}) + \frac{\text{p} \cdot \#\text{ larvae per bucket (52)}}{\#\text{ plants per bucket (9)}}$$

where p is the probability that a larva moves between plants. Using the number of larvae found in the test plants (average of 0.375 larvae per plant, $n = 6$), the number of larvae surviving from each plant was adjusted before the survival data were analysed. If more larvae were found on a plant than eggs inserted, survival was bounded at 100% to permit the use of binomial errors. The data from 25–26-day-old larvae were not adjusted, as these larvae were not observed to have moved between petioles let alone between plants.

As larval head capsule size may be correlated to pupation survival and/or adult fecundity, the effect of treatments on head capsule size was analysed. Larval head capsule sizes were analysed separately for each harvest, and separately for each instar within each harvest. This was done to avoid numerical problems when fitting the model, e.g. there were no second instar larvae from high nutrient plants by 40 days, but the majority of larvae after 20 days were second instar.

The proportion of insects in each of five stages (instars 1, 2 and 3, pupae with larval head capsules, and pupae with adult head capsules) was used to test the effects of nutrient and egg density on larval developmental rate. The response variable was the proportion of individuals in a given stage found in each plant. If there was an interaction among stage identity and the explanatory variable, then it was concluded that this explanatory variable had a significant effect on the proportion of individuals found in that stage. Plant identity was included as a random effect and data were analysed using binomial errors.

Results

Nutrients

In preliminary investigations, 98% of the variation in plant nitrogen concentration between pools was explained by water nutrient level. Thirty-two leaves were analysed from the plants in the actual experiment, half from plants with 12 eggs and half from control plants. Plant nutrient concentration was again strongly affected by water nutrient concentration, but there was no indication of an effect of egg density (2.28% ± 0.11 in leaves from low nutrient pools, 3.90% ± 0.23 from high nutrient pools). Therefore, two significantly different levels of leaf nitrogen were realised by the treatments, and so leaf nitrogen was included as a factor affecting larval development and mortality.

Plant growth rate

Plants grew faster in high nutrient pools (table 1, fig. 1). Plant growth rate was also affected by an interaction among number of eggs and date of harvest. However the number of eggs inserted had a smaller effect on plant growth rate than nitrogen or date of harvest. There was no effect of plant starting weight on plant growth rate.

Leaf production

Leaf production was affected by an interaction among nutrient level and time (table 1), with plants in high nutrient pools producing leaves roughly twice as fast as plants in low nutrient pools (low 0.121 ± 0.013 leaves day^{-1} (±1 standard error) after 20 days, 0.142 ± 0.005 after 40 days; high 0.216 ± 0.018 after 20 days and 0.286 ± 0.018 after 40 days). Leaf production rate was not found to be affected by number of eggs inserted.

Weevil survival probability

A high hatch rate was observed *in vitro* (90%, $n = 72$), in line with other studies (e.g. 98% (Abjar & Bashir, 1984) and 92% (Shih *et al.*, 1994)). The rate of recovery of larvae from plants after 10–11 days was slightly lower than *in vitro*: 80% ($n = 66$). However, this recovery rate was not found to be affected by number of eggs inserted or plant weight ($LR_{4,7} = 0.95$, $P > 0.1$). With only two pools used to test the success of inserting eggs, it was not possible to use mixed effect models to assess the effect of nutrient status. However, if plants were treated as independent sampling units, there was no effect of water nutrient status on the success of inserting eggs ($t_{23.5} = 1.21$, $P > 0.1$) (the analysis uses fractional degrees of freedom to take account of unequal variance). Consequently, egg mortality is treated as a random source of mortality in the larval survival analysis.

Data were transformed using equation 1 prior to analysis. Larval survival significantly decreased as egg density increased, increased with initial plant size and decreased between harvests (fig. 2, table 1). Survival was not significantly affected by nutrients.

Weevil development

Two hypotheses were tested; first that plant nutrient status, larval density and plant size affect larval size within an instar, and second that they affect larval development rate.

Larval size was measured using head capsule width (to the nearest 0.01 mm, fig. 3). In agreement with other observations of *N. eichhorniae* larvae (DeLoach & Cordo, 1976b; Shih *et al.*, 1994), head capsule widths fall into discrete groups: 0.24–0.34 mm for instar 1; 0.36–0.5 mm for instar 2;

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Table 1. Main results of the analyses. The explanatory variables are shown in the form they fit into the minimum adequate model. In each case, the significance of the explanatory variable is tested by comparing the fit of the model with that variable removed (but everything else in the minimum adequate model present) with the maximal model.

Response	Explanatory variables	LR Test	*P*	Direction
Plant nutrient concentration	Water nutrient concentration	$LR_{1,5} = 38.81$	**	+ve
Plant growth rate	Water nutrient concentration	$LR_{1,8} = 14.69$	**	+ve
	+Number of eggs: Date of harvest	$LR_{1,8} = 5.44$	*	fig. 1
	Plant starting weight	$LR_{8,19} = 3.52$	ns	NA
Leaf production rate	Water nutrient concentration: Date of harvest	$LR_{1,11} = 7.84$	**	see text
	Number of eggs	$LR_{4,15} = 5.40$	ns	NA
Weevil survival	Number of eggs	$LR_{6,10} = 11.68$	*	−ve
	+Plant starting weight	$LR_{6,10} = 11.42$	*	+ve
	+Date of harvest	$LR_{6,10} = 21.48$	**	−ve
	Water nutrient concentration	$LR_{10,18} = 6.93$	ns	NA
Weevil development rate first harvest	Number of eggs: Water nutrient concentration	$LR_{13,15} = 468.2$	*	see text
	Plant starting weight	$LR_{15,27} = 15.14$	ns	NA
Weevil development rate second harvest	Number of eggs	$LR_{12,27} = 628$	**	−ve
	+Water nutrient concentration	$LR_{12,27} = 47.2$	**	+ve
	+Plant starting weight	$LR_{12,27} = 29.3$	*	+ve
	Number of eggs: Water nutrient concentration	$LR_{15,27} = 10.66$	ns	NA

The probabilities are shown as: ns $P > 0.05$; * $P < 0.05$; ** $P < 0.01$.

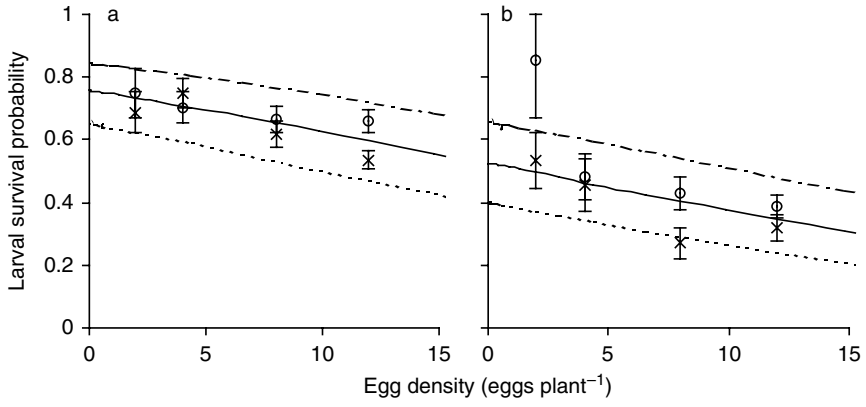

Fig. 2. Effect of *Neochetina eichhorniae* egg density on larval survival, (a) after 25–26 days, and (b) after 45–46 days. Points are average larval survival per plant, × for high and o for low nutrient plants. The fitted relationships are for plants of initial weight 40 g (bottom line/lowest initial weights), 160 g (middle line/experimental average), and 300 g (top line/highest initial weight). The survival probabilities for the 45–46 day larvae were adjusted to correct for larval migration. The data used to produce the fitted relationships for the 45–46 day larvae, were also adjusted so that survival probabilities were bounded at unity.

and 0.52–0.95 mm for third instar larvae, and pupae with intact larvae head capsules. Instar 3 larvae appear to fall into two head capsule groups (fig. 3), but we have no explanation for this.

After 10–11 days, larval size was not affected by number of eggs inserted or nutrient concentration ($LR_{5,8} = 0.63$, $P > 0.1$). There was also no significant effect of nutrients on larval size if plants were treated as independent units ($t_{37.5} = 1.08$, $P > 0.1$). After 25–26 days, almost all larvae were in their second instar (484 out of 529). These second instar larvae tended to be smaller if the egg density was high ($LR_{7,12} = 30.13$, $P < 0.01$), plants weighed less ($LR_{7,12} = 12.89$, $P < 0.05$), or the water was low in nutrients ($LR_{7,12} = 24.18$, $P < 0.01$). After 45–46 days, 16% of insects found were pupae, but several of these had intact larval head capsules and so were included in the analysis. Head capsule width was larger for larvae in high nutrient plants (0.70 mm ± 0.01 vs. 0.76 mm ± 0.01, $F_{1,108} = 18.94$, $P < 0.001$), but was not affected by egg density ($F_{1,107} = 3.025$, $P > 0.05$) or plant weight at sampling ($F_{1,107} = 0.55$, $P > 0.1$). However, residual errors were not normally distributed (head capsule widths of third instar larvae are not normally distributed, fig. 3).

The influence of nutrients, plant weight and egg density on the rate of larval development was tested using the distribution of larvae among development stages. The analysis was again split into different harvests to avoid numerical problems.

After 25–26 days, the proportion of larvae in each stage was not affected by plant starting weight, but was affected by an interaction among egg density and nutrient status (table 1). For both nutrient levels, larvae were less developed at higher egg densities. After 45–46 days, the rate of development was again slower at higher egg densities, and lower nutrients, but the interaction among these factors was

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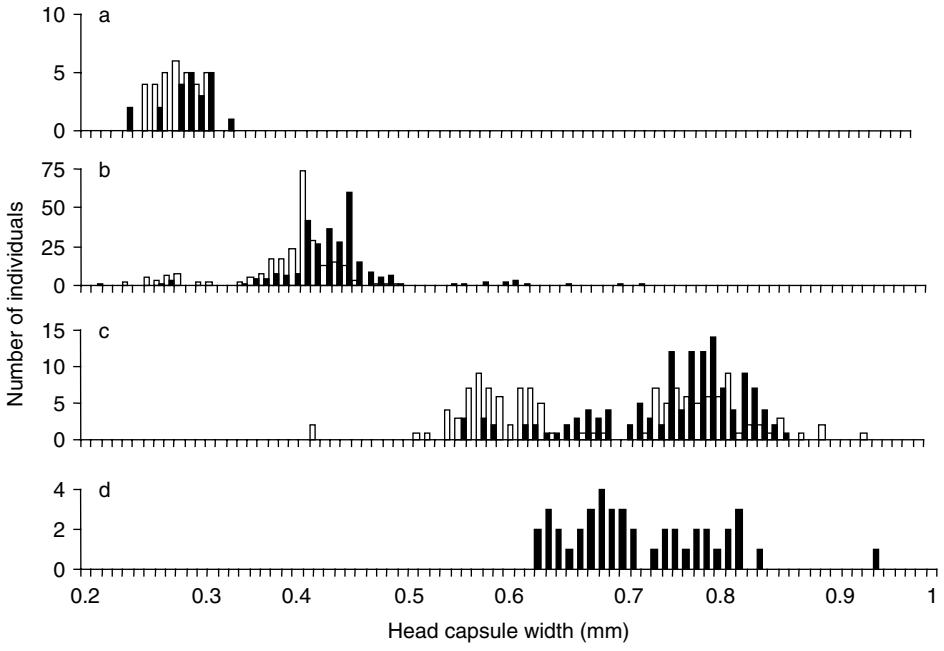

Fig. 3. Head capsule distributions for: (a) 10–11-day-old larvae, (b) 20–26-day-old larvae, (c) 45–46-day-old larvae and (d) 45–46-day-old pupae of *Neochetina eichhorniae*. Eight pupae were too developed for their larval head capsule width to be measured (all from high nutrient pools). □, low nutrients; ■, high nutrients.

not significant (table 1). The rate of development was also found to be slower in smaller plants.

Discussion

The experiment demonstrated that early, but not late larval stages of *Neochetina eichhorniae* experienced density-dependent mortality. First and second instar larvae did not move from the petioles in which they were laid, whereas later instars moved between petioles and between plants. This greater mobility makes them less subject to competition for food, and less subject to leaf mortality. In this experiment, dead larvae were found in badly damaged, waterlogged petioles. Larvae had tunnelled up the petiole towards the leaf and were unable to tunnel back down as other larvae had destroyed the lower part of the petiole. As leaf mortality has been shown to be affected by the weevils (Center & Van, 1989; Van & Center, 1994), it would be expected that at high larval densities, larvae have a higher probability of being stranded in dead and dying leaves, i.e. an interaction between the insects and the leaf dynamics can be used to explain why the density dependence seen in this experiment affects early larval stages.

This suggests that population regulation was occurring before larvae were large enough to cause serious damage to the plant. If the severity of density dependence depends on the current state of mature leaves, then there will be a delay between the production of new material, the alleviation of density-dependent mortality, and an increase in the population of damaging late instar larvae. As the generation time of *Neochetina* spp. is over two months (Julien *et al*., 1999), agents will not respond adequately to changes in the plant population, particularly if the leaf dynamics are already disrupted. This provides a mechanistic explanation for why foliar herbicides and *Neochetina eichhorniae* appear to act in a non-complementary way (Center *et al*., 1999a).

Minimizing damage to leaves at key points in the year could allow biological control to be more effectively integrated with chemical control. In seasonal environments, populations of *Neochetina* spp. can often show defined generational structure (e.g. Grodowitz *et al*., 1991). If foliar herbicides are applied (or if leaves are damaged by frost) when there is a relatively large number of young larvae, then this may severely reduce weevil populations. However, if chemicals are used when the weevil population is predominately late instar, pupae or adults, then the negative impact of leaf mortality on the size of the weevil population could be reduced. A similar effect may be achieved by using sub-lethal doses of herbicides, or leaving some areas unsprayed to provide a reserve for the biological control agent.

During the present experiment, water nutrient levels were maintained at field levels, and plants in the experiment were representative of medium and high nutrient plants (~2.2% dry weight nitrogen and 3.9% respectively, cf. observed maximum range of 0.7–5.0% (Wilson *et al*., 2005)). Higher levels of nitrogen in the water led to a higher plant nutrient status, plant growth rate and leaf turnover. This supports other observations that the speed of increase in an infestation will be much greater at high nutrients (reviewed by Wilson *et al*., 2005). Heard & Winterton (2000), also showed that the population growth rate of *N. bruchi* was higher at higher nutrients. Similarly, in the present experiment, *N. eichhorniae* larvae developed faster at higher nutrients, and larvae of a particular instar in high nutrient plants tended to be larger than those in low nutrient plants.

The effect of nutrients on the interaction between the plant and control agent is less clear. No significant interaction was found between nutrient concentration and

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density-dependent mortality, or between nutrient concentration, larval density and damage. This suggests that nutrients may have little effect on the equilibrium density of the plant and the herbivore under stable conditions. However, high nutrient leaves tend to have broader petioles (Gopal, 1987), and so may allow a higher larval density (at low densities most leaves have floats, but the nutrient effect is independent of this), but in the present experiment, similar plants were chosen at both nutrient levels and so this potential effect could not be explored.

The weevils did not have a large effect on plant growth or on the plant nutrient content (table 1, fig. 1). The number of larvae per plant (even after adjusting for larval mortality) was greater than in some field systems where control has been successful (Deloach & Cordo, 1983; Center & Durden, 1986), and in some experimental systems that showed a significant reduction in plant growth (Forno, 1981; Chikwenhere, 2000). However, only towards the end of the present experiment were large larvae feeding on the meristem and rootstock. If the experiment had been continued the effect of this damage on plant growth rate may have become more apparent.

The eventual level of control in a classical biological control system can only be understood by understanding how the control agents' populations are regulated (e.g. Dent, 1991). The present experiment has highlighted the potential importance of larval development in regulating *N. eichhorniae* population size. In particular, management options that disrupt the leaf dynamics of water hyacinth will have a disproportionate adverse impact on the existing biological control agent, and may prevent these agents from controlling the weed. These effects, and those concerning the role of nutrients on the control of water hyacinth, will be analysed in future papers using mathematical models.

Acknowledgements

This work was funded by a NERC grant to JRUW, and the IMPECCA Programme through the Environment, Peace and Stability Facility (EPSF) of DANIDA (Danish International Development Assistance). The authors would like to thank René Djessou, Gabriel Kouke and Andre Aïvodji for assistance with field-work.

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(Accepted 13 October 2005)