Biological Control 135 (2019) 16–22

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

# Variability in weed biological control: Effects of foliar nitrogen on larval development and dispersal of the alligatorweed flea beetle, *Agasicles hygrophila*

Nathan E. Harms[a,b,*], James T. Cronin[b]

[a] US Army Engineer Research and Development Center, 3909 Halls Ferry Rd., Vicksburg, MS 39180, USA
[b] Department of Biological Sciences, 202 Life Sciences Building, Louisiana State University, Baton Rouge, LA 70803, USA

ARTICLE INFO

*Keywords:*
Aquatic weed
Temporal variability
Density dependence
Host quality
Foliar nitrogen

ABSTRACT

Host quality can have dramatic effects on performance of biological control agents but its importance is understudied. We used a combination of field measurements and laboratory experiments to determine the range of foliar nitrogen (FN) that larvae of the alligatorweed flea beetle (*Agasicles hygrophila*) are exposed to in the field and its importance to larval development and dispersal. Seasonal variability in FN was assessed at field sites spanning southern to northern Louisiana every 2–3 weeks during the growing season for four years. In a series of laboratory experiments, alligatorweed FN was manipulated to examine its influence on larval development and survival (under different temperature regimes), adult biomass, and dispersal of the biological control agent, *A. hygrophila*. There was strong seasonality of FN in field sites with peak levels (4–8% dry weight nitrogen; DW N) recorded early during each year, declining during summer, and slightly increasing again in the fall which coincides with flea beetle activity. Foliar nitrogen and rearing temperature had strong independent effects on larval development rate. High FN increased survival by 40%, decreased developmental time by 15%, and resulted in 11% larger adults. Increasing temperature reduced survival by 43%, shortened developmental time by 28%, and led to 15% smaller adults. In the dispersal experiment we were unable to detect an interaction between FN and conspecific density on larval dispersal, though results suggested that FN may lessen effects at moderate densities. Mean dispersal was more than double in the low versus high FN treatment (51% vs. 23%). Larval density and nitrogen both affected larval weight; high nitrogen plants produced 33% larger larvae over the duration of the experiment and larval fresh weight decreased by 38% from low (one larva plant$^{-1}$) to high (twenty larvae plant$^{-1}$) density treatments. We demonstrated that increasing nitrogen in leaf tissues shortens larval *A. hygrophila* developmental time and increases survival to adulthood, regardless of exposure temperature during development. It also suggests that foliar nitrogen may have important effects on biological control of alligatorweed, particularly as a result of seasonal variation in temperature and plant nutrition at field sites, and could contribute to observed variation in *A. hygrophila* efficacy in the field.

## 1. Introduction

The importance of plant quality (e.g., leaf toughness, moisture, defensive chemistry) and nutrition (e.g., nitrogen, phosphorus, potassium) to herbivorous insects is well established (Scriber and Slansky Jr., 1981; Awmack and Leather, 2002). Herbivore physiology (e.g., development and fecundity), behavior (e.g., host choice and movement) and population dynamics (Denno and McClure, 1983; Helms and Hunter, 2005) are directly tied to local host plant conditions (Scriber and Slansky Jr., 1981; Awmack and Leather, 2002; Van Hezewijk et al., 2008) and may vary with phenological, herbivory (Hunter et al., 1996; Larsson et al., 2000), or stress-induced changes in host plants (Uyi et al., 2018). Weed biological control agents, because they are restricted to a single host species, are particularly vulnerable to changes in host quality, especially if the life stage that feeds on the plant is immobile or a poor disperser and cannot seek out higher-quality hosts. Variability in quality or nutrition may occur spatially, such as over soil moisture or fertilizer gradients, or even between parts of a single clone (Wheeler and Center, 1996; Spencer et al., 2005; Spencer et al., 2010). Changes in nutrition may also occur over a season or between years. For example, many plants show a decline in quality (nitrogen) with age (Scriber and Slansky Jr., 1981), a pattern which may explain

* Corresponding author at: US Army Engineer Research and Development Center, 3909 Halls Ferry Rd., Vicksburg, MS 39180, USA.
*E-mail address:* Nathan.E.Harms@usace.army.mil (N.E. Harms).

https://doi.org/10.1016/j.biocontrol.2019.05.002
Received 27 February 2019; Received in revised form 24 April 2019; Accepted 2 May 2019
Available online 03 May 2019
1049-9644/ Published by Elsevier Inc.

seasonality in some biological control successes (e.g., Coulson, 1977; Spencer et al., 2010; Harms and Shearer, 2017).

A clearer picture is emerging regarding the importance of nitrogen availability to weed biological control. Studies have shown that increased host nitrogen typically leads to improved control agent performance. For example, nitrogen amendment, through foliar application, was used to improve control efficacy of giant salvinia (*Salvinia molesta* D.S. Mitchell; Salviniaceae) in Australia and Papua New Guinea by enhancing establishment and population growth of *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae) (Room and Thomas, 1985; Room et al., 1989). Other studies have shown that variable nitrogen levels in host plants can affect density-dependent processes such as development, mortality, or dispersal (Wilson et al., 2007). However, to our knowledge the only published examination of the interaction between host nitrogen and density-dependence in an external-feeding biological control agent found that enhanced nitrogen increased development rate but not survival of *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) on water hyacinth (*Eichhornia crassipes* (Mart.) Solms; Pontederiaceae) (Wilson et al., 2007). In other systems, higher nutrient availability in the environment may differentially improve plant performance over agents such that plant compensation to herbivory under elevated nutrient conditions ultimately limits control (Coetzee and Hill, 2012). That increased nitrogen in host plants can improve agent performance has also been exploited in mass-rearing programs with the goal to produce large numbers of high quality biological control agents for release (Blossey and Hunt, 1999; Wheeler, 2001; Harms et al., 2009). Combined with an understanding of seasonal variability in reproductive status, insects reared on high nitrogen plants should demonstrate increased fecundity upon release, thus increasing likelihood of establishment (Blossey and Hunt, 1999; Van Hezewijk et al., 2008).

Biological control agents are not only exposed to hosts of varying nutritional status during development, but to varying temperatures as well. Seasonal temperature patterns can be limiting to agents if developmental thresholds are surpassed. However, the ways in which ambient temperature and host nutrition interact to influence herbivore development and population dynamics are unknown for many systems but could be important, particularly near upper and lower thermal limits of the agent. Thus the interaction between nitrogen and ambient temperature may explain why control agents persist or go regionally extinct, and may reveal reasons for changes in seasonal patterns of control. Harms and Shearer (2017) suggested that seasonal patterns in alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb; Amaranthaceae) control may have been related to the interaction between seasonal variation in plant quality and ambient temperature. Varying temperature and nitrogen levels had variable effects on performance of the water hyacinth bug, *Eccritotarsus catarinensis* Carvalho (Hemiptera: Miridae) with the largest reductions in fitness occurring at high temperatures and low nitrogen (Ismail et al., 2017).

Despite levels of control that have been achieved in the southeastern U.S., alligatorweed plants growing in terrestrial environments or in the northern region of its introduced range remain largely undamaged (Coulson, 1977; Harms and Shearer, 2017). Early investigations into variable control by the alligatorweed flea beetle, *Agasicles hygrophila* Selman and Vogt (Coleoptera: Chrysomelidae), focused primarily on structural and nutritional differences between plants growing in terrestrial or aquatic environments and led to limited examination of phosphate deficiency effects on adult feeding (Maddox and Rhyne, 1975; Coulson, 1977). However, studies of effects of nitrogen on larval development are lacking. To understand the importance of variation in host nitrogen for biological control of alligatorweed, we combined field measurements of plant nitrogen with a series of laboratory experiments. First, we collected and analyzed alligatorweed leaves from sites across Louisiana over four years to determine seasonal variation in foliar nitrogen. We then examined the effects of alligatorweed foliar nitrogen and temperature on *A. hygrophila* immature developmental rate and survival. Finally, we tested whether foliar nitrogen and larval density interact to affect larval development or dispersal from host plants. We first predicted that foliar nitrogen in alligatorweed at field sites would be highest in spring and early summer, coinciding with peak population growth of A. hygrophila (Harms and Shearer, 2017). We then predicted that increasing nitrogen levels in host plants would increase development rates and survival of *A. hygrophila* and mediate negative effects of high temperatures on larvae during laboratory experiments. Additionally, we predicted that nitrogen levels would influence larval dispersal such that plants with higher foliar nitrogen would support more larvae. We report here the variation in alligatorweed nutritional conditions potentially encountered by biological control agents in the field and multiple ways that host nutrition may influence *A. hygrophila* performance. Finally, we discuss implications of our findings for regions with poor alligatorweed control.

## 2. Materials and methods

### 2.1. Study system

*Agasicles hygrophila* was first released in the southeastern United States in 1960s to control alligatorweed and has widely been considered the world's first successful aquatic weed biological control program (Coulson, 1977; Buckingham, 1996). Initial releases of *A. hygrophila* were made in all southeastern US states, largely resulting in suppression of alligatorweed to the point where it was no longer considered a nuisance. Cofrancesco (1988) conducted a survey of southeastern infestations nearly 20 years after original biological control introductions and found minimal levels of alligatorweed in coastal areas which largely coincided with presence of control agents. However, alligatorweed infestations can be extensive in areas outside the distribution of *A. hygrophila* (e.g., in Arkansas, Tennessee, Alabama), which is thought to be primarily limited by winter temperatures (Coulson, 1977; Julien et al., 1995).

### 2.2. Experimental procedures

This work was conducted at the Department of Biological Sciences, Louisiana State University (LSU), Baton Rouge, LA and at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS. Plants for all experiments were collected from a roadside ditch in Baton Rouge, LA (N 30.35°, W 91.14°) in 2015 and propagated multiple times prior to experimentation to minimize maternal environmental effects. Plants were cultured hydroponically in either a greenhouse at LSU or indoor environmental growth chambers (27 °C, 14:10 light:dark) at the ERDC. Regardless of location, plants were grown in a series of 20-L plastic buckets filled with 15-L full-strength (200 mg/L N) Hoagland's solution (Hoagland and Arnon 1950). Nutrients were exchanged every other week and reverse osmosis water was added periodically to maintain water levels in between nutrient exchanges.

For experiments, a modified Hoagland's nutrient solution stock was created with zero nitrogen and amended with $NH_4NO_3$ to reach desired nutrient levels, maintaining constant levels of other macro- and micronutrients (P: 32 mg/L; K: 170 mg/L; Fe: 3 mg/L; Ca: 100 mg/L; Mg: 30 mg/L; S: 93 mg/L; B: 0.5 mg/L; Mo: 0.01 mg/L; Mn: 0.5 mg/L; Zn: 0.05 mg/L; Cu: 0.02 mg/L; Cl: 177 mg/L). Three final nutrient solutions were created by this technique, a low-N (2 mg/L N), medium-N (20 mg/L N), and high-N (200 mg/L N) solution. Foliar nitrogen levels generated by growing alligatorweed in these media were comparable to the range of foliar nitrogen in plants collected at field sites in Louisiana. Plants were cultured in appropriate nutrient media for at least eight weeks prior to beginning an experiment. Generally, nutrients were exchanged every two weeks for the first month of culture, then weekly prior to and during the experiment. Leaf samples were collected from cultures three times during the experiment (initial, mid-way, end of experiment) to verify foliar nitrogen levels differed among the

**Table 1**
Sites where alligatorweed leaves were collected to determine foliar nitrogen concentrations.

| Site | Lat | Long | Waterbody type | Years sampled |
|---|---|---|---|---|
| Choctaw landing | 29.85 | −90.68 | River | 2016, 2017, 2018 |
| Chevreuil Bayou | 29.91 | −90.73 | River | 2016, 2017, 2018 |
| Blind River | 30.09 | −90.78 | River | 2016, 2017, 2018 |
| Maurepas WMA | 30.15 | −90.81 | Swamp | 2016, 2017, 2018 |
| Blackwater Conservation Area | 30.54 | −91.09 | Wetland | 2016, 2017, 2018 |
| Greenwood Community Park | 30.57 | −91.17 | Pond | 2016, 2017 |
| Simmesport Pond | 30.97 | −91.81 | Pond | 2016, 2017, 2018 |
| Spring Bayou WMA | 31.14 | −92.01 | River | 2016, 2017, 2018 |
| Lake St. Joseph | 32.08 | −91.23 | Lake | 2016, 2017, 2018 |
| Bayou Macon | 32.09 | −91.56 | River | 2016, 2017 |
| Openwood Pond | 32.40 | −90.79 | Pond | 2016 |
| Poverty Point Reservoir | 32.53 | −91.49 | Reservoir / Lake | 2016, 2017, 2018 |

treatment levels and were consistent over time. Nitrogen analysis was performed by the Soil and Plant Sciences laboratory at the Agricultural Chemistry Laboratory at LSU and confirmed that we were able to induce foliar nitrogen differences in our culture plants: Low = 2.37 ± 0.06% DW N, Medium = 3.49 ± 0.16% DW N, High = 6.43 ± 0.11% DW N (ANOVA, df = 2, F = 251.33, P < 0.001).

### 2.3. Variation in foliar nitrogen at alligatorweed field locations

Field sites in Louisiana were visited every 2–3 weeks during the majority of the growing season in 2015–2018. Eleven sites were located in Louisiana and one in Mississippi (Openwood pond). Sites consisted of ponds, rivers/bayous, wildlife management area wetlands and lakes (Table 1). Approximately 10 g fresh alligatorweed leaves were collected, primarily from the third and fourth apical nodes, then combined for each site and each sample date. The sampling area within a site was limited to approximately 5 $m^2$ and the same area was sampled during each visit to minimize variation in FN stemming from variation in local conditions within a site. Leaves were dried in a forced-air oven at 60 °C then FN was determined as above for experimental plants.

### 2.4. Effects of foliar nitrogen and temperature on Agasicles hygrophila larval development

To determine the independent and interactive effects of foliar nitrogen and temperature on larval development and survival, a 3x3 factorial experiment was conducted. *Agasicles hygrophila* egg masses were field-collected from the Blind River, LA (N 30.0949°, W −90.7785°), incubated in Petri dishes sealed with parafilm at 23 °C and observed daily for hatching. Once sufficient neonates were hatched (within a 24hr period), the experiment was initiated. A single larva was placed in a 30 ml plastic cup on a leaf of varying foliar nitrogen (Low, Medium, High) then sealed with a plastic lid before placing at one of three temperatures (23, 26, 30 °C). Temperatures were chosen to be within the optimal (23–25 °C) and upper (30 °C) range suitable for development of *A. hygrophila* (Stewart et al., 1999) which corresponds to spring and summer temperatures in the southern US range. A total of 20 flea beetles per treatment combination was used. Temperatures and photoperiod (14:10 light:dark) were maintained within controlled-growth chambers until adult emergence. Cups were monitored daily for insect survival, pupation, and adult emergence. Leaves were replaced every two days such that the beetles were never resource limited. Dates of pupation and adult emergence were recorded and used to determine development duration. Dead larvae and pupae were discarded and survival was determined for larvae, pupae, and overall. Additionally, we sexed and weighed newly-eclosed adults within 24 h to determine whether nitrogen, temperature, or sex affected adult mass. In particular, since the adult stage is the long-distance dispersing stage, sex and size of the dispersing individual may affect spread rate and distance of *A. hygrophila* through flight strength or fecundity (Dingle et al., 1980; Berger et al., 2008).

### 2.5. Effects of foliar nitrogen and larval density on development and dispersal

Density-dependence in *A. hygrophila* development rate was determined for two foliar nitrogen levels (2,200 mg/L N). Larval densities of 1, 5, or 20 larvae $plant^{-1}$ were chosen to be representative of average and maximum values observed at field sites during the growing season (Harms and Shearer, 2017). Beginning six weeks prior to the experiment, plants were cultured in appropriate nutrient conditions as described above. One week before the experiment began, approximate 10-cm-long plant fragments were collected from greenhouse cultures and placed individually in nutrient solution to root. Once roots were observed, plant fresh weight (g) was obtained and they were planted in 1000 ml tubs with 200 ml fine sand and 200 ml nutrient solution (as described above). Egg masses were collected three days before the experiment from the Blind River, LA, incubated as previously described, and observed for hatching. Neonates (< 1 day old) were placed in experimental containers at an abundance of one, five, or twenty larvae per plant. Experimental containers were covered in fine mesh (~200 μm), and placed in a Conviron environmental growth chamber at 23 °C, a temperature determined during the first experiment to produce high survival (see below). Photoperiod within chambers was set to 14:10 (light:dark). There were eight replicate experimental units per treatment combination.

The density-dependence experiment lasted seven days. Larvae were collected and separated into those that were on the plant and those that were off the plant (i.e., were collected from the sides or roof of the container), counted and removed from containers to be weighed. Proportion of larvae remaining on the plant was determined.

### 2.6. Statistical approach

To analyze variation in alligatorweed FN at field locations, a polynomial regression was fit for each year of the study to FN values for all study locations and dates. For the first experiment, we used two-way ANOVA to test for effects of temperature and nitrogen on *A. hygrophila* development time and adult biomass. To test for differences in survival, we used a generalized linear model with a binomial distribution of the response variable (survived or died) and logit link function (Bolker et al., 2009). Temperature (T; 23, 26, 30C), foliar nitrogen (FN; Low, Medium, High), and their interaction (T*FN) were independent variables in these models. Likewise, two-way ANOVAs were used for the experiment on the effects of conspecific density and FN on larval fresh mass and the proportion of dispersing larvae. Density (D; 1, 5, 20 larvae per plant), FN (low, high), and their interaction (D*FN) were independent variables. Proportion dispersing was arcsine square-root transformed (Ellison and Gotelli, 2004) and larval fresh weight was ln-

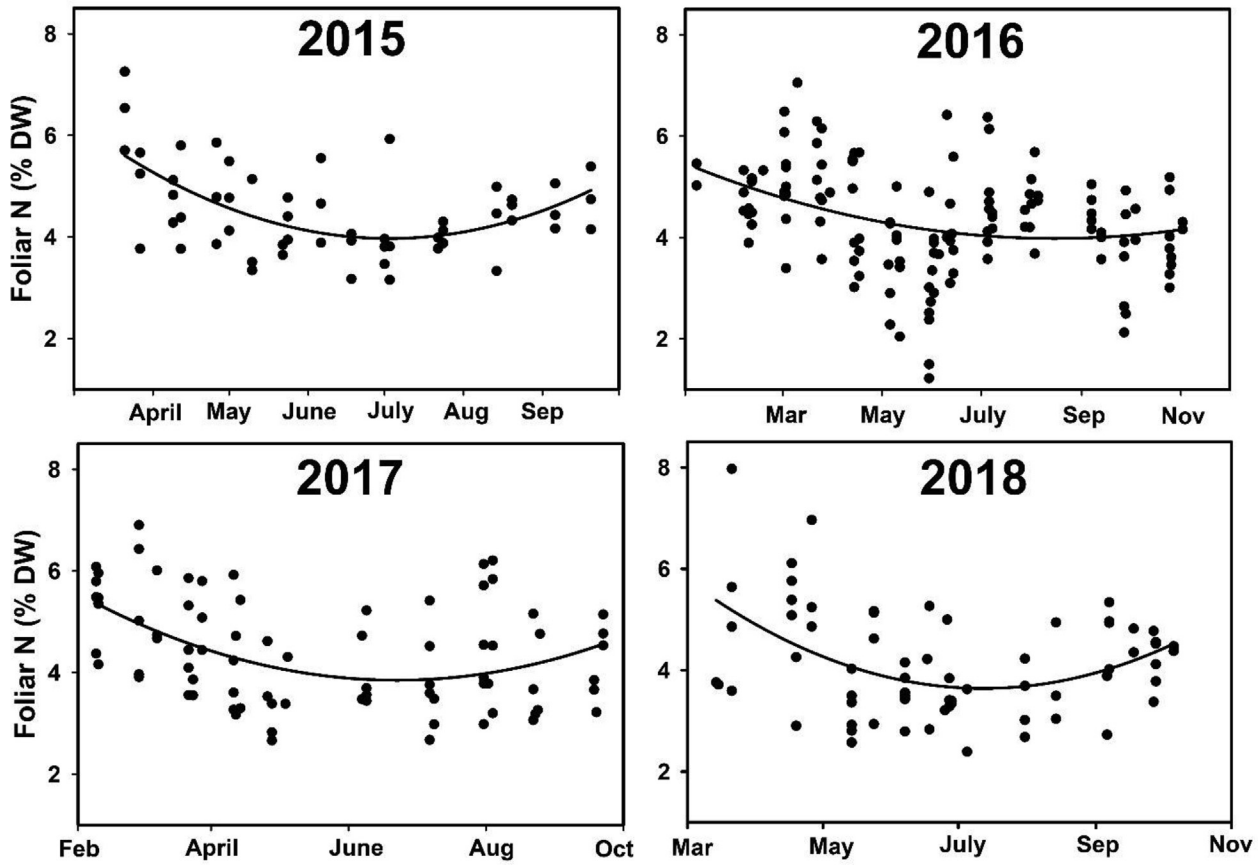


**Fig. 1.** Seasonal variation in foliar nitrogen at alligatorweed field sites in Louisiana during 2015–2018. Solid lines are best-fit curves determined by nonlinear regression (2015: FN = 269,581 + −12.8x + 0.0002x$^2$, $P < 0.001$, $R^2 = 0.33$; 2016: FN = 51,938 + -10.4x + 0.0001x$^2$, $P = 0.002$, $R^2 = 0.18$; 2017: FN = 156,454 + −7.3x + 0.00008x$^2$, $P < 0.001$, $R^2 = 0.21$; 2018: FN = 225207 + −10.40x + 0.0001x$^2$, $P = 0.002$, $R^2 = 0.18$). Points are FN values for each site and date.

transformed to achieve normality and homogeneity of variances. For each ANOVA, post-hoc mean separation was determined by Tukey HSD test. Statistical tests were performed using SigmaPlot, version 12.3 (Systat Software, Inc., San Jose CA) or Statistica, version 12 (Stat Soft, Inc., Tulsa OK).

## 3. Results

### 3.1. Variation in foliar nitrogen at alligatorweed field locations

Alligatorweed FN varied between sites, seasonally, and between years but seasonal patterns were consistent each year (Fig. 1). Foliar nitrogen peaked during the spring, declined during the summer, and then increased slightly during the late summer/fall. On several occasions (e.g., Blind River, LA on March 21, 2018), FN values as high as 8% DW were recorded. Of the five largest FN values, all occurred from February to April. The amount of variation in FN between locations was considerable on some census dates. For example, during the first census of 2018, FN ranged from 3.7 to 7.9% DW (Fig. 1). However, there was no seasonal pattern in the level of variation observed. Although we did not sample sediment or water nutrients for this study, the differences in FN between sites was most likely related to local nutrient conditions.

### 3.2. Effects of foliar nitrogen and temperature on Agasicles hygrophila larval development

Developmental duration of *A. hygrophila* ranged from 14 ± 0.3 days at high N and high temperature (30 °C) to 23 ± 0.5 days at low N and low temperature (23 °C), and was shortened by both increased FN and temperature (Fig. 2; Table 2). Increasing FN from low to high decreased development duration by 16%, regardless of temperature. In the high FN treatment, developmental duration was shortened by 28% from 23 °C to 30 °C. Although there was no significant interaction between treatments detected for survival, survival increased by 40% from low (57%) to high (80%) FN and decreased by 44% with increasing temperatures from 23 °C (85%) to 30 °C (49%) (Fig. 2). Adult mass increased by 11% from low (0.0060 ± 0.0002 g) to high (0.0066 ± 0.0002 g) FN but decreased 15% from low (0.0068 ± 0.0002 g) to high (0.0058 ± 0.0003 g) temperature.

### 3.3. Effects of foliar nitrogen and larval density on development

Larval dispersal was zero in the low density (1 larva per plant) treatments (Fig. 3). As such, we statistically compared only the medium (5 larvae per plant) and high (20 larvae per plant) treatments. Excluding the low density treatment, dispersal was not significantly different between medium and high densities or FN (Table 2; Fig. 2A), despite a two-fold increase in mean dispersal between high and low FN plants at intermediate density (0.51 ± 0.11 vs 0.23 ± 0.12). At high (20 larvae/plant) larval density, dispersal was high (0.48 – 0.58 of larvae), regardless of nitrogen level. Differences in larval fresh mass were due to both density and foliar nitrogen (Fig. 3B). Increased foliar nitrogen in leaves led to 23% larger larvae, and larval biomass was highest (0.0084 ± 0.0005 g) at intermediate density. Interestingly, FN only had a significant effect on larval biomass at high densities; larval size was 64% larger in high (0.0068 ± 0.00065 g) than low (0.0041 ± 0.00065 g) FN.

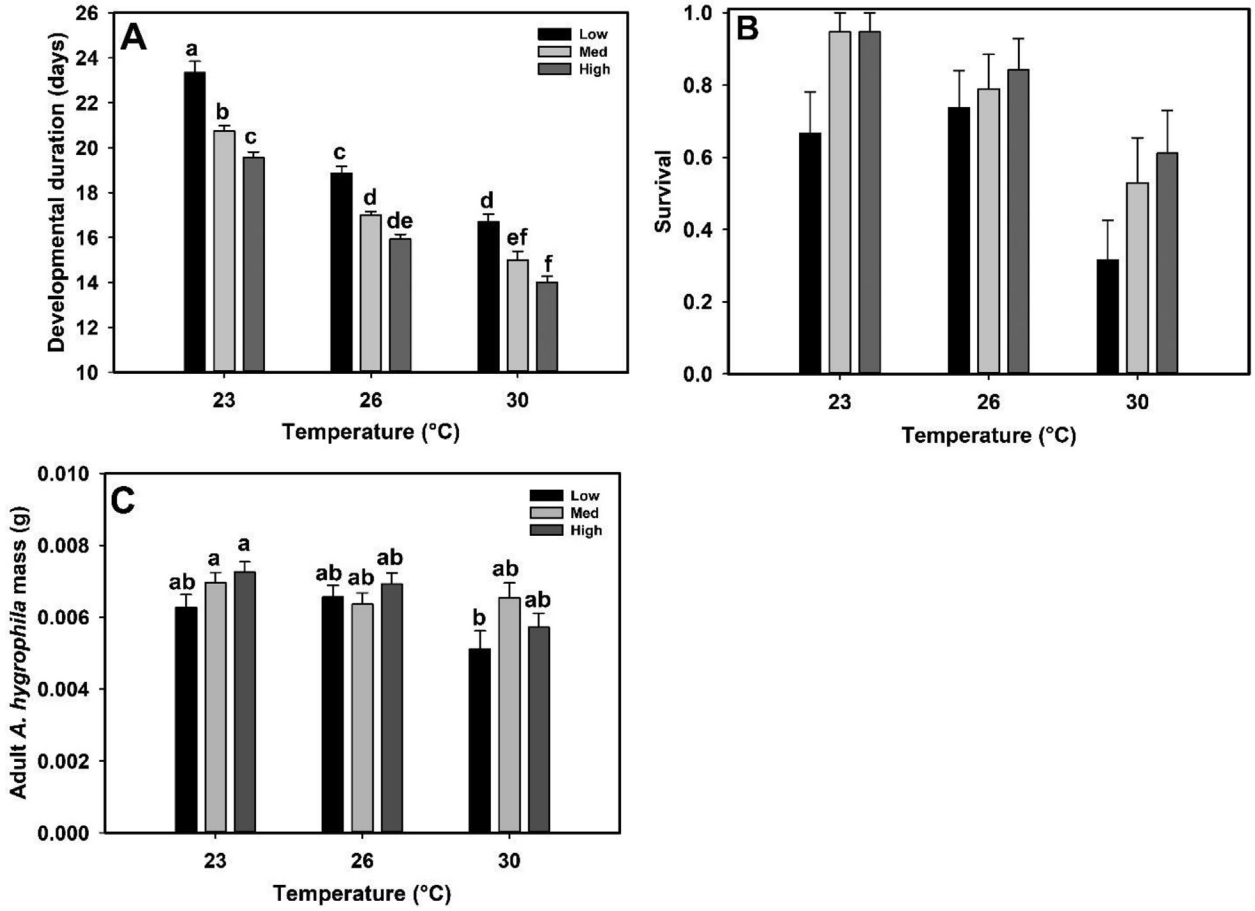


**Fig. 2.** Mean ( ± SE) developmental duration from egg hatch to adult (A) survival (B), and adult mass (C) for *A. hygrophila* at combinations of foliar nitrogen and temperature. Low = 2 mg/L N, Med = 20 mg/L N, High = 200 mg/L N. Letters above error bars indicate Tukey's significantly different means for ANOVA tests.

**Table 2**
Results of statistical tests to determine differences in various larval traits caused by a combination of temperature (T) and foliar nitrogen (FN) (left) or density and foliar nitrogen (right) on *Agasicles hygrophila* during two experiments.

| Temperature and nitrogen experiment | | | | | Density and nitrogen experiment | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Adult mass | Effect | df | F | *P* | Larval mass | Effect | df | F | *P* |
| | T | 2 | 6.76 | 0.002 | | D | 2 | 9.90 | < 0.001 |
| | FN | 2 | 3.15 | 0.047 | | FN | 1 | 9.00 | 0.005 |
| | T*FN | 4 | 1.49 | 0.21 | | D*FN | 2 | 0.16 | 0.19 |
| Developmental duration | Effect | df | F | *P* | Dispersal | Effect | df | F | *P* |
| | T | 2 | 292.16 | < 0.001 | | D | 1 | 1.03 | 0.32 |
| | FN | 2 | 77.36 | < 0.001 | | FN | 1 | 0.22 | 0.64 |
| | T*FN | 4 | 0.99 | 0.47 | | D*FN | 1 | 1.64 | 0.21 |
| Survival | Effect | df | *z* | *P* | | | | | |
| | T | 2 | 19.2 | < 0.001 | | | | | |
| | FN | 2 | 8.8 | 0.012 | | | | | |
| | T*FN | 4 | 2.5 | 0.64 | | | | | |

## 4. Discussion

Among a growing list of studies for other targets of weed biological control (e.g., Room et al., 1989; Center and Dray Jr., 2010; Coetzee and Hill, 2012; Ismail et al., 2017), our study is the first to show that alligatorweed varies spatially and temporally in FN and that this variation has important fitness and life history consequences for its biological control agent, *A. hygrophila*. Plant nitrogen effects on insect herbivores can be significant, with positive effects on larval and adult physiology and behavior. This may explain variation in the population dynamics of some biological control agents in the field in relation to spatial or temporal (i.e., seasonal) variation in host plant quality (Mattson Jr., 1980). For instance, *A. hygrophila* overwintering on host plants (southern USA sites) generally follow the pattern of rapid population increase early in the year (i.e., spring) when temperatures are mild (Harms and Shearer, 2017) and host plant nutrition is high (the current study). However, individuals which disperse from low to high latitude sites arrive during a time when temperature may be limiting (i.e., summer) or host plant quality is insufficient to support population growth (Harms and Shearer, 2017), thus providing inadequate control in those areas. We found independent but not interactive effects of temperature and host nitrogen on developmental duration or survival,

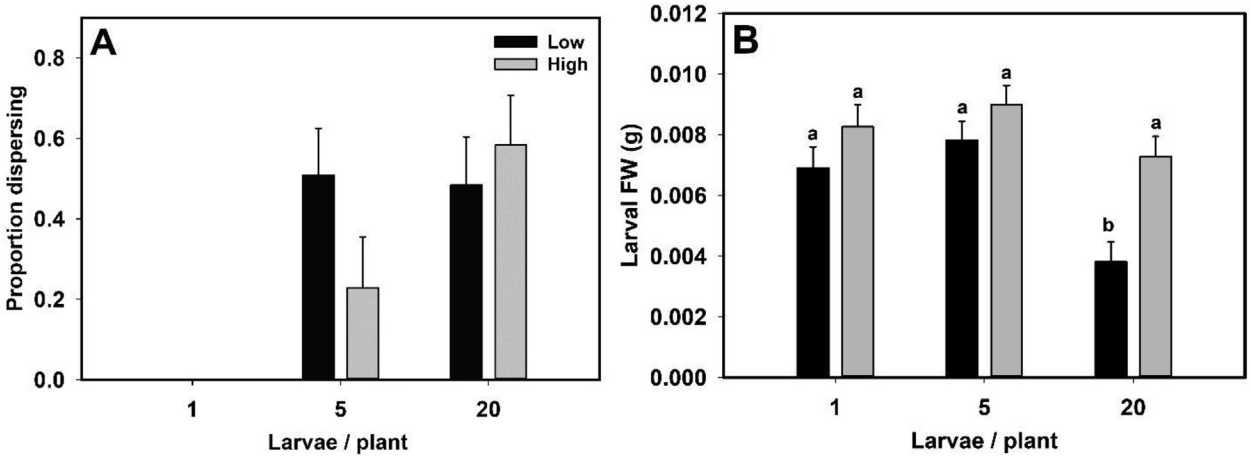


**Fig. 3.** A) Mean ( ± SE) proportion of *A. hygrophila* larvae dispersed and B) larval fresh weight, relative to larval density and foliar nitrogen. The lowest larval density (one larva plant$^{-1}$) is not visible in A) because there was zero dispersal from plants in all replicates. Letters above error bars indicate Tukey's significantly different means.

which is in contrast with other studies that have examined the effects of the interaction on biological control agents (e.g., Ismail et al., 2017). However, variability of the nitrogen effect on survival was ten times higher at low and high than medium temperature ($\sigma^2_{survival}$ = 0.02 at 23° and 30 °C, $\sigma^2_{survival}$ = 0.002 at 26 °C). We anticipated that negative effects of high temperature stress on immature development would be disproportionately reduced by increasing foliar nitrogen, but the positive effect of nitrogen was consistent across all temperatures (Fig. 2). This could be in part due to the range of temperatures we chose for our experiment. If had we chosen a wider range of temperatures (i.e., those closer to developmental thresholds of *A. hygrophila*), we may have detected interactions between temperature and FN in development and survival. However, survival of larvae in the 30 °C, low FN treatment (30%) was similar to that reported by Stewart et al. (1999) (20%) at the same temperature. During our study, the effect of FN on survival at 30 °C was significant by a factor of two (30% at low FN and 60% at high FN) and underlines the importance of including plant quality measurements in baseline biology studies of weed biological control agents. The range of FN we used for developmental studies was within the range observed at field sites during our surveys (Fig. 1). In fact, FN in our high treatment was less than that recorded from plants in the field, suggesting that the importance of FN for *A. hygrophila* development and survival, and ultimately population growth, may be greater in the field than we've estimated.

Local variability in nutrients available to host plants may lead to control in some parts of the site but not others. Although research into nutritional deficiencies has been conducted to address alligatorweed inter-plant variability in attack rate by *A. hygrophila*, past examinations focused on feeding attraction and adult consumption of plants, mostly ignoring importance of nitrogen to larvae (Maddox and McCready, 1975; Maddox and Rhyne, 1975). Levels of nitrogen are often higher in young, newly-developing leaves of many species (Mattson Jr., 1980). Regrowth of alligatorweed following a defoliation event is likely to be high in nitrogen and nutritious for *A. hygrophila* populations. Regrowth tissues on alligatorweed plants treated with herbicides were attacked by *A. hygrophila* at higher rates than untreated plants, presumably due to high nutritional content of young leaves (Coulson, 1977). This positive feedback may only support *A. hygrophila* populations for a limited time though, until either plants no longer have regenerative capacity due to depleted carbohydrate reserves or summer temperatures become limiting on agents. However, quality of alligatorweed regrowth following defoliation has received some attention with regards to the induction of increased chemical and physical defenses in response to feeding by *A. hygrophila* or other herbivores (Liu et al., 2018; Yu and Fan, 2018). An examination of induced defensive responses to larval feeding, especially as they may be influenced by environmental heterogeneity (e.g., available nitrogen or ambient temperatures), would further enhance our understanding of the complex spatial and temporal interactions between agents and hosts.

Regional variability in nutrition/quality of plants may also be a factor influencing dispersal activity from low to high latitude sites since typical spring defoliation in low latitude sites may lead to temporarily reduced-quality plants. Larvae fed on high nitrogen plants accumulated more mass (experiment #2) and became larger adults (experiment #1). Adult insect size and flight ability are often correlated (Dingle et al., 1980; Kaufmann et al., 2013) but whether body size in *A. hygrophila* is related to long-distance dispersal ability has not been studied despite the well-known occurrence of annual long-distance dispersal into areas outside the overwintering zone of *A. hygrophila* (Coulson, 1977; Buckingham, 1996; Harms and Shearer, 2015). We did not measure dispersal in adult beetles, but it may be valuable to determine whether larval conditions (i.e., FN and conspecific density) contribute to the likelihood and distance of adult dispersal after emergence. If larger individuals made up the majority of dispersers, then collection and comparison of adults between low and high latitude sites soon after their arrival should yield body size differences between the two, with high latitude sites harboring larger individuals than low latitude sites. Although larvae are not the primary dispersal stage, we demonstrated the propensity for dispersal once larval density increases above one individual per plant, but the dispersal likelihood was reduced by half under moderate larval density, on high nitrogen plants. Thus, seasonal variation in foliar nitrogen of alligatorweed at field sites may lead to associated variation in density dependent dispersal of *A. hygrophila*, independent of other environmental variables such as temperature or predation.

Spatial and temporal variability in FN may generate inconsistent patterns of control, even in areas where biological control is thought likely to succeed. For example, control of the floating aquatic weed, giant salvinia (*Salvinia molesta*) has been shown to be highly contingent on nutritional quality of the plant when establishing biological control agents (Room and Thomas, 1985). This may be particularly important to consider when introducing *A. hygrophila* into areas where they do not overwinter (e.g. Arkansas, Tennessee), and time their introduction to coincide with high plant quality. Incidentally, the time of year (spring; Fig. 1) when plant quality is highest coincides with mild temperatures and may provide optimal chance of establishment and control in those areas. Future experiments could be designed to test the importance of nutrition and temperature on establishment of *A. hygrophila* by manipulating the timing of agent release at northern sites, documenting plant nitrogen, ambient temperatures, establishment success, and

ultimately reduction in infestation level.

## Author contribution

NH and JC conceived the experiments, NH collected the data, analyzed the data, and prepared the manuscript with input from JC.

## Acknowledgements

The authors would like to thank the following for assistance in the laboratory or field: Stacey Springfield, Tri Tran, Andrew Flick, Rachel Harman, Warwick Allen, David Lattuca, Judy Shearer, and Julie Nachtrieb. Thanks also extend to Rodrigo Diaz, Gary Dick, Julie Nachtrieb, the editor, and two anonymous reviewers for comments and suggestions to improve this manuscript. This work was conducted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Louisiana State University (NEH), and supported by the US Army Engineer Research and Development Center Aquatic Plant Control Research Program. The funding source had no role in study design, writing the manuscript, or decision to submit to Biological Control. Citation of trade names does not constitute endorsement or approval of the use of such commercial products.

## References

Awmack, C.S., Leather, S.R., 2002. Host plant quality and fecundity in herbivorous insects. Ann. Rev. Entomol. 47, 817–844.
Berger, D., Walters, R., Gotthard, K., 2008. What limits insect fecundity? Body size- and temperature-dependent egg maturation and oviposition in a butterfly. Funct. Ecol. 22, 523–529.
Blossey, B., Hunt, T.R., 1999. Mass rearing methods for *Galerucella calmariensis* and *G. pusilla* (Coleoptera: Chrysomelidae), biological control agents of *Lythrum salicaria* (Lythraceae). J. Econ. Entomol. 92, 325–334.
Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.-S.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24, 127–135.
Buckingham, G.R., 1996. Biological control of alligatorweed, *Alternanthera philoxeroides*, the world's first aquatic weed success story. Castanea 61, 232–243.
Center, T.D., Dray Jr., F.A., 2010. Bottom-up control of water hyacinth weevil populations: do the plants regulate the insects? J. Appl. Ecol. 47, 329–337.
Coetzee, J.A., Hill, M.P., 2012. The role of eutrophication in the biological control of water hyacinth, *Eichhornia crassipes*, in South Africa. Biocontrol 57, 247–261.
Cofrancesco, A.F., 1988. Alligatorweed survey of ten southern states. Department of the Army, Waterways Experiment Station, Corps of Engineers, Environmental Laboratory.
Coulson, J.R., 1977. Biological control of alligatorweed, 1959-1972: a review and evaluation. Department of Agriculture, Agricultural Research Service.
Denno, R., McClure, M., 1983. Variable Plants and Herbivores in Natural and Managed Systems. Academic Press.
Dingle, H., Blakley, N.R., Miller, E.R., 1980. Variation in body size and flight performance in milkweed bugs (*Oncopeltus*). Evolution 34, 371–385.
Ellison, G.N., Gotelli, N., 2004. A Primer of Ecological Statistics. Sinauer, Sunderland, Massachusetts, USA.
Harms, N., Grodowitz, M.J., Nachtrieb, J.G., 2009. Mass-rearing Cyrtobagous salviniae Calder and Sands for the management of Salvinia molesta Mitchell. US Army Engineer Research and Development Center.
Harms, N.E., J.F. Shearer, 2015. Apparent herbivory and indigenous pathogens of invasive flowering rush (Butomus umbellatus L.) in the Pacific Northwest. US Army Corps of Engineers Engineer Research and Development Center, Vicksburg, MS.
Harms, N.E., Shearer, J.F., 2017. Early-season dynamics of alligatorweed biological control by *Agasicles hygrophila* in Louisiana and Mississippi. J. Aquatic Plant Manage. 55, 89–95.
Helms, S.E., Hunter, M.D., 2005. Variation in plant quality and the population dynamics of herbivores: there is nothing average about aphids. Oecologia 145, 196–203.
Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without soil. Circular. California Agricultural Experiment Station 347.
Hunter, M.D., Malcolm, S.B., Hartley, S.E., 1996. Population-level variation in plant secondary chemistry, and the population biology of herbivores. Chemoecology 7, 45–56.
Ismail, M., Compton, S.G., Brooks, M., 2017. Interaction between temperature and water nutrient levels on the fitness of *Eccritotarsus catarinensis* (Hemiptera: Miridae), a biological control agent of water hyacinth. Biol. Control 106, 83–88.
Julien, M.H., Skarratt, B., Maywald, G., 1995. Potential geographical distribution of alligator weed and its biological control by *Agasicles hygrophila*. J. Aquatic Plant Manage. 33, 55–60.
Kaufmann, C., Reim, C., Blanckenhorn, W.U., 2013. Size-dependent insect flight energetics at different sugar supplies. Biol. J. Linn. Soc. 108, 565–578.
Larsson, S., Ekbom, B., Björkman, C., 2000. Influence of plant quality on pine sawfly population dynamics. Oikos 89, 440–450.
Liu, M., Zhou, F., Pan, X., Zhang, Z., Traw, M.B., Li, B., 2018. Specificity of herbivore-induced responses in an invasive species, *Alternanthera philoxeroides* (alligator weed). Ecol. Evol. 8, 59–70.
Maddox, D., McCready, R., 1975. Preliminary studies of the effects of an induced phosphate deficiency on carbohydrate-nitrogen ratios in Alternanthera philoxeroides [Centrospermae: Amaranthacea] and on the feeding of Agasicles hygrophila [Col.: Chrysomelidae]. Biocontrol 20, 409–419.
Maddox, D., Rhyne, M., 1975. Effects of induced host-plant mineral deficiencies on attraction, feeding, and fecundity of the alligatorweed flea beetle. Environ. Entomol. 4, 682–686.
Mattson Jr, W.J., 1980. Herbivory in relation to plant nitrogen content. Annu. Rev. Ecol. Syst. 11, 119–161.
Room, P., Julien, M., Forno, I., 1989. Vigorous plants suffer most from herbivores: latitude, nitrogen and biological control of the weed *Salvinia molesta*. Oikos 92–100.
Room, P.M., Thomas, P.A., 1985. Nitrogen and establishment of a beetle for biological control of the floating weed salvinia in Papua New Guinea. J. Appl. Ecol. 22, 139–156.
Scriber, J., Slansky Jr., F., 1981. The nutritional ecology of immature insects. Annu. Rev. Entomol. 26, 183–211.
Spencer, D.F., Ksander, G.G., Whitehand, L.C., 2005. Spatial and temporal variation in RGR and leaf quality of a clonal riparian plant: *Arundo donax*. Aquat. Bot. 81, 27–36.
Spencer, D.F., Tan, W., Whitehand, L.C., 2010. Variation in *Arundo donax* stem and leaf strength: implications for herbivory. Aquat. Bot. 93, 75–82.
Stewart, C.A., Chapman, R.B., Emberson, R.M., Syrett, P., Frampton, C.M.A., 1999. The effect of temperature on the development and survival of *Agasicles hygrophila* Selman & Vogt (Coleoptera: Chrysomelidae), a biological control agent for alligator weed (*Alternanthera philoxeroides*). N. Z. J. Zool. 26, 11–20.
Uyi, O.O., Zachariades, C., Heshula, L.U., Hill, M.P., 2018. Developmental and reproductive performance of a specialist herbivore depend on seasonality of, and light conditions experienced by, the host plant. PLoS One 13, e0190700.
Van Hezewijk, B.H., De Clerck-Floate, R.A., Moyer, J.R., 2008. Effect of nitrogen on the preference and performance of a biological control agent for an invasive plant. Biol. Control 46, 332–340.
Wheeler, G., Center, T., 1996. The influence of hydrilla leaf quality on larval growth and development of the biological control agent *Hydrellia pakistanae* (Diptera: Ephydridae). Biol. Control 7, 1–9.
Wheeler, G.S., 2001. Host plant quality factors that influence the growth and development of *Oxyops vitiosa*, a biological control agent of *Melaleuca quinquenervia*. Biol. Control 22, 256–264.
Wilson, J.R.U., Rees, M., Ajuonu, O., 2007. 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*. Bull. Entomol. Res. 96, 145–152.
Yu, H., Fan, S., 2018. Differences in physiological traits and resistances of *Alternanthera philoxeroides* after herbivory by generalists and specialists. Aquat. Ecol. 52, 323–332.