

Early-season dynamics of alligatorweed biological control by *Agasicles hygrophila* in Louisiana and Mississippi

NATHAN E. HARMS AND JUDY F. SHEARER*

ABSTRACT

Timing of management can be critical to long-term biological control of weeds, but may vary by location with arrival (or releases) of agents during times when conditions are unsuitable for agent population development. We investigated, during spring and summer, the timing of occurrence and intensity of damage (percentage of leaf area consumed) caused by the biological control agent alligatorweed flea beetle (*Agasicles hygrophila* Selman and Vogt) on alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.] at two sites in southern Louisiana and two sites in northern Louisiana/central Mississippi. Alligatorweed flea beetle population peaks were documented at southern sites in May (7.6 ± 3.45 insects stem⁻¹) and June (3.35 ± 0.25 insects stem⁻¹). Mean leaf damage at southern sites was $21 \pm 2\%$ and maximum leaf damage was 76%, which coincided with the first peak. At northern sites, where annual recolonization must occur because of overwintering limitations, alligatorweed flea beetles first appeared later in the season (June) and insect populations never formed a distinct peak or reached similar abundances to the southern sites. Mean leaf damage at northern sites was $7 \pm 3\%$ and maximum leaf damage was 25%. Alligatorweed in northern sites, subjected to less herbivory, maintained a positive relative growth rate (RGR) of 0.012 ± 0.016 (mm² leaf area [m² water surface area]⁻¹ day⁻¹) during the study period, whereas alligatorweed in southern sites, subjected to early-season damage, had a RGR of -0.027 ± 0.035 . Overall, insect abundance was positively associated with percentage of leaf damage ($R^2 = 0.56$) and negatively associated with RGR ($R^2 = 0.58$). Our data support the notion that an early-season herbivore peak, associated with near total defoliation, was responsible for the reduced RGR and suppression of alligatorweed at sites within the southern range of the alligatorweed flea beetle. Northern sites experienced delayed and lower impact from the insects. Although previous authors have suggested the importance of this early-season phenomenon, this study represents the first quantification of early-season alligator-

weed flea beetle population dynamics and associated plant impacts in the southern United States.

Key words: aquatic weed, biological control, herbivore-plant interactions, management timing, population dynamics, seasonal ecology.

INTRODUCTION

Timing of pest management is important for success in both agricultural and natural systems (Paynter 2003, Carisse and Rolland 2004). In integrated pest management (IPM) programs of weeds, application of management (e.g., herbicides, prescribed burns) may be timed to maximize impact on the target weed population by exploiting particularly vulnerable host phenological stages (McAllister and Haderlie 1985, Pesacreta and Luu 1988, Luu and Getsinger 1990, Owens and Madsen 1998) or coincide with periods of low natural enemy activity, abundance, or susceptibility (Chi 1990, Newman et al. 1998, Mudge et al. 2013). In biological control, timing of agent releases may occur during a period thought to be conducive to establishment, such as when weather is mild or the chance for disturbance is low (Norris et al. 2002, Van Driesche et al. 2008). Some agents benefit from releases during periods when their host is in the appropriate phenological stage, such as during flowering or seed production (e.g., multiple agents of yellow starthistle, *Centaurea solstitialis* L.; Wilson et al. 2003). However, to date, most discussion about optimal release methods centers on the number of releases and quantities of released agents rather than timing of releases (Memmott et al. 1996, Memmott 1998, Grevstad 1999, Shea and Possingham 2000, Grevstad et al. 2011).

Although the timing of impacts may be important for efficacy of biological control, there are few examples in the literature that discuss the effects of damage timing on the host or the importance of release timing for establishment of the agent. Because phenological events are often related to seasonal variables (resource availability, temperature, rainfall, photoperiod, etc.), understanding the importance of damage timing may aid biological control practitioners in developing release or monitoring schedules. A prime example in which the importance of seasonal impacts has been suggested is that of alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.] (Amaranthaceae) and its primary biological control agent, the alligatorweed flea beetle (*Agasicles hygrophila* Selman and Vogt) (Coleoptera: Chrysomelidae) (Coulson 1977, Spencer and Coulson 1976, Vogt et al. 1992).

*First author: Research Biologist, U.S. Army Engineer Research and Development Center, Vicksburg, MS 39180 and Graduate Student, Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70810. Second author: Research Plant Pathologist, U.S. Army Engineer Research and Development Center, Vicksburg, MS 39180. Corresponding author's E-mail: Nathan.E.Harms@usace.army.mil. Received for publication August 17, 2016 and in revised form March 2, 2017.

Alligatorweed is a semiaquatic perennial weed species present throughout the southeastern United States (USDA NRCS 2015). A biological control program for alligatorweed was initiated in the 1950s and '60s and produced three host-specific agents, of which the alligatorweed flea beetle received the most attention because of the severe and rapid defoliation caused by the agent (Buckingham 1996). Although alligatorweed is found from the Gulf Coast north into Arkansas (USDA NRCS), it is thought that the alligatorweed flea beetle only controls infestations in areas where average winter temperatures remain above 10 to 11.1 C (Coulson 1977, Julien et al. 1995) and herbivore populations are able to overwinter and peak early in the subsequent growing season (Spencer and Coulson 1976, Coulson 1977). In areas outside the overwintering climate of the alligatorweed flea beetle, insects must immigrate annually to recolonize alligatorweed (Vogt et. al 1992). It has mostly been considered that in these outer areas two other introduced agents (*Arcola malloi* Pastrana and *Amynothrips andersonii* O'Neill) are tolerant to low temperatures and provide more control than the alligatorweed flea beetle (Spencer and Coulson 1976, Vogt et. al 1992). This system offers the opportunity to study an agent (alligatorweed flea beetle) in which 1) timing of attack has been suggested to be important for control and 2) variable attack timing is displayed because of overwintering limits and the need to recolonize sites in its northern range (Spencer and Coulson 1976, Coulson 1977, Julien et al. 1995, Stewart et al. 1999). Whether variability of control by the flea beetle is primarily a function of overwintering, phenology of alligatorweed in different locations, or a combination of the two is not fully understood.

The objective of the current study was to quantify the early-season timing and intensity of feeding by the alligatorweed flea beetle at sites in southern Louisiana and northern Louisiana/central Mississippi. Our prediction was that alligatorweed-alligatorweed flea beetle dynamics would follow a pattern of early-season, high-intensity damage (insect populations would peak once with corresponding intensity of plant damage) at southern sites whereas northern sites would display the same pattern but with a lag due to the time required for immigrating individuals to arrive, establish, and build new populations (Coulson 1977). We provide here documentation of alligatorweed-alligatorweed flea beetle early-season population dynamics.

MATERIALS AND METHODS

Site selection

Overwintering of the alligatorweed flea beetle is reported to occur where mean winter air temperature stays above 10 to 11.1 C so we chose our study sites with that in mind. Four sites were chosen, two in southern areas where the alligatorweed flea beetle should successfully overwinter and two northern sites that occur beyond the overwintering range such that annual recolonization through immigration would be required (Figure 1). Mean daily temperatures of the preceding winter (from 1 November 2014 until 1 March 2015) were calculated for each site using daily temperature

data obtained from an online database (see Weather Underground 2016), then compared to published estimates of climatic limits of the alligatorweed flea beetle. Northern sites had a mean daily winter temperature of 8.6 C whereas southern sites had a mean daily winter temperature of 11.6 C during winter 2014 to 2015. This temperature information, combined with past qualitative observations of alligatorweed flea beetle presence (unpubl. data) at these sites, met our selection criteria. Northern sites were Lake St. Joseph, Louisiana (32°4'39"N, 91°13'58"W) and Openwood Lake, Mississippi (32°23'46"N, 90°47'37"W) and southern sites were Maurepas Wildlife Management Area, Louisiana (30°8'59"N, 90°48'24"W) and Blind River, Louisiana (30°5'42"N, 90°46'43"W) (Figure 1). Sites consisted of lakes (Openwood, St. Joseph), a wetland (Maurepas), and a river (Blind River). Alligatorweed abundance and coverage at sites was visually assessed prior to the study and determined to be similar among sites. Within sites, we limited our study areas to approximately 5-m² patches. Samples were collected within 1.5 m of the shore and in water < 1m deep.

Sites were visited every 3 wk from March 2015 until the presence of the alligatorweed flea beetle was documented to assess abundance of flea beetles and associated herbivore impact on alligatorweed. Thereafter, sites were visited at 2-wk intervals (\pm 2 d) with the exception of July and August sampling which was done at 3wk intervals for both southern and northern sites. Our study period lasted from March until September at southern sites and March until August at northern sites. We chose these study dates because our intention was to capture early growing-season dynamics. Sampling intervals corresponded with first insect presence, so northern and southern sample schedules were different for most of the study.

Alligatorweed flea beetle population dynamics

At each census date, 10 plants per site were examined *in situ* and the number of larvae and adult alligatorweed flea beetles were recorded. In order to obtain an accurate count, care was taken not to disturb insects during examination. Insect abundance is reported both on a per-stem and per-square-meter basis. To calculate insects per square meter we multiplied the number of insects per stem by the number of stems in a square meter on that date. In addition to larval and adult abundances, alligatorweed flea beetle egg masses per stem were counted on each date. The first recorded presence of egg masses, larvae, or adults at sites was used to estimate the timing of insect arrival within the 2- to 3-wk window between censuses.

Two additional biological control agents (*Arcola malloi* and *Amynothrips andersonii*) are present in the southern United States, so we documented evidence of their presence or absence during our study by observing associated damage (collapsed stems with leaves attached indicating *Arcola malloi*, or curled apical leaves, *Amynothrips andersonii* [Van Driesche et al. 2002]) and following up by focused searches upon observation of damage. Although we observed these agents in the field, their presence at our sites during this study was rare so we have omitted them from discussion.

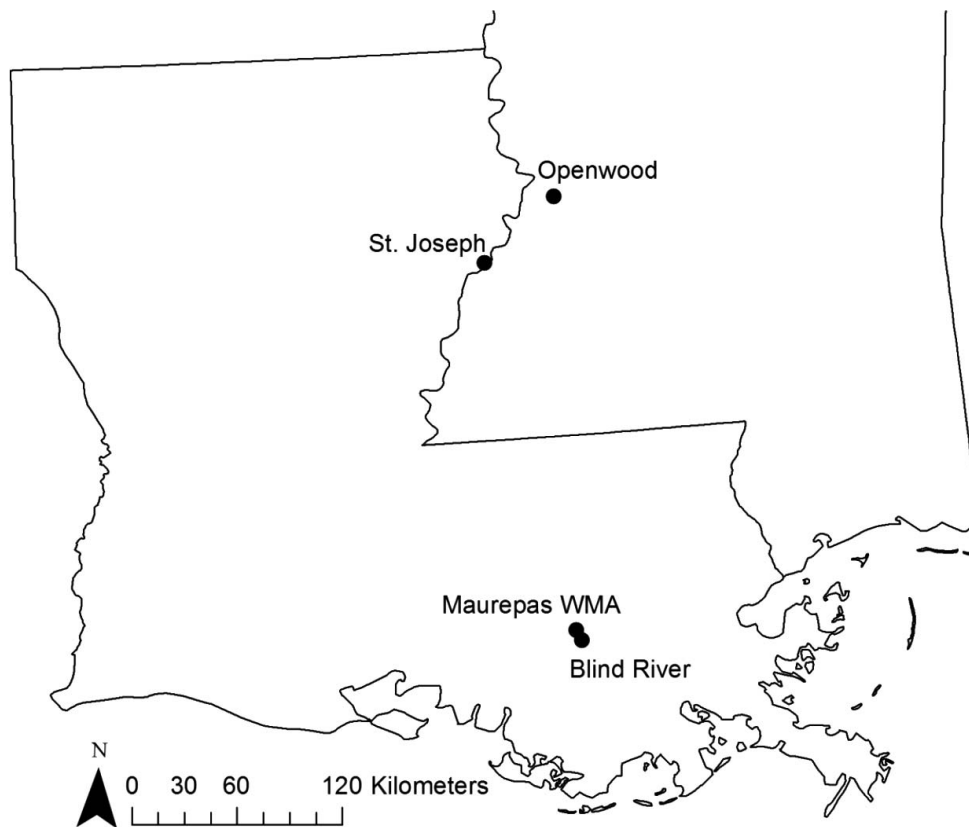


Figure 1. Locations of study sites in Louisiana and Mississippi.

Alligatorweed leaf damage

Ten alligatorweed plants per site were haphazardly chosen and measured to estimate growth. Above-water plant parts were examined, and we considered each emergent stem to represent an individual plant. Number of leaves per plant, stem density, and leaf areas were determined. Stem density (stems m^{-2}) was determined by placing a 0.25-m^2 polyvinyl chloride quadrat within the mat at three haphazardly chosen locations and counting the number of emergent stems within the frame. Approximately 10 g fresh weight of alligatorweed leaves was collected per site and census date and used to estimate leaf damage. To standardize our leaf collections for this analysis, we sampled fully developed leaves from third and fourth apical nodes of the plants. Leaves were collected without regard to damage level.

From each collection, 33 to 49 leaves were haphazardly chosen and imaged on a flatbed scanner at 1,200 dpi. Images were processed using ImageJ software (NIH 2014) to estimate undamaged and damaged leaf area. These measurements were used to calculate the following:

$$\begin{aligned} \% \text{ Leaf damage} \\ &= (1 - [\text{damaged leaf area/whole leaf area}] \times 100). \end{aligned}$$

Scans were standardized by using adaxial leaf surfaces in the analysis. On 19 May at Maurepas, nearly all plants were defoliated and not enough leaves were present to collect

samples. Therefore, we conservatively estimated that leaf damage was 95% on this date.

Alligatorweed relative growth rate

Leaf area index (LAI) was used to assess alligatorweed growth rate during our study. LAI is a measure of light-intercepting leaf area and can be used to estimate primary production potential (Jonckheere et al. 2004, Campillo et al. 2010). LAI for each site and date was calculated as follows:

$$\begin{aligned} \text{LAI} &= \text{no. leaves plant}^{-1} \times \text{mean area leaf}^{-1} (\text{mm}^2) \\ &\quad \times \text{no. plants } m^{-2}. \end{aligned}$$

This provides a value that represents the total undamaged alligatorweed leaf area (mm^2) per square meter of water surface. Alligatorweed relative growth rate (RGR) was then calculated using LAI:

$$\text{RGR} = \frac{(\ln[\text{LAI}\{t + 1\}] - \ln[\text{LAI}\{t\}])}{\text{no. days in sampling interval}},$$

where $\text{LAI}(t)$ = initial LAI, $\text{LAI}(t + 1)$ = LAI at subsequent sampling date. RGR was then examined over time for all study sites and averaged to compare overall RGR between regions. Units for relative growth rates are mm^2 undamaged leaf (m^2 water surface area) $^{-1}$ day $^{-1}$. For simplicity of presentation, we present RGR without units.

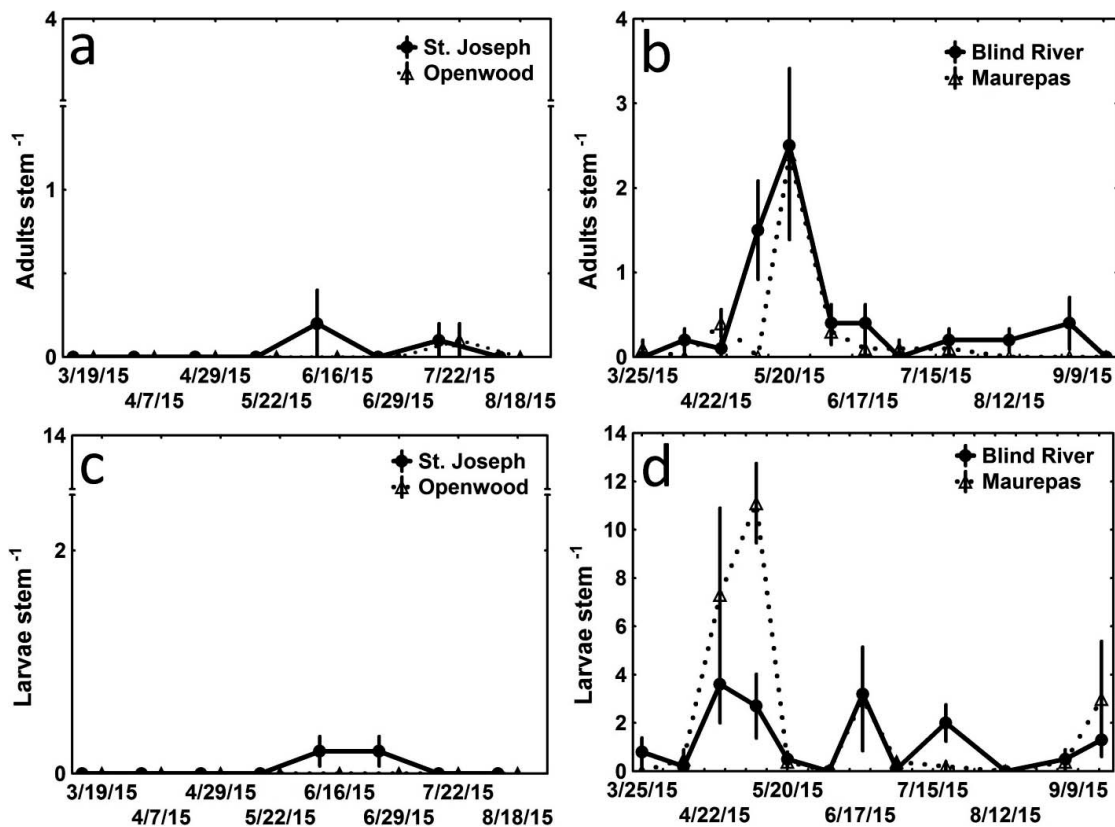


Figure 2. Mean (\pm SE) adult and larval abundance at (a,c) northern and (b,d) southern sites. (a,c) Axis breaks were included for northern sites.

Statistical approach

To determine the relationship between insect abundance and RGR, and insect abundance and percentage of leaf damage we used least-squares regression. Overall RGR was compared between regions using a *t* test. To compare insect abundance (larvae, adults) between southern sites over time, ANOVA was used. In order to achieve homogeneity of variances, count data (egg, larval, adult abundance) were $\log(+0.5)$ transformed prior to analyses. Because zeros made up the majority of counts at northern sites, we excluded northern sites from the insect abundance statistical analyses and discuss them qualitatively. If significance was found with ANOVA, Tukey's HSD was used to separate means. Sample schedules in regions were different; southern sites were sampled more frequently than northern sites. Because of this, we make comparisons between sites and dates within each region but directly compare regions only when examining general trends in RGR or insect presence/absence. All statistical analyses were performed using Statistica, version 12.¹

RESULTS AND DISCUSSION

Alligatorweed flea beetle population dynamics

Alligatorweed flea beetle occurred later in northern sites than in southern sites (Figure 2). Although insects (larvae) were recorded on the first sample date (25 March) in southern

sites, at the northern sites, they were not recorded until 16 June at St. Joseph (larvae and adults) and 22 July at Openwood (eggs and adults), despite abundant plant material in northern sites during earlier sample dates. This finding supports our assumption that insects do not overwinter in northern sites and immigrate there during the summer (Coulson 1977). We cannot definitively state that overwintering occurred in southern sites, but insect presence in March coupled with mean winter temperatures above published thresholds provides strong support for overwintering at these sites. Vogt et al. (1992) documented arrival of the alligatorweed flea beetle at northern Louisiana sites in April (1974), May (1975, 1976), or not at all (1977), depending on the severity of the preceding winter. Clearly, there is considerable variability in timing of arrival, which has implications for both short- and long-term control at these sites.

Overall, mean alligatorweed flea beetle abundance in northern sites was 2% of the abundance at southern sites (mean \pm SE: 2.1 ± 0.4 vs. 0.05 ± 0.04 insects stem⁻¹, respectively). Peak larval abundance at southern sites occurred on 8 May at Maurepas (11.1 ± 1.64 larvae stem⁻¹; $\sim 2,923$ larvae m⁻²; $F[11, 216] = 3.75$, $P < 0.001$; Figure 2c). A lesser peak occurred at Maurepas on 18 June (3 ± 2.1 larvae stem⁻¹; 1,110 larvae m⁻²). At Blind River, peak larval abundance was lower than at Maurepas but reached 3.6 ± 1.6 larvae stem⁻¹ (732 larvae m⁻²) on 24 April. Peak adult abundance at southern sites occurred at both Maurepas (2.4 ± 1.0 adults stem⁻¹, 488 adults m⁻²) and Blind River (2.5 ± 0.84 adults stem⁻¹, 550 adults m⁻²) on 20 May ($F[11, 216] =$

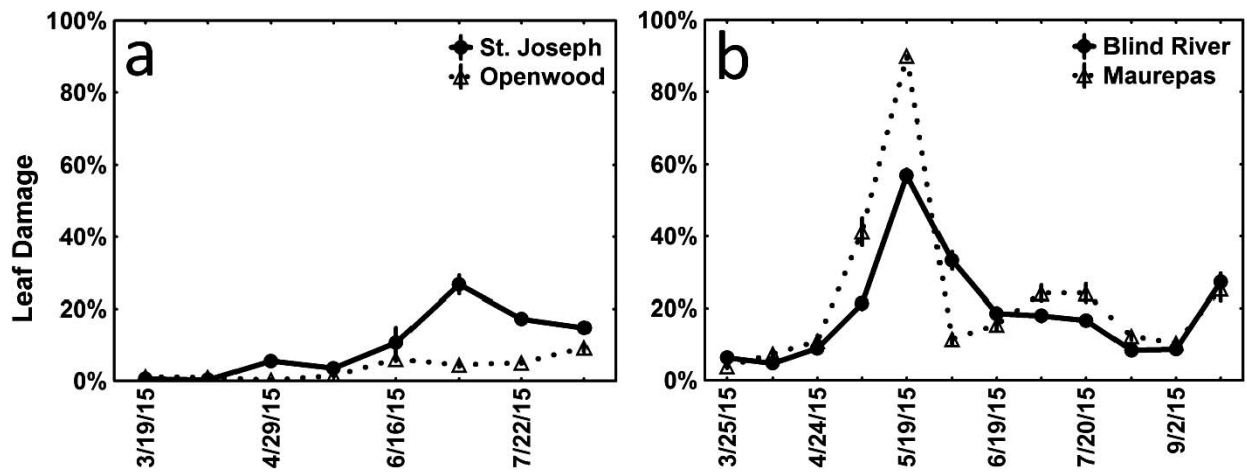


Figure 3. Mean (\pm SE) percent leaf damage at (a) northern and (b) southern sites. The asterisk denotes a conservative estimate of leaf damage on that date.

2.21, $P < 0.01$; Figure 2a). Peaks in adult abundance were considerably higher than those reported in New Zealand (~ 250 adults m^{-2} ; Stewart et al. 2000) where climate was thought to be limiting, but peak larval abundance was similar (2,970 larvae m^{-2}). Other studies documenting peak insect abundance as it relates to control of alligatorweed are lacking.

Alligatorweed leaf damage

Alligatorweed in northern sites had relatively minor leaf damage during the study period (Figure 3a). Maximum leaf damage at northern sites was $25 \pm 2\%$ and was recorded at St. Joseph on 29 June. At other dates, mean leaf damage was always less than 20% in northern sites and averaged $7 \pm 3\%$. Southern sites experienced a high-intensity damage event, which began in late April and continued until early June with its peak on 19 May ($> 90\%$ at Maurepas and $55 \pm 1\%$ leaf damage at Blind River; Figure 3b). Overall, southern sites had $21 \pm 2\%$ mean leaf damage. Adult alligatorweed flea beetle abundance was strongly positively associated with

percentage of leaf damage on the sampling date (Figure 4a) whereas larval abundance was strongly positively associated with percentage of leaf damage on the subsequent sampling date (Figure 4b).

Alligatorweed RGR

Mean alligatorweed RGR at northern sites was positive (0.012 ± 0.016) but negative at southern sites (-0.027 ± 0.035). However, the difference between the north and south was not statistically significant (t test, $df = 15$, $P = 0.39$). The minimum and maximum RGRs in southern sites occurred on 8 May (-0.29) and 4 June (0.16), and in northern sites on 22 May (-0.014) and 19 March (0.10). Minimum RGR in southern sites coincided with the climb to peak insect abundance (Figure 2B) and leaf damage (Figure 3) on 8 and 19 May, respectively. Overall, insect abundance (total insects $stem^{-1}$) was negatively associated with RGR ($R^2 = 0.54$, $P < 0.001$). The lack of statistical difference in mean RGR between regions is likely due to high variability in RGR in southern populations (coefficient of variation [CV] = 4.12

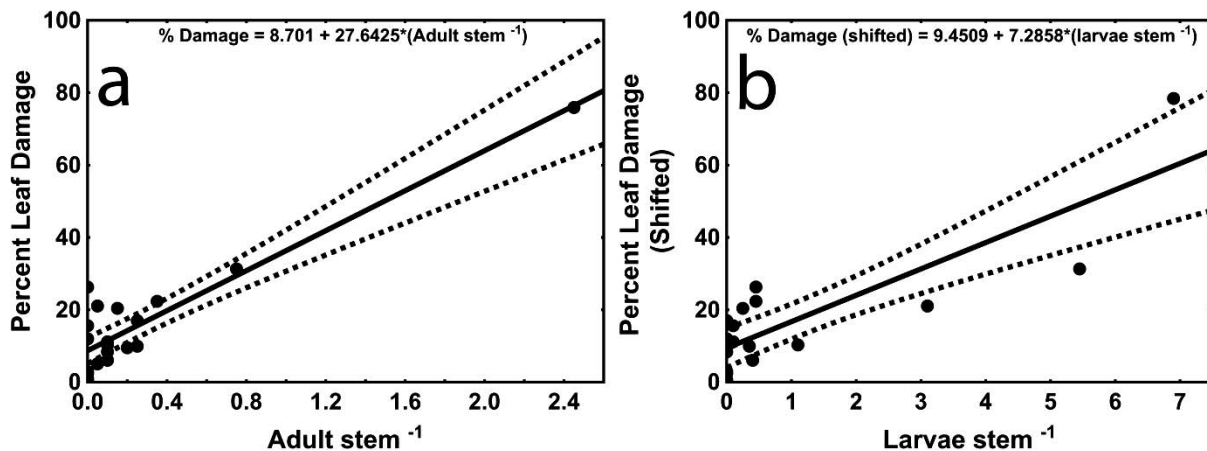


Figure 4. (a) Percentage of leaf damage as a function of adult alligatorweed flea beetle abundance ($R^2 = 0.83$, $P < 0.001$) and (b) percentage of leaf damage on the subsequent sampling date as a function of larval abundance ($R^2 = 0.71$, $P < 0.001$). Dotted lines represent 95% confidence bands around the line of best fit.

in southern populations; $CV = 3.39$ in northern populations). Although we cannot determine the cause of variability in southern RGR, a plausible explanation is that greater variability in insect abundance (rise and fall of populations) and damage translated into the greater variability in alligatorweed RGR.

The relative success of biological control at the southern sites (and lack of success at the northern sites) can likely be attributed to timing, explained in three (not mutually exclusive) ways: 1) southern sites, but not northern sites, are within the overwintering area of the alligatorweed flea beetle and allow rapid insect population growth when temperatures rise in the spring; 2) air temperature at southern sites when the insects first become active is relatively mild, allowing for peak activity and plant damage, while northern temperatures are much higher, and thus limiting (Stewart et al. 1999, Xin et al. 2009) when insects arrive from southern locations; 3) plant quality is highest early in the year (N. E. Harms et al., unpubl. data) and declines during the season due to limited resources, thus providing highest value food early in the season when insect populations are growing in southern sites. The implications of this early-season higher plant quality are that insect performance and impact should be similar between northern and southern sites if insects were introduced (or immigrated) to northern sites early in the season. The actual explanation is probably a combination of all the above and we will address the role of each in successful biological control in the future.

Since the introduction of the alligatorweed flea beetle into the United States more than 40 yr ago, regional alligatorweed abundance and distribution have been dramatically reduced in most areas (Gangstad 1976, Spencer and Coulson 1976, Buckingham 1996). In our study, at sites where alligatorweed had negative RGRs (i.e., southern sites), we observed a large alligatorweed flea beetle population peak early (Figure 2), whereas alligatorweed at northern sites had little impact from herbivory (and positive mean RGR during the study period). We predicted this, given the earlier suggestions that early-season dynamics were important for successful and sustained biological control of alligatorweed (Coulson 1977, Vogt et al. 1992). However, despite presence of alligatorweed flea beetles at all sites during the study, insect populations failed to increase in northern sites. Vogt et al. (1992) documented the variable timing of impacts by alligatorweed flea beetles across the Lower Mississippi River valley, but until now there has been a lack of quantitative data to document insect population dynamics and associated impacts to early-season alligatorweed populations.

A unique feature of alligatorweed biological control is the ability of the alligatorweed flea beetle to disperse long distances each spring/summer and recolonize areas outside its overwintering zone. Such long-distance annual dispersal coupled with variable impacts due to arrival timing provides an opportunity to study natural systems where biological control varies with temporal (within and between years) or spatial (local or regional) scale. Although low winter temperatures in northern sites may be responsible for lack of overwintering (and lack of early-season damage to plants)

of alligatorweed flea beetles, we do not fully understand the role plant phenology or nutrition play in insect population growth (and subsequent impacts to alligatorweed) later in the growing season. However, it has been documented that nutrition in many aquatic weeds varies considerably during the growing season, a pattern that has been reviewed for its potential to inform management timing (Pesacreta and Luu 1988). In alligatorweed, crude protein declines during the summer months from a maximum in spring (Boyd and Blackburn 1970; N. E. Harms et al., unpub. data). Additionally, slight differences in winter severity from year to year may translate into large differences in alligatorweed control if mild winters lead to insect overwintering in more northern areas or shortened dispersal distances from overwintering sites and thus earlier colonization and impacts at northern sites.

We chose to examine early-season alligatorweed biological control because 1) there is a history of successful control by the introduced agents, and 2) previous observations indicated a seasonal pattern of control. Although this study represents only one biological control system during one season, the importance of seasonality in biological control should be further examined for other target weeds. Knowing the timing of important phenological events is useful when designing release or monitoring schedules for agents; if, for example, temperature or plant nutrition follow seasonal patterns then releases can be optimized to maximize the likelihood of establishments and impacts. Additionally, incorporating seasonal ecology into overseas exploration for new agents may improve agent detection and provide useful biological information to inform potential future releases in the United States.

SOURCES OF MATERIALS

¹Statistica, version 12, Statsoft, Inc., Tulsa, OK 74104.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the assistance of Sam Kirk, Schuyler Cool, Jan Freedman, Julie Nachtrieb, and Jonathan Winslow. We thank Rodrigo Diaz, Jim Cronin, and two anonymous reviewers for critical review of this manuscript. This work was funded by the U.S. Army Engineer Aquatic Plant Control Research Program under management of Linda Nelson.

LITERATURE CITED

- Boyd CE, Blackburn RD. 1970. Seasonal changes in the proximate composition of some common aquatic weeds. *Hyacinth Control J.* 8:42-44.
- Buckingham GR. 1996. Biological control of alligatorweed, *Alternanthera philoxeroides*, the world's first aquatic weed success story. *Castanea* 61:232-243.
- Campillo C, García MI, Daza C, Prieto MH. 2010. Study of a non-destructive method for estimating the leaf area index in vegetable crops using digital images. *HortScience* 45:1459-1463.
- Carisse O, Rolland D. 2004. Effect of timing of application of the biological control agent *Microsphaeropsis ochracea* on the production and ejection pattern of ascospores by *Venturia inaequalis*. *Phytopathology* 94:1305-1314.

- Chi H. 1990. Timing of control based on the stage structure of pest populations: A simulation approach. *J. Econ. Entomol.* 83:1143–1150.
- Coulson JR. 1977. Biological control of alligatorweed 1959–1972: A review and evaluation. U.S. Department of Agriculture, Tech. Bull. 1547, Washington DC. 106 pp.
- Gangstad EO. 1976. Biological control operations on alligatorweed. *J. Aquat. Plant Manag.* 14:50–53.
- Grevstad FS. 1999. Factors influencing the chance of population establishment: Implications for release strategies in biocontrol. *Ecol. Appl.* 9:1439–1447.
- Grevstad FS, Coombs EM, McEvoy PB. 2011. Revisiting release strategies in biological control of weeds: Are we using enough releases? pp. 368–376. In: XIII International Symposium on Biological Control of Weeds, Waikoloa, HI.
- Jonckheere I, Fleck S, Nackaerts K, Muys B, Coppin P, Weiss M, Baret F. 2004. Review of methods for in situ leaf area index determination: Part I. Theories, sensors and hemispherical photography. *Agric. For. Meteorol.* 121:19–35.
- Julien MH, Skarratt B, Maywald GF. 1995. Potential geographic distribution of alligator weed and its biological control by *Agasicles hygrophila*. *J. Aquat. Plant Manag.* 33:55–60.
- Luu KT, Getsinger KD. 1990. Seasonal biomass and carbohydrate allocation in waterhyacinth. *J. Aquat. Plant Manag.* 28:3–10.
- McAllister RS, Haderlie LC. 1985. Seasonal variations in Canada thistle (*Cirsium arvense*) root bud growth and root carbohydrate reserves. *Weed Sci.* 33:44–49.
- Memmtt J, Fowler SV, Harman HM, Hayes LM. 1996. How to best release a biological control agent. pp. 19–26. In: V. C. Moran and J. H. Hoffmann (eds.). Proceedings of the IX International symposium on Biological Control of Weeds, Stellenbosch, South Africa.
- Memmtt J, Fowler SV, Hill RL. 1998. The effect of release size on the probability of establishment of biological control agents: gorse thrips (*Sericothrips staphylinus*) released against gorse (*Ulex europaeus*) in New Zealand. *Biocontrol Sci. Technol.* 8:103–115.
- Mudge CR, Harms NE, Nachtrieb JG. 2013. Interactions of herbicides, surfactants, and the giant salvinia weevil (*Cyrtobagous salviniae*) for control of giant salvinia (*Salvinia molesta*). *J. Aquat. Plant Manag.* 51:77–83.
- Newman RM, Thompson DC, Richman DB. 1998. Conservation strategies for the biological control of weeds. pp. 371–396. In: P. Barbosa (ed.). Conservation biological control. Academic Press, New York.
- [NIH] National Institutes of Health. 2014. ImageJ Image Analysis Software. <https://imagej.nih.gov> accessed November 1, 2015.
- Norris RJ, Memmtt J, Lovell DJ. 2002. The effect of rainfall on the survivorship and establishment of a biocontrol agent. *J. Appl. Ecol.* 39:226–234.
- Owens CS, Madsen, JD. 1998. Phenological studies of carbohydrate allocation in hydrilla. *J. Aquat. Plant Manag.* 36:40–44.
- Paynter Q. 2003. Integrated weed management: Effect of herbicide choice and timing of application on the survival of a biological control agent of the tropical wetland weed, *Mimosa pigra*. *Biol. Control* 26:162–167.
- Pesacreta GJ, Luu KT. 1988. Feasibility of relating phenology and carbohydrate partitioning to improve aquatic plant control. U.S. Army Engineers Waterways Experiment Station, Miscellaneous Paper A-88-7, Vicksburg, MS. 28 pp.
- Spencer NR, Coulson JR. 1976. The biological control of alligatorweed, *Alternanthera philoxeroides*, in the United States of America. *Aquat. Bot.* 2:177–190.
- Stewart C, Chapman R, Barrington A, Frampton C. 1999. Influence of temperature on adult longevity, oviposition and fertility of *Agasicles hygrophila* Selman & Vogt (Coleoptera: Chrysomelidae). *N. Z. J. Zool.* 26:191–197.
- Stewart C, Chapman R, Frampton C. 2000. Growth of alligator weed (*Alternanthera philoxeroides* (Mart.) Griseb.(Amaranthaceae)) and population development of *Agasicles hygrophila* Selman & Vogt (Coleoptera: Chrysomelidae) in northern New Zealand. *Plant Prot. Q.* 15:95–101.
- [USDA NRCS] U.S. Department of Agriculture Natural Resources Conservation Service. 2015 Plants Database. <http://plants.usda.gov>. Accessed October 17, 2015.
- Van Driesche R, Hoddle M, Center T. 2008. Control of pests and weeds by natural enemies: An introduction to biological control. Wiley Blackwell, Oxford, UK. 484 pp.
- Van Driesche R, Lyon S, Blossey B, Hoddle M, Reardon R. 2002. Biological control of invasive plants in the eastern United States. U.S. Forest Service Publication FHTET-2002-04, Morgantown, WV. 424 pp.
- Vogt GB, Quimby PC Jr, Kay SH. 1992. Effects of weather on the biological control of alligatorweed in the lower Mississippi Valley region, 1973–1983. U.S. Department of Agriculture, Agricultural Research Service, Tech. Bull. 1766, Washington, D.C. 143 pp.
- Wilson LM, Jette C, Connett J, McCaffrey J. 2003. Biology and biological control of yellow starthistle. 2nd ed. U.S. Department of Agriculture, Forest Service FHTET-1998-17, Morgantown, WV. 78 pp.
- Weather Underground. 2016. www.wunderground.com. Accessed February 27, 2016.
- Xin Z, JianWei F, Fanghao W, Jianjing G, JinJun W. 2009. Effects of brief high temperature exposure on reproductive characteristics of *Agasicles hygrophila* (Coleoptera: Chrysomelidae). *Acta Entomol. Sin.* 52:1110–1114.