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Growth and developmental performance of the milfoil weevil on distinct lineages of Eurasian watermilfoil and a northern x Eurasian hybrid

KYLE R. BORROWMAN[1], ERIC. P. S. SAGER[2,3], AND RYAN A. THUM[4]

ABSTRACT

Recent identification of two genetically distinct lineages of Eurasian watermilfoil (*Myriophyllum spicatum* L.) and hybrid *M. spicatum* x *M. sibiricum* Kom. populations in Ontario, Canada has lead to emerging concerns surrounding the efficacy the milfoil weevil [*Euhrychiopsis lecontei* (Deitz)] as a biological control agent. In the summer of 2011 we conducted an experiment in a climate controlled growth chamber to determine the growth and developmental performance of *E. lecontei* on two distinct *M. spicatum* lineages (EWM1, EWM2) an *M. spicatum x M. sibiricum* hybrid (HYB) and *M. sibiricum* (NWM). We observed an increased overall survivorship of *E. lecontei* on HYB in comparison to EWM2 and NWM. Additionally, greater mass at emergence and shorter larval stage duration was observed for *E. lecontei* reared on HYB and EWM1 in comparison to NWM. Although not statistically significant, an increased survivorship of *E. lecontei* was observed on HYB in comparison to EWM1. This suggests a dominance or hybrid susceptibility to herbivory for HYB in comparison to its parental congeners rather than an additive or intermediate relationship observed in previous studies. This may be due in part to increased nutritional quality; HYB had a higher shoot tissue N and P content than all other milfoil types. *E. lecontei* reared on EWM2 showed poor development including low overall survivorship and larval stage duration which may have been greatly influenced by poor plant response to growth in the environmental chamber. The results of this experiment expand current knowledge surrounding the influence of host plant preference and host plant quality on the growth and development of herbivorous insects and can be used to inform future biological control applications.

Key words: biological control, developmental performance, hybridization, invasive species, milfoil weevil.

INTRODUCTION

Since its widespread establishment throughout North America, Eurasian watermilfoil (*Myriophyllum spicatum* L.; herein referred to as EWM) has become one of the most invasive submersed macrophyte species on the continent (Smith and Barko 1990). In addition to classical control techniques such as mechanical harvesting and herbicide application, the use of a native phytophagous insect, the milfoil weevil [*Euhrychiopsis lecontei* (Deitz)] has become commercially available for EWM management.

Effective biological control of EWM is dependent on successful growth, development and reproduction of the milfoil weevil (Newman 2004). Host range expansion of the milfoil weevil to include EWM has occurred over a short generational timespan and has led to increased fitness and developmental performance compared to individuals reared on its original native host, northern watermilfoil (*Myriophyllum sibiricum* Kom.; herein referred to as NWM) (Newman et al. 1996; Sheldon and Jones 2001). Specifically, milfoil weevils have been identified to exert higher overall survivorship, quicker development, greater mass at adult emergence and higher ovipositional preference when reared on EWM over NWM (Newman 1996, Newman et al. 1997, Solarz and Newman 2001). Reasons speculated for this increased fitness includes shifts in host plant preference (Solarz and Newman 2001), nutritional quality of the plant (Creed 2000), and release from defense compounds and resiliency of its native host NWM (Creed 2000, Solarz and Newman 2001, Newman 2004).

Hybridization of NWM and EWM provides new challenges for lake managers because of the potential for increased resiliency to herbivory and increased invasiveness expressed through hybrid vigor (Roley and Newman 2006, Schierenbeck and Ellstrand 2009). Roley and Newman (2006) compared the developmental performance of milfoil weevils on an EWM x NWM hybrid to its parental congeners. They found a higher rate of survivorship on EWM than NWM with intermediate survivorship on the hybrid. These results suggest the potential for increased invasiveness of the hybrid through resiliency to herbivory and thus sustained hybrid vigor through clonal expansion (Moody and Les 2002, Roley and Newman 2006).

In addition to hybridization, two distinct lineages of EWM (EWM1 and EWM2) have been identified in Ontario and across North America (Zuellig and Thum 2012, Borrowman et al. 2014). In some cases, biotypic variation has led to differences in the success of biological control agents. One such example includes the use of the Australian hydrilla leaf-mining fly (*Hydrellia balciunasi* Bock) as a biological control agent of hydrilla (*Hydrilla verticillata*) in the United States (Grodowitz et al. 1997). Although the Australian leaf-

*First author, Environmental and Life Sciences Graduate Program, Trent University, 1600 West Bank Drive, Peterborough, ON, Canada, K9J 7B8. Second author, Environmental and Resource Sciences Program, Trent University, 1600 West Bank Drive, Peterborough, ON, Canada, K9J 7B8 and Ecological Restoration Program, Fleming College, 200 Albert St. South, Lindsay, ON, Canada K9V 5E6. Third author, Annis Water Resources Institute, Grand Valley State University, 750 W. Shoreline Dr. Muskegon, MI, 49441. Corresponding author's E-mail: kyleborrowman@trentu.ca. Received for publication October 20, 2013 and in revised form July 30, 2014.

mining fly is capable of successful establishment on Australian hydrilla, the fly showed poor establishment and lowered developmental performance on biotypes present in North America (Grodowitz et al. 1997, Cuda et al. 2008).

The influence of plant quality of the target host has also become a major topic of interest for terrestrial and aquatic biological control applications (Newman 2004, Cuda et al. 2008). Plant quality in terms of nutritional availability, palatability and the presence of defense compounds has been given special attention because of their influence on the development of herbivorous insects (Awmack and Leather 2002). Nutritional availability has been identified to influence the growth, development, oviposition and survival of many herbivorous insects (Awmack and Leather 2002, Mattson 1980). For example, Center and Dray (2010) showed a positive relationship between the concentration of nitrogen available to water hyacinth [*Eichhornia crassipes* (Mart.) Solms] and the ovarian development of two water hyacinth weevil species, *Neochetina eichhorniae* and *Neochetina bruchi*. Similarly, Wheeler and Center (1997) noticed a 50% increase in development rates and increased mass of the hydrilla weevil (*Bagous hydrillae* O'Brien) when reared on hydrilla with 3.5% N content over hydrilla with 2.0% N content. However, limitations of other dietary requirements (i.e. P or Fe), host quality and the release of secondary defense compounds have also been considered to influence herbivorous insect success (Creed 2000, Newman 2004, Perkins et al. 2004, Cuda et al. 2008, Guenther et al. 2011).

The milfoil weevil has been identified to occur naturally on hybrids and both EWM lineages identified in Ontario (Borrowman et al. 2014). The survivorship and developmental performance of the milfoil weevil on these various lineages and hybrids is an important consideration for the success of biological control applications. In addition, the influence of plant quality on the developmental performance and success of the milfoil weevil has been suggested but has not specifically been investigated (Creed 2000, Newman 2004, Cuda et al. 2008). The objective of this study is to 1) investigate the developmental performance of the milfoil weevil on these distinct lineages and hybrids of EWM and 2) compare shoot quality, including C, N and P for each milfoil type and its influence on weevil growth.

METHODS

Experimental Set Up

Milfoil shoot collection and preparation. In total, five populations of milfoil were compared in this experiment consisting of NWM, two distinct lineages of EWM and a NWM x EWM hybrid (HYB). The two lineages of EWM used in this experiment include two populations of EWM1 and one population of EWM2. Shoots were collected in late May 2011 from populations previously identified through molecular analysis (Borrowman et al. 2014). EWM2 was collected from Richard Lake (Sudbury Region), HYB from Lower Buckhorn Lake (Peterborough County) and NWM was collected from Pigeon Lake (Peterborough County). Since EWM2 did not occur within the same geographic location or environmental conditions (ie. eutrophic vs oligotrophic lakes) as HYB and NWM, EWM1 was collected from both regions to control for any localized adaptation to the geographic/environmental conditions. These lakes were Big Bald Lake (named EWM1a; Peterborough County) and McFarlane Lake (named EWM1b; Sudbury Region).

Once collected, shoots of each population were inspected for previous herbivore damage and rinsed gently but thoroughly to remove any epiphytic algae and detritus. Shoots ~30 cm in length were planted in 20 L white polyethylene pails (50 shoots per pail) and grown in an environmental growth chamber for two weeks at the Environmental Sciences Building at Trent University in Peterborough ON. The shoots were planted in 2 cm of homogenized sediment collected from the Otonabee River adjacent to Trent University property and covered with 1cm of sand. The pails were filled with river water from the Otonabee River and aerated. Conditions within the chamber consisted of a constant temperature of 24 C with a 16 h photoperiod (3 h at ~400 μmol m^{-2} s^{-1}, 10 h at ~700 μmol m^{-2} s^{-1} followed by 3 h at ~400 μmol m^{-2} s^{-1}).

After two weeks, 24 shoots of each population were transplanted into 10 cm plastic flower pots, stabilized in aquarium gravel and lowered into clear acrylic columns (60 cm in height, 10 cm in diameter) at a frequency of four shoots per column with six replicate columns per population. Each column was filled with river water sourced from the Otonabee River adjacent to Trent University property north of Peterborough ON. Columns were given a number that corresponded with a location evenly spaced along the outer perimeter of one of two tables in the environmental chamber. Shoots that were not transferred into columns were used for analysis of carbon, nitrogen and phosphorus content as well as confirmation of milfoil type through DNA analysis.

Milfoil weevil egg incubation and transfer to plant. Weevil eggs used in this experiment were provided by EnviroScience Inc. These eggs were reared from a stock of adult milfoil weevils collected from lakes in Summit County, Ohio, that are ultimately used for commercial biological control. EWM shoots with eggs on the upper meristems were obtained from this commercial lab, put in re-sealable bags with water and placed in a cooler with ice for transport to the Environmental Sciences Building at Trent University in Peterborough ON.

Upon arrival, eggs were removed from the apical meristem and upper leaves of milfoil of which they were oviposited and placed in ice cube trays for incubation. Since the type of milfoil they are exposed to can influence feeding preference and oviposition preference (Solarz and Newman 2001), upper meristematic tissue from each population was placed in separate ice cube trays to provide food for hatching larvae prior to transfer into columns.

Milfoil weevils were transferred into columns as larvae because of difficulty in transplanting eggs. Egg incubation trays were placed under a dissecting microscope to search for hatched larvae every four hours. Once found, larvae were transferred into columns at a ratio of one larva per plant. To minimize disturbance to the plants and larvae, all larvae for one column (4 larvae) were transferred at the

same time. In addition, aeration tubes were removed for one day to minimize turbulence in the water.

Larvae were transferred into columns over a period of 3 d. Once the transfer was completed for a column, daily observations commenced. During the larvae transferring stage of the experiment, some larvae did not respond positively to the host and did not survive the first few days. Shoots that showed no apparent larval damage or no change in damage over the first 3 days were provided with a second weevil larva.

Determining shoot quality. Remaining shoots of each milfoil type that were not transferred into columns were used to determine shoot quality (tissue carbon, nitrogen and phosphorus). Five composite samples for each population consisting of the upper 15 cm of five shoots were dried, homogenized using a SPEX Sample Prep 8000D mixer/mill stainless steel ball mill grinder and analyzed for carbon, nitrogen and phosphorus content. Carbon and nitrogen were analyzed using an Elementar Vario Macro CNS Analyzer and expressed as % shoot C and % shoot N. Phosphorus content was determined using nitric acid digestion with methods outlined by Hutchinson et al. (1999). Once digested, samples and standards were diluted 1 : 10 with B-Pure and analyzed using a Perkin Elmer Optima 7000 ICP-OES. Results were expressed as shoot tissue %P.

Shoot tissue content of %C, %N and %P were all LOG transformed. Shoot %N and %P met assumptions of normality and homogeneity of variances by milfoil type (K–S test, $P > 0.05$; Levene's, $P > 0.05$) and were compared across milfoil type using One-way ANOVAs. Percent shoot C did not meet the assumptions of homogeneity of variances and a Kruskal-Wallis test for non-parametric data was used to compare %C by milfoil type (K–S test, $P > 0.05$; Levene's $F_{4,19} = 9.01$, $P < 0.005$).

DNA analysis/milfoil type confirmation. The upper 3 cm of five shoots from each population were rinsed thoroughly in deionized water, placed in separate re-sealable bags and frozen in liquid nitrogen. Once frozen, these samples were stored in a freezer at −80 C and transported to Grand Valley State University's Robert B. Annis Water Resources Institute in Muskegon, MI, USA for genetic analysis. Shoots were analyzed to confirm that each population used in the experiment was properly identified/represented. The shoots were analyzed using AFLP methods outlined in Zuellig and Thum (2012) followed by scoring results using GeneMapper 4.0 (Applied Biosystems). All populations collected accurately represented each milfoil type.

Weevil growth and development. Determining the developmental performance and survival of weevils on various milfoil types was similar to methods outlined by Roley and Newman (2006) and Mazzei et al. (1999). This consisted of recording daily observations of larval feeding, burrowing, visual shoot damage and current weevil lifestage (larva, pupa, adult). Observations were continued throughout the duration of the experiment until adults emerged from the pupal chambers.

The amount of time needed for milfoil weevils to complete larval and pupal stages as well as survival rate throughout each life stage was determined through these observations and compared across replicate columns within and across each population. Since we used larvae and not eggs, the final survivorship following the experiment was determined by dividing the number of adult weevils that emerged by the total number of larvae used in the experiment ($n = 24$ per population).

Upon emergence from pupation, adult weevils were removed from the column, blotted dry using a paper towel and weighed using a microbalance to the nearest 0.001 mg. After weighing, adult weevils were preserved and stored in a freezer at −20 C.

One-way ANOVAs were used to compare weevil development including: survivorship, mass at adult emergence and larval burrowing distance by milfoil type. In addition, Kruskal-Wallis tests for non-parametric data were used to compare larval and pupal life stage duration by milfoil type since they did not pass tests of normality and/or homogeneity of variances. All proportion data of life stage survival were arcsine(square root [x]) transformed to meet assumptions of normality and homogeneity of variances (Adult: Kolmogorov Smirnov Test; $P > 0.05$; Levene's $F_{4,25} = 1.37$, $P = 0.27$; Larval: K-S Test; $P > 0.05$; Levene's $F_{4,25}$=1.38, $P = 0.27$; Pupal: K-S Test; p $P > 0.05$; Levene's $F_{4,25} = 2.15$, $P = 0.10$). Statistical software used for these and all analyses consisted of Statistica 9 (Stat Soft.).

Post experiment shoot measurements. Once all adult weevils emerged from a column, the shoots were removed and examined to determine the shoot length and total length of larval burrowing. Shoot length was determined using a measuring tape accurate to the closest mm. Since larvae often exit and re-enter the stem throughout the burrowing/feeding process, larval burrowing was determined by measuring the length of each burrowing chamber using a digital caliper accurate to 0.01 mm.

The total shoot length of each shoot and the burrowing length of each successfully emerged weevil were compared to determine if differences occurred based on milfoil type present. Total shoot length and larval burrowing length met assumption of normality and homogeneity of variance and was compared by milfoil type using a One-way ANOVA (K–S Test, $P > 0.05$; shoot length, Levene's $F_{4,115} = 0.16$, $P = 0.95$; larval burrow, Levene's $F_{4,68} = 0.52$, $P = 0.72$).

RESULTS AND DISCUSSION

Milfoil weevils reared on HYB showed significantly higher rates of survivorship and shorter larval duration in comparison to NWM and EWM2. In total, HYB had the highest overall survivorship from egg to adult ($n = 21$) followed by EWM1*b* ($n = 19$), EWM1*a* ($n = 14$), EWM2 ($n = 11$) and NWM ($n = 9$) (One-way ANOVA; $F_{4,25} = 4.258$, $P < 0.01$; Tukey HSD, $P < 0.05$) (Table 1). However, there were no significant differences between milfoil types when comparing survivorship through the larval stage (One-way ANOVA; $F_{4,25} = 0.65$, $P = 0.63$) or the pupal stage (One-way ANOVA; $F_{4,25} = 2.47$, $P = 0.07$) (Table 1).

The larval stage duration of weevils reared on HYB, EWM1a and EWM1b were significantly shorter than weevils reared on EWM2 and NWM (Kruskal-Wallis Test; $H_{4,73} = 27.42$, $P < 0.001$; Non-parametric post-hoc analysis, $P <$

Table 1. Mean duration to complete larval and pupal lifestages, adult mass at emergence ±1SE and percentage of survival through each lifestage by milfoil type. Significant differences in each column are denoted by superscript letters (significant at $P < 0.05$). Survivorship and adult mass were compared using One-way ANOVAs whereas lifestage duration was determined using Kruskal-Wallis tests for non-parametric data.

		Successful lifestage completion		
Milfoil type	n	Larvae (days)	Pupae (days)	Adult mass (mg fresh weight)
NWM	24	11.0±0.5[a]	8.8 ± 0.4[a]	0.951 ± 0.029[a]
% survival		75%	50%	37.5%[a]
HYB	24	8.3 ± 0.3[b]	8.0 ± 0.2[a]	1.150 ± 0.031[b]
% survival		92%	95%	87.5%[b]
EWM1*a*	24	7.9 ± 0.3[b]	8.0 ± 0.1[a]	1.221 ± 0.046[b]
% survival		75%	78%	58.3%[ab]
EWM1*b*	24	8.2 ± 0.4[b]	8.1 ± 0.1[a]	1.244 ± 0.039[b]
% survival		88%	86%	75.0%[ab]
EWM2	24	10.1 ± 0.5[a]	8.6 ± 0.3[a]	1.137 ± 0.054[ab]
% survival		92%	50%	45.8%[a]

0.05). Pupal stage duration was marginally not significantly different by milfoil type (Kruskal-Wallis Test; $H_{4,73} = 8.88$, $P = 0.06$) (Table 1).

The fresh weight of weevils at adult emergence differed significantly by milfoil type (One-way ANOVA; $F = 6.08$, $P < 0.001$) where weevils reared on HYB, EWM1*a* and EMW1*b* were significantly larger than those reared on NWM (Tukey HSD post hoc; $P < 0.05$). Mass of weevils reared on EWM2 were marginally not significantly different than all other milfoil types (Tukey HSD post hoc; $P = 0.07$) (Table 1).

Additionally, the total length of larval burrowing did not significantly differ by milfoil type with a grand mean of 6.22 cm ± 0.35 SE (One Way ANOVA; $F_{4,68} = 1.55$, $P = 0.20$) (Table 2).

Role of host plant preference in the success of milfoil weevil development. Shifts in host plant preference of herbivorous invertebrates occur in both aquatic and terrestrial systems and is often attributed to increases in development and fitness of the insect (Agosta 2006). Previous studies have shown that adult milfoil weevils reared on EWM have an increased preference for oviposition on EWM over NWM followed by increased overall developmental performance including greater survival, size and quicker larval development (Newman et al. 1997, Sheldon and Jones 2001, Solarz and Newman 2001). Similarly, the results of our experiment express lower developmental performance on NWM in comparison to EWM1 and HYB. Milfoil weevil eggs used in this experiment were oviposited on EWM1 populations used in commercial culturing for biological control, thus low survivorship was expected on NWM for this study because of this shift in host plant. Nonetheless, NWM was included to compare weevil development on HYB to both parental congeners. Our results are consistent with previous studies that suggest NWM resistance to herbivory may be contributing to lowered survivorship of the weevil in comparison to its novel hosts (Newman et al. 1997, Sheldon and Jones 2001, Solarz and Newman 2001, Roley and Newman 2006).

Table 2. The mean shoot length (cm) ±1SE and mean larval burrowing length (cm) ±1SE post experiment. Significant differences in each column are denoted by superscript letters (significant at $P < 0.05$). One-way ANOVAs were used to compare all parameters below by milfoil type.

Milfoil type	Total length (cm)	Larval feeding (cm)
NWM	29.5 ± 0.8[a]	4.16 ± 0.66[a]
HYB	40.2 ± 0.9[b]	6.07 ± 0.73[a]
EWM1*a*	32.4 ± 0.7[c]	6.33 ± 0.63[a]
EWM1*b*	36.4 ± 0.8[d]	7.11 ± 0.74[a]
EWM2	30.4 ± 0.8[a]	6.60 ± 0.97[a]

The role of host plant preference in the relationship of the milfoil weevil to HYB is relatively unknown, however previous studies have considered interspecific hybridization to play an important role in the success of herbivorous insects (Fritz et al. 1994, Messina et al. 1996). Since insect response to hybrid hosts is extremely variable, Fritz et al. (1994) defined four possible scenarios of insect performance patterns on hybrid plants in comparison to their parental congeners which include: 1) additive performance, intermediate between parental species; 2) dominance, hybrid is similar to one parental species; 3) hybrid susceptibility, hybrid is more susceptible to herbivory; and 4) hybrid resistance, both parental species are more susceptible to herbivory than the hybrid (Fritz et al. 1994). Although Roley and Newman (2006) observed an additive (intermediate) response of the hybrid to herbivory in comparison to its parental species, the results of our experiment are more suggestive of the dominance or hybrid susceptibility hypothesis proposed by Fritz et al. (1994) where the hybrid performed similar to one parental species or better than both parental species. Developmental performance of the weevil on HYB may have been attributed to increased plant fitness and nutritional value observed in comparison to other milfoil types. It is likely that hybrid vigor contributed to the increased plant fitness of HYB observed through its initial growth in the environmental chamber prior to weevil introduction (Schierenbeck and Ellstrand 2009).

Differences in plant quality by milfoil type. The results of our experiment are consistent with previous studies outlining the positive influence of host plant quality and nutrition on the survivorship, developmental performance, mass at adult emergence and fecundity of herbivorous insects in both terrestrial and aquatic systems (Mattson 1980, Awmack and Leather 2002, Van Hezewijk et al. 2008, Center and Dray 2010).

Prior to weevil introduction, HYB had a significantly higher %N content (2.01% ± 0.08 SE) than all other milfoil types ranging from 1.51% ± 0.02 SE to 1.67% ± 0.09 SE (One-way ANOVA, $F_{4,19} = 8.08$, $P < 0.001$) (Tukey HSD; $P < 0.05$) (Figure 1b). HYB also had a significantly higher % shoot P (0.207% ± 0.012 SE) than all other milfoil types, which ranged from 0.124% ± 0.008 SE to 0.149% ± 0.002 SE (One-way ANOVA, $F_{4,19} = 15.23$, $P < 0.001$) (Tukey HSD; $P < 0.05$) (Figure 1c). Furthermore, % shoot C was significantly lower in NWM (35.4% ± 0.1 SE) than EWM1*a* (38.1% ± 0.2 SE) and EWM1b (37.9% ± 0.2 SE) (Kruskal-Wallis test; $H_{4,25} = 18.37$, $P = 0.001$; Non-parametric post-hoc analysis, $P < 0.05$) (Figure 1a).

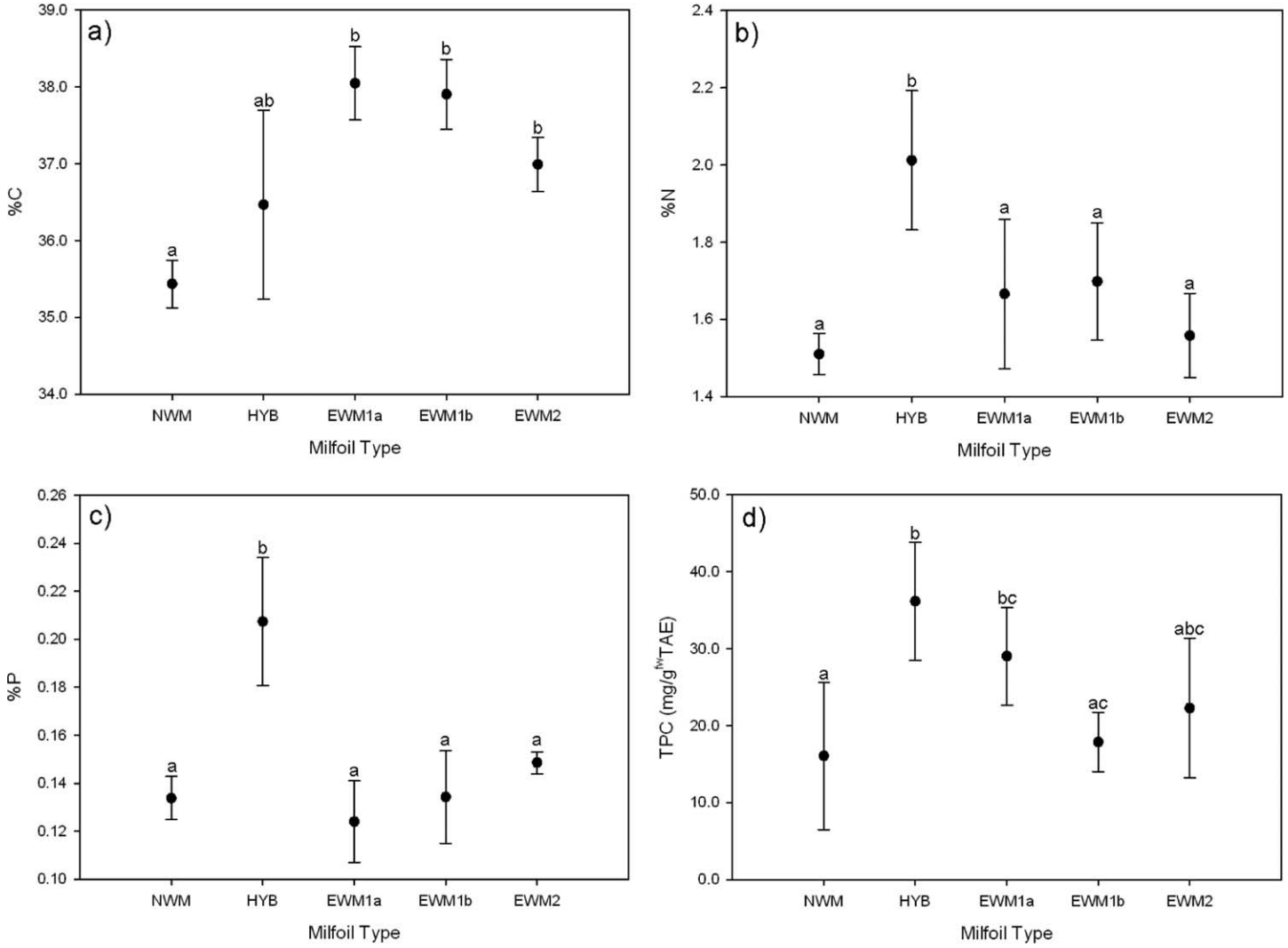

Figure 1. The mean values of a) %C, b) %N and c) %P for each milfoil type prior to milfoil weevil introduction. Tests were based on log transformed data and points represent mean values and error bars represent ±1 standard deviation. Different lettered superscripts denote significantly different populations.

These results are consistent with previous studies which discuss the importance of dietary N content and its positive influence on the developmental performance of other weevil species used in terrestrial and aquatic biological control (Mattson 1980, Wheeler and Center 1997, Awmack and Leather 2002, Newman 2004, Cuda et al. 2008, Van Hezewijk et al. 2008). Because of the design of this experiment we are not able to differences in overall developmental performance were directly caused by specific elements (i.e. N or P); however it is interesting to note that NWM and EWM2 not only had the lowest tissue N and P content, but also the lowest overall survivorship and longer larval development times than all other milfoil types, whereas the opposite was observed for HYB.

Another important consideration for these results is the phenotypic response of milfoil type to the environmental growth chamber including differences in shoot growth, comparatively low % shoot N and P content and plant health prior to collection. The % shoot N and P observed in our experiment were low in comparison to other studies (Grace and Wetzel 1978, Spencer and Ksander 1999, Gross 2003, Marko et al. 2008). Low overall tissue N and P content in all shoots used for this experiment may have been caused by allocation of nutrients needed for root development (Grace and Wetzel 1978). Root development on the lower leaf nodes of the newly fragmented shoots used in this experiment was noticed following the initial two-week growth period (Kyle Borrowman, personal observation). After this two-week period, samples were collected to determine shoot C, N, and P. Thus, lower nutrient concentrations within shoot tissue may have been the result of downward translocation of shoot C, N and P for root development (Grace and Wetzel 1978).

In addition, shoot growth varied significantly by milfoil type. During the initial two weeks, HYB grew noticeably taller than all other milfoil types whereas little change in plant growth was observed for EWM2 and NWM. Similarly, following the experiment, shoot length of HYB was significantly greater than EWM1b and EWM1a; all of which were significantly greater than EWM2 and NWM (One Way

ANOVA; $F_{4,115} = 31.55$; $P < 0.001$) (Tukey HSD; $P<0.05$) (Table 2). This initial growth by HYB may be attributed to increased fitness and quicker physiological response through hybrid vigor (Schierenbeck and Ellstrand 2009). However, it is very likely that this rapid growth could also be attributed to greater fitness of the HYB population related to in-lake characteristics such as nutrient availability prior to collection or because of phenotypic response to conditions within the environmental chamber. It is important to note noticeable increase in plant growth may have had a positive influence on habitat quality for weevil development (Roley and Newman 2006). Roley and Newman (2006) identified that larger stem width in the upper 2 cm of the plant can have a positive influence on weevil mass and survival. Although this was not measured in our experiment, more suitable habitat may have been available to weevils reared on HYB in comparison to other milfoil types because of this noticeable growth (Roley and Newman 2006).

Furthermore, decreased weevil performance on EWM2 was surprising because of high weevil populations at the source lake used for shoot collection. In a 2010 survey, a high natural weevil population was observed in stands of EWM2 consisting of 0.7 weevils per stem (Borrowman et al. 2014). Although biotypic variation has been identified to impact the developmental performance of biological control agents (Grodowitz et al. 1997, Cuda et al. 2008), it is unlikely that host preference plays a major role on the poor development of weevils on EWM2 considering high weevil density observed on EWM2 in 2010 and the successful development on an interspecific hybrid of EWM1 and NWM. Low developmental success on EWM2 is more likely caused by poor plant quality expressed through low nutrient quality of the plant and poor phenotypic response to conditions within the environmental chamber.

CONCLUSIONS

The results of this experiment expand current knowledge surrounding the influence of host plant preference and host plant quality on the growth of herbivorous insects. Our results are consistent with previous research outlining growth and developmental fitness of weevils reared on EWM and HYB over NWM. In addition to specific taxonomic groups of milfoil (EWM, HYB, NWM), differences in genotypic and phenotypic variation may be an important consideration for the developmental performance of the weevil. Unlike Roley and Newman (2006), our results do not show an intermediate developmental performance of the weevil on HYB but rather a similar development to the invasive EWM1 suggesting a dominance or hybrid susceptibility to herbivory as outlined by Fritz et al. (1994). Rather than subscribing to one of the four scenarios outlined by Fritz et al. (1994), it is possible that genotypic variation of milfoil hybrids can allow a wide range of hybrid susceptibility to herbivory. It is also important to note that hybrid resistance of milfoil to herbivory has not yet been identified. As suggested by Moody and Les (2007) the role of genotypic variation of milfoil hybrids on the invasiveness and response to herbivory needs to be investigated further.

Host quality and nutrient availability also appeared to play a major role in the developmental performance of the milfoil weevil in this experiment. Hybrid plants had a higher overall fitness in comparison to all other milfoil types. This increased fitness may be caused by physiological advantages related to hybrid vigor of the plant and may have played an important role in weevil development. Conversely, poor development of weevils on EWM2 surprising because of high weevil populations on EWM2 identified in previous field surveys (Borrowman et al. 2014). This poor weevil development is likely caused by poor phenotypic response of EWM2 to conditions within the growth chamber.In addition to host plant quality and nutrient availability, the role of secondary phenolic compounds on overall plant quality and palatability of EWM is somewhat understood. Further investigation into the role of secondary compounds in the developmental performance and feeding of milfoil weevils would broaden the knowledge and understanding surrounding the success of biological control applications.

From a management perspective, prior knowledge of a distinct EWM lineage or hybrid genotype's response to herbivory could be an important factor in determining the efficacy of a biological control program or other aquatic plant management techniques. These results provide lake managers with a better understanding of factors affecting the efficacy of biological control. Identifying the milfoil taxon to be managed and the associated in-lake weevil populations can provide insight into the potential success of a biological control program. Like most studies investigating the weevil-milfoil relationship, our focus was on the developmental performance of the weevil. Further investigation into the relationship of the weevil with a focus on the plant response to herbivory would help build this current foundation and compliment future management applications.

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