

LEAF DAMAGE AND GENDER BUT NOT FLOWER DAMAGE AFFECT FEMALE FITNESS IN *NEMOPHILA MENZIESII* (HYDROPHYLLACEAE)¹

ANDREW C. MCCALL^{2,3}

²Center for Population Biology, Department of Entomology, University of California-Davis, Davis, California 95616 USA

Researchers can answer questions about the evolution or maintenance of separate sexes using dioecious plant systems. Because females in these species typically put more resources into reproductive effort than male plants, researchers have hypothesized that females may be less tolerant of the stresses found in marginal habitats. Herbivory can act as a biotic stressor that reduces resources in plants much like a marginal habitat can. Females may be limited by resources, and may thus be less tolerant to herbivory than males. Here, I explore the relationships between florivory, leaf herbivory, and gender in a gynodioecious, annual plant, *Nemophila menziesii* (Hydrophyllaceae, sensu lato). I performed a crossed design experiment examining the main effects and interactions of plant gender, artificial leaf damage, and artificial flower damage on components of female plant fitness. Leaf damage decreased fruit set and females made significantly more fruit than hermaphrodites. However, contrary to theory, I found little evidence for a gender by damage interaction for either type of artificial herbivory. Based on these results, I propose more work exploring the effects of both source and sink damage in dioecious species to help elucidate where and when different sexual morphs are favored by natural selection.

Key words: dioecy; floral herbivory; florivory; gynodioecy; *Nemophila menziesii*.

For many years, researchers have been intrigued by the evolution and maintenance of dioecy in angiosperms (Lloyd and Webb, 1977; Renner and Ricklefs, 1995; Sakai and Weller, 1999). Several hypotheses regarding the environmental conditions that favor female or male persistence have come from theoretical work on gender specialization (Bierzychudek and Eckhart, 1988; Pickering and Hill, 2002). In particular, because female plants in dioecious or gynodioecious species pass their genes through seed, which is more costly to produce than pollen, some workers have hypothesized that females may be more limited by resources than male morphs (Bierzychudek and Eckhart, 1988; Delph, 1999). Thus, females may be less successful in marginal habitats than those morphs that retain male function.

Several studies have shown either that females are less successful in poor habitats than males (Lloyd and Webb, 1977; Cox, 1981; Pickering and Hill, 2002; but see Sakai, 1990; Barr, 2002) or that physiological responses to stress affect females more than males (Dawson and Bliss, 1989). There are some advantages to being female as well, with females sometimes producing more seed than male morphs or having a greater proportion of outcrossed progeny than hermaphrodites in gynodioecious species (Sakai et al., 1997). However, leaf herbivory is rarely considered in these studies (but see Cole and

Ashman, 2005), although damage to leaves can be considered a resource-limiting biotic interaction, similar to an abiotic stressor such as drought. Damage to leaves can also reduce female fitness components (Marquis, 1984) and can affect flower characters such as corolla size (Mothershead and Marquis, 2000), pollen viability (Quesada and Stephenson, 1995; Strauss et al., 1996), or nectar quantity (Strauss et al., 1996).

Herbivory is rarely limited to leaf tissue. Damage to flowers or reproductive tissues can have direct and immediate impacts on gamete survival (Olesen, 1992; Weiss, 1996; Wolfe, 2002). Additionally, damage to reproductive tissues may have indirect negative effects on plant fitness through reduced pollinator visitation and pollen export (Neff and Simpson, 1990; Karban and Strauss, 1993; Krupnick and Weis, 1999). These indirect effects may be the result of reduced flower size or symmetry following florivory. For example, bumblebees are known to prefer symmetric flowers vs. asymmetric flowers (Møller and Sorci, 1998; Rodriguez, 2004). Despite these potentially large effects, floral herbivory has received considerably less attention than leaf herbivory (McCall and Irwin, 2006).

Interactions between floral and leaf herbivory have remained relatively unexplored. It is possible that leaf damage may limit a plant's ability to compensate for different types of floral damage or that the combined action of floral and leaf damage may affect fitness in a nonadditive manner. Furthermore, these interactions may help contribute to the relative success of females and hermaphrodites in the field, although little information regarding this scenario is available.

In dioecious species, females that must outcross to produce seed may be less tolerant of petal damage than hermaphrodites, because damaged flowers may be less attractive to pollinators (Ashman et al., 2004). Alternatively, petals may be more important for male function than for female function, because more pollinator visits are usually necessary to export all the pollen from a flower than are necessary to fertilize all the ovules in a flower (Bell, 1985; Stanton and Preston, 1988). Thus, petal damage may also have negative effects on the male

¹ Manuscript received 10 March 2006; revision accepted 19 January 2007.

The author thanks R. Karban, S. Strauss, and K. Rice for providing advice and encouragement during this project. Three anonymous reviewers helped substantially improve an earlier version of this work. C. Covington and R. Gerstemberger provided fantastic field support, and P. Connors and the UC-Davis Bodega Marine Reserve graciously allowed the author to work at BMR. Funding was provided by the Ford Foundation, the Bodega Marine Reserve, the UC-Davis Entomology Department, the UC-Davis Center for Population Biology, and the UC-Davis Section of Evolution and Ecology.

³ Author for correspondence (e-mail: MCCALLA@DENISON.EDU); phone: 740-587-8554; present address: Department of Biology, Denison University, Granville, OH 43023 USA

component of fitness in hermaphrodites. Female plants may be more tolerant of leaf damage up until flowering compared to hermaphrodites (Cole and Ashman, 2005), but less tolerant of leaf damage after fruiting when females put relatively more resources into fruit than do hermaphrodites or males (Ågren et al., 1999). For example, in the gynodioecious plant *Hebe subalpina*, leaf removal prior to anthesis reduced fruit production more in hermaphrodites than in females (Delph, 1990). Because of these mechanisms, some researchers have hypothesized that the maintenance of females in some gynodioecious plant populations may be the result of hermaphrodite-biased herbivory (Wolfe, 1997; Marshall and Ganders, 2001; Ashman, 2002). Unfortunately, we are still lacking data to conclusively evaluate this hypothesis (Ashman, 2002).

Despite the hypotheses regarding the relative effects of leaf and floral damage and the potential evolutionary consequences of sex-biased resistance or tolerance to damage, few studies have manipulated both leaf and petal damage in a gynodioecious plant in the field. A simultaneous exploration of the effects of both leaf and floral damage in such plants would be particularly valuable because damage to both carbon sources (leaves) and sinks (flowers) can be manipulated simultaneously, and interactions among gender, leaf damage, and floral damage may be examined. The distribution of genders that we observe in nature may then be partially explained by these interactions, especially if the sexes are differentially affected by either type of damage.

The gaps in our understanding of how leaf and floral damage interact and how different genders in gynodioecious plants may be affected by these antagonisms led me to conduct a large field experiment designed to answer two questions: (1) Does leaf or flower damage affect components of female fitness? (2) Does leaf or flower damage affect females and hermaphrodites in different ways?

MATERIALS AND METHODS

Study system—*Nemophila menziesii* (Hooker and Arnott, Hydrophyllaceae, sensu lato) is an annual plant native to California and the Pacific Northwest of the United States. This self-compatible, gynodioecious species produces entirely female and entirely hermaphroditic plants. Females produce anthers, but lack functional pollen (Cruden, 1967, 1972). Based on a 2003 field survey, hermaphrodites of *N. menziesii* produce a mean of 6.62 (± 0.48 , 1 SE, median = 5, $N = 150$) flowers per season, yielding a mean of 32.9 (± 4.42 , 1 SE) seeds produced per plant. Females produced a mean of 5.35 (± 0.74 , 1 SE, median = 5, $N = 23$) flowers during a season, yielding a mean of 44.6 (± 9.68 , 1 SE) seeds per plant (A. McCall, unpublished data). Although self-compatible, hermaphrodites in the study population seldom set fruit in the greenhouse (1.8%, $\pm 0.79\%$ of all flowers setting fruit, 1 SE, $N = 45$; A. McCall, unpublished data). Flowers usually open in mid-late February and finish flowering by April or May, depending on local site conditions, principally soil moisture availability. Fruit dehiscence typically occurs in late May or June (A. McCall, unpublished data).

Natural florivory rates can be heavy, with a mean of 21.7% ($\pm 3.2\%$, 1 SE) of flowers damaged per plant in some years (A. McCall, unpublished data). The major floral herbivores of *N. menziesii* at BMR during the peak of flowering (March–May) are larvae of *Platyrepia virginalis* (Lepidoptera: Arctiidae) and *Orgyia vetusta* (Lepidoptera: Lymantriidae). *Platyrepia virginalis* larvae preferentially eat the petals of flowers, both in the field and under laboratory conditions (A. McCall, unpublished manuscript). Leaves in the field are eaten by an unknown Coleopteran larva and the larvae of *O. vetusta*, with most damage occurring prior to the onset of flowering. These antagonists damaged a mean of 32.3% ($\pm 2.7\%$, $N = 50$, range = 0–80%) leaves per plant in 2003 (A. McCall, unpublished data).

Study area—I conducted all experiments and observations at the Bodega Marine Reserve (BMR), in Sonoma County, California, USA during the 2005 field season. The two field sites, Horseshoe Cove and Mussel Point, contained large patches of *N. menziesii* and were surrounded by typical California coastal grassland with introduced annual grasses and a variety of native forbs (described in detail by Barbour et al., 1973). As is common on the California coast, BMR has a Mediterranean climate, with wet winters and much drier summers. For 1967–2002, the mean December precipitation was 14.31 mm (± 1.74 , 1 SE), and the mean June precipitation was 0.61 mm (± 0.12), with a mean annual rainfall of 85.33 mm (± 5.85).

Pollinators—I collected the three major pollinators found at BMR on *N. menziesii* during the peak of flowering in 2003; these were identified by Dr. Robbin Thorp of the UC-Davis Bohart Museum of Entomology as *Habropoda miserabilis* (Anthophoridae), *Bombus vosnesenskii* (Apidae), and *Andrena saccata* (Andrenidae). These insects made up the great majority of floral visitors that actually contacted both anthers and stigmatic lobes in *N. menziesii* (A. McCall, personal observation). These observations are qualitatively similar to pollinator data that Cruden (1967, 1972) collected on *N. menziesii* in its southern range; we both found that solitary bees made many of the pollination visits.

The effects of leaf damage, floral damage, sex, and site—To test the effects of petal damage, leaf damage, gender, and site, I conducted a four-factor, partially crossed experiment at two separate sites. Plants from one site, Horseshoe Cove, had previously shown significant pollen limitation in the presence of artificial petal damage only, suggesting that pollinators discriminated against damaged flowers at this site. Another difference between the sites was that there were no female plants found in the experimental transects at Horseshoe Cove, whereas females were more common at Mussel Point. In contrast, plants at Mussel Point had no pollen limitation with or without artificial petal damage (A. McCall, unpublished manuscript). These sites were separated by a strip of coastal shrubland approximately 1.2 km in length, so pollen can likely travel between the sites. The potential difference in pollinator service between these sites was suitable for this study because pollinator environment may interact with manipulations that affect flower display or size. Because I was interested in these particular sites as a result of the different effects pollinators had on damaged versus control plants, I considered site as a fixed factor in the statistical analyses.

On 25 December 2004, I marked 378 plants at Mussel Point and 162 plants at Horseshoe Cove along 20 m linear transects for a total of 540 plants. I then randomly assigned each plant to one of four treatments using artificial clipping damage: (1) 25% leaf damage only, (2) 25% petal area removal only, (3) both 25% leaf and 25% petal damage, and (4) unmanipulated controls. A total of 36 female plants, all at the Mussel Point site, and 504 hermaphrodites were chosen for the study.

To attain the 25% leaf damage for the appropriate treatments, I removed 50% of the leaf area along the midrib of 50% of all leaves produced until flowering started. This type of damage is typical of *O. vetusta* leaf herbivory, with natural damage rarely cutting the leaf midrib. For the flower damage treatment, I removed approximately 50% of the petal mass of 50% of all open flowers throughout the season. One-half of each petal was removed with a hole punch, creating a standard pattern of corolla damage across different plants. This type and amount of damage mimics the effects and patterns of natural florivore damage and produces significantly asymmetric and smaller corollas than unmanipulated controls (A. McCall, unpublished manuscript). All flower damage was applied after anthesis, but prior to stigma maturation so that petal damage, if important, would affect pollen receipt on the stigma. I also counted the number of leaves produced by each plant 1 month after germination for use as a covariate in the statistical analyses because larger plants usually produce more fruit (A. McCall, unpublished data).

All flowers were marked with a piece of colored thread, and I counted the total number of flowers and fruit produced by each plant at the end of the season, on 5 April 2005. In *N. menziesii*, fruit production is correlated with seed production (Pearson's $r = 0.65$, $P < 0.001$, $N = 162$; A. McCall, unpublished data), so fruit number should be an appropriate proxy for lifetime female fitness.

Data analysis—Many plants died prior to flowering, which is not unusual for plants at BMR (A. McCall, unpublished data). To determine if any of the experimental factors affected mortality prior to flowering, I performed a logistic regression with mortality prior to flowering as the dependent, binary factor and

TABLE 1. The effects of various explanatory factors on the mortality of *Nemophila menziesii* at the Bodega Marine Reserve using a logistic regression model. In this model, the regression coefficient for each explanatory variable is tested for statistical significance (null = coefficient is zero) and is approximately Chi-square distributed. Boldfaced values are significant at the $\alpha = 0.05$ level.

Effect	Mortality	
	χ^2	P
Leaf damage	0.00	0.992
Flower damage	0.00	0.992
Gender	10.98	0.0009
Site	6.27	0.0122
Leaf \times flower	3.51	0.061
Leaf \times gender	<0.0001	0.993
Leaf \times site	2.89	0.089
Flower \times gender	<0.0001	0.993
Flower \times site	0.59	0.441
Leaf \times flower \times gender	0.03	0.855
Leaf \times flower \times site	3.80	0.079
Initial leaf no.	7.61	0.0058

each of the fixed factors and all of the interactions, except those including site by gender, as the dependent variables. I analyzed the effects of the explanatory variables using logistic regression in JMP. The regression coefficient of each independent variable is approximately chi-square distributed, and the significance of each coefficient was tested using the chi-square test. Site by gender was not considered because one site, Horeshoe Cove, had no females in the sample population. To examine the effects of the treatments on components of female fecundity, I also performed separate ANCOVAs with total flowers produced and total fruit number as response variables and leaf number as a covariate.

If a plant died before flowering, it was scored as producing zero flowers and fruit. For main effects, I used leaf damage (fixed), flower damage (fixed), site (fixed), and gender (fixed). Not all interactions among main effects were included in the model because the Horseshoe Cove site had no female plants. Thus, interactions including site and gender were not considered. I log-transformed both response variables to improve the homogeneity of variances among factor values. As required for the ANCOVA model, there were no significant interactions between the covariate and any of the main effects for either response variable (data not shown). Because I measured two separate variables, flower production and fruit set, using the same ANCOVA model, P values were adjusted using the sequential Bonferroni method (Rice, 1989).

All statistics were performed with JMP version 6.02 for Macintosh (SAS Institute, Cary, North Carolina, USA).

RESULTS

Mortality—Site identity had a strong effect on plant mortality prior to flowering, with plants at Mussel Point nearly twice as likely to die as plants at Horseshoe Cove (26% vs. 15%, Table 1). Gender also affected survival to flowering, with hermaphrodites having a threefold higher mortality rate than females (23% vs. 8%, Table 1). There were no significant effects of leaf or flower treatment on mortality and no significant interactions among any factors, although the leaf by flower treatment interaction was marginally significant at the $\alpha = 0.05$ level (Table 1). Larger plants were significantly more likely to survive to flowering than smaller plants. Examining the extremes of initial leaf number, 62% of the plants having four initial leaves survived to flower ($N = 114$), while 100% of the plants having nine initial leaves survived to flower ($N = 6$) (Table 1, data not shown).

Components of maternal fitness—The ANCOVA showed that plants with leaf damage produced 20% fewer flowers than

TABLE 2. The effects of independent variables on lifetime fruit production and lifetime flower production in *Nemophila menziesii* at Bodega Marine Reserve. Boldfaced values are significant at the $\alpha = 0.05$ level.

Effect	Flower number		Fruit number	
	F	P	F	P
Leaf damage	4.26	0.039	5.37	0.021
Flower damage	0.27	0.606	0.10	0.919
Gender	11.86	0.0006	11.29	0.0008
Site	2.14	0.144	25.58	<0.0001
Leaf \times flower	0.75	0.388	0.0009	0.977
Leaf \times gender	2.33	0.127	1.94	0.164
Leaf \times site	0.04	0.847	0.006	0.941
Flower \times gender	0.02	0.899	0.11	0.742
Flower \times site	2.79	0.095	0.03	0.859
Leaf \times flower \times gender	0.96	0.329	0.03	0.855
Leaf \times flower \times site	0.02	0.886	0.01	0.908
Initial leaf no.	28.90	<0.0001	24.28	<0.0001

plants without damage (controls = 4.04 ± 0.25 , 1 standard error of the mean (SE); leaf damaged = 3.35 ± 0.24 , 1 SE; Table 2) and females produced 60% more flowers than hermaphrodites (hermaphrodites = 3.54 ± 0.67 , 1 SE; females = 5.78 ± 0.18 , 1 SE; Table 2). As expected, initial leaf number had a strong, significant positive relationship with flower number (Table 2). Plants with leaf damage produced almost 30% fewer total fruit than unclipped controls (controls = 2.66 ± 0.17 , 1 SE; leaf damaged = 2.07 ± 0.17 , 1 SE; Table 2, Fig. 1), and females produced a mean of one more fruit throughout the season than hermaphrodites (hermaphrodites = 2.29 ± 0.13 , 1 SE; females = 3.31 ± 0.47 , 1 SE; Table 2, Fig. 2). Site identity also significantly affected fruit set, with plants at Horseshoe Cove producing 55% more fruit than plants at Mussel Point (Horseshoe Cove = 3.54 ± 0.67 , 1 SE; Mussel Point = 5.78 ± 0.18 , 1 SE; Table 2, Fig. 3). Finally, there were no significant interactions between gender and leaf damage, between gender and flower damage, or among leaf damage, flower damage, and gender (Table 2).

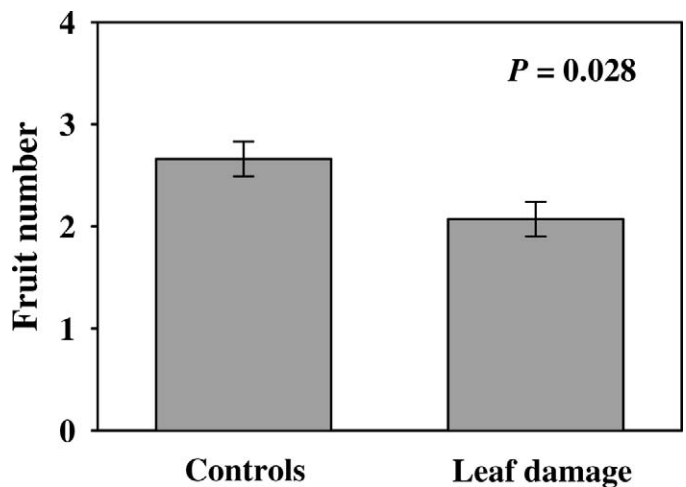


Fig. 1. The effects of artificial leaf clipping on fruit set in naturally occurring *Nemophila menziesii* plants at the Bodega Marine Reserve. Bars show means and 1 SE about the mean.

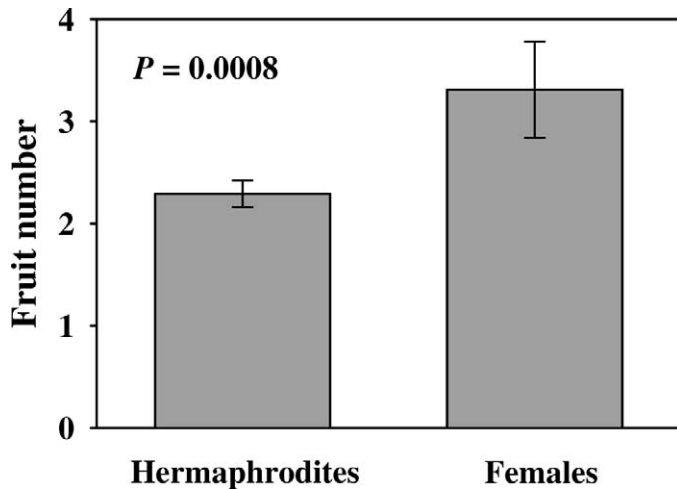


Fig. 2. The effects of gender on lifetime fruit set in naturally occurring *Nemophila menziesii* at the Bodega Marine Reserve. Bars show means and 1 SE about the mean.

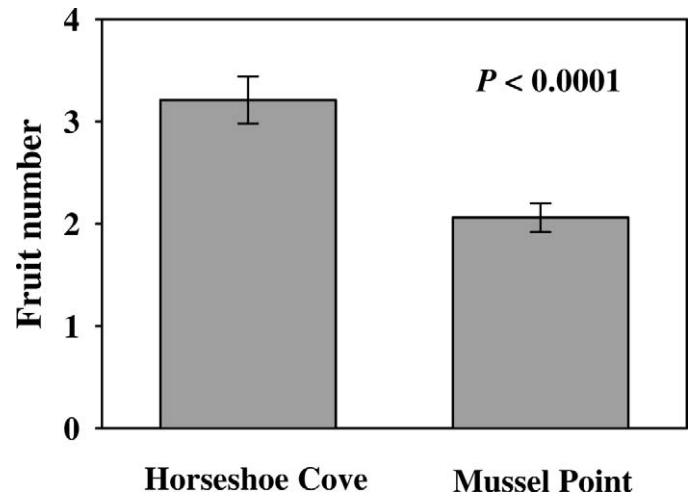


Fig. 3. The effects of different study sites of naturally occurring *Nemophila menziesii* on total fruit set within Bodega Marine Reserve. Bars show means and 1 SE about the mean.

DISCUSSION

This study aimed to determine how leaf damage, flower damage, and gender affect female fitness. It is clear from the results that leaf damage is more important than flower damage at BMR, and there was little evidence that any type of damage affected the genders differentially (Table 2). Gender by itself was a strong predictor of female fitness, with females surviving longer and producing more fruit than hermaphrodites at both sites (Tables 1 and 2). The absence of any interactions among gender and damage is inconsistent with the hypothesis that females may suffer less from herbivory prior to flowering than males or hermaphrodites (Delph, 1990; Ashman, 1994). A lack of significant interactions among gender and damage is also inconsistent with the hypothesis that females may be less tolerant of damage during flowering or fruiting. These results, however, are consistent with the finding that *N. menziesii* females and hermaphrodites are not differentially distributed according to moisture levels across several populations (Barr, 2004). Thus, *N. menziesii* females may not be as sensitive to either lowered moisture levels or reduced resources via herbivory than other dioecious species that have been studied.

Some caution should be taken when making inferences about any gender by damage interactions because there were only 36 females in the entire study, reducing the power to detect significant effects. In fact, a posthoc power analysis ($\alpha = 0.05$, fruit number as the response) for finding a significant gender by flower damage effect yielded a power of 0.11, power analysis for finding a significant gender by leaf damage effect yielded a power of 0.09, and power analysis for finding a significant gender by leaf damage by flower damage interaction yielded a power of 0.05. Thus, there is ample evidence that a lack of interactions among these factors may be due to a lack of statistical power.

An alternative reason for a lack of a gender by damage interaction may be due to the timing of damage; I only damaged leaves prior to flowering. If females have differential reductions in fitness compared to hermaphrodites only when damage occurs at fruiting, the damage treatment may have been insufficient to cause significant negative effects. Damage to *N.*

menziesii leaves, however, typically occurs early in the season prior to the onset of flowering, with little leaf damage occurring during fruit set (A. McCall, personal observation). Thus, the imposed damage, although perhaps not timed to cause the greatest fitness decrease, was realistic in terms of the actual temporal patterns of herbivory.

In many dioecious or subdioecious plants females produce more fruit than plants that retain male function (Delph, 1999, but see Antos and Allen, 1999). Males may produce more flowers than females because increased display size may increase male function more than female function. In this study, females produced more fruit and flowers throughout the season than hermaphrodites, which is inconsistent with the hypothesis that floral display is linked more tightly to male function rather than female performance. It is important to note, however, that there are usually only two or three flowers open at once on a given plant (A. McCall, unpublished data). Thus, lifetime flower production may not be an accurate measure of the day-to-day floral presentation that would affect pollinator visitation.

Site identity also had a large effect on female fitness, with plants at Mussel Point consistently producing fewer seeds and fruit than plants at Horseshoe Cove. The lack of interaction between damage and site was not expected; previous work in 2003 had suggested that pollen limitation in the face of artificial petal damage occurred at Horseshoe Cove but not at Mussel Point (A. McCall, unpublished manuscript). It is possible that, like herbivory, pollinator service varies temporally and spatially at BMR, with evidence of pollinator limitation in one particular year not necessarily being a good predictor of service in later years.

One limitation of this study is that it was conducted over a single field season. Florivory rates and pollinator service vary across seasons at BMR just as they do in other systems (A. McCall, unpublished data), so it is possible that the effects I observed could be quite different in a different year. For example, if a very warm winter causes early germination and flowering of *N. menziesii* and precludes normal pollinator service, herbivory may be of relatively little importance in the long-term. In fact, the mean February–March temperature for

the year of the study, 2005, was marginally greater than the same time period for the years between 2003–2006 (2003 = 12.76°C, ± 1.1 SE; 2004 = 11.78°C ± 1.1 ; 2005 = 15.48°C ± 1.1 ; 2006 = 11.22°C ± 1.1 ; $F_{3,16} = 2.82$, $P = 0.07$), although data from more years is necessary before I can conclusively state that 2005 was particularly abnormal in temperature. Finally, I also did not measure the effects of damage on male function. Petal damage may affect male function more than female function (Bell, 1985), so it is possible that a gender by damage interaction was overlooked by not measuring either pollen production or export.

Because females were less likely to die than hermaphrodites and also produced more flowers and fruit than hermaphrodites, it remains unexplained why so few females were found. It may be the case, as some researchers have suggested, that female frequency in *N. menziesii* is negatively associated with resource quality (Delph, 1990). Barr (2004) found that the *N. menziesii* female advantage in seed production over hermaphrodites was more pronounced in dry sites than in wet sites in one population near BMR, but that there was no significant relationship between female frequency and soil moisture across a range of populations. For the particular sites in the present study, I have no information on the soil moisture differences between Horseshoe Cove and Mussel Point, although they do not seem qualitatively different in soil moisture when the soil in each site is overturned by hand (A. McCall, personal observation). Nevertheless, it is possible that unknown abiotic factors are major determinants of female success at BMR and that there is differential distribution of females based on these factors. This unequal distribution could add unexplained variance to experiments that look at the effects of herbivory on the different sexes and could be remedied by planting known numbers of females and hermaphrodites at different sites prior to experimental manipulations.

These results contribute to the relatively few studies that have examined the effects of herbivory on different genders in gynodioecious plants. Seminal work on tolerance to herbivory and gynodioecy has been performed with the model system *Fragaria virginiana* (wild strawberry). In a field study, spittlebug damage to carbon sources negatively affected hermaphrodites more than females, but in a greenhouse study, artificial flower clipping negatively affected female fitness more than hermaphrodites (Ashman et al., 2004; Cole and Ashman, 2005). The results from the present study do not suggest such differential tolerance based on gender, but there are important differences between *N. menziesii* and *F. virginiana*. First, the flower clipping study in *F. virginiana* was performed in the greenhouse, which may not mimic the field conditions that the plant normally experiences. If the typically harsher conditions in the field were applied, it is possible that hermaphrodite tolerance would decrease to match female tolerance levels. Second, *N. menziesii* is an annual plant and *F. virginiana* is a perennial plant. Perennials may forego reproduction one year in order to store resources for use in a later season (Simons and Johnston, 2003). *Fragaria virginiana* females, in response to flower clipping, may shunt resources to storage for later use, thus resulting in reduced tolerance to clipping in the year that damage is sustained. Thus, even if females and hermaphrodites suffer the same absolute amount of resource reduction, reduced tolerance may only be realized in females. *Nemophila menziesii* females, on the other hand, have no such alternative and most likely put all possible resources into maturing fruit, even if significant amounts of

floral damage occur, possibly resulting in little differential tolerance to floral herbivory between the genders.

In summary, this study shows that artificial damage to leaves is relatively more important than petal damage in this particular population and that there is little evidence that either flower or leaf damage affects the different genders in different ways. Gender itself is an important predictor of female fitness in this species, regardless of damage type, and physical site plays a large role in determining female fitness. It is still too early to make generalizations regarding the differential effects of herbivory on the sexes (Cole and Ashman, 2005), but it is hoped that this study will spur more research on the roles that herbivory, both on sources and sinks, play in determining the relative success of different gender morphs in nature.

LITERATURE CITED

- ÅGREN, J., K. DANNELL, T. ELMQVIST, L. ERICSON, AND J. HJÄLTEN. 1999. Sexual dimorphism and biotic interactions. In M. A. Geber, T. E. Dawson, and L. F. Delph [eds.], *Gender and sexual polymorphism in flowering plants*, 217–246. Springer-Verlag, Berlin, Germany.
- ANTOS, J. A., AND G. A. ALLEN. 1999. Patterns of reproductive effort in male and female shrubs of *Oemleria cerasiformis*: a 6-year study. *Journal of Ecology* 87: 77–84.
- ASHMAN, T. L. 1994. A dynamic perspective on the physiological cost of reproduction in plants. *American Naturalist* 144: 300–316.
- ASHMAN, T. L. 2002. The role of herbivores in the evolution of separate sexes from hermaphroditism. *Ecology* 83: 1175–1184.
- ASHMAN, T. L., D. H. COLE, AND M. BRADBURN. 2004. Sex-differential resistance and tolerance to herbivory in a gynodioecious wild strawberry. *Ecology* 85: 2550–2559.
- BARBOUR, M. G., R. B. CRAIG, F. R. DRYSDALE, AND M. T. GHISELIN. 1973. *Coastal ecology of Bodega Head*. University of California Press, Berkeley, California, USA.
- BARR, C. M. 2004. Soil moisture and sex ratio in a plant with nuclear-cytoplasmic sex inheritance. *Proceedings of the Royal Society of London, B, Biological Sciences* 271: 1935–1939.
- BIERZYCHUDEK, P., AND V. ECKHART. 1988. Spatial segregation of the sexes of dioecious plants. *American Naturalist* 132: 34–43.
- COLE, D. H., AND T. L. ASHMAN. 2005. Sexes show differential tolerance to spittlebug damage and consequences of damage for multi-species interactions. *American Journal of Botany* 92: 1708–1713.
- COX, P. A. 1981. Niche partitioning between sexes of dioecious plants. *American Naturalist* 117: 295–307.
- CRUDEN, R. W. 1967. Genecological studies of *Nemophila menziesii* H. and A. (Hydrophyllaceae). Ph.D. dissertation, University of California, Berkeley, California, USA.
- CRUDEN, R. W. 1972. Pollination biology of *Nemophila menziesii* with comments of the evolution of oligolectic bees. *Evolution* 26: 373–389.
- DAWSON, T. E., AND L. C. BLISS. 1989. Patterns of water use and tissue water relations in the dioecious shrub, *Salix arctica*: the physiological basis for habitat partitioning between the sexes. *Oecologia* 79: 332–343.
- DELPH, L. F. 1990. Sex-differential resource allocation patterns in the subdioecious shrub *Hebe subalpina*. *Ecology* 71: 1342–1351.
- DELPH, L. F. 1999. Sexual dimorphism in life history. In M. A. Geber, T. E. Dawson, and L. F. Delph [eds.], *Gender and sexual dimorphism in flowering plants*, 149–174. Springer-Verlag, Berlin, Germany.
- KARBAN, R., AND S. Y. STRAUSS. 1993. Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. *Ecology* 74: 39–46.
- KRUPNICK, G. A., AND A. E. WEIS. 1999. The effect of floral herbivory on male and female reproductive success in *Isomeris arborea*. *Ecology* 80: 135–149.
- LLOYD, D. G., AND C. J. WEBB. 1977. Secondary sex characteristics in plants. *Botanical Review* 43: 177–216.

- MARQUIS, R. J. 1984. Leaf herbivores decrease fitness of a tropical plant. *Science* 226: 537–539.
- MARSHALL, M., AND F. R. GANDERS. 2001. Sex-biased seed predation and the maintenance of females in a gynodioecious plant. *American Journal of Botany* 88: 1437–1443.
- MCCALL, A. C., AND R. E. IRWIN. 2006. Florivory: the intersection of pollination and herbivory. *Ecology Letters* 9: 1351–1365.
- MØLLER, A. P., AND G. SORCI. 1998. Insect preference for symmetrical artificial flowers. *Oecologia* 114: 37–42.
- MOTHERSHEAD, K., AND R. J. MARQUIS. 2000. Fitness impacts of herbivory through indirect effects on plant–pollinator interactions in *Oenothera macrocarpa*. *Ecology* 81: 30–40.
- NEFF, J. L., AND B. B. SIMPSON. 1990. The roles of phenology and reward structure in the pollination biology of wild sunflower (*Helianthus annuus* L., Asteraceae). *Israel Journal of Botany* 39: 197–216.
- OLESEN, J. M. 1992. Flower mining by moth larvae vs. pollination by beetles and bees in the cauliflorous *Sapranthus palanga* (Annonaceae) in Costa Rica. *Flora* 187: 9–15.
- PICKERING, C. M., AND W. HILL. 2002. Reproductive ecology and the effect of altitude on sex ratios in the dioecious herb *Aciphylla simplicifolia* (Apiaceae). *Australian Journal of Botany* 50: 289–300.
- QUESADA, M. B. K., AND A. G. STEPHENSON. 1995. Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. *Ecology* 76: 437–443.
- RENNER, S. S., AND R. E. RICKLEFS. 1995. Dioecy and its correlates in the flowering plants. *American Journal of Botany* 82: 596–606.
- RICE, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43: 223–225.
- RODRIGUEZ, I., A. GUMBERT, N. H. DE IBERRA, J. KUNZE, AND M. GIURFA. 2004. Symmetry is in the eye of the ‘beeholder’: innate preference for bilateral symmetry in flower-naïve bumblebees. *Naturwissenschaften* 91: 374–377.
- SAKAI, A. K. 1990. Sex ratios of red maple (*Acer rubrum*) populations in northern Lower Michigan. *Ecology* 71: 571–580.
- SAKAI, A. K., AND S. G. WELLER. 1999. Gender and sexual dimorphism in flowering plants: a review of terminology, biogeographic patterns, ecological correlates, and phylogenetic approaches. In M. A. Geber, T. E. Dawson, and L. F. Delph [eds.], *Gender and sexual dimorphism in flowering plants*, 1–32. Springer-Verlag, Berlin, Germany.
- SIMONS, A. M., AND M. O. JOHNSTON. 2003. Suboptimal timing of reproduction in *Lobelia inflata* may be a conservative bet-hedging strategy. *Journal of Evolutionary Biology* 16: 233–243.
- STANTON, M. L., AND R. E. PRESTON. 1988. Ecological consequences and phenotypic correlates of petal size variation in wild radish, *Raphanus sativus* (Brassicaceae). *American Journal of Botany* 75: 528–539.
- STRAUSS, S. Y., J. K. CONNER, AND S. L. RUSH. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. *American Naturalist* 147: 1098–1107.
- WEISS, M. R. 1996. Pollen-feeding fly alters floral phenotypic gender in *Centropogon solanifolius* (Campanulaceae). *Biotropica* 28: 770–773.
- WOLFE, L. M. 1997. Differential flower herbivory and gall formation on males and females of *Neea psychotrioides*, a dioecious tree. *Biotropica* 29: 169–174.
- WOLFE, L. M. 2002. Why alien invaders succeed: support for the escape-from-enemy hypothesis. *American Naturalist* 160: 705–711.