

## SHORT COMMUNICATION

# Petal herbivory by chrysomelid beetles (*Phyllotreta* sp.) is detrimental to pollination and seed production in *Lepidium papilliferum* (Brassicaceae)

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**Abstract.** 1. Insect herbivory directed at flowers can decrease fruit and seed production by decreasing the attractiveness of a damaged flower to potential pollinators, by disrupting the transfer of pollen between pollinators and stigmas, or both.

2. Effects of petal herbivory by a chrysomelid beetle (*Phyllotreta* sp.) on pollination and seed production in *Lepidium papilliferum* (Brassicaceae) were examined.

3. Under natural conditions, flowers with a hole chewed in a petal produced fruit and seed at a significantly lower rate than undamaged flowers (44% vs. 80% respectively). However, when damaged and undamaged flowers were hand pollinated, there was no significant difference in fruit or seed set (84% vs. 80% respectively).

4. Petal herbivory in *L. papilliferum* disrupts the effectiveness of insect-mediated pollination, but it does not physically inhibit pollination or seed production.

**Key words.** Chrysomelidae, florivory, herbivory, *Lepidium papilliferum*, pollination.

## Introduction

Insect herbivory can decrease a plant's fitness by disrupting its growth, survival, and reproduction. When herbivory is focused on reproductive structures of a plant the damage can be especially detrimental because it directly affects reproductive success (Zammit & Hood, 1986; Schemske & Horvitz, 1988; Evans *et al.*, 1989; Wallace & O'Dowd, 1989; English-Loeb & Karban, 1992; Pellmyr & Huth, 1994; Krupnick & Weis, 1999). Florivory, which includes damage to the petals of a flower, may reduce pollen dispersal and seed production by disrupting the mutualistic relationship between a flowering plant and its animal pollinators.

Whereas numerous studies have shown that damage caused by insect herbivory alters floral characters and reduces rates of pollinator visitation to flowers of damaged plants (Karbon & Strauss, 1993; Lohman & Berenbaum, 1996; Lehtilä & Strauss, 1997; Krupnick *et al.*, 1999; Mothershead & Marquis, 2000), only a few studies have examined the consequences of florivory on seed production. In one notable example, Mothershead and Marquis (2000) found that herbivory on flowers of *Oenothera macrocarpa* (Onagraceae) in the bud stage reduced the mature flower's corolla diameter, and that the two sphingid moth species that pollinate

*O. macrocarpa* preferred flowers with larger corollas. Reduced visitation to bud-stage damaged flowers caused a 68% decrease in fruit set of the mature flower relative to undamaged flowers. Although Mothershead and Marquis (2000) showed that early damage to flowers alters floral development, leading to a decrease in pollinator visitation and seed production, they did not document the effects of herbivory on mature flowers.

The present study examines whether feeding damage to mature flowers by chrysomelid beetles reduces the likelihood of pollination and fruit set in *Lepidium papilliferum* [(L. Henderson) A. Nels. & J. F. Macbr.], a rare mustard (Brassicaceae) endemic to sagebrush steppe habitat in south-western Idaho. Within sagebrush steppe, *L. papilliferum* inhabits microsites known as 'slick spots', which are areas of high clay and salt concentration and high water retention that most other plants find inhospitable (Meyer, 1995; Quinney, 1998). Reaching 5–40 cm in height, the plant has numerous, multiflowered inflorescences that terminate at the branches. The small flowers have white petals, and filaments of the anthers are covered with club-shaped hairs. Flower numbers range from several score to several thousand per plant, depending on plant size. Blooming typically begins in early to mid May and peaks in early June. By the end of the flowering period, large amounts of orbicular, flattened seed about 2 mm in length are produced. Each mature fruit produces two seeds. Robertson and Klemash (2003) found that approximately 70–80% of flowers on a plant successfully fruit and set seed under natural conditions.

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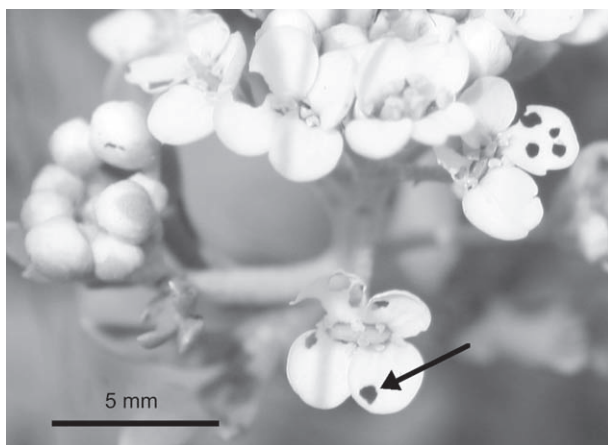
Recent studies indicate that *L. papilliferum* is reliant on insects for pollination (Robertson & Klemash, 2003; Robertson, 2004) and that fruit production is greater when pollination is outcrossed rather than selfed (Robertson & Ulappa, 2004). Robertson and Klemash (2003) noted that at least 25 families of insect visit the plant's flowers for pollen and nectar. Subsequent study has revealed that only a few types of insects contribute substantially to pollination, most notably tachinid and bombilyiid flies, sphecid and vespid wasps, and apid and halictid bees (Robertson *et al.*, 2005).

Although *L. papilliferum* is reliant on insects for pollination, several kinds of insects visiting the plant's flowers are herbivores. The most abundant herbivore is a chrysomelid beetle, *Phyllotreta* sp. (hereafter referred to as *Phyllotreta*), which chews holes in the flower's petals. The damage caused by *Phyllotreta* ranges from a single small ( $\approx 0.5$ -mm diameter) hole chewed in a petal to the loss of complete petals (Fig. 1). Damage by chrysomelid feeding appears to occur on petals in a random fashion.

The objective of this study was two-fold: First, to determine whether petal herbivory in *L. papilliferum* reduces seed production in affected flowers under natural conditions. Second, to establish whether reductions in seed production in damaged flowers, should they exist, were caused by (1) a reduced capacity of damaged flowers to produce seed, independent of pollinator visitation rates, or (2) by a change in the effectiveness of insect-mediated pollination.

## Methods

The study was conducted from May to July 2005 using individual *L. papilliferum* plants located throughout south-western Idaho. In early May, prior to blooming, 72 plants were randomly selected and enclosed within cylindrical cages constructed from 10-mm hardware cloth covered in 0.25-mm white mesh and measuring 10–25 cm in diameter and 10–40 cm in height. The



**Fig. 1.** Flowers of *Lepidium papilliferum* showing feeding damage by *Phyllotreta* beetles (arrow, as well as similar damage visible on other flowers).

cages prevented insect access to plants, thereby ensuring that the flowers used in our experiments were unpollinated.

Once *L. papilliferum* was in bloom, sweep nets were used to collect *Phyllotreta* from *Sisymbrium altissimum* (Brassicaceae) plants at the same study sites. Although *Phyllotreta* also occurred on *L. papilliferum* at the sites, beetles were collected from *S. altissimum* to avoid damaging study plants with the sweep nets. Five *Phyllotreta* were released into each cage and left undisturbed for 24 h. Caged plants were then inspected and a string was tied around one damaged flower (i.e. a flower with a single hole chewed in the petal) and one undamaged flower on each plant. To aid later recognition, a knot was tied at the end of the string around the damaged flower but not the undamaged flower.

Plants were randomly assigned to one of two experiments. In experiment 1, cages were permanently removed from the plants to expose both damaged and undamaged flowers to pollinators and test whether damaged flowers were less likely to produce fruit than undamaged flowers. In experiment 2, the damaged and undamaged flowers on each plant were pollinated by hand using pollen from flowers located 50 m away – Robertson and Ulappa (2004) found that fruit set in *L. papilliferum* is highest at this outcrossing distance. Cages were replaced over plants immediately after hand pollination to prevent access by insects. This manipulation was designed to determine if damaged flowers had reduced capacity to produce fruit, independent of visitation and pollen deposition by insects.

Fourteen days after exposing flowers to insects or hand pollination, the number of flowers that had fruited in each of the experiments was counted. Approximately one-third of the plants died during this period, which is a normal occurrence for *L. papilliferum* at this time of year (I. C. Robertson, unpublished). This left a total of 25 pollinator-exposed plants and 25 hand-pollinated plants for analysis. Fruits were dissected for the presence of seed, but no measurements of the seeds were taken. A flower was scored as having set seed if a mature seed-bearing fruit was produced, and as failing to set seed if the flower had withered off the plant. Effects of treatment were analysed in two separate binary logit regressions using the GENMOD procedure in SAS v. 9.1 (SAS Institute, 2005). Because the data were clustered (i.e. damaged and undamaged flowers occurred on the same plant), generalised estimating equations (GEE) were used to interpret the test statistics (Allison, 1999). Effects of treatment were considered significant when  $P < 0.05$ .

## Results

### *Experiment 1: pollination by insects under natural conditions*

Seed-bearing fruits always contained two seeds. Flowers suffering damage (i.e. a hole chewed in a single petal) from *Phyllotreta* set seed at a significantly lower rate than undamaged flowers on the same plant (Table 1). Eighty per cent (20/25) of undamaged flowers produced fruit when exposed to pollinators, whereas only 44% (11/25) of damaged flowers produced fruit. The odds of fruit production decreased 80% (95% CI = 41–94%) for damaged flowers when compared with undamaged flowers (Table 1).

**Table 1.** Results of binary logit regression analysis using generalised estimating equations (GEE) for relationship between seed set of damaged and undamaged flowers exposed to pollinators. The GEE accounted for damaged and undamaged flowers being clustered on the same plant. Odds of flowers producing seed were modelled. Zeros indicate class variable serving as reference category.

Parameter	d.f.	Estimate	SE	z	P	Odds ratio
Intercept	1	1.39	0.50			
Damaged flowers	1	-1.63	0.56	-2.90	0.0037	0.196
Undamaged flowers	0	0.00	0.00			

### Experiment 2: hand pollination

Although damaged flowers were less likely to set seed when exposed to insects, this reduction was not because the flowers were incapable of producing seed. There was no significant difference in seed production between damaged and undamaged flowers when flowers were hand-pollinated (Table 2). Eighty per cent (20/25) of the undamaged hand-pollinated flowers set seed, whereas 84% (21/25) of damaged hand-pollinated flowers set seed. Clearly, flowers damaged by *Phyllotreta* feeding were capable of producing seed. No attempt was made to germinate the seeds.

## Discussion

*Lepidium papilliferum* flowers damaged by herbivory were significantly less likely to set seed than undamaged flowers on the same plant under natural conditions. However, damaged and undamaged flowers were equally capable of producing seed when hand pollinated. This result indicates that petal herbivory disrupts the effectiveness of insect-mediated pollination, but does not physically inhibit pollination or seed production.

In a similar study, Krupnick and Weis (1999) found that herbivore-damaged *Isomeris arborea* (Capparaceae) flowers had abnormally low stigmatic pollen loads and produced fewer seeds than undamaged flowers. The low stigmatic pollen loads in *I. arborea* suggest that a disruption in pollen transfer by insects may have caused reduced seed set. However, unlike the findings for *L. papilliferum*, hand pollination of damaged

**Table 2.** Results of binary logit regression analysis using generalised estimating equations (GEE) for relationship between seed set of damaged and undamaged hand-pollinated flowers. The GEE accounted for damaged and undamaged flowers being clustered on the same plant. Odds of flowers producing seed were modelled. Zeros indicate class variable serving as a reference category.

Parameter	d.f.	Estimate	SE	z	P	Odds ratio
Intercept	1	1.39	0.50			
Damaged flowers	1	0.27	0.61	0.45	0.6544	1.312
Undamaged flowers	0	0.00	0.00			

*I. arborea* flowers did not increase their rate of seed production. This result indicates that pollen limitation was not the primary cause of reduced seed set in *I. arborea*. Instead, Krupnick and Weis (1999) determined that herbivory destroyed gametes and made flowers less capable of setting seed. The contrast between the results for *L. papilliferum* and *I. arborea* emphasises the need for both hand pollination and natural exposure to pollinators when assessing the causes and consequences of florivory on pollination and fruit production.

There are several ways by which petal herbivory may have altered the effectiveness of insect-mediated pollination in *L. papilliferum*. Damaged flowers may have been visited less frequently by pollinators, or by a suite of insects that were less efficient than the pollinators attending undamaged flowers. Decreased pollinator visitation rates to damaged flowers are well documented (e.g. Karban & Strauss, 1993; Krupnick *et al.*, 1999; Mothershead & Marquis, 2000), as are changes in pollinator communities when floral characteristics are altered (Johnson *et al.*, 1995; Conner & Rush, 1996). Damaged flowers may fail to provide insects with the visual cues that attract visitation (e.g. symmetrical flowers: Bell, 1985; Moller & Eriksson, 1995), or they may decrease the area on which to alight, prompting some insects to avoid damaged flowers. Feeding damage may also betray lower nectar or pollen rewards in the flowers of affected plants (Frazee & Marquis, 1994; Quesada *et al.*, 1995; Lohman & Berenbaum, 1996; Strauss *et al.*, 1996; Krupnick *et al.*, 1999). For pollinator insects visiting *L. papilliferum* flowers, the ability to discriminate against flowers with low nectar or pollen rewards may be critical because foraging involves a risk of predation (Robertson & Klemash Maguire, 2005). Finally, it is possible that damaged flowers inhibit the transfer of pollen from insects to stigmas, resulting in reduced pollination success. Discriminating among these alternatives requires further study. Nevertheless, this study clearly demonstrates that petal herbivory in *L. papilliferum* reduces the effectiveness of insect-mediated pollination and seed production.

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## References

- Allison, P.D. (1999) *Logistic Regression Using the SAS System: Theory and Application*. SAS Institute Inc., Cary, North Carolina.
- Bell, G. (1985) On the function of flowers. *Proceedings of the Royal Society of London B*, **224**, 223–265.
- Conner, J.K. & Rush, S. (1996) Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Oecologia*, **105**, 509–516.
- English-Loeb, G.M. & Karban, M. (1992) Consequences of variation in flowering phenology for seed head herbivory and reproductive success in *Erigeron glaucus* (Compositae). *Oecologia*, **89**, 588–595.

- Evans, E.W., Smith, C.C. & Gendron, R.P. (1989) Timing of reproduction in a prairie legume: seasonal impacts of insects consuming flowers and seeds. *Oecologia*, **78**, 220–230.
- Frazee, J.E. & Marquis, R.J. (1994) Environmental contribution to floral trait variation in *Chamaecrista fasciculata* (Fabaceae: Caesalpinioideae). *American Journal of Botany*, **81**, 206–215.
- Johnson, S.G., Delph, L.F. & Elderkin, C.L. (1995) The effect of petal-size manipulation on pollen removal, seed set, and insect–visitor behavior in *Campanula americana*. *Oecologia*, **102**, 174–179.
- Karban, R. & Strauss, S.Y. (1993) Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. *Ecology*, **74**, 39–46.
- Krupnick, G.A. & Weis, A.E. (1999) The effect of floral herbivory on male and female reproductive success in *Isomeris arborea*. *Ecology*, **80**, 135–149.
- Krupnick, G.A., Weis, A.E. & Campbell, D.R. (1999) The consequences of floral herbivory for pollinator service to *Isomeris arborea*. *Ecology*, **80**, 125–134.
- Lehtilä, K. & Strauss, S.Y. (1997) Leaf damage by herbivores affects attractiveness to pollinators in wild radish, *Raphanus raphanistrum*. *Oecologia*, **111**, 396–403.
- Lohman, D.J. & Berenbaum, M.R. (1996) Impact of floral herbivory by parsnip webworm (Oecophoridae: *Depressaria pastinacella* Duponchel) on pollination and fitness of wild parsnip (Apicaceae: *Pastinaca sativa* L.). *American Midland Naturalist*, **136**, 407–412.
- Meyer, S.E. (1995) *Autecology and Population Biology of Lepidium papilliferum*. Unpublished report on file at State of Idaho Military Division, Army National Guard, Boise, Idaho.
- Moller, A.P. & Eriksson, M. (1995) Pollinator preference for symmetrical flowers and sexual selection in plants. *Oikos*, **73**, 15–22.
- Mothershead, K. & Marquis, R.J. (2000) Fitness impacts of herbivory through indirect effects on plant–pollinator interactions in *Oenothera macrocarpa*. *Ecology*, **81**, 30–40.
- Pellmyr, O. & Huth, C.J. (1994) Evolutionary stability of mutualism between yuccas and yucca moths. *Nature*, **372**, 257–260.
- Quesada, M., Bollman, K. & Stephenson, A.G. (1995) Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. *Ecology*, **76**, 437–443.
- Quinney, D. (1998) *LEPA* (*Lepidium papilliferum*). Unpublished report on file at State of Idaho Military Division, Army National Guard, Boise, Idaho.
- Robertson, I.C. (2004) Importance of outcrossing for fruit production in slickspot peppergrass, *Lepidium papilliferum* L. (Brassicaceae). *Western North American Naturalist*, **64**, 265–268.
- Robertson, I.C. & Klemash Maguire, D. (2005) Crab spiders deter insect visitations to slickspot peppergrass flowers. *Oikos*, **109**, 577–582.
- Robertson, I.C. & Klemash, D. (2003) Insect mediated pollination in slickspot peppergrass, *Lepidium papilliferum* L. (Brassicaceae), and its implications for population viability. *Western North American Naturalist*, **63**, 333–342.
- Robertson, I.C., Novak, S., Leavitt, H. & Stillman, A. (2005) *Lepidium Papilliferum*: Analysis of Pollination, Herbivory, and Genetic Structure of Populations. Unpublished report on file at State of Idaho Military Division, Army National Guard, Boise, Idaho.
- Robertson, I.C. & Ulappa, A.C. (2004) Distance between pollen donor and recipient influences fruiting success in slickspot peppergrass, *Lepidium papilliferum*. *Canadian Journal of Botany*, **82**, 1705–1710.
- SAS Institute (2005) *SAS, Version 9.1*. SAS Institute, Inc., Cary, North Carolina.
- Schemske, D.W. & Horvitz, C.C. (1988) A test of the pollinator limitation hypothesis for a neotropical herb. *Ecology*, **69**, 200–206.
- Strauss, W.Y., Conner, J.K. & Rush, S.L. (1996) Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. *American Naturalist*, **147**, 1098–1107.
- Wallace, D.D. & O’Dowd, D.J. (1989) The effect of nutrients and inflorescence damage by insects on fruit-set by *Banksia spinulosa*. *Oecologia*, **79**, 482–488.
- Zammit, C. & Hood, C.W. (1986) Impact of flower and seed predators on seed-set in two *Banksia* shrubs. *Australian Journal of Ecology*, **11**, 187–193.

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