Search methods for egg and larval stages of Puget & Fender's blue butterflies (*Icaricia icarioides blackmorei* & *I. i. fenderi*) for purposes of experimental studies.

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Life history

Puget and Fender's blues are subspecies of the widespread Boisduval's Blue (*Icaricia icarioides*). Fender's Blue (*I. i. fenderi*) is Federally Endangered, and is found in oak woodland and prairie habitat in the Willamette Valley of Oregon (Pyle 2002). Puget Blue (*I. i. blackmorei*) is a Washington State Candidate, and is found in prairies in the Puget Trough of Washington (Pyle 2002). Both butterflies have the same life cycle and feeding habits, and all life stages are visually similar. Eggs are laid on lupine leaves in May and June, during the butterfly's flight period. Larvae hatch and feed on lupine leaves for one or two weeks, and enter diapause as second or third instar larvae. Larvae emerge from diapause in late February or March and feed for six to eight weeks. The mature, fourth instar larva pupates near the soil surface. Adults eclose in mid-May. Males are blue (Figure 1a) and females are brown (Figure 2a) above, and both sexes are light gray below with black markings (Figures 1b, 2b and 2c). Adults are surveyed using common methods for adult butterfly population assessments. Pupae are virtually impossible to survey in the wild, as they are small (around 10 mm) and hidden in duff and possibly underground.

Similar Species

Silvery Blue (*Glaucopsyche lygdamus*) is a common butterfly and is often confused with Fender's and Puget blue adults. Silvery Blue eggs are indistinguishable from Fender's and Puget blue eggs except for their location: Silvery Blue eggs are almost always laid on lupine buds and flowers (Figure 15), while Fender's and Puget blue eggs are laid exclusively on lupine leaves (Figures 3a-c). Silvery Blue larvae are also similar to Fender's and Puget blues, but are distinguished by a broad, dark dorsal stripe, varying colors from green to pink and purple, and are most often found on lupine flowers or seed pods rather than leaves (Figures 16a-d). Silvery Blue butterflies also differ from Fender's and Puget blue in that the larvae completely develop in one season and overwinter as pupae. In early spring, Fender's and Puget blue larvae are generally larger (third and fourth instar) than Silvery Blue larvae (first and second instar), while in June and July the stages are reversed, with Silvery Blue larvae being larger (third and fourth instar) than Fender's and Puget blue larvae (first and second instar).

Egg identification and survey method

Puget and Fender's blue eggs are 0.7 mm in diameter and greenish-white (Figures 3a-c). Eggs are laid singly, not in clusters, although two to five eggs are sometimes found on one leaflet. Do not confuse these eggs with the orange or creamy brown eggs sometimes found on lupine from other insects, such as Hemipterans. Hatched Puget and Fender's blue eggs look like small white beads (Figure 3b). Hatched eggs are often surrounded by "windows", a unique type of feeding

damage by early instar larvae where they chew tiny holes in leaflets and feed on the inside of the leaflet, producing a small clear area of the leaf (Figures 4a and 4b).

Survey Puget and Fender's blue eggs after the peak flight period, when most eggs have been laid, and before the host lupines have senesced. Observers should focus on the underside of leaves, where most eggs are laid. Occasionally an egg is laid on a stem or on the upper surface of a leaf. The most efficient method to count eggs is as follows:

1. Grasp a single stem of the plant, and search all the leaves on the stem, starting from the bottom and working upwards.
2. Grasp each leaf at the base of the leaflets, gently turn it sideways, and rotate or "twirl" the leaf between the thumb and forefinger to search the underside of each leaflet for eggs.
3. Scan the upper surface of each leaf and the stem for "misplaced" eggs.
4. Repeat search with another stem.
5. Keep track of the stems searched by placing a pin flag or flagging tape between the searched stems and unsearched stems of the plant.
6. Record the number of hatched and unhatched eggs as well as pre-diapause larvae (Figures 3a-d).

Survival of Puget and Fender's blue eggs to post-diapause larvae is 5-10% (Schultz and Crone 1998). Based on these data, 1.5 x 1.5 meter plots need no fewer than 20-25 eggs or pre-diapause larvae per plot to have a statistically detectable effect when assessing post-diapause larvae. If plots have fewer than 25 eggs, then they can be supplemented with eggs and pre-diapause larvae from outside the plots.

1. Scan lupines outside of the plots for "windows"; this is the most efficient way of finding larvae to transfer to other plots. Fresh windows have a green hue while old windows are yellowish or brown.
2. Carefully pinch off the leaf with the larva (it is a good idea to place your hand or a jar under the leaf while doing this, because the larvae frequently fall off).
3. Prop the leaf over some lupine stems near the base of the plant in the plot you wish to supplement. Preferably, place the larva directly on the plant to ensure the wind does not blow away the leaf and larva.
4. Alternatively, unhatched eggs can be used to supplement plots. There are two ways to do this:
    a. Place leaves containing unhatched eggs at the base of the lupine plant to be supplemented. This is not as accurate however, as the leaves may dry before the egg hatches, or the leaves containing transferred eggs may blow away from the plot.
    b. Place filter paper or a paper towel in a small plastic container and lightly mist with water. Place the leaflets containing unhatched eggs on the damp paper (Figure 3a) and cover with a lid or mesh. Check eggs daily and transport newly

hatched larvae to the plots to be supplemented. Change the paper as needed to keep the leaves fresh and not moldy.

Larva identification and survey method

Fender's and Puget blue pre-diapause larvae range from 2 to 5 mm. Larvae are light green after hatching (Figure 3d), and turn pinkish-brown when preparing to diapause (Figures 5a-c). Once they emerge in the spring and begin feeding, they turn green again, and reach up to 16 mm before pupating. Do not confuse them with larger moth larvae sometimes seen on lupine (Figures 12a and 12b).

To assess effects on Puget and Fender's blue larvae, post-diapause larvae are surveyed after an experimental treatment such as an herbicide application. A common error in surveying post-diapause larvae is surveying too late, once most larvae have pupated. Monitor experimental plots starting in mid- or late-March to identify when the larvae emerge from diapause. Larvae prefer feeding on developing leaves (LaBar, personal observations). Indicators that larvae have likely pupated include fully developed lupine leaves with old feeding damage (Figures 9a-c), developing lupine flowers, and emergence of Silvery Blue adults (LaBar, personal observations).

Treat plots at least five days prior to the planned survey date(s). The most accurate assessment is to pair control and treatment plots to account for spatial and temporal variability; therefore, survey paired plots at the same time or one after the other. When searching for post-diapause larvae, first identify any feeding damage that may lead to the discovery of larvae. In the event that few or no larvae are found in plots, the number of feeding damage instances can be recorded and used as a rough indicator of larval survival. Feeding damage includes windows, munched leaves, and munched stems.

1. Windows – small clear patches in the leaves made by early instar larvae, usually pre-diapause but occasionally early post-diapause larvae (Figures 4a and 4b).
2. Munched leaves – fresh feeding is usually seen on developing leaves, where the side of the leaf has been chewed so that two or more leaflets are eaten (Figures 6a-d, 7a and 7b). Old feeding is indicated by fuzzy or wilted tips of leaflets (Figures 8a-c and 9a), wilted leaves (Figures 11a and 11b) or missing leaflets on fully opened leaves (Figures 9b, 9c, and 11b).
3. Munched stems – usually caused by late instar larvae. Fresh feeding is usually still green, while old feeding sometimes has a fuzzy appearance (Figures 10a-c) or has light brown scar tissue. Do not confuse with scar tissue along the length of stems from other damage, or with rodent damage, where stems are chewed off less than an inch from the ground. Munched stems often occur when larvae begin with "munched leaf" behavior and continue to chew through the base of developing leaves.

To search for larvae:

1. Scan the lupine plant for obvious signs of larval feeding before touching the plant. Larvae quickly drop to the ground when disturbed, so it is best to look for larvae before touching the plant.

2. Gently part the lupine plant (push aside some lupine stems) and scan for any larvae immediately visible within the plant.
3. Search nodes of the plant where new leaves are developing. Post-diapause larvae are frequently found close to the ground feeding on developing lupine leaves (LaBar, personal observations; Figures 7a, 7b, and 14b).
4. Scan the underside of leaves and stems. Early post-diapause larvae are sometimes found feeding on developed leaves (creating windows), and late instar larvae are sometimes found chewing holes in the side of stems.
5. After searching all the lupine stems, part the stems and carefully search the base of the plant, being careful not to potentially squish any hiding larvae. If there is loose moss or other debris at the base of the plant, carefully move it aside to search for hiding larvae.

The presence of ants on or near lupine plants is another sign of the presence of Puget and Fender's blue larvae (Figures 6a, 13a-b and 14a-b) as well as Silvery Blue larvae (Figures 16b and 16d). Ant tending is common in many Lycaenid butterfly species, a relationship where ants protect larvae from predators and parasites in return for sugary secretions (Fraser et al. 2001). The secretion is produced by the dorsal nectary organ on the eighth segment of the larva. Two tentactular organs (Figure 14a) located in the ninth segment also play a role in attracting ants to the larva.

Because of low success in finding post-diapause Puget and Fender's blue larvae for recent research (LaBar 2012), a suggestion for future larval searches is to examine lupine plants in the late evening or at night, as some larvae of *Icaricia* species feed at night and rest in duff or ant tunnels during the day (Pyle 2002).

References

Fraser, A.M., A.H. Axen, and N.E. Pierce. 2001. *Assessing the quality of different ant species as partners of a myrmecophilous butterfly.* Oecologia 129:452-460.

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Pyle, R.M. 2002. *The Butterflies of Cascadia*. Seattle Audubon Society, Seattle, WA.

Schultz, C.B. and E.E. Crone. 1998. *Burning prairie to restore butterfly habitat: a modeling approach to management tradeoffs for the Fender's blue*. Restoration Ecology 6:244-252.


(a)

(b)

Figure 1. Adult Fender's blue males at Cardwell Hills, southwest of Corvallis, Oregon.


(a)

(b)

(c)

Figure 2. Adult Fender's blue females at Cardwell Hills, southwest of Corvallis, Oregon.


(a)

(b)

(c)

(d)

Figure 3. (a, c) Unhatched Puget blue eggs, (b) hatched Puget blue egg, and (d) first instar larva (3mm) from Johnson Prairie, near Tenino, WA.


(a)

(b)

Figure 4. "Window" feeding damage from early instar larvae; (a) at Johnson Prairie, near Tenino, WA, (b) in the greenhouse at Washington State University Vancouver.


(a)

(b)

(c)

Figure 5. Puget blue larva in diapause at Johnson Prairie, near Tenino, WA. Scale shown in (b) is one centimeter.


(a)

(b)

(c)

(d)

Figure 6. Post-diapause larvae feeding on developing leaves (arrows indicate area of feeding). (a) Ant-tended larva at Scatter Creek Wildlife Area south of Olympia, WA, (b, c, d) at Johnson Prairie, near Tenino, WA.


(a)

(b)

Figure 7. Mature Fender's blue larva feeding on lupine (ruler shows centimeters) at Basket Slough NWR. This is "munched leaf" behavior (arrows indicate area of feeding), where the larva is feeding on a developing leaf.


(a)

(b)

(c)

Figure 8. "Munched leaf" feeding damage (white circles). This damage is a few days old.


(a)

(b)

(c)

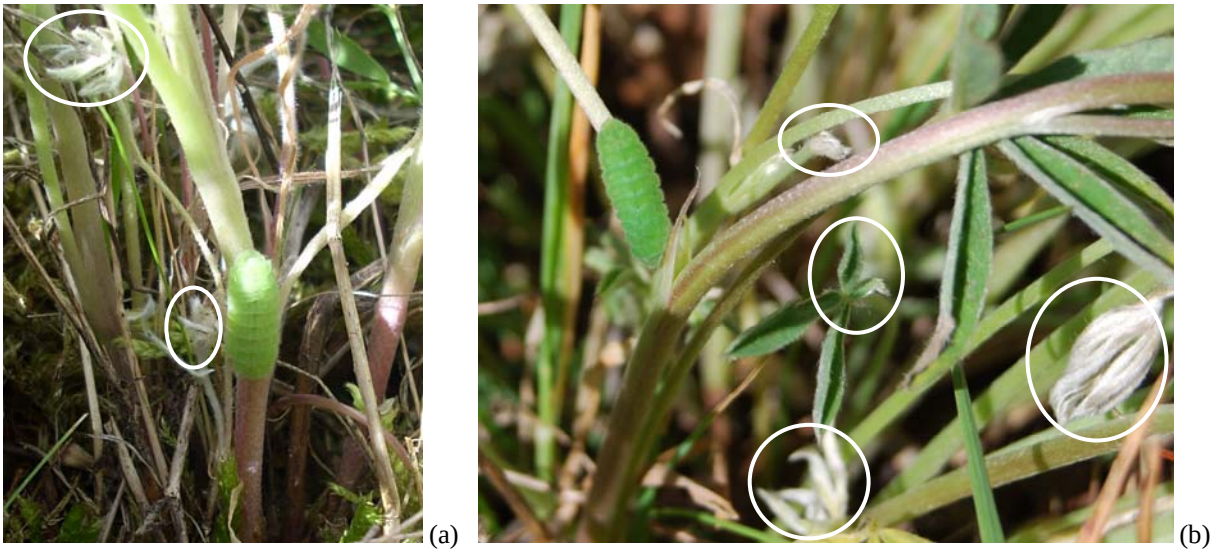
Figure 9. "Munched leaf" feeding damage (white circles). This damage is at least a week old.


(a)

(b)

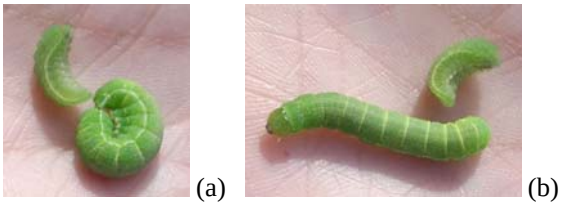
(c)

Figure 10. "Munched stem" feeding damage (white circles). This damage is caused by late instar larvae, and includes stems and leaf petioles that are partly or entirely severed.



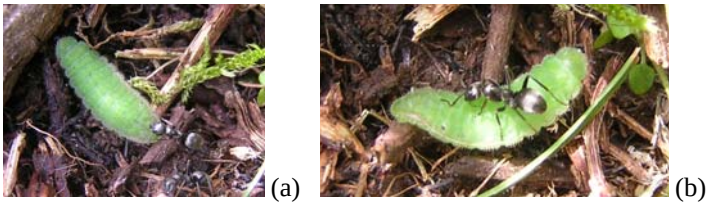
(a) (b)

Figure 11. Mature Fender's blue larvae and older feeding signs at Basket Slough NWR. (a) Larva resting on lupine stem with head facing up; (b) larva crawling on plant with head facing down. White circles indicate larval feeding signs.



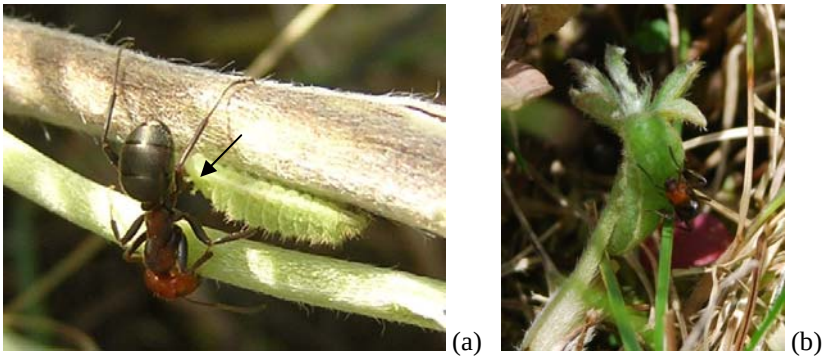
(a) (b)

Figure 12. Comparison between Fender's blue larva (top) and moth larva (bottom).



(a) (b)

Figure 13. Late instar Fender's blue larva tended by ant at the base of a lupine at Basket Slough NWR.



(a) (b)

Figure 14. Larvae tended by ants at (a) Scatter Creek Wildlife Area and (b) Johnson Prairie, near Tenino, WA. (a) Everted tentacular organs (two tubular organs that attract ants, black arrow); (b) late instar larva tended by ant and feeding on developing lupine leaf.

Figure 15. Silvery blue eggs (black arrows) on developing lupine flowers, at Johnson Prairie, near Tenino, WA.

(a)

(b)

(c)

(d)

Figure 16. Silvery blue larvae at Johnson Prairie, near Tenino, WA. (a) Mid-instar larva, (b) mid-instar larva tended by ants, (c) late instar larva feeding on leaves, (d) late instar larva feeding on lupine flower and tended by ants.