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Authors: Tangren, Sara A., and Frye, Christopher T.

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# Clover Stem Borer Infestation in Sundial Lupine: Recognition and Consequences

Sara A. Tangren<sup>1,3</sup> and Christopher T. Frye<sup>2</sup>

<sup>1</sup>University of Maryland Extension, Home & Garden Information Center, 12005 Homewood Road, Ellicott City, MD 21042

<sup>2</sup>Wildlife and Heritage Service, Department of Natural Resources, PO Box 68, 909 Wye Mills Road, Wye Mills, MD 21679

<sup>3</sup>Corresponding author: [invasive.slayer@gmail.com](mailto:invasive.slayer@gmail.com)

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## ABSTRACT

The clover stem borer (CSB; *Languria mozdardi*) is a native North American beetle whose larvae develop inside the stems of a broad range of herbaceous host plants, including sundial lupines (*Lupinus perennis*). Sundial lupine is an imperiled species in many of the jurisdictions where it occurs, and three rare butterfly species depend on it as a larval host plant. As such, sundial lupines are the focus of many conservation projects. Larval damage to the inside of lupine stems reduces pod production, and is thus a threat to the long-term health of lupine populations. Infestations tend to go unnoticed, partly because so much of the insect's life cycle is spent inside the plant stem. Our description of field indicators is intended to help professionals working on lupine conservation projects recognize CSB activity. In Maryland, sites infested with CSB are connected to the anthropogenic meadow network, whereas sites that are not infested are separated from it by a forest buffer.

*Index terms:* anthropogenic meadows; *Languria mozdardi*; *Lupinus perennis*; pod set; stem borer

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## INTRODUCTION

This study adds a rare plant, sundial lupine (*Lupinus perennis* L.), to the list of known larval host plants for the native beetle, clover stem borer (CSB; *Languria mozdardi* Latr.). Sundial lupine occurs in the eastern United States and is considered extirpated, critically imperiled, imperiled, or vulnerable in 17 of the 29 states within its natural range (NatureServe 2019). Several insect species use the sundial lupine as a larval host plant, feeding externally on stems, leaves, flowers, and fruits. These insects include imperiled butterflies such as the Karner blue (*Lycaeides melissa samuelis* Nabokov), the frosted elfin (*Callophrys irus* Godart), and the persius duskywing (*Erynnis persius persius* Scudder). Conservation of these wildflowers and the butterflies that depend on them requires that we create, restore, and maintain landscapes that support viable sundial lupine populations.

The clover stem borer is a beetle native throughout much of North America (Quebec–Manitoba to Florida–Texas and northern Mexico; Bugguide 2019). This study adds sundial lupine to a lengthy list of CSB larval host plants (Wildermuth and Gates 1920; Fothergill et al. 2013), and describes the internal and external appearance of sundial lupine stems occupied by CSBs. The lack of published work on this topic may be because of the cryptic nature of stem-boring insects, or because the damage to the plant's vascular system is not outwardly apparent until late in the plant's life cycle, when pods are ripening.

The purpose of this paper is to determine whether CSB infestation results in decreased reproductive capacity of sundial lupine stems. Additionally, we provide descriptions and illustrations of the appearance of CSB infestation, and report on the extent of CSB infestation in Maryland sundial lupine populations.

## METHODS

To determine the distribution and frequency of CSB activity in sundial lupine, we collected stems from eight sites across Maryland between 2014 and 2018. Our primary goal was to construct a model of the impact of CSB infestation on lupine reproductive capacity. We estimated the number of stems needed based on the determining variable (two levels of infestation) and the response variable (typically 0–15 pods per stem). This suggests an adequate sample size of around 300 stems. This quantity would be too damaging to collect from any single lupine population at one time, so we distributed stem collection over 8 sites and 5 years. Resources and site circumstances made it impossible to sample each population every year. Stem sampling also provided information on infestation rates. Only large effects were of interest: Is the infestation absent, mild, or rampant? Preliminary field work had shown that a sample of 10 stems was sufficient to provide this information. Stems were collected after flowering had completed and pods were mature and mostly dispersed.

Lupine distribution at Maryland sites is patchy with dense clumps of plants interspersed with larger areas of unoccupied habitat. Populations are typically small, although census of individuals is difficult because it is impossible to determine without excavating plants; a dense clump of lupine may contain multiple ramets of a single large individual or several genets in close proximity (Grigore and Tramer 1996). With one exception (Site 3), lupines occur in linear habitats often measuring less than 200 m<sup>2</sup>. In these linear habitats we walked a single transect paralleling the population and sampled stems by turning away from the plants and tossing a wire flag into the lupine. The trajectory of the imbalanced object was predictable enough that it usually landed in the sampling area, but unpredictable enough

that there was no bias over which clump of lupines would be hit. We collected the stem nearest the end of the wire, walked a number of steps proportional to the transect length divided by five, and repeated this process until the end of the transect was reached. We chose to divide the transect into five sections to ensure sampling throughout the population. No fewer than 10% of the flowering clumps were sampled. In instances where discrete clumps of plants were isolated from other clumps, we sampled each discrete patch by tossing the flag into the patch. At Site 3, a larger, nonlinear population within an open inland sand dune, we walked five different transects through the population with stops at intervals proportional to the transect length divided by four. The number of stems that were sampled varied over years as a result of deer browse, vole damage, construction, and herbicide damage.

Stems were cut near ground level, and cool-stored for later examination. Lupine reproductive capacity, represented by the number of pods per stem, was recorded. Stems were examined for external evidence of insect activity, then dissected longitudinally. Oviposit wounds, eggs, larvae, pupae, adults, frass, and damaged pith were recorded.

The impact of CSB on lupine reproductive capacity can only be estimated using stems that would otherwise be capable of producing pods. Sometimes we could not use stems because circumstances other than CSB activity had eliminated, or nearly eliminated, the possibility of pod production. For example, white-tailed deer (*Odocoileus virginianus* Zimmerman) graze of lupine stems is prevalent at some of the study sites (Frye 2012). Grazed stems could not be used for statistical analysis of the impact of stem borer activity on pod production because grazing removes the raceme. Similarly, sites where pod set was strongly suppressed by herbicides (Site 2) or shade and low population size (Site 6) were excluded from statistical analysis of pod set.

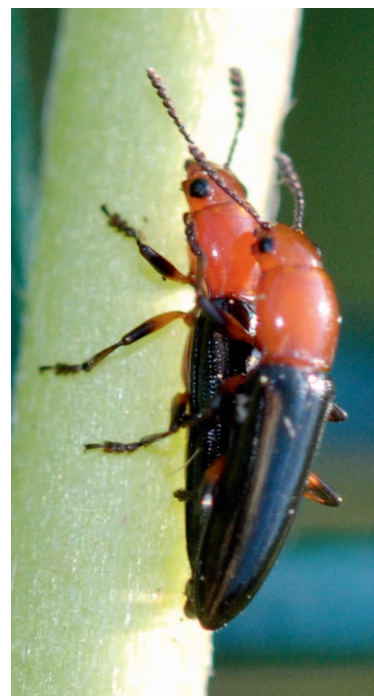
Based on the extent of internal damage, each stem was assigned to one of three categories:

- little-to-no damage: brown pith <3 cm, larva  $\leq 4$  mm
- moderate damage: brown pith 3–4 cm, larva 4–5 mm
- extensive damage: brown pith >4 cm and/or larva  $\geq 5$  mm

Some stems could not be categorized due to decay or data transcription errors. For statistical analysis of pod set, stems with “little-to-no” internal damage were compared to stems with “extensive” internal damage.

Statistical analysis of pod set was conducted in R 3.5.1 (R Core Team 2018). The pod set data are not normally distributed, and when compared to a Poisson distribution are both overdispersed and zero-inflated. For statistical analysis of the impact of CSB activity on pod set, *pscl* (Zeileis et al. 2008; Jackman 2017) and *MASS* (Venables and Ripley 2002) R packages were used to create a zero-inflated negative binomial (ZINB) mixture model. Statistical analysis of stem cracking and of connection to the anthropogenic meadow network (the meadows that would return to canopy closure were it not for mowing along roadsides and under utility lines) were conducted using JMP (Pro 14.1, SAS Institute).

Lupine stems containing late instar CSB larvae or pupae were placed in containers for rearing. Successfully reared CSB adults are maintained in the author’s personal collection.



**Figure 1.**—Clover stem borers mating on lupine stem, 2 June 2011, Worcester County, Maryland. At only 1 mm wide, they successfully sidle behind lupine stems.

## RESULTS AND DISCUSSION

### Recognition of Clover Stem Borer Activity in Sundial Lupine

Despite the shiny red head and thorax (Figure 1), adult CSBs were rarely observed among the lupine plants. Adults land and rest below the foliage, making them difficult to detect at eye level. Even when the viewer is near ground level, they are still hard to see because they sidle to avoid detection. Eggs, larvae, and pupae are even more difficult to detect than adults because of the cryptic habits of the immature stages, which complete their development entirely enclosed in the host plant stem (Ellsbury 1991). It is therefore helpful for the conservation professional to be familiar with the field indicators of CSB activity, as described below.

### Mating and Oviposition

CSB oviposition activity varied considerably from site to site and year to year (Table 1). During our field work, we observed the beetles mating and ovipositing on lupine stems as soon as buds colored up in late April (Figures 1, 2).

Of 190 random stems, only one did not have oviposit observations due to the deteriorated condition of the stem surface. Among the remaining 189 stems, we observed 73 with oviposit wounds. It was common for a stem to have two or three wounds, with eight the maximum observed on a single stem. Although wounds were found all along the stems, the most frequent location was at the raceme base. Typical oviposit wounds consisted of a disk 2 mm wide, where the female had chewed the stem surface away (Figure 3). Within this area there was often a smaller and slightly deeper disk of about 1 mm, and centered within that was a puncture of <0.5 mm

**Table 1.**—Percent of lupine stems with oviposit wounds by site and year; – indicates missing data.

Site	2014		2015		2016		2017		2018	
	%	N	%	N	%	N	%	N	%	N
1 – utility line	100%	11	67%	9	44%	9	100%	10	–	–
2 – railroad shoulder	60%	10	11%	9	–	–	29%	7	100%	6
3 – open sand roads	–	–	–	–	–	–	63%	19	70%	10
4 – utility line	100%	19	–	–	86%	7	–	–	50%	10
5 – utility line	38%	26	48%	21	–	–	–	–	–	–
6 – forested sand road	–	–	0%	9	–	–	0%	5	0%	8
7 – forested camp site	0%	10	–	–	–	–	0%	9	0%	6
8 – crushed stone road	0%	10	0%	10	0%	8	0%	8	0%	8

diameter. Fresh, green, oviposit wounds were only observable on stems harvested for dissection. Within 24 hr a wound becomes tan, and is thus more readily observed (Folsom 1909). Our observations are similar to those of Wildermuth and Gates (1920), who described CSB oviposition in alfalfa (*Medicago sativa* L.). However, Pemberton et al. (1996), who described oviposition in clover (*Trifolium* spp.), wrote that the wounds were mere tiny, pin-sized holes.

During dissection of stems for this study, and in field work preceding this study, we found only 15 CSB eggs. These were located in a chamber inside the stem immediately across from the oviposit wound (Figure 4). Chambers were three or four times the volume of the egg, which is an orange ovoid 1–2 mm long. Several prior studies mention the egg as curved (e.g., Folsom 1909), whereas all eggs we observed were unbent. Thirteen of the eggs were solo, with just one chamber

containing a pair. Pemberton et al. (1996) also observed that eggs in clover were usually deposited singly, whereas Wildermuth and Gates (1920) describe the eggs in alfalfa as usually occurring in pairs.

In the random sample of 180 stems with good oviposit and internal damage information, open, vertical cracks formed in 14 stems. These appear to be the result of the oviposit wound elongating over time (Figures 3, 5), exposing the hollow of the stem. Eighty-six percent of stems with open vertical cracks also had visible CSB oviposit wounds  $\chi^2$  (1,  $N = 180$ ) = 16.2,  $p < 0.0001$ . It is unclear what relationship open cracks might have to a CSB occupying the stem, for example, whether it may benefit the plant by allowing larval predators inside, or benefit the developing beetle by allowing it to exit upon maturity. By summer, damaged stems may bend or break at the site of the oviposit wound, leaving whole plants looking as if they have been trampled. Folsom (1909) described a similar phenomenon in clover.

### CSB Larvae Occupy Lupine Stems and Cause Internal Damage

We dissected 138 stems that contained CSB larvae. Larvae were encountered in stems during all phenological phases, from earliest bud color-up well into senescence.

Double occupancy was only observed four times (three stems containing two larvae, one stem containing an egg and a larva). However, it was not uncommon for a stem to contain evidence of multiple occupants over time. For example, one stem contained only a single larva at the time of dissection, and



**Figure 2.**—On 25 April 2011, this oviposition event was observed in Baltimore County, Maryland. An adult female CSB was near a fresh, surficial, circular wound on a lupine stem. She paced up and down the stem near this wound for 2 min, when three male beetles appeared and attempted to mount her. Within a minute only one male remained, and 3 min later copulation commenced. Three minutes after that, the male departed and the female returned to the vicinity of the stem wound. Ten minutes later she chewed at the wound for roughly 30 sec, then she turned 180° and positioned the tip of her abdomen on the hole for another 30 sec. She repeated the chewing and positioning activities in cycles for another 10 min, after which she departed. At no time during this process did this female insert her head inside the hole, as observed by Wildermuth and Gates (1920).



**Figure 3.**—Clover stem borer oviposit wounds on sundial lupine stems. From left to right: a young wound not fully browned yet, four typical wounds, a wound that is developing a vertical crack.



**Figure 4.**—Dissection of the stem at far left in Figure 3 reveals a clover stem borer egg just under 2 mm long.

evidence that four other larvae had occupied the stem previously.

As CSB larvae eat, they leave winding tunnels through the white pith. Neonate larvae cause little to no vascular or structural damage and leave no visible frass. The larger larvae



**Figure 5.**—Clover stem borer wounds sometimes elongate into vertical cracks, which become brown, hardened, and open. The beetle shown here stayed within a few centimeters of the crack for 12 min after she was noticed by the primary author. 24 May 2014, Anne Arundel County, Maryland.



**Figure 6.**—Late instar clover stem borer from sundial lupine stem. Larva is just over 8 mm long. Lower photo by Suzanne Klick.

observed were 8–10 mm long and 1 mm wide (Figure 6). Late instar larvae eat into the vascular framework of the stem. The consequences are easily observed in the field on a hot sunny day, when occupied lupine stems wilt while their unoccupied neighbors do not.

The inside of a lupine stem occupied by a late instar CSB larva contains numerous frass pellets. CSB frass pellets are black, separate, widely scattered, and about 0.1 mm diameter. The frass does not persist long after the departure or demise of the insect. Damaged pith becomes brown and may harden.

#### CSB Pupa and Emergence of Adult from Stem

Only two pupae were encountered during stem dissections (Figure 7). Wildermuth and Gates (1920) provide an excellent illustration of the pupa. They described the beetles as staying within alfalfa stems for days or even weeks after maturation, and that the beetles chew their way out, leaving a small, irregular exit hole. Fothergill et al. (2010) also observed CSB exit holes in soy stems (*Glycine max* L.). We observed only one lupine stem that appeared to have a separate exit hole bored from the inside out. As a lupine stem grows, the oviposit wound enlarges and may provide a convenient exit.

We have not sought to determine what adult beetles do after they exit lupine stems. Folsom (1909) observed that, in Illinois, CSBs overwinter as adults in the leaf litter and rubbish on the ground of the clover fields.



**Figure 7.**—Clover stem borer pupa, 7 mm long. Found in dormant lupine stem on 26 July 2015, Caroline County, Maryland.

Over the course of this study, all six beetle larvae that were successfully reared to adulthood were CSB.

### Other Stem-Boring Insects

While dissecting stems from our Coastal Plain sites, we uncovered 43 moth larvae. We were unsuccessful in rearing them to adulthood and have not identified them to species. Leg positions, a large, reddish, bilobate head capsule, and a thicker body are obvious physical characteristics that differentiate these moth larvae from beetle larvae. The frass of these moth larvae consisted of pellets that were larger, more aggregated, and much paler than the frass of CSB larvae. The moth caterpillars were also different in that they moved more slowly, hid in the base of the stem, and attempted to knit their dissected stem back together.

One stem collected from the Delmarva Peninsula on 11 June 2018 contained tunneling and frass unlike that of any other stem dissected. The entry was through an abrasion at the ground surface, and the tunneling headed downward, into the hardened, woody root, below the cut made to collect the sample. Small, scattered, black frass pellets were present, but these were larger than those characteristic of CSBs.

### CSB Infestation and the Anthropogenic Meadow Network

As part of an effort to determine an appropriate conservation rank, we searched across the state to find as many of Maryland's remaining lupine populations as possible. The data are also useful for understanding the relationship between CSB infestation and any forest that buffers a site from CSB populations in the anthropogenic meadow network (Table 2). Of the 18 lupine sites we found, only 11 were large enough to afford collection of stems for dissection. At one of the smaller sites (I) we were able to confirm infestation by field observation of adults outside the lupine stems. In the resulting sample of 12 sites, all 3 sites with forest buffers lacked CSB infestation, while all 9 sites without buffers were infested. The probability of this occurring by chance alone is 0.0045 (Fisher's Exact Test).

Two arguments could be made for removing Site L from the calculation of probability. The forest buffer is much thinner than at J and K. Also, L is the only site that has a very dusty, crushed-stone road (Figure 8). Stone dust is regularly used to prevent insect damage on crops, and it could be that dust from the road would have deterred any CSB that broke through the thin forest

**Table 2.**—Connectivity of lupine sites to anthropogenic meadow network. Forest buffer measured from aerial photographs at thinnest point.

Site	Site description	Forest buffer (m)
<b>Infested populations</b>		
A	utility line	0
B	railroad shoulder	0
C	logged area/sand and asphalt roads	0
D	utility line	0
E	utility line and sand roads	0
F	asphalt bike trail	0
G	asphalt road and sand roads	0
H	sand and gravel mine	0
I	asphalt road and utility line	0
<b>Not-infested populations</b>		
J	sand road through large forest	700
K	asphalt road through large forest	400
L	crushed stone road through subdivision	30

buffer. Even setting L aside, the probability of finding such results by chance alone is 0.0182.

In the context of local ecology, this result makes sense. Maryland is part of the Eastern Deciduous Forest where naturally occurring upland meadows are isolated openings surrounded by forest, a condition that would work against colonization by CSB. Such meadows may be perpetuated by fire or storm, but are generally short-lived due to canopy regeneration. Either situation would work against the build-up of CSB populations. By contrast, our modern network of roadside and utility corridor meadows are interconnected, fire-suppressed, and persist for many decades, even centuries.

Wildermuth and Gates (1920) also noted that CSB infestations in crops increase in proximity to unkempt areas of farm fields where their numerous host plants (i.e., *Phleum pratense* L., *Arctium minus* Bernh., *Melilotus* spp., *Ambrosia* spp., *Erigeron* spp.) abound, and recommended that, for protection of alfalfa crops, weedy areas should be kept mown. Conservation professionals may wish to consider the role of forest buffers when designing or maintaining lupine projects.



**Figure 8.**—Lupines at Site 8 receive regular dusting from traffic travelling on nearby crushed stone roads. This may deter CSB activity.



**Figure 9.**—A high proportion of stems randomly sampled for this study dropped all buds and blossoms, therefore producing zero pods. This clump would be at peak bloom if not for the dropped blossoms.

### Clover Stem Borers Reduce Sundial Lupine Reproductive Capacity

The mean pod set of the 189 random stems (one stem with immature pods was removed from analysis) was 3.15, with a variance of 11.31, and 58 of those stems (30.7%) had dropped all buds, flowers and nascent pods (Figure 9). Among species that flower sequentially from the base of a raceme upward, as lupines do, it is normal for *some* flowers to drop from the raceme without producing fruit. Surplus flowers provide many evolutionary advantages (e.g., larger floral display, male contribution to gene pool, bet-hedging), and when they have finished serving their purpose they wither and fall off. Resource limitation (e.g., water, nutrients) can also cause flowers and fruits to drop. The distal flowers on racemes are particularly prone to blossom drop due to structural limitations in the transport of water and nutrients (Jadeja and Tenhumberg 2018). While dropping surplus blossoms is to be expected, dropping all blossoms eliminates reproductive capacity.

The ZINB model with the best fit included damage level, site, and year in both the zero-inflated and negative binomial components (Table 3). Although year and site were useful for improving model fit, their interpretation provides little biolog-

**Table 3.**—Comparison of zero-inflated negative binomial models. Model fit was improved by removing the two sites where pod set is reduced by factors other than CSB infestation. The best fit was obtained by including all three terms as regressors in both components of the ZINB model.

Count model regressors	Zero-inflation regressors	Irrelevant sites	–log likelihood	AIC
yr+dam+site	yr+dam+site	included	–437.7	929.4
yr+dam+site	yr+dam+site	removed	–387.7	821.4
yr+dam+site	yr+site	removed	–389.7	823.4
yr+site	yr+dam+site	removed	–389.3	822.7
yr+site	yr+site	removed	–392.5	827.1

ical insight because most sites went unsampled for more than one year.

The logistic portion of the ZINB model indicates that stems with extensive internal damage contributed more heavily to the zero-inflation shown in Figure 10. A flowering stem with extensive internal damage was 3.4 times more likely to drop all its blossoms and become an excess zero (a zero count beyond that which can be fit with the negative binomial curve alone) than a stem with little-to-no internal damage ( $Z = -2.042$ ,  $p < 0.05$ , two-tailed).

The negative binomial component of the ZINB model, which predicts pod set for stems that were not excess zeros (Figure 11), indicates that stems with little-to-no damage produce on average 1.47 more pods than do stems with extensive damage, but this coefficient is not significant at the 0.05 level ( $Z = 1.802$ ,  $p = 0.072$ , two-tailed).

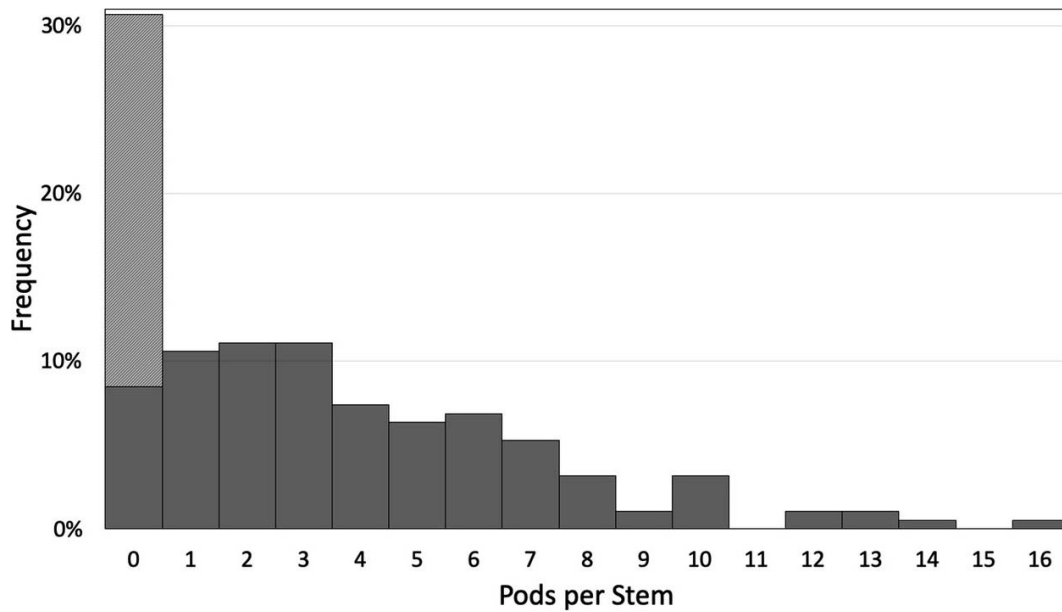
The overall effect is that stems with extensive internal damage produced an average of  $2.00 \pm 2.98$  pods, whereas stems with little-to-no internal damage produced an average of  $3.45 \pm 3.40$  pods.

The average number of peas per pod was similar for stems with little-to-no internal damage ( $2.14 \pm 0.788$ ) and stems with extensive damage ( $2.09 \pm 1.252$ ). Accordingly, ZINB regression of peas produced per stem closely paralleled that of pods produced per stem, and will not be reported further here.

### Conclusions

Predation by clover stem borers is an inconspicuous activity, but one that compromises stem function and is associated with reduced reproductive capacity. Extensive damage is associated with an increased probability of stems dropping all flowers, thus producing zero pods. Professionals managing lupine conservation projects need to be able to recognize the signs of CSB infestation. Without close examination, including dissection of stems, CSB infestations will typically go unnoticed. Manifestations of CSB damage that are visible outside the stem include a distinctive oviposit wound, and in some cases vertical cracking of the stem. Dissection of an infested stem may reveal a CSB egg, larva, pupa, or adult, as well as tunneling, frass, and browning and hardening of the stem interior.

In Maryland, lupine sites that were part of the modern network of transportation corridor and utility line meadows were all infested with CSB, whereas lupine sites with a forest buffer were not. Professionals may wish to consider creation or protection of forest buffer when designing or managing lupine conservation projects.

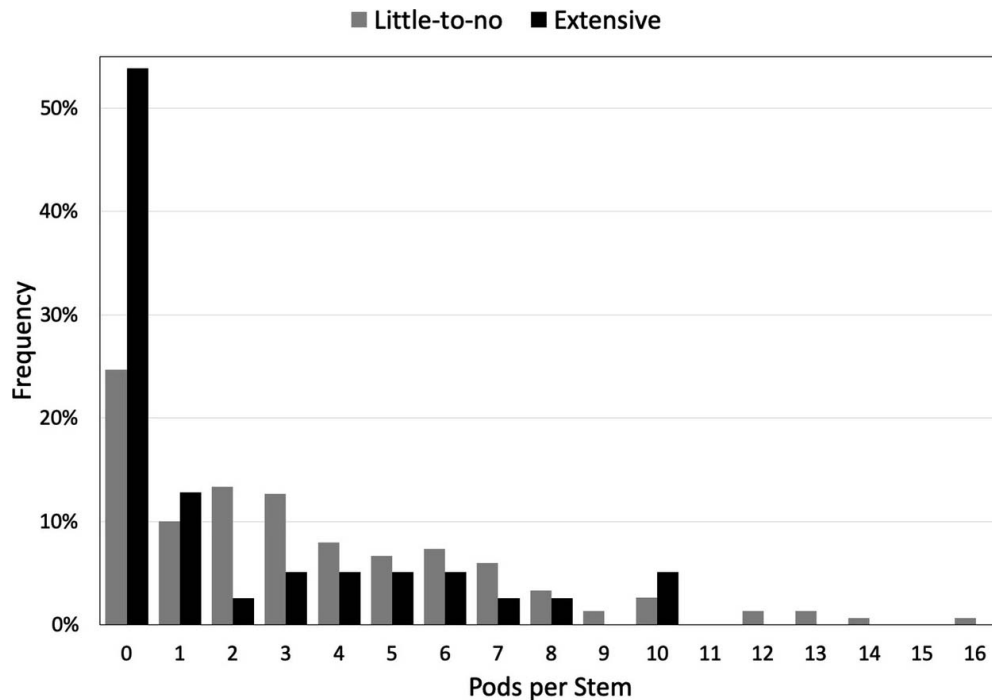


**Figure 10.**—Pod set data for 189 random stems collected from six sites between 2014 and 2018. The ZINB model predicted 16 zeros to fit the negative binomial distribution (dark gray). The additional 42 zeros (indicated in cross-hatch), are the zero-inflation as analyzed by the ZINB model using a logistic regression.

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**Figure 11.**—Pod set for 189 random stems sampled from six sites, 2014 to 2018. Zeros dominate pod set data for both stem damage categories. Extensively damaged stems have both more stems that dropped all blossoms, thus producing zero pods, and lower pod set among stems that did produce pods. The highest pod sets of above 10 pods per stem are exclusive to stems with no internal damage.

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*Sara Tangren is an Instructor for the University of Maryland Extension, where her favorite work is teaching Master Gardeners, Master Naturalists, and landscape professionals about native plants. Prior to her academic career, Sara was a wetland delineator, and a native plant and native seed producer. She has been teaching and conducting native plant and soil research since 1996.*

*Christopher Frye is the State Botanist for the Wildlife and Heritage Service in Maryland. Chris's work with Service includes all aspects of plant conservation in the state. His academic work focuses on the relationships between plant mating systems and evolutionary fitness.*

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