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NATURAL HISTORY AND DEMOGRAPHY OF THE IMPERILED REDWOOD FOREST SPECIALIST *PEDICULARIS DUDLEYI* (DUDLEY'S LOUSEWORT, OROBANCHACEAE)

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ABSTRACT

Dudley's Lousewort (*Pedicularis dudleyi* Elmer) is a rare and apparently declining species confined to two populations associated with Redwood forests in coastal California. In this study, we aimed to determine whether pollination, seed dispersal, or seed germination were important limiting factors for population growth of *P. dudleyi* at Portola Redwoods State Park, the northernmost of the two known populations. We conducted a full census of the population, performed pollinator and seed disperser observations, conducted a pollinator exclusion study, and conducted a litter removal experiment and a greenhouse seed germination study. Fewer than 500 reproductive individuals were found in the census, confirming the extremely small population size at the State Park. Both pollination by native bees (*Bombus* spp. Latreille 1802) and seed dispersal by yellowjackets (*Vespula alascensis* Packard 1870) appear to be robust. The litter removal results were inconclusive and no seeds successfully germinated. Combining age class data from the census, poor overall recruitment in the litter removal trial, and poor seed germination, we infer that low establishment of new individuals and populations is a primary threat to the species and should be the focus of future management goals.

Key Words: California, conservation, pollination, rare species, seed dispersal, vespichochory.

At least 45 rare vascular plants are known to be associated with California Redwood forests (Sholars and Golec 2007), and because rare plants associated with redwood habitats exhibit wide variability in distribution, habitat, and life history, understanding their persistence requires species-level assessments (Sholars and Golec 2007). Not all rare plants respond to threats in the same way; the response largely depends on each species' habitat requirements and life history (Oostermeijer 2003; Sholars and Golec 2007). In order to develop effective conservation guidelines for rare plants, it is critical that the life stages which have a large effect on population size are identified (Schemske et al. 1994). Several life history and reproductive biology components, including pollinator abundance, pollinator diversity, number of reproductive plants, rates of pollination, seed production, germination, seedling establishment, and growth can influence rare plant persistence (Yates and Ladd 2005; Sholars and Golec 2007). Additionally, understanding plant-insect interactions can inform population growth rates due to effects on reproduction, growth, and survival (Schemske et al. 1994; Fishman and Hadany 2010). Habitat destruction can also result in dispersal limitation if relationships between specific seed-dispersing organisms and the plant are disrupted (Oostermeijer 2003). Large mammals can threaten rare plants through herbivory, overgrazing, and trampling, resulting in increased mortality rates or decreased fecundity (Oostermeijer 2003). Herbivory is not usually the primary factor leading to the decline of a threatened plant species; however, habitat fragmentation or degradation can often result in increased sensitivity to herbivory (Oostermeijer 2003).

This study investigates the pollination, seed dispersal, life history, and demography of *Pedicularis dudleyi* Elmer (Dudley's Lousewort, Orobanchaceae) a rare redwood forest endemic, as an effort to aid the conservation of the species. *Pedicularis dudleyi* is currently restricted to two populations, one occurring at Portola Redwoods State Park and adjacent private property in San Mateo County ("Portola population") and the other along the North Fork Little Sur River in the Los Padres National Forest and adjacent private property in Monterey County

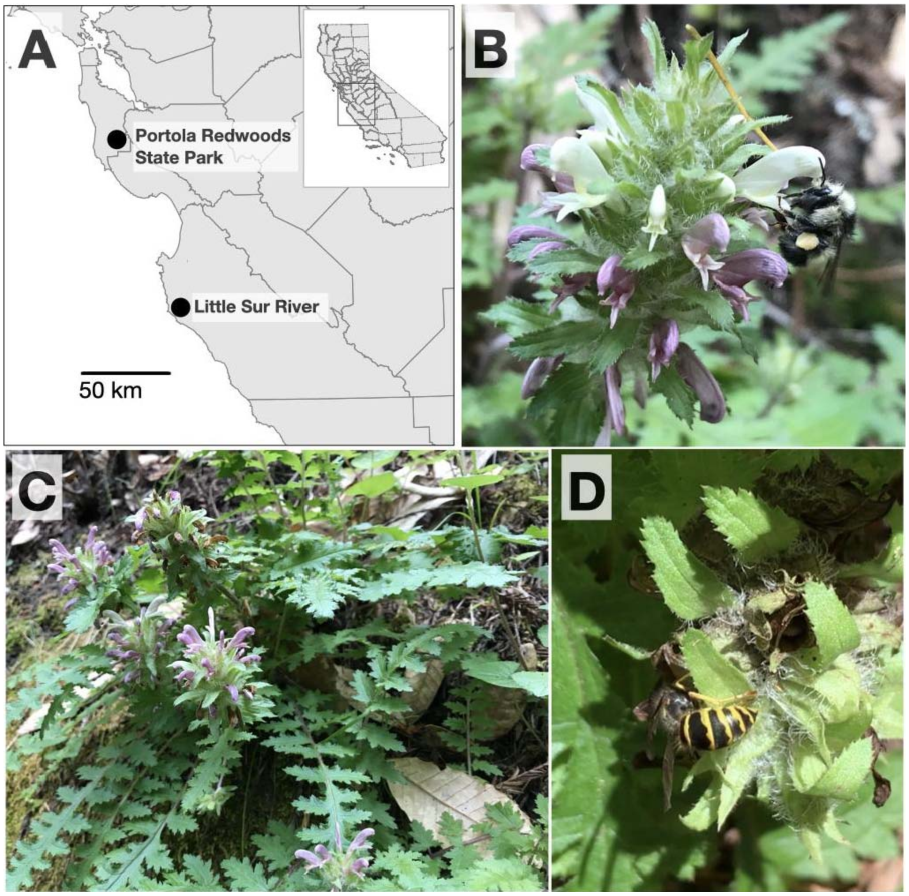

FIG. 1. A. Distribution of extant populations of *P. dudleyi*. B. Flowering stalk of *P.dudleyi* with one of its primary pollinators, the native bumblebee *Bombus edwardsii*. C. Mature individuals of *P. dudleyi* in flower. D. Seed disperser *Vespula alascensis* preparing to enter a capsule of *P. dudleyi*.

("Little Sur River population"; Fig. 1). Two herbarium specimens from the late 19th century (CAS-BOT-BC266671, labeled, without collector name or number, "Aptos, April 1884", and CAS-BOT-BC266670, labeled "By road in redwoods San Lorenzo River, M. R. Dudley, May 7, 1893") document putative stations of the species between these two extant populations, and we speculate (though without supporting evidence) that the heavy logging throughout the late 19th and early 20th century may have contributed to the decline of the species. Over the last few decades, monitoring efforts suggest steady declines in both the San Mateo and Monterey populations (Kim Kuska, personal communication), and E. Sprague, in her unpublished PhD dissertation on the ecology of *Pedicularis*, described the Portola population as occurring "along two or three miles of the creek banks and in the immediately adjacent forest" (Sprague 1959) which is markedly larger than the current population.

The species appears to be a close relative of both the widespread *P. densiflora* Benth. and the rare *P. rigginsiae* D.J.Keil, a species restricted to the Hearst Ranch in San Luis Obispo county that was interpreted until recent study to be a third population of *P. dudleyi* (Keil 2019). Unlike *P. densiflora*, which has a classic elongate, red tubular corolla associated with hummingbird pollination, the flowers of *P. dudleyi* emerge with white corollas which then mature to a lavender color, and have modifications to the corolla, pistil and anthers commonly associated with bee pollination. These morphological traits, and bee pollination behavior, were observed, illustrated, and discussed by Sprague (1959, 1962a), who noted that the flowers were pollinated by two bumble bee species, *Bombus edwardsii* Cresson 1879 and *B. sitkensis* Nylander 1848, with the former being a more frequent visitor.

The species appears to prefer moderately disturbed sites (Sprague 1959), with most of the current populations occurring along roadbanks (Portola population), flood plains above creeks (Little Sur population), or in campgrounds (both populations). Root parasitism is common across California *Pedicularis* species and Sprague (1959, 1962b), based on field observations, suggested that *Vaccinium ovatum* Pursh, *Notholithocarpus densiflorus* (Hook. & Arn.) Manos, C.H.Cannon & S.Oh or *Ceanothus thyrsiflorus* Eschsch. might be hosts for *P. dudleyi*.

In this study, we (1) present baseline demographic data for *P. dudleyi*, (2) attempt to determine whether insufficient pollination or seed dispersal might be limiting population growth, and (3) investigate whether leaf litter buildup influences the establishment of *P. dudleyi* in the redwood forest understory.

METHODS

Population Census

A complete census of known populations of *P. dudleyi* in Portola Redwoods State Park was performed June–August 2019. The population extends to private property adjacent to the park and that portion of the population was not surveyed. Individuals were assigned to a total of 22 subpopulations, each separated by a minimum of 10 m but typically separated by at least 50–100 m. For the census, individuals were assigned to the following age classes: Reproductive Adult (flowering), Mature Adult (similar or equal in size to flowering plants, but without reproductive structures), Immature Plant (substantially smaller than adults, as estimated by number of leaves and/or leaf size), and Seedling (cotyledons present). The criteria for our Immature Plant category were leaves less than 10 cm and fewer than five leaves. Plants in this category were markedly different from the two adult categories and we assume that these individuals are immature. For reproductive individuals, the number of inflorescence stalks was recorded, as was the proportion of stalks from which flowers were removed via herbivory. Using these data, we calculated an indirect and very rough estimate of field germination rate by estimating number of seeds produced per subpopulation (number of seeds per fruit × number of fruits per inflorescence × number of inflorescences per subpopulation) and regressing that against number of seedlings per patch to derive a relationship (regression slope) of estimated seeds produced relative to seedlings observed across subpopulations.

Pollinator Observations

Pollinator observations were made 1 May–16 May 2019 and between 6:30 (approximately 15 min after daybreak) and 14:30. Observations were made at the largest subpopulation in Portola Redwoods State Park, which at the time of the survey had approximately 270 inflorescences with open flowers. The patch occurs on a vertical, moss-covered road cut that ranges in height from approximately 1.5 m to 2 m and extends for approximately 16 m in length. *Pedicularis dudleyi* can produce multiple spikes, each of which produces 5–35 flowers, with an average of approximately 20 flowers per inflorescence. The flowers on each indeterminant inflorescence mature sequentially and on a typical inflorescence, approximately 5–6 flowers will be apparently receptive at any given time. Maintaining visual contact with the entire patch was easily accomplished, and when a potential pollinator entered the patch, the insect could be approached, and in most cases filmed easily with a handheld video camera. Potential pollinator visits were infrequent enough that it was rare that more than one potential pollinator was present in the patch at the same time. During the survey, an observer would stand with the entire patch in view until a potential pollinator came to the patch. The observer would make a video recording of the insect over its entire visit until it left the patch.

Videos were later analyzed to determine the species of potential pollinators, as well as the number of flowers, inflorescences, and plants visited by each insect and the duration of their visit to the *P. dudleyi* patch. The corolla of *P. dudleyi* has petals fused distally into a hood-shaped structure with the stamens and stigma enclosed in the hood. If a large insect (i.e., the size of a honeybee or larger) inserts its head into the opening of the corolla, the thorax or upper abdomen can contact the stamens and/or stigma. A pollination attempt was therefore recorded as an insect inserting its head into the corolla with the thorax or abdomen touching the corolla hood. If an insect landed on the corolla without appearing to touch the corolla hood, that flower visit was not recorded.

Only two species, the native and locally abundant bumble bees *Bombus edwardsii* and *B. sitkensis*, were captured on video using pollinating behavior as described above. Vouchers for the two species were captured and deposited in the J. Gordon Edwards Entomology Museum at San Jose State University. The bees would typically fly to the patch and then visit several to many flowers on one or more plants. To determine the relative importance of the two bee species, the videos were scored with each pollinator visit to a patch as a replicate. We tested whether either species visited significantly more plants per visit, more inflorescences per visit, and flowers per visit. We also tested whether either species visited significantly more flowers per plant or flowers per inflorescence. These data were not normally distributed, so a Kruskal Wallis test was used. These and all subsequent analyses were performed in R (version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria).

Pollinator Exclusion

Two pollinator exclusion experiments were performed. The first was to test the hypothesis that pollinators are necessary to transfer pollen from flower to flower for fertilization and the second was to test whether the plants are self-compatible (i.e., produce seeds when pollinated with pollen from the same plant). In 2019, pollinator exclusion bags made of mesh cloth were secured over entire inflorescences prior to flowering. No more than one inflorescence was bagged per plant. Twenty-one individual inflorescences were selected randomly for bagging (by arbitrarily selecting inflorescences and then using coin flips to determine which were selected) and 20 individual inflorescences from the same subpopulations were selected for an unbagged treatment. Once fruits in nearby individuals at similar stages of development had fully ripened, the bagged and unbagged inflorescences were scored for the number of individual fruits in three categories: fully matured, no fruit development (i.e., withered pistil), or incomplete fruit development (i.e., pistil had begun to enlarge, but aborted prior to seed maturation). In 2020, 18 inflorescences from the same population were bagged prior to flowering. Once flowers opened, all open flowers (typically 5–6 per inflorescence) were hand-pollinated by removing anthers with fine forceps and transferring pollen to stigmas of other flowers on the same inflorescence. Afterward, bags were re-installed to prevent insect pollination. At maturation, hand-pollinated flowers were collected and fruits were counted and scored as described in the previous experiment.

Both pollinator exclusion experiments tested whether the treatments (pollinators excluded and self-pollination) differed from an unbagged treatment in the proportion of flowers that matured into fully developed fruits. Replicates were individual inflorescences and values being compared were the proportion of flowers per inflorescence that developed into fruits. It should be noted that we did not remove stamens from flowers in the unbagged treatment, so it is not possible to determine whether pollination in that treatment was caused by strictly outcrossing. These data were not normally distributed, so a Kruskal Wallis test was used, with post hoc pairwise comparisons made using a non-parametric Dunn test.

Seed Dispersal/Predation Observations

Preliminary observations indicated that two insects, the ant *Formica argentea* Wheeler and the yellowjacket *Vespula alascensis* Packard 1870, were potential seed dispersers. To test this, we used two forms of video observation. We used GoPro cameras set up at fruiting plants to estimate the frequency of visits. We also used hand-held video cameras to capture behavior of animals as they visited the fruits. Recordings were made near mid-day (11:00–15:00) on 17 and 18 July 2019 and 10:00–15:00 from 6–11 July 2020. Specimens of both species were captured for identification.

Videos were analyzed to determine the number of visits made by each species, the behaviors associated with visits to the fruits, and to determine whether any other animals visited the fruits. A total of 37.3 hr of video footage (29.8 hr from GoPros, 7.5 hr from handheld) was captured and analyzed. Data were then tabulated to determine the relative importance of the putative seed dispersers.

After observing yellowjacket behavior, we determined that seed coats from seeds visited by the yellowjackets appeared to have been damaged by the visiting yellowjackets. To better understand whether yellowjackets might be responsible for damaging the seed coats, we collected a total of 161 seeds and noted whether the fruits were fully opened or not (which allows access of yellowjackets to seeds) and whether the outer seed coats had been chipped away.

Litter Removal

A litter removal trial was performed to determine whether removing litter promoted germination or establishment success in the 2020 growing season. Forty-six individual plants were selected for the experiment, with the requirement that each experimental plants were at least 1 m apart. Plants were randomly assigned to a control group ($n = 22$) which was not manipulated and a treatment group ($n = 24$). In all cases, individuals were assigned to two groups: vertical substrate or horizontal substrate. For those in the vertical class, a 50×50 cm quadrat ($0.25\ m^2$) was placed on the horizontal ground directly below the vertical surface from which the plant was growing. For those in the horizontal class, the quadrat was placed on top of the substrate with the plant at the center of the quadrat. For the treatment plots, all loose litter from the plot was removed so that only fully decomposed organic material (approximately 2–5 cm deep in most cases) remained above the mineral soil. Litter removal was performed in June 2019 after the plants were pollinated, but prior to maturation of the seeds and fruits. The following growing season, each plot was checked to determine the number of seedlings that had germinated.

The litter exclusion experiment tested whether controls differed from treatment plots in the number of seedlings that germinated in the growing season following litter removal. Data were analyzed with a Wilcoxon Mann-Whitney test (a non-parametric analog of a standard t-test).

Germination Trial

A germination trial was performed to test the following four hypotheses: (1) Germination rates differ between seeds from hand pollinated (self-fertilized) flowers and controls; (2) Germination rates differ between seeds with the outer seed coat putatively disrupted by seed dispersers and controls; (3) Germination rates differ between seeds subjected to cold stratification (15 d at ca. -15°C) and controls; and (4) Germination rates differ between seeds sown in native soil and seeds sown in native soil that was sterilized prior to planting. Seeds from six treatment groups were planted. The six groups were (a) hand-pollinated with self-pollen, (b) hand-pollinated with self-pollen + stratification, (c) stratification only, (d) seed coat disrupted, (e) unsterilized soil, and (f) control. Each treatment consisted of 25 seeds. The seeds were sown into a mixture of 75% native soil from the collection site and 25% calcined clay aggregates (Turface for Plants, Bonsai Supply, Inc., Fort Lauderdale, FL), which improves both water

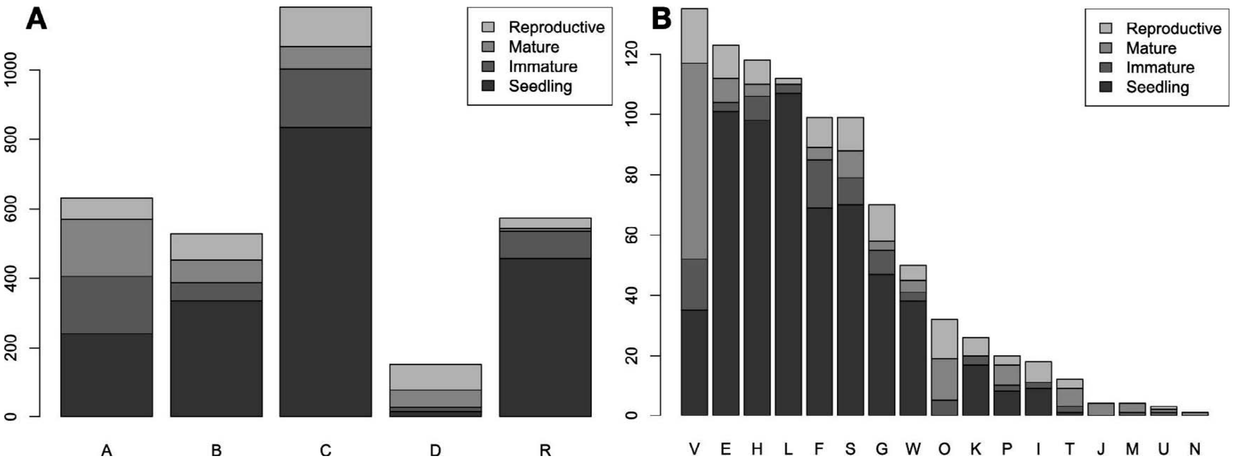

FIG. 2. Proportions of age classes among the 22 subpopulations of *P. dudleyi* in Portola Redwoods State Park. A. Populations with >20 Reproductive Adult individuals. B. Populations with <20 Reproductive Adult individuals. See Methods for age class definitions.

retention and drainage. Sterilized soil was subjected to 2 hr of steam sterilization. After sowing, seeds were placed in a seed germinator with a cycle of 11 hr light at 15°C and 13 hr dark at 10°C, conditions which approximate those at Portola Redwoods State Park during the winter/early spring months when the seeds germinate in nature. Soil was kept moist as necessary with purified water throughout the experiment.

RESULTS

Population Census

A complete census was conducted during the 2019 growing season for all known populations of *P. dudleyi* in Portola Redwood State Park, but did not include adjacent private property which is known to harbor additional individuals. In the park, 468 flowering individuals, 1,041 mature (i.e., 2nd year or older) but non-reproductive individuals, and 2484 seedlings were recorded.

The population in 2019 was widely dispersed into 22 discrete subpopulations ranging in size from 0 to 112 flowering individuals (median = 9). Proportions of age classes varied widely among subpopulations (Fig. 2), with five of the 22 subpopulations producing no seedlings in 2019, while three had no flowering individuals in the same year. The number of seedlings in each subpopulation was positively correlated with the number of inflorescences (Fig. 3); the number of seedlings per subpopulation ranged from 1–835 (median = 36.5).

Using data collected during the census on numbers of seedlings and inflorescences, we can provide an indirect and very rough estimate of percent germination. Across subpopulations, the mean number of inflorescences per reproductive plant was 3.12, and in a haphazard sample of 23 inflorescences from one population, there were an average of 17.1 flowers per inflorescence (range = 4–38, SD = 10.4). Seed production per fruit was not quantified, but ranged from one to four seeds with most fruits producing 2–3 seeds. Regressing seedlings against inflorescences in each subpopulation ($R^2 = 0.654$, $P < 0.0001$) resulted in a slope of 2.07, or an average of two seedlings per inflorescence (with the assumption that the number of inflorescences produced in a patch is relatively stable year-to-year). Given the values above, this results in a rough average of 42.5 seeds per inflorescence (17 × 2.5), which suggests a germination rate of approximately 4.7% (2.07/42.5). This is an indirect and quite coarse estimate, but provides a starting point for future work.

To determine whether herbivory of the flowering stalks prior to seed maturation might be an important factor in reducing the seed production, we included in our census a count of the number of flowering stalks that had been removed: Across the 22 sites, we observed between 0–50 inflorescences

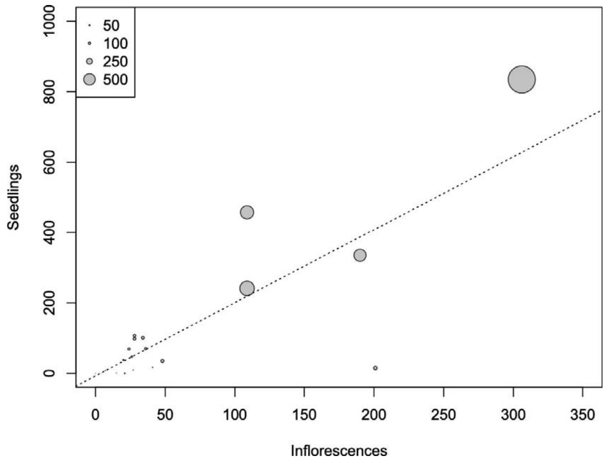

FIG. 3. Relationship of number of inflorescences in 2019 to seedlings in 2019 among the 22 subpopulations of *P. dudleyi* at Portola Redwoods State Park (slope = 2.077, $R^2 = 0.654$, $P < 0.0001$). Size of points indicates the number of individuals in each subpopulation.

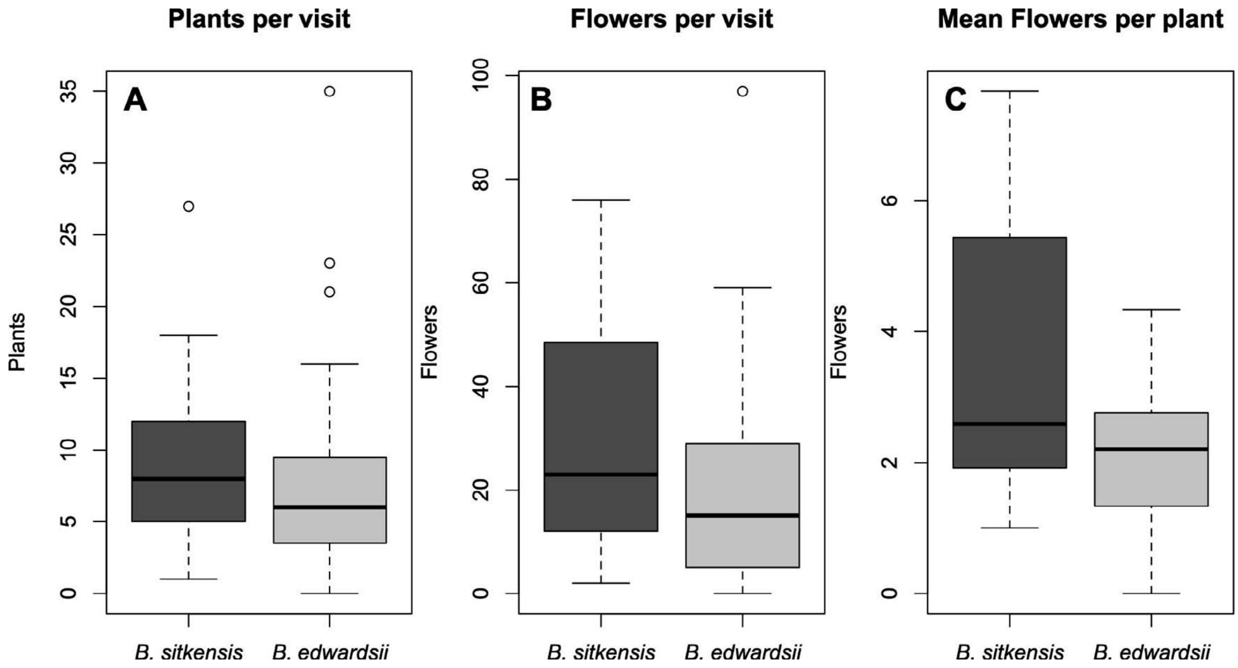

FIG. 4. Pollinator behavior of the two primary pollinators of *P. dudleyi*- the bumblebees *Bombus edwardsii* and *B. sitkensis*. Results are from 23 visits to a *Pedicularis* patch by *B. edwardsii* and 23 visits by *B. sitkensis*. A. Medians and interquartile ranges of the number of plants visited (Kruskal Wallis test, P = 0.544). B. Medians and interquartile ranges of the number of flowers visited (Kruskal Wallis test, P = 0.371). C. Medians and interquartile ranges of the Mean number of flowers per plant visited (Kruskal Wallis test, P = 0.047)

(median = 1) removed per population, ranging from 0–25% of the total number of flowering stalks per site (median = 3.6%) lost to herbivory.

Pollinator Observations

Pedicularis dudleyi is primarily pollinated by two native bees, *Bombus sitkensis* and *Bombus edwardsii.* Anecdotal observations were made of honeybee (*Apis mellifera* Linneaus 1758) visits, but these were rare and did not occur during any of the surveys. Other insects were observed in the vicinity of flowers, but pollinating behavior (i.e., contact with stigmas and/or anthers) was not observed. The bees exhibited relatively consistent pollinating behavior, in which they would arrive at the discrete patch of *Pedicularis* under observation, visit one or more flowers, then fly away and leave the patch. Thus, each of these patch visits was used as a replicate.

A total of 46 patch visits were observed (n = 23 for *B. edwardsii* and n = 23 for *B. sitkensis*). The two *Bombus* species visited patches of *Pedicularis* with similar frequency and exhibited fairly similar behaviors (Fig. 4). Overall, the mean number of plants visited by a bee of either species during a patch visit was 9.1 (min 1, max 35) and the mean number of individual flowers visited was 27.3 (min 1, max 97). A Kruskall Wallis test revealed no significant difference between the two bee species in either number of flowers visited ($\chi^2 = 1.888$, $P = 0.169$) or number of plants visited ($\chi^2 = 0.354$, $P = 0.552$) on each patch visit. The only detectable difference in the behavior of the two bee species was in a tendency for *B. sitkensis* to visit more flowers per plant (mean = 3.5) than *B. edwardsii* (mean = 2.2), which was significantly different with a Kruskall Wallis test ($\chi^2 = 3.96$, $P = 0.0467$).

Both species had a relatively high fidelity to *Pedicularis* flowers while visiting the patch under observation (although members of the genus are widely regarded as generalists). Of the 46 patch visits observed, 36 (78%) included visits to only *Pedicularis* flowers and no other flowering species while on the patch. Nine patch visits included at least one visit to one or more *Viola ocellata* Torr. & A.Gray individuals and one patch visit included a visit to a single *Lysimachia latifolia* (Hook.) Cholewa individual.

Pollinator Exclusion

The pollinator exclusion experiment revealed strong differences among the exclusion treatment, the exclusion plus hand pollination treatment, and the naturally pollinated (unbagged) treatment. In the unbagged group, an average of 79.6% of flowers matured into fully developed fruits, as compared to 47.2% for those that were hand pollinated and excluded from insect pollinators, and 0.0% that were excluded from pollinators with no hand pollination (Fig. 5). All three of these treatments were significantly different from one another with a Kruskal Wallis test ($\chi^2 = 45.796$, $df = 2$, $P < 0.0001$) and all

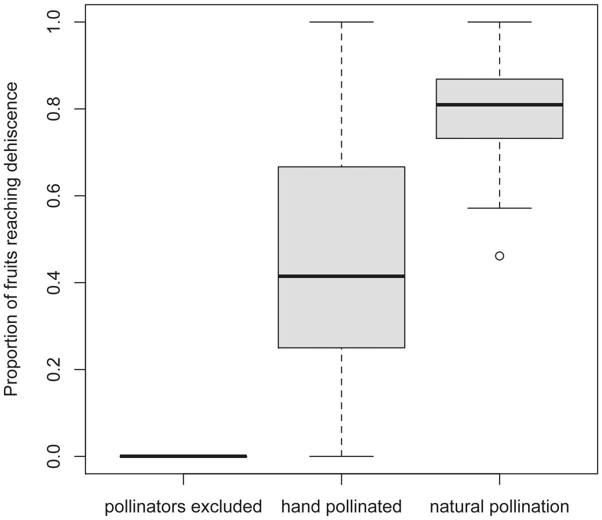

FIG. 5. Medians and interquartile ranges of the differences in fruit maturation in inflorescences of *P. dudleyi* with pollinators excluded (n = 21), pollinators excluded plus hand pollination (n = 18) and naturally pollinated (pollinators not excluded, n = 20). The three treatments are all significantly different from one another ($P < 0.01$ in post hoc pairwise comparisons. See text for details).

three post hoc pairwise comparisons were significantly different (Dunn test, $\alpha = 0.05$).

Seed Dispersal/Predation Observations

Wasps (*Vespula alascensis*) were recorded visiting fruits 21 times during the unattended GoPro recordings (0.82 visits/hr), and 21 times during the handheld camera recordings. Ants were recorded visiting fruits 5 times in the GoPro footage (0.17 visits/hr), and seven times during the handheld camera recordings. No other insects or other animals were observed visiting the fruits.

Wasps appeared to target *P. dudleyi* fruiting stalks, and upon landing would browse the dehisced fruit capsules. Occasionally, they would insert the upper half of their body including the mandibles, head, and thorax into open fruit capsules. They would grasp seeds of *P. dudleyi* with their mandibles, pulling seeds out of seed pods and rotating them several times between their forelegs and mandibles before flying away holding seeds. Approximately 52% of wasp visits to fruits resulted in the wasp flying away with a seed in their mandibles.

Ants were seen occasionally visiting inflorescence heads of *P. dudleyi*, but they appeared to be much less focused on the fruits than the wasps had been. Upon visiting an infructescence, ants would scan the openings of the fruits. Those that investigated fruit capsules further also inserted the upper half of their body in fruit capsules, however ants were never observed removing seeds from the fruits.

Several direct observations were made of wasps burying their heads into mature fruits, in some cases removing the seed and in others, simply appearing to interact with the seeds. In three cases, we collected the fruits that had just been visited and found the outermost layer of the testa to be scraped away. The testa comprises a very hard, dark brown inner layer overlain by a thin, brittle, and relatively fragile light brown outer layer. To further investigate this phenomenon, we microscopically observed an additional 161 seeds that were still held within fruits. Of these, 29 were held within fully matured and dehisced fruits which had failed to fully open (and thus expose the seeds), while 132 were in fully opened fruits. In the opened fruits, 62 (47%) had scrape marks similar to those observed after following wasp visits, while the remaining 53% had intact outer layers. Among the seeds in the fruits that failed to fully open, 100% had intact outer layers.

Litter Removal

In the 0.25 m^2 litter removal plots, the number of seedlings ranged from 0–47, with an overall mean of 3.8 seedlings (SD = 7.4). Sixteen plots (34.7%) had zero seedlings. The litter removal plots (mean = 2.5, SD = 3.9) had fewer seedlings than the control plots (mean = 5.2, SD = 9.7), but the difference was not statistically significant (Wilcoxon Mann-Whitney test: W = 333, P = 0.436). Flat and vertical plots were distributed equally between control and treatments, and there was not a significant difference in number of seedlings between flat and vertical plots (Wilcoxon Mann-Whitney test: W = 376, P = 0.371).

Germination Trial

After 24 wk in the seed germinator, no seeds germinated from any of the treatments or control. Several seedlings of other species from the soil collection site did emerge from treatments with unsterilized soil, indicating that conditions were appropriate for other species from the field site.

DISCUSSION

Pedicularis dudleyi is a species of conservation concern because of its extreme rarity. In this study, we conducted a baseline census to which future census data can be compared and provide the first empirical data to address factors that could potentially be influencing the future viability of the population.

Our data suggest that pollinators and/or pollination are not limiting the reproduction or population growth of *P. dudleyi*. Two native bees, *Bombus edwardsii* and *B. sitkensis* are regular visitors. These two were previously known to be pollinators of *P. dudleyi* from a small observational study by Sprague (1959, 1962a) at the same population. Although a number of other potential pollinators were observed in the vicinity of *P. dudleyi* flowers over the course of our study, none commonly visited flowers and none were clearly seen pollinating flowers. For example, several butterfly visits included nectar harvesting, but the butterflies do not come into contact with either style or anthers. At one subpopulation, several honeybees were seen entering flowers. It is unclear though, with their smaller body size relative to the native bees, whether they are effectively pollinating when they enter the flowers. The honeybees were only observed at one population, whereas the two native bees are ubiquitous throughout the area.

Both *Bombus edwardsii* and *B. sitkensis* are common throughout Portola Redwoods State Park, and appear to be regular visitors to *P. dudleyi*. They also tend to visit numerous flowers and numerous individual plants and visit very few other flowering plant species in between. There are currently no data indicating the extent to which bees could potentially cross pollinate across the twenty-two subpopulations, which is important because most of the subpopulations consist of only a few individuals (median number of flowering individuals per subpopulation = 9). Fortunately, the hand-pollination experiment indicates that experimentally self-pollinated flowers produce fruit and visually normal seeds (although at a level significantly lower than naturally pollinated flowers), suggesting that some degree of selfing may be possible if pollen from different plants is not available. The pollinator exclusion data also clearly speak to the importance of maintaining the existing native bee populations, because none of the inflorescences from which pollinators were excluded produced a single fruit. Collectively, these results suggest that pollination in its current state appears to be robust for *P. dudleyi*. Although it is not clear whether the spatial separation of subpopulations is cause for concern, a lack of effective pollination is not likely to be driving the decline of *P. dudleyi*.

Seed dispersal of *P. dudleyi* has not been studied previously, however naturalists familiar with the plant (Kim Kuska, personal communication) have suggested that potential dispersers could include deer, which appear to be responsible for herbivory of flowering stalks, or banana slugs, yellowjackets, or ants. In our preliminary observations and then quantitative studies on ants and wasps, only yellowjackets were seen to remove seeds from fruits. We observed ants foraging on and around *P. dudleyi* plants and even entering dehisced fruits with seeds in them, but found no evidence that they remove seeds. Banana slugs are abundant throughout the area and were observed foraging in and around *P. dudleyi* populations. During the flowering season, they were frequently observed eating withered brown corollas of the *P. dudleyi* plants. During the fruiting season in mid-summer, however, slugs are not as active in the populations and no slugs were observed interacting with fruiting stalks of *P. dudleyi*. Similarly, herbivory of flowering stalks, putatively by deer, occurred in the flowering season before seeds mature. *Pedicularis dudleyi* has relatively large seeds, ca. 1–1.5 mm × 2–3 mm and there is a substantial period of seed maturation of more than a month between when the last flowers wither and when the fruits dehisce.

Visitation by the yellowjacket *Vespula alascensis* was frequent in mid-summer, and in our survey conducted at peak fruiting season, 52% of wasp visits to *P. dudleyi* fruiting stalks resulted in the wasp flying away with a seed. *Vespula alascensis* is a common wasp in the Santa Cruz Mountains and is known to be a scavenger for protein and sugar sources in addition to feeding on live prey (Kimsey and Carpenter 2012). Burge and Beck (2019) summarized the literature on instances of vespichochory with respect to *Vespula* and found several instances involving plant genera (*Trillium* L., *Vancouveria* C.Morren & Decne., *Calycanthus* L.) native to California. Burge and Beck (2019) performed an ecological study on dispersal of *Calycanthus* by *Vespula* and were able to demonstrate that a seed organ in *Calycanthus* is attractive to the wasps. Important to this dispersal system is the fact that fruits remain upright so that seeds do not fall out at maturity. Burge and Beck (2019) further noted observations that *Vespula* also actively remove seeds from *Aristolochia californica* Torr. fruits and fly away with them. The attractive tissue in *Aristolochia* is not an eliasome, but a strip of tissue filling a furrow in the seed coat (Burge and Beck 2019).

Our observations indicate no presence of an eliasome or analogous structure in *Pedicularis dudleyi*, however there is a brittle outer testa layer which is scraped away in a high proportion of the seeds observed in the field. We hypothesize that the outer testa layer is attractive to *V. alascensis* and instigates seed dispersal by that species. If true, this provides a mechanism for seed dispersal that should be considered in the management of the species. It also adds to a growing body of literature supporting the importance of vespichochory in California native plants. With respect to management of *P. dudleyi*, *V. alascensis* is common and seed dispersal appears to be relatively frequent. However, there are no estimates for how far the wasps carry the seeds nor any information on what happens to the seeds after the wasps depart with them, and these would be useful avenues of further research.

Seed germination and establishment of *P. dudleyi* remains poorly understood, but we provide preliminary information based on indirect estimates from census data, a litter removal experiment, and a seed germination greenhouse experiment. From our indirect estimates based on field census data, it appears that germination and persistence into the first rainy season is rather low, with only around 5% of seeds forming seedlings, under the assumption that germination reflects only the previous year's seed production without contribution from a seedbank. This is likely an overestimate, given the regular removal of seeds by wasps at a the rate we observed of 0.8 infructescence visits per hour with 52% of visits resulting in removal of a seed. Cotyledons emerge early in the rainy season and then persist throughout the summer. In a very small proportion of individuals, (not quantified, but certainly 5% or less) one or more true leaves is formed. We interpreted the formation of a true leaf to be an indication that the seedling had established and would persist into the next growing season, but it remains unclear the extent to which this is true, and if so, what proportion of seedlings at the cotyledon stage persist to the true leaf stage. This is an important avenue for future research.

Our litter exclusion trial did not provide evidence that a thick litter layer reduces germination or first year establishment. Based on our observations of extreme litter buildup and an apparent preference of extant individuals and populations for areas with low levels of litter buildup (e.g., roadbanks) we interpret this finding with some skepticism. Variation in litter depth and native seed bank among subpopulation sites may have contributed to the absence of a pattern in this study, and follow-up studies are in progress to replicate the results in the preliminary study reported here.

The germination trial resulted in no successful germinations across five treatments and a control group. The presence of seedlings in the field indicates that seed germination occurs in nature and the experiment we performed must not have correctly mimicked natural conditions. While the experiment did not succeed in testing differences among treatment groups, it did indicate that any breeding programs in the future will need to go through an experimental phase to identify appropriate germination conditions. It also suggests, consistent with field observations, that seed germination may be a productive area for future studies attempting to determine the causes of rarity for this species. Many species, especially those that are adapted to some level of disturbance (as might be the case in *P. dudleyi*) can exhibit prolonged seed dormancy (Baskin and Baskin 1998). Understanding the nature of this dormancy, as well as the relationship between disturbance and germination, will likely be key steps in developing an effective conservation strategy.

Additional unknown aspects of the life history include estimates of time (in years) between germination and first reproduction, and lifespan of the plants. Two out-planting experiments can provide an initial window into these important life-history parameters. Attempts to establish new populations were made by K. Kuska in 2011 and 2015. During the 2019 and 2020 field seasons, most of the extant individuals planted in 2015 had full rosettes similar in size to those of reproductive individuals, but were not yet flowering, although a few had single flowering stalks. Most of the individuals planted in 2011 had perished at some point prior to the 2019 and 2020 growing seasons. Those that persisted were mostly robust individuals with multiple flowering stalks. In one case, an individual planted at the top of a slope in 2011 had produced a large shadow of seedlings and young plants down the slope, one of which was flowering in 2019 and 2020. Together these findings suggest that a substantial proportion of individuals may not reach reproductive maturity for at least 5–6 yr, while it is possible for an individual to grow to reproductive maturity and then produce offspring which also reach reproductive maturity within a span of less than 10 yr. It also suggests that site-specific variation in environmental conditions may play a large role in the number of seasons it takes to reach reproductive maturity, however further study is warranted.

In summary, much remains unknown about the life history of this very rare plant. The primary threat, however, does appear to be associated with low germination and low seedling establishment in the field. In this study we were able to determine that pollination and seed dispersal appear not to be limiting, and anecdotally, there appear to be no important threats from invasive species. We therefore recommend that management efforts be directed toward establishment of new populations. Current populations occur most frequently in lightly disturbed areas along roadsides and in a campground, and establishment of new populations that are less vulnerable to accidental disturbance from park visitors and maintenance crews would likely be advantageous for the species.

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Literature cited

Baskin, C. C. and J. M. Baskin. 1998. Seeds; ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego, CA.

Burge, D. O. and J. Beck. 2019. Dispersal of spicebush (*Calycanthus occidentalis*, Calycanthaceae) by yellow jackets (genus *Vespula*; Hymenoptera: Vespidae). Madroño 66:41–46.

Fishman, M. A. and L. Hadany. 2010. Plant-pollinator population dynamics. Theoretical Population Biology 78:270–277.

Keil, D. J. 2019. *Pedicularis rigginsiae* (Orobanchaceae), a new species from an area of botanical endemism in central-coastal California. Phytoneuron 2:1–8.

Kimsey, L. S. and J. M. Carpenter. 2012. The Vespinae of North America (Vespidae, Hymenoptera). Hymenoptera Research 28:37–65.

OOSTERMEIJER, J. G. B. 2003. Threats to rare plant persistence. Pp. 17–58 *in* C. A. Brigham and M. W. Schwartz, Population viability in plants: conservation, management, and modeling of rare plants. Springer-Verlag, Amsterdam, Netherlands.

SCHEMSKE, D. W., B. C. HUSBAND, M. H. RUCKELSHAUS, C. GOODWILLIE, I. M. PARKER, AND J. G. BISHOP. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75:584–606.

SHOLARS, T. AND C. GOLEC. 2007. Rare plants of the redwood forest and forest management effects. United States Department of Agriculture, Forest Service. Website https://www.fs.usda.gov/treesearch/pubs/28258 [accessed 15 April 2020].

SPRAGUE, E. 1959. Ecological life history of California species of *Pedicularis*. Unpublished Ph.D. Dissertation, Claremont Graduate School, Claremont, CA.

SPRAGUE, E. 1962a. Pollination and evolution in *Pedicularis* (Scrophulariaceae). Aliso 5:181–209.

SPRAGUE, E. 1962b. Parasitism in *Pedicularis*. Madroño 16:192–200.

YATES, C. J. AND P. G. LADD. 2005. Relative importance of reproductive biology and establishment ecology for persistence of a rare shrub in a fragmented landscape. Conservation Biology 19:239–249.