CALYX VARIABILITY, PLASTICITY, AND THEIR RELATIONSHIPS TO SEED WEIGHT AND INFLORESCENCE STRUCTURE IN PHACELIA SUBSECT. HUMILES (HYDROPHYLLACEAE)

Author: Lee, Gregory J.

Source: Madroño, 70(2) : 79-89

Published By: California Botanical Society

URL: https://doi.org/10.3120/0024-9637-70.2.79

MADROÑO, Vol. 70, No. 2, pp. 79–89, 2023

CALYX VARIABILITY, PLASTICITY, AND THEIR RELATIONSHIPS TO SEED WEIGHT AND INFLORESCENCE STRUCTURE IN *PHACELIA* SUBSECT. *HUMILES* (HYDROPHYLLACEAE)

GREGORY J. LEE
Red Butte Garden and Arboretum, University of Utah, 300 Wakara Way,
Salt Lake City, UT 84108

ABSTRACT

Phacelia (Hydrophyllaceae) Juss. is a primarily western North American genus of over 200 annual and perennial species. Historically, calyces in *Phacelia* subsect. *Humiles* have provided important taxonomic characters with published descriptions giving mature calyx sizes as ranges rather than a specific number, and with little attention paid to the intra- or interspecific biological significance of this variation. This paper tests the hypothesis that *Phacelia* sepals are phenotypically plastic organs and that their post-accrescent size in *Phacelia* subsect. *Humiles* is related to seed production in associated fruits. Examination of ten taxa within *Phacelia* subsect. *Humiles* revealed that calyx size variation within a species was positively correlated with seed weight per capsule for nine of the ten taxa examined. Experimental data showed the correlation was not due to allometry. In defoliation experiments plants grown with all leaves removed post-germination were able to flower and produce seed solely through cotyledon, stem, and calyx photosynthesis, with the sepals expanding in size well beyond normal parameters. This and other data demonstrated that the sepals have developmental plasticity, are important photosynthetic organs, that the seed-weight/sepal-size correlation represents a functionally important source-sink relationship, and that pedicel length and inflorescence density are frequently related to calyx morphological characteristics.

Key Words: accrescence, calyx, *Phacelia*, photosynthetic reproductive structures, plasticity, sepals.

Many floral and fruiting parts are photosynthetic (Antlfinger et al. 1979; Aschan and Pfanz 2003; Raven and Griffiths 2015; Brazel and Ó'Maoiléidigh 2019). Agricultural literature is replete with examples of photosynthetic reproductive structures (PRS) contributing to a plant's reproductive carbon economy (Simkin et al. 2020). Ear photosynthesis in awned wheat varieties contributed 33–42% of the grain's total carbon in Evans and Rawson (1970) and up to 65% of the photosynthate for grain filling in Sanchez-Bragado et al. (2016). The contribution of ear photosynthesis to grain filling and yield has been reported in wheat (Biscoe et al. 1975; Li et al. 2006; Tambussi et al. 2007; Maydup et al. 2012; Sanchez-Bragado et al. 2014), oats (Jennings and Shible 1968); and in barley (Bort et al. 1994). Flinn et al. (1977) showed pod photosynthesis in *Pisum arvense* L. reduced the fruit's demand for photosynthate from the rest of the plant by 16-20%, and Lovell and Lovell (1970) found that 60% of pod photosynthate in *Pisum sativum* L. was exported into seeds within 24 hours of fixation. The contribution that PRS makes to the final yield in some crop plants has been reported as high as 75% (Buttrose and May 1959; Frey-Wyssling and Buttrose 1959; Allen et al. 1971; Inanga and Kimura 1974). A small sampling of crop plants shown to have significant photosynthetic contributions from PRS include alfalfa (Wang et al. 2016); cotton (Hu et al. 2012; Hu et al. 2014); cucumber (Marcelis et al. 1995); grapes (Lebon et al. 2005; Vaillant-Gaveau et al. 2011); tomato (Hetherington et al. 1998; Smillie et al. 1999); and persimmon (Yonemori et al. 1996). Sepal removal from flax (*Linum usitatissimum* L.) reduced fruit yield by up to 88% (Deshmikh et al. 1976; Tomar et al. 1979).

In wild land plants, PRS contributions to carbon gain have been most extensively studied in *Arabidopsis* Heynh. (e.g., Leonardos et al. 2014; Gnan et al. 2017). Earley et al. (2009) found that inflorescences of five different *Arabidopsis thaliana* (L.) Heynh. genotypes contributed more on average (55%) to lifetime plant carbon gain than leaves, with percentages ranging from to 36% to 93% amongst the different genotypes. A sampling of other wild land plants for which PRS have been documented to be important contributors to fruit or whole plant carbon gain include *Aciphylla* J.R.Forst. & G.Forst. (Hogan et al. 1998); *Ambrosia* L. (Bazzaz and Carlson 1979); *Bowiea* Haw. (Martin and Naidoo 2018); *Cymbidium* Sw. (Dueker and Arditti 1968); *Encelia* Adans. (Werk and Ehleringer 1983); *Floerkea* Willd. (Mokhtar 2005); *Galanthus* L. (Aschan and Pfanz 2006); *Helleborus* L. (Aschan et al. 2005; Herrera 2005; Guitián and Larrinaga 2014); *Paris* L. (Kun et al. 2013); *Ranunculus* L. (Galen et al. 1993); and *Spiranthes* Rich. (Antlfinger 1997). See reviews in Aschan and Pfanz (2003) and Brazel and Ó'Maoiléidigh (2019).

Despite the aforementioned research, little attention has been paid to the functional significance of taxonomically important variation in PRS characters and what these differences may mean for the ecology and diversification of undomesticated species. Herrera (2005) notes that "investigation of the post-floral functions of perianth parts other than those related to seed dispersal are strikingly scarce" and "further investigations on a variety of species in diverse ecological scenarios are needed to assess the functional significance and possible adaptive value of perianth persistence."

Phacelia Juss. provides an excellent system to investigate non-seed dispersal post-floral functionality of calyces. Within the genus, considerable diversity exists amongst species in sepal size, shape, and degree of accrescence. In many species, individual sepals within a calyx are unequal in size. This variation has frequently been used as a taxonomic character (e.g., Constance 1951; Howell 1943, 1945). In the 10th edition of The Jepson eFlora (Jepson Flora Project 2022), 92 of 94 California *Phacelia* species listed are described as having sepals which lengthen after flowering, *Phacelia ramosissima* Douglas ex Lehm. and *P. campanularia* A.Gray being exceptions, and the key to species in the genus includes 25 couplets which use some aspect of sepal size or morphology other than indumentum to distinguish species. In every species in the Jepson eFlora, sepal length is given as a range, indicating variability in this character. Despite the calyx's importance to taxonomic understanding of the genus, little to no attention has been paid to the relationship between calyx size and total seed weight per fruit and between calyx morphology and other inflorescence characteristics and the adaptive function of these relationships.

Phacelia subsect. *Humiles* (Walden and Patterson 2012) represents an assemblage of over 30 annual taxa and one, *Phacelia franklinii* (R.Br.) A.Gray, which has been described as annual/biennial (L. Constance, UC Berkeley, personal communication). It was formerly known as the *Phacelia* Humiles Group (sensu Constance 1963; Lee 2022). All are restricted to western North America, 23 are endemic to California, and all have accrescent sepals. This paper presents evidence that sepals in this taxon are phenotypically plastic organs, that their calyx size after accrescence is positively correlated with total seed weight per associated fruit, and that sepal accrescence, sepal size variation within a calyx, pedicel length, and inflorescence density may be components of adaptive syndromes to enhance calyx photosynthetic contributions to developing fruits.

METHODS

Calyx Size in Relationship to Seed Number and Weight

Twenty-eight *Phacelia stebbinsii* Constance & Heckard, 28 *P. marcescens* Eastw. ex J.F.Macbr. and six *P. quickii* J.T.Howell plants from four, four, and two populations, respectively were field-collected in 1980 and 1981 with each individual plant's calyces and associated seeds divided into lots based on number of seeds produced per fruit. The calyces and seeds of each seed number lot were weighed as a group to obtain the average calyx weight and average total seed weight per fruit to assess if average calyx size was related to the number of seeds in a fruit and total seed weight per fruit.

The correlation between calyx size and total seed weight per associated capsule was further tested by individually weighing 25 field-collected calyces and their associated seeds, henceforth referred to as a fruiting structure, from one population of each of ten taxa in *Phacelia* subsect. *Humiles*. These fruiting structures were on plants previously collected to provide seed for hybridization and cytogenetic studies. These particular species' populations were used because my collections of them contained a sufficient number of fruiting structures. The fruiting structures were randomly drawn from an envelope containing hundreds of fruiting structures derived from a minimum of six different individuals per taxon. The plants sampled were field-collected from locations listed in Appendix 1. The individual plants used in the study all had high levels of seed set and largely unopened fruit.

Allometry

The study site for this experiment was located in Placer County 100 meters south of Highway 20, 0.4 km east of the Nevada County line. A 107 cm long by 104 cm wide by 54 cm high insect exclusion box constructed from 3/4" by 3/4" wooden molding and mosquito netting was placed over part of a dense uniform-sized *Phacelia marcescens* population as it initiated flowering. The box's function was to reduce fruit set to test if the total seed weight-calyx-weight correlation was allometric (i.e., positively correlated for developmental reasons). If calyx size and total seed weight per fruit are allometrically related, then under conditions of low fruit set, the positive correlation between them should be as strong as under conditions of high fruit set. If the relationship between them could be decoupled by a dramatic reduction in the percentage of flowers developing fruits acting as photosynthetic sinks, that would indicate the relationship was not allometric. *Phacelia marcescens* was chosen for this experiment because its capsules retain their seed at maturity which made fruit and seed collection easy, and its self-compatible flowers, which are not markedly autogamous (Lee, 2022), ensured pollinator exclusion would significantly reduce but not eliminate seed set.

After fruit set, 56 plants were collected, 28 covered by the insect exclusion box to reduce pollination and fruit set, and 28 control plants from outside the box. Control plants were taken from the box's northern edge so they would have experienced shading similar to within-box plants. Each harvested plant was placed in a separate envelope. Mean fruit set in the control plants was 75% (SD = 8%). Mean fruit set in the treatment group (12%, SD = 8%) was too low to make within-plant correlations so only one intact fruiting structure was sampled per plant from both groups. The fruiting structure chosen for each plant was the first one with seed in an intact capsule to shake out of each envelope. Calyces were dried at 70°C for 72 hours and weighed to the nearest 0.1 mg.

Defoliation

Phacelia orogenes Brand seeds collected along the Eagle Lake Trail in Mineral King, Tulare Co. and *P. quickii* seeds collected 0.2 miles southwest of Glacier Point, Yosemite National Park were grown in a growth chamber with all leaves removed as they formed. Plants were made "leafless," by removing all leaf buds as they appeared. At no time did they have leaf tissue that reached beyond 1 mm in length, leaving them dependent upon cotyledon, stem, and floral photosynthesis. Additional plants which had no leaf tissue removed were grown for *Phacelia orogenes* to provide an experimental control. The growth chamber environmental regime was 16 hours light (450–550 $\mu E/m^{-2}/s^{-1}$) at 17°C, followed by eight hours of dark at 11°C. *Phacelia orogenes* and *P. quickii* were chosen for this experiment because previous work demonstrated that under long-day conditions they could flower subsequent to the first or second pair of leaves forming. Flowers were hand pollinated using a forceps to pluck stamens with dehiscing anthers and to then touch those anthers to another plant's punctiform stigma.

For calyx size comparisons in the leaf removal experiment, calyces from four *Phacelia orogenes* plants, two with leaves removed and two with leaves left intact, were removed and weighed at anthesis. This was repeated with four other plants after seed set and just prior to senescence, at which time the sampled calyces were at their maximum size. Calyces were dried and seeds weighed as described above. Calyx dry weight was used as a surrogate for photosynthetic capacity (Herrera 2005) throughout this paper because measuring length was less precise than weight, and sepal shape and thickness were potentially confounding factors.

Carbon Allocation

The carbon invested in calyces versus leaves was examined using 22 additional *Phacelia marcescens* plants from the population used in the allometry experiment. They were field-collected when their fruiting structures had largely matured, but before seed dispersal had started to occur. They were dried as described above and each plant's total calyx dry weight was compared to its total leaf dry weight.

RESULTS AND DISCUSSION

Calyx Size in Relationship to Seed Number and Weight

In the first study of the relationship between calyx size and seed production, total seed weight per capsule increased as seed number increased in the fruits of *Phacelia stebbinsii*, *P. marcescens*, and *P. quickii*. Calyx size typically increased as total seed weight increased (Appendix 2).

In the second study, there was a positive correlation between calyx dry weight and total seed weight per capsule for all ten taxa examined (Fig. 1). *Phacelia humilis* Torr. & A.Gray var. *humilis* was the sole case where the relationship was not statistically significant. These positive correlations indicate a resource-based relationship between calyx size and fruit size.

Allometry

A possible alternative explanation for the relationship between calyx size and total seed weight per capsule could be allometry, where the size of one organ is developmentally tied to the size of another.

In order to rule out allometry as a possible explanation, pollinators were excluded from a group of *Phacelia marcescens* plants to reduce seed set. In the control group where pollinators were not excluded, mean fruit set was 75% (SD = 8%) and calyx dry weight was strongly positively correlated with total seed weight per fruit [$r_s = 0.77$; P (2-tailed) < 0.001; $y = 1.4126x + 1.003$]. In the treatment group where pollinators were excluded, mean fruit set was reduced to 12% (SD = 8%) and the correlation between calyx dry weight and total seed weight per capsule fell to $r_s = 0.21$ [P (2-tailed) $= 0.28$; $y = 0.5146x + 5.499$] (Fig. 2).

If the calyx size and seed weight per capsule positive correlation was due to allometry, then low fruit set should not affect it. If the correlation represents a functional source-sink relationship, when fruit set is low then calyx-produced photosynthate from unfertilized flowers could provide an alternate carbon source for fertilized flowers' seeds, thereby lessening the effect an individual calyx might have on its associated capsule's yield (expressed as total weight of seed produced). This experiment's results support a source-sink hypothesis because the relationship between calyx size and fruit weight was uncoupled under low fruit-set conditions when photosynthate from unfertilized flowers calyces presumably would be more available to developing fruits throughout the plant. This interpretation is further supported by the slope in the regression lines. The slope for the regression line in the low fruit set group is just 36% of the size of the slope in the high fruit set group, indicating that calyx size in low fruit set conditions has a smaller effect on total seed weight of the associated fruit than it does in high fruit set conditions.

Defoliation

The *P. orogenes* plants grown without leaves thrived in the growth chamber, exceeding in size many field-collected specimens (Fig. 3). In fruit, sepals from leafless *P. orogenes* plants became significantly

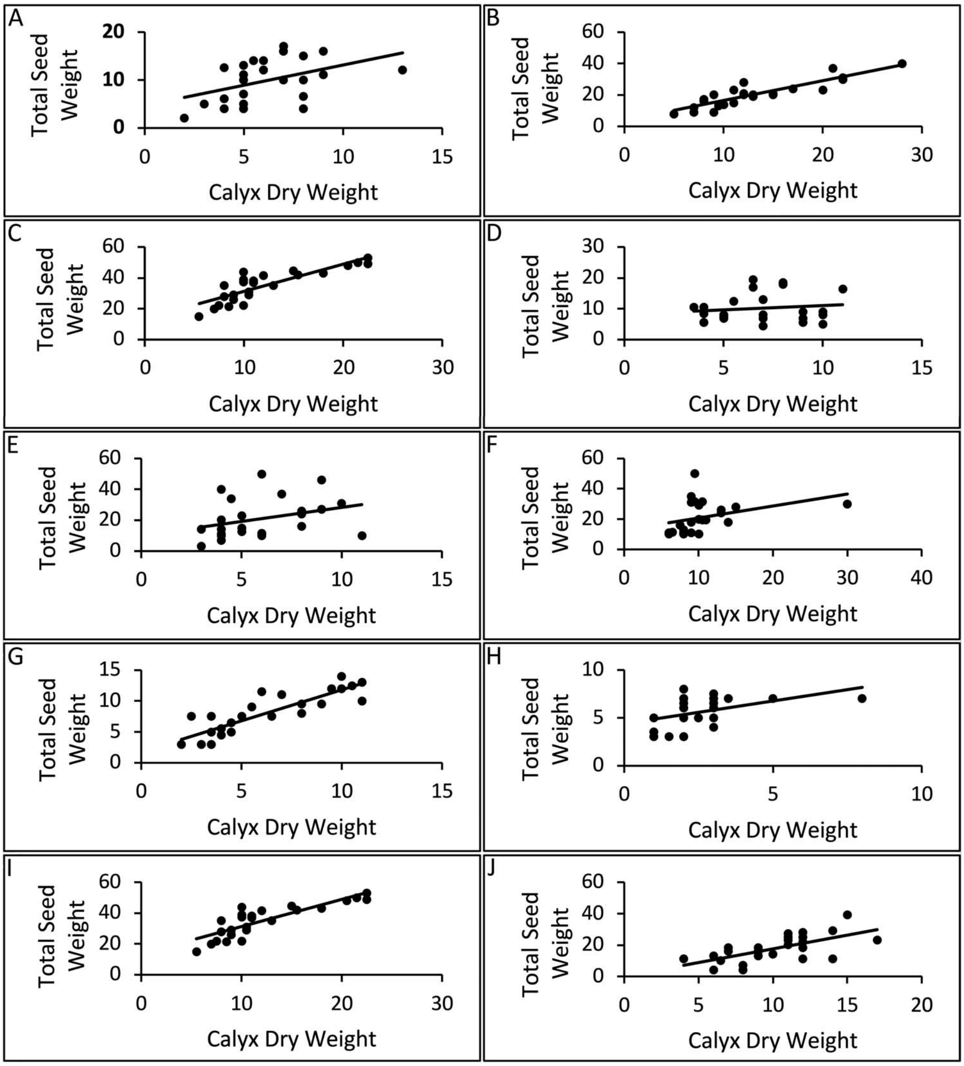

FIG. 1. Spearman rank correlation coefficients (r_s), P values, regression lines and equations for calyx dry weight (10^{-4} G) versus total seed weight per fruit (10^{-4} G) for ten different taxa in *Phacelia* subsect. *Humiles*. A. *Phacelia austromontana* $r_s = 0.475$; $p < 0.01$; $y = 0.84x + 4.70$; B. *Phacelia congdonii* $r_s = 0.864$; $p < 0.001$; $y = 1.26x + 3.95$; C. *Phacelia divaricata* $r_s = 0.632$; $p < 0.001$; $y = 1.13x + 11.12$; D. *Phacelia humilis* $r_s = 0.077$; $p > 0.10$; $y = 0.28x + 8.25$; E. *Phacelia humilis* var. *dudleyi* $r_s = 0.399$; $p < 0.025$; $y = 1.82x + 10.13$; F. *Phacelia purpusii* $r_s = 0.550$; $p < 0.005$; $y = 0.79x + 12.88$; G. *Phacelia quickii* $r_s = 0\ .877$; $p < 0.001$; $y = 1.01x + 1.77$; H. *Phacelia stebbinsii* $r_s = 0.518$; $p < 0.005$; $y = 0.47x + 4.39$; I. *Phacelia vallicola* $r_s = 0.475$; $p < 0.01$; $y = 1.76x + 13.65$; J. *Phacelia verna* $r_s = 0.652$; $p < 0.001$; $y = 1.75x + 0.10$. N = 25 for each sample. Collection locations in Appendix 1.

larger than the control group's sepals even though they were smaller at anthesis (Table 1). Both sets of plants produced viable seed. Although measurements were not made, in leafless *P. quickii* plants growth was dramatically reduced and not all plants survived to produce seed, but those that flowered did expand their sepals to well beyond their normal sizes (Fig. 4). The increase in size of both species' calyces when leaves were removed further demonstrates the plasticity *of Phacelia* sepals and that the degree of accrescence can increase when the plant is stressed by defoliation and would benefit from activation of an alternate source of photosynthate.

Carbon Allocation

One measure of the importance of calyces as photosynthetic structures in *Phacelia* is the amount of carbon invested in calyces versus leaves. In *P. marcescens*, which has smaller calyces than most species in *Phacelia* subsect. *Humiles*, average total dry weight of calyces from 22 field-collected *P. marcescens* plants

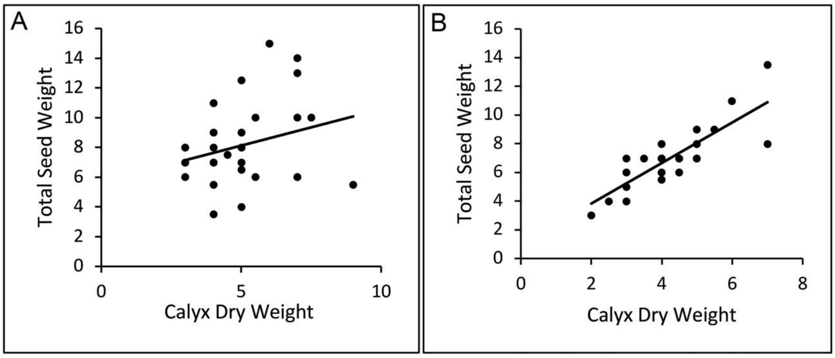

FIG. 2. Calyx dry weight (10^{-4} G) versus total seed weight per fruit (10^{-4} G) for low and high fruit set *Phacelia marcescens* plants: (A) low fruit set (inside box): $r_s = 0.21$; $P = 0.28$; $y = 0.515x + 5.499$; (B) high fruit set (outside box): $r_s = 0.77$; $p < 0.001$; $y = 1.413x + 1.003$. N = 28 for each sample.

was comparable to the dry weight of each plant's leaves over a wide range of plant sizes (Table 2). In 13 of the 22 individuals examined, total dry calyx weight per plant actually exceeded total leaf dry weight per plant and overall averaged 106.43% (SD = 35.22%, n = 22) of the leaves' total dry weight. While there was considerable variation in the calyx dry weight to leaf dry weight ratio amongst individual plants (Table 2), the overall relationship between the two was surprisingly linear over a wide range of plant sizes (Fig. 5).

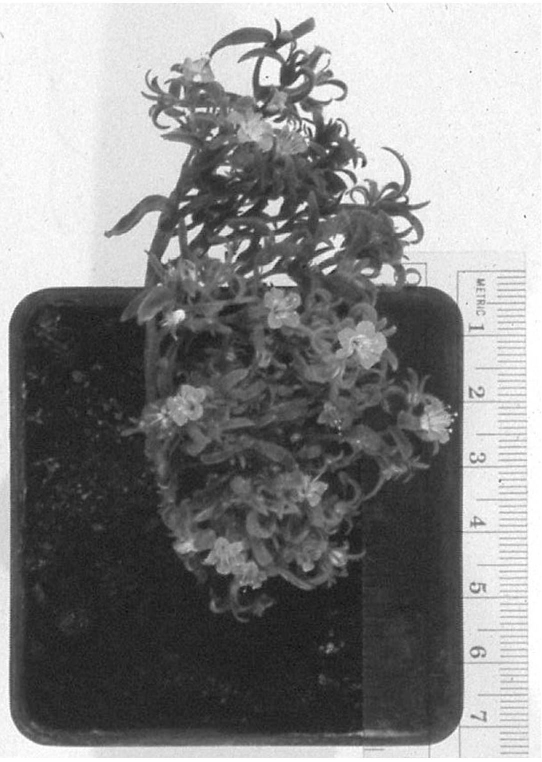

FIG. 3. Growth chamber grown *Phacelia orogenes*. The plant was completely defoliated at all times post-germination. All of the foliaceous tissue in the photo are sepals which were abnormally large for the species.

Morphology

Many species in *Phacelia* subsect. *Humiles* have calyces with sepals which are markedly unequal in size. In every instance of markedly unequal sized sepals, the smallest one is adaxial and located between the capsule and peduncle with the larger ones located abaxially. The smallest sepal is thus in the most shaded position and the largest are in positions presumably receiving the most illumination. In these otherwise actinomorphic flowers, unequal-sized sepals may represent a modification for enhancing calyx photosynthesis.

Phacelia subsect. *Humiles* species with equal-sized sepals, e.g., *P. curvipes* Torr. ex S.Watson, *P. davidsonii* A.Gray, and *P. douglasii* (Benth.) Torr., typically have longer pedicels than species such as *P. quickii*, *P. purpusii* Brandegee, and *P. mohavensis* A.Gray with unequal-sized sepals. In taxa with equal-sized sepals, adaxial sepal-shading may be minimized, not by reducing that sepal's size, but by moving it away from the peduncle. In compact inflorescences, adaxial sepal-shading could still be a problem, but in *Phacelia* subsect. *Humiles* long pedicels and equal-sized sepals are often associated with less congested inflorescences, or with inflorescences, if distally congested, that are proximally laxer in fruit, as in *P. orogenes*. This is consistent with the observation that calyx shading should be less in distal flowers than for those located in more basal positions in the inflorescence. Another common pattern is unequal calyx lobes, short pedicels, and uncongested inflorescences in fruit, as in *P. vallicola* Congdon ex Brand., *P. pringlei* A.Gray, and *P. leonis* J.T.Howell. *Phacelia humilis* var. *humilis* and *P. humilis* var. *dudleyi* J.T.Howell are unusual in *Phacelia* subsect. *Humiles* as they have equal-sized sepals, short pedicels, and compact inflorescences, a poor combination of characteristics for reducing sepal shading. They were also the taxa in

TABLE 1. KRUSKAL-WALLIS TEST COMPARING CALYX DRY WEIGHT AT ANTHESIS AND AFTER ACCRESCENCE FOR *PHACELIA OROGENES* PLANTS GROWN WITH AND WITHOUT LEAVES. At anthesis the plants with leaves had significantly heavier calyces ($H = 10.49$; $P < 0.025$) while after accrescence, the calyces of the plants grown without leaves were significantly heavier ($H = 36.98$; $P < 0.001$).

Developmental stage	Leaf status	Plant #	Median weight (mg)	Mean rank	N
At anthesis	With leaves	1	0.6	18.8	9
		2	0.6	16.8	11
	Without leaves	3	0.4	6.5	4
		4	0.4	6.5	4
After accrescence	With leaves	1	1.4	10.4	10
		2	1.6	16.5	15
	Without leaves	3	2.6	37.5	14
		4	2.9	42.2	17

which the calyx size-fruit yield correlation was weakest (Fig. 1). For the most part, though, the observed inflorescence character combinations would appear to increase the light-harvesting potential of calyces, but whether or not this is functionally significant is beyond the scope of this paper.

Physiological Ecology

Photosynthesis that maximizes carbon gain and minimizes water loss is a documented attribute of some photosynthetic reproductive structures. For example, Earley et al. (2009) found that inflorescences in different genotypes of *Arabidopsis thaliana* contributed on average 55% of lifetime carbon gain, but only 25% to lifetime water loss. Bort et al. (1996) found that the awns of lemmas, which are modified sepals, increased water- use- efficiency (WUE) in barley. Weyhrich et al. (1995) found that awns increased WUE in wheat. However, unlike agricultural crops, the open habitats most *Phacelia* species occupy become very hot and dry as the growing season progresses. Therefore, if *Phacelia* calyces have greater WUE than their leaves, this could explain the relatively large late-season investment of carbon in calyces as compared to leaves.

Phacelia sepals are generally smaller than their leaves and morphologically, the calyx resembles a palmately dissected leaf. The temperature of small leaves and dissected larger leaves stays closer to ambient air temperatures than undivided large leaves because a thinner boundary layer enables heat to be convected away more effectively, thereby reducing the need for transpirative cooling and increasing WUE (Bannister 1976). In addition, *Phacelia* flowers are usually elevated relative to leaves, presumably for pollinator attraction, but this also elevates them above soil surfaces (and positions them well for light interception). As air temperature is usually highest at the soil surface and decreases upward (Geiger, 1950), *Phacelia* inflorescences may be experiencing a lower air temperature microenvironment than leaves. Calyces would therefore require less water per surface area than leaves for transpirative cooling, enhancing plant WUE.

The variability in and length of *Phacelia* pedicels and peduncles may also contribute to thinner boundary layers and/or better light interception. The plasticity of sepals, pedicels and peduncles; their relationship to seed production; the water use efficiency WUE of sepals versus leaves; the percentage of total lifetime carbon gain contributed by sepals; the effect of partial defoliation and its timing, such as might occur in nature, on sepal accrescence; the movement and distribution of calyx-produced photosynthate in the plant; and determining the prevalence and importance of the phenomenon described in this paper in other sections of the genus are all avenues for further research. In summary, the taxonomically important calyx characteristics of accrescence, size, and sepals equal or unequal in combination with pedicel length and inflorescence density merit investigation to determine the

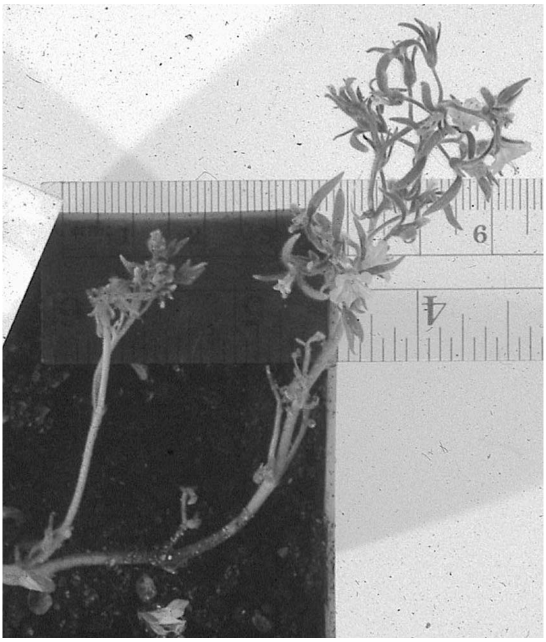

FIG. 4. Growth chamber grown *Phacelia quickii*. The plant was completely defoliated at all times post-germination. Note the abnormally large sepals developed subsequent to defoliation.

TABLE 2. TOTAL LEAF DRY WEIGHT (10^{-4} g) AND TOTAL CALYX DRY WEIGHT (10^{-4} g) FOR FIELD-COLLECTED *PHACELIA MARCESCENS* PLANTS.

Plant #	Weight of leaves	Weight of calyces	Calyces: leaves weight ratio
1	98	102	1.041
2	77	43	0.558
3	148	63	0.426
4	448	437	0.975
5	81	98	1.210
6	669	738	1.103
7	661	1145	1.732
8	2271	2771	1.220
9	308	394	1.279
10	798	762	0.955
11	233	332	1.425
12	169	159	0.941
13	35	28	0.800
14	38	23	0.605
15	512	533	1.041
16	630	505	0.802
17	1031	1259	1.221
18	259	125	0.483
19	659	773	1.173
20	542	880	1.624
21	618	910	1.472
22	679	964	1.420

extent to which they represent adaptive syndromes to enhance calyx photosynthesis.

CONCLUSIONS

In *Phacelia* subsect. *Humiles*, variability in the size of the mature calyx lobes within an individual and between individuals of each species is functionally related to seed production. Calyx size in fruit and total weight of the seed produced in the associated capsule are positively correlated. In previous work on photosynthetic reproductive structures, isotope studies have demonstrated the importance of sepals in providing photosynthate to seeds in a wide variety of species and that sepal removal reduces yield in a variety of species. Herrera (2005) demonstrated a positive, non-allometric linear relationship between calyx size and mean seed mass in associated follicles by manipulating calyx size in *Helleborus foetidus* L. The novel approach of this study in *Phacelia* was to test the effect on seed production and calyces of removing foliar instead of calyx tissue and to examine the relationships between mature calyx size and total seed weight per fruit within individual plants, within a population, between populations of the same species, and in different species within the same genus. This demonstrated that *Phacelia* sepals are phenotypically plastic organs capable of expansion to compensate for lost foliar tissue and that their final size is positively correlated with the total weight of the seed produced in the associated fruit. Sepal photosynthesis is an unexplored potential driving force for the structure of *Phacelia* calyces and inflorescences. This taxonomic characteristic of the genus has functional significance and this functional significance has practical implications. The use of sepal length measurements in the construction and use of taxonomic keys should take into account the variability and plasticity of *Phacelia* sepals lest it lead to misleading couplets and specimen mis-identifications (Lee, 2022).

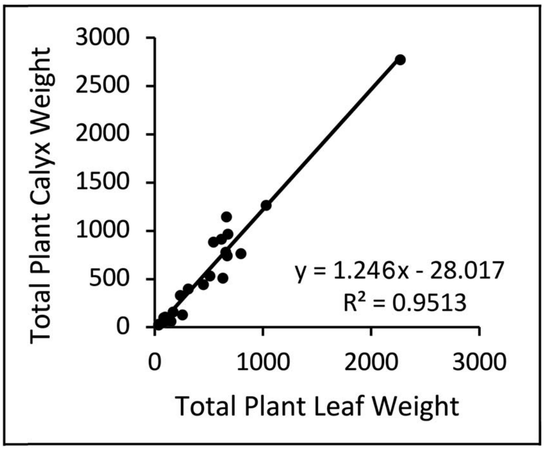

FIG. 5. Total calyx dry weight (10^{-4} g) versus total leaf dry weight per plant (10^{-4} g) at fruit maturation for 22 field-collected *Phacelia marcescens* plants.

ACKNOWLEDGMENTS

I wish to thank Donald Kyhos for his advice and support during this project; the late Lincoln Constance for many invaluable insights into systematics of *Phacelia*; John Harris, Lisa Palermo, and Julie Chiemi Uyehara for their friendship and support during the years when data for this paper was generated; Jason Baker for help with the paper's graphics, and Ann Deneris for her support and encouragement to finally publish this research. This study was supported in part by DEB-7908608 from the National Science Foundation and a Grant-in-Aid of research from Sigma Xi.

LITERATURE CITED

ALLEN, E. J., D. G. MORGAN, AND W. J. RIDGEMAN. 1971. A physiological analysis of the growth of oilseed rape. Journal of Agricultural Science 77:339–341.

ANTLFINGER, A. E. AND L. E. WENDEL. 1997. Reproductive effort and floral photosynthesis in *Spiranthes cernua* (Orchidaceae). American Journal of Botany 84:769–78.

ASCHAN, G. AND H. PFANZ. 2003. Non-foliar photosynthesis - a strategy of additional carbon acquisition. Flora 198:81–97.

———, AND H. PFANZ. 2006. Why snowdrop (*Galanthus nivalis* L.) tepals have green marks? Flora 201:623–632.

———, H. PFANZ, D. VODNIK, AND F. BATIC. 2005. Photosynthetic performance of vegetative and reproductive structures of green hellebore (*Helleborus viridus* L. agg). Photosynthetica 43:55–64.

BANNISTER, P. 1976. Introduction to physiological plant ecology. A Halsted Press Book, John Wiley and Sons, New York, NY.

Bazzaz, F. A. and R. W. Carlson. 1979. Photosynthetic contribution of flowers and seeds to reproductive effort of an annual colonizer. New Phytologist 82:223–232.

Biscoe, P.V., J. N. Gallagher, E. J. Littleton, J. L. Monteith, and R. K. Scott. 1975. Barley and its environment. IV. Source of assimilate for the grain. Applied Ecology 12:295–318.

Bort, J., A. Febrero, T. Amaro, and J. Araus. 1994. Role of awns in ear water-use efficiency and grain weight in barley. Agronomie 14:133–139.

Brazel, A. J. and D. S. Ó'Maoiléidigh. 2019. Photosynthetic activity of reproductive organs. Journal of Experimental Botany 70:1737–1753.

Buttrose, M. S. and L. H. May. 1959. Physiology of cereal grains: I. The source of carbon for the developing barley kernel. Australian Journal of Biological Sciences 12:40–52.

Constance, L. 1951. *Phacelia*. Pp. 486-518 *in* Illustrated Flora of the Pacific States, Vol. 3. Stanford University Press, Stanford, Palo Alto, CA.

———, 1963. Chromosome numbers and classification in Hydrophyllaceae. Brittonia 15:273–285.

Deshmikh, P. S., D. P. S. Tomar, and S. K. Sinha. 1976. Contribution of sepals to seed weight per boll in linseed (*Linum usitatissimum* L.). Photosynthetica 10:136–139.

Dueker, J. and J. Arditti. 1968. Photosynthetic $^{14}CO_2$-fixation by green *Cymbidium* (Orchidaceae) flowers. Plant Physiology 43:130–132.

Earley, E. J., B. Ingland, J. Winkler, and S. J. Tonsor. 2009. Inflorescences contribute more than rosettes to lifetime carbon gain in *Arabidopsis thaliana* (Brassicaceae). American Journal of Botany 96:786–792.

Evans, L. T. and H. M. Rawson 1970. Photosynthesis and respiration by the flag leaf and components of the ear during grain development in wheat. Australian Journal of Biological Sciences 23:245–254.

Flinn, A. M., C. A. Atkins, and J. S. Pate. 1977. Significance of photosynthetic and respiratory exchanges in the carbon economy of the developing pea fruit. Plant Physiology 60:412–418.

Frey-Wyssling, A. and M. S. Buttrose. 1959. Photosynthesis in the ear of barley. Nature 184:2031–2032.

Galen, C., T. E. Dawson, and M. L. Stanton. 1993. Carpels as leaves: meeting the carbon cost of reproduction in an alpine buttercup. Oecologia 95:187–193.

Geiger, R. 1950. The Climate Near the Ground. Harvard University Press, Cambridge, MA.

Gnan, S., T. Marsh, and P. X. Kover. 2017. Inflorescence photosynthetic contributions to fitness releases *Arabidopsis thaliana* plants from trade-off constraints on early flowering. PLoS One 12:e0185835.

Guitián, J. and A. R. Larrinaga. 2014. The role of post-floral persistent perianth in *Helleborus viridis* subsp. *occidentalis* (Ranunculaceae). Nordic Journal of Botany 32:852–857.

Herrera, C. M. 2005. Post-floral perianth functionality: contribution of persistent sepals to seed development in *Helleborus foetidus* (Ranunculaceae). American Journal of Botany 92:1486–1491.

Hetherington, S. E., R. M. Smillie, and W. J. Davies. 1998. Photosynthetic activities of vegetative and fruiting tissues of tomato. Journal of Experimental Botany 49:1173–1181.

Hogan, K. P., M. B. Garcia, J. M. Cheeseman, and M. D. Loveless. 1998. Inflorescence photosynthesis and investment in reproduction in the dioecious species *Aciphylla glaucescens* (Apiaceae). New Zealand Journal of Botany 36:653–660.

Howell, J. T. 1943. *Sertulum greeneanum*. American Midland Naturalist 30:6–39.

———, 1945. Studies in *Phacelia* – revision of species related to *P. douglasii*, *P. linearis*, and *P. pringlei*. American Midland Naturalist 33:460–494.

Hu, Y. Y., Y. L. Zhang, H. H. Luo, W. Li, R. Oguchi, D. Y. Fan, W. S. Chow, and W. F. Zhang. 2012. Important photosynthetic contribution from the non-foliar green organs in cotton at the late growth stage. Planta 235:325–336.

———, Y. L. Zhang, X. P. Yi, D. X. Zhan, H. H. Luo, C. W. Soon, and W. F. Zhang. 2014. The relative contribution of non-foliar organs of cotton to yield and related physiological characteristics under water deficit. Journal of Integrative Agriculture 13:975–989.

Inanga, S. and A. Kimura. 1974. Studies on dry matter production of the rape plant (*Brassica napus* L.) I. Changes with growth in rates of photosynthesis and respiration of a rape population. Proceedings of Crop Science Society of Japan 43:261–266.

The Jepson Flora Project (eds.). 2022. Jepson eFlora The Jepson Herbarium, University of California, Berkeley, CA. Website http://ucjeps.berkeley.edu/eflora/ [accessed 17 August 2022].

Jennings, V. M. and R. M. Shibles. 1968. Genotypic differences in photosynthetic contributions of plant parts to grain yield in oats. Crop Science 8:173–175.

Kun, Y., Q. Fan, Y. Wang, J. Wei, Q. Ma, D. Yu, and J. Li. 2013. Function of leafy sepals in *Paris polyphylla*: photosynthate allocation and partitioning to the fruit and rhizome. Functional Plant Biology 40:393–399.

Lebon, G., O. Brun, C. Magne, and C. Clement. 2005. Photosynthesis of the grapevine (*Vitus vinifera*) inflorescence. Tree Physiology 25:633–639.

Lee, G. J. 2022. Comparative morphology, biosystematics, and reproductive ecology of *Phacelia stebbinsii, P. marcescens,* and *P. quickii*. Madroño 69:16–23.

Leonardos, E. D., S. A. Rauf, S. M. Weraduwage, E. F. Marilla, D. D. Taylor, and B. J. Micallef-Bernard. 2014. Photosynthetic capacity of the inflorescence is a major contributor to daily C gain and the responsiveness of growth to elevated CO_2 in *Arabidopsis thaliana* with repressed expression of mitochondrial-pyruvate-dehydrogenase-kinase. Environmental and Experimental Biology 107:84–97.

Li, X. J., H. G. Wang, H. B. Li, L. Y. Zhang, N. J. Teng, Q. Q. Lin, J. Wang, T. Y. Kuang, Z. S. Li, B. Li, A. M. Zhang, and J. X. Lin. 2006. Awns play a dominant role in carbohydrate production during the grain filling stages in wheat (*Triticum aestivum* L.) Physiologia Plantarum 127:701–709.

Lovell, P.H. and Lovell, P.J. 1970. Fixation of CO_2 and export of photosynthate by the carpel in *Pisum sativum*. Physiologia Plantarum 23:316–322.

Marcelis, L. F. M. and L. R. B. Hofman-Eijer. 1995. The contribution of fruit photosynthesis to the carbon requirement of cucumber fruits as affected by irradiance, temperature and ontogeny. Physiologia Plantarum 93:476–483.

Martin, C. E. and G. Naidoo. 2018. Dependence of the South African geophyte *Boweia volubis* on its inflorescence for plant carbon gain. South African Journal of Botany 118:183–187.

Maydup, M. L. M. Antonietta, J. J. Guiamet, and E. A. Tambussi. 2012. The contribution of green parts of the ear to grain filling in old and modern cultivars of bread wheat (*Triticum aestivum* L.): evidence for genetic gains over the past century. Field Crops Research 134:208–215.

MOKHTAR, I. B. AND G. HOULE. 2005. Photosynthate contribution by sepals alleviates the carbon cost of reproduction in the spring ephemeral *Floerkea proserpinacoides* (Limnanthaceae). Annals of the Missouri Botanical Garden 92:438–444.

RAVEN, J. A. AND H. GRIFFITHS. 2015. Photosynthesis in reproductive structures: costs and benefits. Journal of Experimental Botany 66:1699–1705.

SANCHEZ-BRAGADO, R., G. MOLERO, M. P. REYNOLDS, AND J. L. ARAUS. 2014. Relative contribution of shoot and ear photosynthesis to grain filling in wheat under good agronomical conditions assessed by differential organ $\delta^{13}C$. Journal of Experimental Botany 65:5401–5413.

———, G. MOLERO, M. P. REYNOLDS, AND J. L. ARAUS. 2016. Photosynthetic contribution of the ear to grain filling in wheat: a comparison of different methodologies for evaluation. Journal of Experimental Botany 67:2787–2798.

SIMKIN, A. J., M. FARALLI, S. RAMAMOORTHY, AND T. LAWSON. 2020. Photosynthesis in non-foliar tissues: Implications for yield. The Plant Journal 101:1001–1015.

SMILLIE, R. M., S. E. HETHERINGTON, AND W. J. DAVIES. 1999. Photosynthetic activity of the calyx, green shoulder, pericarp, and locular parenchyma of tomato fruit. Journal of Experimental Botany 50:707–718.

TAMBUSSI, E. A., J. BORT, J. J. GUIAMET, S. NOGUÉS, AND J. L. ARAUS. 2007. The photosynthetic role of ears in C_3 cereals: metabolism, water use efficiency and contribution to grain yield. Critical Reviews in Plant Sciences 26:1–16.

TOMAR, D. P. S., P. S. DESHMUKH, AND S. K. SINHA. 1979. Importance of sepals in fruit and seed development in linseed (*Linum usitatissimum* L.). Euphytica 28:739–745.

VAILLANT-GAVEAU, N., P. MAILLARD, G. WOJNAROWIEZ, P. GROSS, C. CLEMENT, AND F. FONTAINE. 2011. Inflorescence of grapevine (*Vitus vinifera* L.): a high ability to distribute its own assimilates. Journal of Experimental Botany 62:4183–4190.

WALDEN, G. K. AND R. PATTERSON. 2012. Nomenclature of subdivisions within *Phacelia* (Boraginaceae: Hydrophylloideae). Madroño 59:211–222.

WANG, H., L. HOU, M. WANG, AND P. MAO. 2016. Contribution of the pod wall to seed grain filling in alfalfa. Scientific Reports 6:1–7.

WERK, K. S. AND J. R. EHLERINGER. 1983. Photosynthesis by flowers in *Encelia farinosa* and *Encelia californica* (Asteraceae). Oecologia 57:311–315.

WEYHRICH R. A., B. F. CARVER, AND B. C. MARTIN. 1995. Photosynthesis and water-use efficiency of awned and awnletted near-isogenic lines of hard red winterwheat. Crop Science 35:172–176.

YONEMORI, K., A. ATAI, R. NAKANO, AND A. SUGIURA. 1996. Role of calyx lobes in gas exchange and development of persimmon fruit. Journal of the American Society for Horticultural Science 121:676–679.

APPENDIX 1

COLLECTION LOCATIONS FOR FIG. 1

Unless otherwise indicated, all counties are in California.

Phacelia austromontana J.T.Howell, Inyo Co. White Mt. Road, 11.5 km from intersection with Hwy 168. *P. congdonii* Greene, Tulare Co. Hwy 245, 1.8 km north of Badger. *P. divaricata* (Benth.) A.Gray, Marin Co. Avenida Miraflores, Tiburon. *P. humilis* Torr. & A.Gray var. *humilis*, Mono Co. 8 km north of Lee Vining and 1 km west of Hwy 395. *P. humilis* var. *dudleyi* J.T.Howell, Kern Co. near summit of Mt. Breckenridge. *P. marcescens* Eastw. ex J.F.Macbr, Placer Co. 0.5 km east of Nevada Co. line on Hwy 20. *P. orogenes* Brand, Tulare Co. Trail to Eagle Lake, Mineral King. *P. purpusii* Brandegee, El Dorado Co. 3 km from Hwy 50 on Plum Creek Road. *P. quickii* J.T.Howell, El Dorado Co. Wentworth Springs Road, 4 km north of intersection with Ice House Road, behind Gerle Creek campground. *P. stebbinsii* Constance & Heckard, El Dorado Co. north-facing slope above spillway below Junction Dam. *P. vallicola* Congdon ex Brand, El Dorado Co. along staircase above Jaybird Powerhouse, northwest of Riverton. *P. verna* Howell, Douglas Co., Oregon, 4.3 km north of Hwy 42 on road to Reston.

APPENDIX 2

MEAN CALYX DRY WEIGHT AND MEAN SEED WEIGHT PER CAPSULE FOR CALYCES AND CAPSULES OF *PHACELIA STEBBINSII*, *P. MARCESCENS,* AND *P. QUICKII* ACCORDING TO THE NUMBER OF SEEDS PRODUCED PER FRUIT. In each group the seeds and calyces were weighed collectively, not individually. In each column, semicolons separate groups based on the number of seeds in that group's capsules starting with 0-seeded capsules on the left. All locations are in California.

Species; Collection location	Year	Plant #	No. seeds/ capsule	Number of calyces weighed in each category of seeds per capsule	Mean calyx weight in each category (mg)	Mean total seed weight per capsule in each category (mg)
P. stebbinsii; Jaybird Powerhouse, NW of Riverton; El Dorado Co.	1980	1	0; 1	50; 50	4.10; 5.58	0; 9.12
		2	0; 1	30; 50	3.10; 6.10	0; 7.76
		3	0; 1	50; 50	3.86; 5.02	0; 6.68
		4	0; 1	50; 50	2.84; 4.98	0; 6.42
	1981	1	0; 1	22; 50	4.05; 6.20	0; 5.68
		2	0; 1	38; 42	4.53; 5.40	0; 5.63
		3	0; 1	50; 45	5.35; 6.09	0; 6.38
P. stebbinsii; west face of Wentworth Mountain; El Dorado Co.	1980	1	0; 1; 2	10; 50; 10	2.00; 2.48; 2.70	0; 4.88; 8.50
		2	0; 1	19; 50	1.89; 2.46	0; 5.18
		3	0; 1	20; 50	2.65; 2.88	0; 5.72
		4	0; 1	18; 50	1.50; 2.10	0; 5.44
		5	0; 1; 2	16; 50; 8	2.20; 2.54; 3.25	0; 5.36; 9.62
	1981	1	0; 1	44; 183	1.66; 2.62	0; 5.42
		2	0; 1; 2	16; 74; 1	1.37; 2.41; 4.00	0; 4.26; 5.00
		3	0; 1	25; 18	3.00; 2.94	0; 6.94
		4	0; 1	57; 64	1.72; 2.69	0; 4.43
P. stebbinsii; north-facing slope above spillway below Junction Dam; El Dorado Co.	1980	1	0; 1; 2	20; 50; 9	2.65; 3.74; 5.33	0; 6.90; 9.78
		2	0; 1	10; 50	2.50; 3.42	0; 6.04
		3	0; 1	25; 26	2.08; 2.57	0; 7.07
	1981	1	0; 1	26; 27	1.42; 2.19	0; 4.22
		2	0; 1	26; 27	2.46; 2.96	0; 4.74
P. stebbinsii; Wentworth Springs Rd., 2.5 mi. N of intersection with Ice House Rd., behind Gerle Creek Campground; El Dorado Co.	1980	1	0; 1; 2	4; 32; 12	2.75; 3.84; 5.67	0; 4.28; 7.50
		2	0; 1; 2	40; 50; 1	2.67; 3.12; 5.0	0; 5.42; 14.0
		3	0; 1	20; 30	2.40; 2.80	0; 5.80
		4	0; 1	40; 50	2.85; 3.74	0; 7.46
		5	0; 1	5; 14	2.40; 3.29	0; 5.43
	1981	1	0; 1	20; 31	4.15; 4.94	0; 7.00
		2	0; 1; 2	4; 31; 1	3.25; 4.32; 8.00	0; 5.32; 10.00
P. marcescens; by Hwy 80, 0.2 miles SW of Fordyce Lake Rd.; Placer Co.	1980	1	0; 1; 2; 3	20; 19; 20; 3	2.20; 2,68; 3.60; 4.00	0; 5.78; 9.40; 13.33
		2	0; 1; 2	18; 50; 12	3.61; 4.94; 5.58	0; 6.90; 9.83
		3	0; 1; 2; 3	3; 19; 16; 3	3.33; 5.00; 3.00; 3.67	0; 5.26; 8.69; 11.00
		4	0; 1; 2	49; 32; 35	2.80; 3.31; 4.54	0; 3.94; 7.43
		5	0; 1; 2	40; 50; 1	2.48; 2.96; 5.00	0; 4.60; 7.00
P. marcescens; Baker Ranch Soda Springs Springs Rd., 14.7 miles SE of Serene Lakes; Placer Co.	1980	1	0; 1	10; 23	2.20; 3.04	0; 5.70
		2	0; 1; 2	6; 43; 2	1.67; 2.95; 6.00	0; 6.53; 11.50
		3	0; 1	3; 20	2.00; 2.85	0; 5.75
		4	0; 1	10; 20	2.00; 3.00	0; 6.45
		5	0; 1	9; 50	2.00; 2.68	0; 6.42
	1981	1	0; 1	13; 13	2.08; 2.54	0; 3.77
		2	0; 1; 2	8; 12; 5	1.50; 2.42; 3.40	0; 5.00; 11.80
		3	0; 1	4; 8	1.25; 1.87	0; 4.25
		4	0; 1; 2	8; 69; 9	1.87; 2.45; 2.67	0; 5.83; 10.64
P. marcescens; Hwy 20, 0.3 miles east of Nevada Co. line; Placer Co.	1980	1	0; 1; 2; 3	30; 50; 38; 10	3.33; 4.22; 4.63; 3.80	0; 6.06; 8.74; 9.00
		2	0; 1; 2; 3	50; 50; 40; 1	3.24; 3.82; 4.42; 5.00	0; 4.92; 7.97; 13.00
		3	0; 1; 2; 3	40; 50; 20; 1	2.90; 3.70; 4.65; 6.00	0; 5.92; 9.10; 13.00

APPENDIX 2. CONTINUED

Species; Collection location	Year	Plant #	No. seeds/ capsule	Number of calyces weighed in each category of seeds per capsule	Mean calyx weight in each category (mg)	Mean total seed weight per capsule in each category (mg)
	1981	1	0; 1; 2	8; 5; 6	3.62; 6.80; 5.67	0; 7.00; 9.83
		2	0; 1; 2	8; 4; 5	1.87; 3.00; 3.20	0; 4.00; 5.80
		3	0; 1; 2	6; 9; 2	3.17; 3.89; 4.50	0; 5.11; 8.50
		4	0; 1; 2; 3; 4	26; 8; 31; 19; 1	2.81; 4.50; 4.90; 5.16; 6.00	0; 5.00; 8.03; 11.00; 11.00
		5	0; 1; 2	25; 34; 9	3.08; 5.06; 6.11	0; 5.56; 10.33
P. marcescens; Intersection of Hwy 20 and Spaulding Lake Rd, 2.1 miles west of *Hwy 80*	1981	1	0; 1; 2; 3	23; 51; 9; 3	2.26; 3.57; 4.44; 5.00	0; 5.47; 7.64; 9.67
		2	0; 1	16; 91	3.50; 4.95	0; 6.30
		3	0; 1	69; 75	3.41; 4.65	0; 4.58
		4	0; 1; 2	10; 12; 4	2.40; 4.42; 6.00	0; 6.27; 10.00
		5	0; 1; 2; 3	8; 25; 20; 3	3.12; 3.84; 4.85; 4.33	0; 4.63; 7.67; 8.33
		6	0; 1; 2; 3	5; 13; 20; 3	4.00; 6.00; 5.60; 5.67	0; 5.38; 8.10; 9.33
P. quickii; W of Loon Lake, El Dorado Co.	1980	1	1; 2; 3; 4	4; 8; 15; 17	3.50; 5.50; 5.87; 7.00	4.25; 7.50; 7.67; 14.59
		2	0; 1; 2; 3; 4	2; 3; 5; 3; 7	5.00; 4.67; 6.40; 6.67; 9.29	0.0; 4.33; 7.00; 10.67; 13.86
		3	0; 1; 2; 3; 4	1; 2; 3; 6; 6	6.0; 4.0; 4.33; 8.00; 8.67	0.0; 4.00; 7.00; 11.67; 15.00
P. quickii; Wentworth Springs Rd., 2.5 mi. N of intersection with Ice House Rd., behind Gerle Creek Campground; El Dorado Co.	1980	1	1; 2; 3; 4	5; 10; 21; 18	5.80; 6.90; 6.48; 7.89	3.00; 6.00; 9.57; 11.50
		2	0; 1; 2; 3; 4	15; 8; 23; 16; 14	5.07; 5.50; 6.00; 7.00; 7.21	0; 3.87; 7.23; 10.53; 13.64
		3	1; 2; 3; 4	4; 8; 12; 13	5.75; 7.00; 7.83; 9.46	3.75; 7.625; 12.92; 17.23