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EFFECTS OF FIRE ON GERMINATION OF *ERICAMERIA FASCICULATA* (ASTERACEAE), A RARE MARITIME CHAPARRAL SHRUB

JON R. DETKA
Division of Science & Environmental Policy, California State University Monterey Bay, 100 Campus Center, Building 53, Seaside, CA 93955-8001
jon_detka@csumb.edu

SUSAN C. LAMBRECHT
Department of Biological Sciences, San José State University, One Washington Sq., San José, CA 95192-0100

ABSTRACT

Knowledge gaps regarding the greenhouse propagation of rare, fire-adapted plant species can impede community level conservation efforts that require fire and active revegetation as management tools. *Ericameria fasciculata* is a rare shrub endemic to the maritime chaparral community of the central California coast and a listed species of concern. Prescribed burning is actively used in maritime chaparral to maintain community composition and conserve several species of concern with known affinities for fire-related conditions. No study has investigated the seed viability and germination requirements for *E. fasciculata*. The goal of this study was to ascertain the (1) greenhouse propagation potential of *E. fasciculata* for planned restoration efforts and (2) to determine if fire-related conditions inhibit or promote *E. fasciculata* germination. Seed dissection and viability testing indicated that a large percentage of seed were empty or inviable. A greenhouse study examined the potential for fire-related germination cues from heating, light, and charate. Heating and charate had negative effects on seed germination. The combination of heating and charate treatments were particularly lethal. Exposure to light or the addition of GA_3 had no influence on germination rates. Results suggest that seed germination of *E. fasciculata* is inhibited by fire and, therefore, this species is dependent on seedling establishment between fire events.

Key Words: Asteraceae, *Ericameria fasciculata*, fire, germination, maritime chaparral.

The ability to propagate rare endemic plant species has become increasingly important with the advent of conservation goals directed at revegetation and restoration of endemic plant communities (U.S. Army Corps of Engineers 1997; Padgett et al. 1999). The legal impetus for such actions in California stem from the ratification of the Endangered Species Act (ESA) and the California Endangered Species Act (CESA). In tandem with these legal requirements, a growing appreciation of native California flora and the intrinsic values associated with species diversity has prompted the need for additional information regarding the propagation of endemic plant species (Emery 1988). Much of this concern for the protection and conservation of rare species also stems from the knowledge that increased anthropogenic influences (e.g., global climate change and habitat fragmentation) are moving at rates that exceed the ability of species with restricted distributions to accommodate (Davis 1989). In response to these biological crises, it has been recommended that urgency be placed on the development of conservation techniques that can be used to actively increase the size and distribution of rare plant populations (Primack and Miao 1992).

Active restoration of rare plant populations by seed broadcasting typically fails to establish self-sustaining populations (Primack 1996). Several hypotheses have been proposed as explanations for the lack of success from introduced seed and general inability to establish self-sustaining regenerative populations. These include the need for (1) suitable habitat/community composition, (2) potentially specialized germination responses and (3) improper seasonal timing of seed collection or distribution (Primack 1996; Willson and Traveset 2000). In addition, seed broadcasting challenges are compounded when working with rare species as the current plant distribution may not adequately represent the conditions required for germination, required germination conditions may be unknown, and wildland seed stock may be in short supply. Therefore, restorationists have shifted their efforts to propagating rare species in greenhouses for reintroduction into wildlands (Gordon-Reedy and Mistretta 1997; Padgett et al. 1999). Active outplanting of greenhouse-propagated plants into suitable unoccupied habitat may increase the dispersal potential of a species with limited seed dispersal capabilities (Primack and Miao 1992).

It is well established that many plant species in fire-prone Mediterranean-type plant communities have unique fire adapted seed life histories (Went et al. 1952; Sweeney 1956; Keeley and Zedler

1978; Keeley 1987). In general, these germination adaptations are responses to the drastically altered environmental conditions that are present following fire events. However, the majority of these studies investigated the role of fire in Southern California inland chaparral dominated by *Adenostoma fasciculatum* Hook. & Arn. (Christensen and Muller 1975a, b; Keeley et al. 1981; Moreno and Oechel 1991; Swank and Oechel 1991; Odion and Davis 2000). There are no clear trends in the degree or trend of seed germination responses among closely related taxa in the maritime chaparral plant community (Davis et al. 1989). Furthermore, most studies have investigated the role of fire on germination of common chaparral shrub species (Keeley and Zedler 1978; Keeley and Keeley 1984; Keeley 1987, 2006; Tyler 1995; Holl et al. 2000).

Woody chaparral plant species with an obligate seeding fire life history are particularly challenging to propagate given that many are reliant on specific combinations of fire-related germination cues for their emergence (Keeley 1987; Emery 1988; Gordon-Reedy and Mistretta 1997; Padgett et al. 1999; Boyd 2007). Fire may impact seed germination by altering the microscale environmental conditions through heating, charate, and changes in available light (Keeley 1987; Davis et al. 1989; Baskin and Baskin 2001)

During a fire, temperatures at the surface of sandy soils can exceed 600°C (Sweeney 1956). However, heating from fire dramatically decreases (50–200°C) with small changes in depth (1–2 cm) and duration (<20 minutes) (Sweeney 1956; Davis et al. 1989). Germination responses of woody chaparral shrubs to dry heating at temperatures similar to near-surface burial (70–120°C) are extremely varied, ranging from increases, decreases, and no effect on germination rates (Baskin and Baskin 2001).

The increased availability of light often associated with the post-burn chaparral environment can have a significant impact on the emergence of seedlings (Sweeney 1956; Keeley 1987). Fire can dramatically reduce canopy vegetation cover and litter, increasing the amount of available light. There are few clear trends in light-facilitated germination response among plants. But, it is generally accepted that smaller seeded species have more seed residing near the surface of soils and are more dependent on light signaling than are larger seeded species (Pons 2000). Keeley (1987) noted 22 species from 15 families of woody California chaparral shrubs that exhibited a significant light-stimulated germination response. Interestingly, several species within families exhibited no consistent trend in light-related germination responses.

Previous studies have demonstrated a wide variety of effects of charred wood on germination of individual species (McPherson and Muller 1969; Keeley and Pizzorno 1986; Keeley 1987; Thanos and Rundel 1995; Tyler 1996). Additionally, studies have elucidated that there are species-specific germination responses to different combinations of heat, light, and charate (Keeley and Keeley 1984; Keeley et al. 1985; Keeley 1987; Tyler 1996).

Ericameria fasciculata (Eastw.) J. F. Macbr. is a stout (<5 dm tall) shrub in the Asteraceae (Hickman 1993), previously classified as *Haplopappus eastwoodiae* H. M. Hall. *E. fasciculata* is listed as a species of concern (List 1B) by the California Native Plant Society (Skinner and Pavlik 1994), and is proposed for listing as an endangered species on the Federal Endangered Species List (CNDDB 2002). The most distinguishing features of *E. fasciculata* are its aromatic, resinous, cylindrical leaves arranged in fasciculate bundles, its pale yellow radiate flower heads that bloom in July and its achenes that are attached to a dense golden-white pappus (Matthews 1997).

The geographic range of *E. fasciculata* is estimated at less than 4,000 hectares (CNDDB 2002). Scattered individuals occur in coastal dune, central maritime chaparral, and coastal closed cone pine forest from 30–270 meters (MSL) elevation in Monterey Co., California, but have historically been most abundant in the central maritime chaparral plant community (Griffin 1976, 1978; Van Dyke and Holl 2003). This central maritime chaparral plant community consists of a diverse array of fire-adapted endemic sclerophyllous shrubs, residing in predominately sandy soils and blanketed by the summer fog of the coastal regions (Griffin 1978).

Several taxa related to *E. fasciculata* have demonstrated a range of post-fire responses making it difficult to infer the potential for fire stimulated seed germination (Keeley and Keeley 1984; Keeley 1987; Tueller and Payne 1987; Holl et al. 2000). Prior to this study, the germination response of *E. fasciculata* seed was not known. This study was prompted primarily in response to the need for information regarding (1) greenhouse propagation potential of *E. fasciculata* for planned restoration efforts and (2) to determine if burning inhibits or promotes *E. fasciculata* seed germination. Field observations noted low occurrences of natural post-burn *E. fasciculata* seedling emergence coupled with a catastrophic mortality rate in the first year following prescribed burning (Detka 2007). In addition, initial attempts to propagate the species without fire-related stimuli in greenhouses yielded mediocre results (Detka personal observation).

Materials and Methods

Seed Collection and Storage

Mature capitula were collected from 29 plants located on the Fort Ord, Parker Flats Reserve,

Monterey, CA (36°38′4.60″N, 121°46′38.78″W) during September 2005 and 2006. A voucher specimen was collected, pressed, and mounted for deposit at the Carl W. Sharsmith Herbarium, San Jose State University, CA. All collected seed was grouped by year and no cleaning or sorting was conducted. Seeds were stored loosely in brown paper bags in unlit standard refrigeration at 5C–10C.

Seed Viability Testing

Prior to propagation trials, three random samples of 300 seeds each were acquired from 2005 and 2006 seed stocks. Seeds were visually inspected under 10× hand lens magnification and sorted into three categories; intact, aborted, and dead. Seeds with obvious external structural deformations, such as being smaller than the mean seed length or width, were imbibed in 1 mM $CaCl_2$ solution at lab temperature for 1 hr and dissected under a dissection microscope to determine if an embryo was present. Those seeds with no embryos present and no signs of physical damage, predation, or fungal attack were recorded as aborted. Seeds that appeared intact with no external deformations, physical damage, or fungus present were grouped as intact. Seeds with obvious physical damage from predation or fungal attack were recorded as dead.

We used a 1% 2,3,5-triphenyl-tetrazolium Chloride (TZ) staining technique (Carolina Biological Supply Co., Burlington, NC) to evaluate collected intact seeds for embryo viability from each seed stock for 2005 and 2006 (Lakon 1949). Prior to TZ staining we soaked intact seeds in a 1 mM $CaCl_2$ solution at laboratory temperature for 1 hour to imbibe seeds to soften the seed coat for dissection. We removed the seed coats of intact seeds under a dissection microscope and inspected for intact embryo material. Seeds containing no embryo or the presence of decayed soft tissues were recorded and pooled in the dead category. Those seeds containing intact embryos were soaked in TZ staining solution for 18 hours. Care was also taken to insure that embryos remained completely submerged in the solution with no contact to air or exposure to light. The presence of a pink to red coloration along portions of the embryo indicated viable seed.

Greenhouse Germination Trials

Greenhouse germination trials were conducted in the late fall following seed collection to examine the role of fire-related cues in the germination of *Ericameria fasciculata*. The fire-related treatments were: (1) pre-sowing heat, (2) powdered charate from *Adenostoma fasciculatum* wood, and (3) light. Initial germination trials had produced poor rates of germination so gibberellic acid (GA_3) treatment was applied.

Seeds were sorted from remaining plant material and inspected for signs of physical damage (i.e., predation, fungal invasion) or obvious deformities. Thirty-two lots of 150 intact seeds were sorted into steel soil tins and were designated to receive orthogonally grouped treatments of heat (70°C–120°C) or no heat, light or dark, charate or no charate, and gibberellic acid (GA_3) or no gibberellic acid (GA_3).

Seeds were dry heated in the open steel tins using a forced convection oven set at 70°C for 1 hr, 100°C for 5 min, and 120°C for 5 min to mimic fire conditions observed by Sweeney (1956) and recommended in Keeley (1987). A control treatment was also designated and received no heating. Immediately following heat treatment we removed seeds from the tins and placed them in 50 ml centrifuge tubes (BD Biosciences, MA).

Sixteen of the 32 centrifuge tubes were designated for the GA_3 treatments. GA_3 treated seeds were imbibed with a mixture of 20 ml of 1 mM $CaCl_2$ solution and 20 ml of 100 ppm GA_3 solution. We designated a control treatment for the remaining 16 centrifuge tubes to receive 40 ml of $CaCl_2$ only. Seeds were soaked in solutions at laboratory temperature (22°C–25°C) for 3 hr. Seeds that were designated for dark propagation treatment were housed in centrifuge tubes wrapped in aluminum foil to prevent light exposure.

Previous greenhouse trials using a sterile pre-moistened soil mixture (4 parts peat, 2 parts perlite, and 2 parts vermiculite) resulted in extremely poor seed germination response across all treatments and this prompted the adoption of Petri dish propagation techniques. Each group of 150 treated seeds were sown into 32 plastic Petri dishes (150 mm × 25 mm) containing two sheets of 150 mm #1 filter paper (Whatman International Ltd., Maidstone, England). Filter paper was pre-moistened with 1 mM $CaCl_2$ solution and any standing solution was removed. Petri dishes were covered with their lids and sealed in re-sealable plastic food storage bags to decrease moisture loss. All Petri dishes were cold stratified for 1 month in an unlit refrigerator at 5°C–10°C.

For seeds receiving charate treatment, 1 g of powdered charred wood was applied evenly on top of Petri dish filter paper prior to pre-moistening. Charate was made by charring fresh cut *A. fasciculatum* stems in a steel burn barrel with a propane torch. Once the stems appeared charred, but not completely reduced to ash, we extinguished the fire by covering the barrel with a lid. Woody charred stems (5–10 mm diameter) were removed and pulverized in a SPEX mill (SPEX CertiPrep, Metuchen, NJ) to produce a fine charate powder.

Analysis of preliminary germination trials had determined that cold treatment improved mean percent germination by 6% in *E. fasciculata* ($t = 3.530$, $df = 4$, $P = 0.024$) (Detka 2007). During preliminary trials, we had noted fungal invasion in both the cold treatment and control. This prompted the testing of a potential pre-sowing disinfection treatment. Results of disinfection solution testing suggested that the solution was effective in reducing fungal invasion, but at a significant cost to seed germination (Detka 2007). Therefore, we did not use disinfection treatments in any future germination trials.

Following cold stratification, Petri dishes were placed on an indoor Juliana grow rack (ACF Greenhouses, Buffalo Junction, VA) at laboratory temperature (22°C–25°C) and out of direct sunlight. We incubated seeds receiving dark treatment on the grow rack shelves in cardboard boxes with removable lids. Ventilation holes were placed on the backside of the boxes to allow sufficient air flow. Seeds undergoing light treatment were placed under 40w fluorescent bulbs (GRO-LUX Wide spectrum, Sylvania LTD., Danvers, MA) under low light (approximately 70–100 $\mu mol\ s^{-1}\ m^{-2}$) for a 13-h photoperiod, as recommended in Comstock et al. (1989).

We surveyed Petri dishes every two days to count germinated seeds and remoisten filter paper with DI water if necessary. Germination was scored based on the first observation of radicle emergence. All dark treatment dishes were surveyed under indirect green light. Monitoring continued for 30 days. We based the monitoring time period on previous growth trial observations that suggested a peak in seedling emergence approximately 10–14 days following removal from cold stratification and a rapid decline in germination thereafter.

Data Analysis

We used two-way ANOVA to determine if differences were evident between the observed proportions of viable seed in 2005 and 2006 seed stock. We used multi-way ANOVA to compare the proportion of seedlings emerging within and between the different propagation treatments. We used SYSTAT v. 10.0 (SYSTAT, San Jose, CA) for all statistical analyses. Levene's test was used to test for homogeneity of variances and the assumption of normality was examined with probability plots of the residuals.

Results

Seed Viability Testing

Results of seed dissection and TZ staining indicated that approximately 10% of *Ericameria fasciculata* seeds were viable. In both the 2005 and 2006 seed stock, empty and dead seeds were more prevalent than viable seed (Fig. 1). There was no significant difference in the proportion of empty, viable, and dead seed condition between the 2005 and 2006 seed stock ($F_{2,12} = 0.514$, $P = 0.611$).

Greenhouse Germination Trials

The addition of GA_3 ($F_{1,64} = 0.269$, $P = 0.606$) and light stimulus ($F_{1,64} = 1.261$, $P = 0.266$) had no significant impact on *E. fasciculata* germination (Table 1).

The use of charate had a deleterious effect on germination ($F_{1,64} = 48.963$, $P < 0.001$) resulting in mean germination responses less than 1% in all cases (Table 1). The interaction of charate and higher temperature treatments (>70°C) had a particularly lethal effect on *E. fasciculata* seed germination ($F_{3,64} = 18.619$, $P < 0.001$) (Table 1). No other significant interactions between main effects were evident. Heat treatments as a main effect had a significant effect on the germination of *E. fasciculata* ($F_{3,64} = 23.147$, $P < 0.001$). Post hoc tests suggested that there is a significant difference in percent germination between seed experiencing lower temperature heat treatments (Control and 70°C) compared to higher temperature (100°C and 120°C) heat treatments ($P < 0.001$). Higher temperature treatments (>70°C) had catastrophic effects resulting in the near elimination (99%) of germination response. The highest rates of germination occurred in seeds that received no heat treatments (control) and no charate (Table 1). Comparison of mean percent germination of seeds receiving no heating and 70°C heat treatment indicated a mean reduction in germination response of 35% with the addition of the 70°C heat treatment.

Discussion

Ericameria fasciculata is found in fire-prone plant communities (Griffin 1978) and yet fire-related germination cues appear to have predominantly negative effects on seed germination. Dry heating conditions similar in temperature to those associated with near-surface burial resulted in marked decreases in germination at temperatures greater than 70°C. In addition, the presence of charate had a particularly deleterious effect on germination. Interestingly, the presence of light had little or no impact on seed germination. This low tolerance for heating and charate and unresponsiveness to light suggests that *E. fasciculata* seed (1) existing prior to fall burning is largely destroyed during fall burns, (2) buried relatively deeper in the near soil surface (>1 cm) may be able to endure exposure from low burn intensities and germinate without exposure to light, and (3) dispersal and subsequent coloniza-

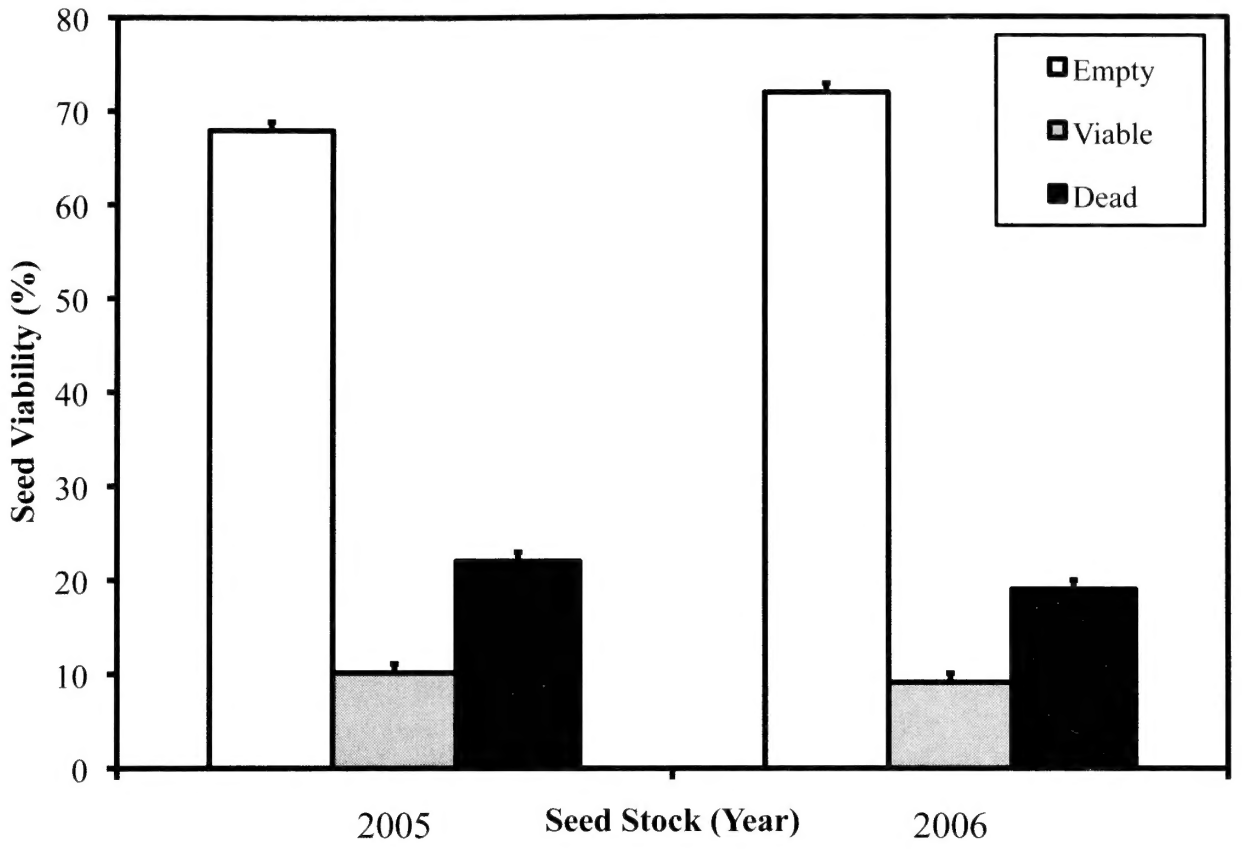

FIG. 1. Mean percent seed viability from 2005 and 2006 seed stocks. Viability percentage is based on results of seed dissection and 2,3,5-triphenyl-tetrazolium chloride (TZ) staining. Mean percentage is based on average of replicate trials (n = 3) for each seed stock. Error bars indicate SEM.

tion may occur from adjacent unburned or low intensity burned sites.

The observed germination responses to fire-related conditions are not uncommon in chaparral species known to utilize an obligate resprouting post-fire strategy and may indicate preferences for niches in the mosaic of post-burn environmental conditions and trade-offs associated with resprouting (Keeley and Zedler 1978; Keeley 1987; Baskin and Baskin 2001; Boyd 2007). For example, Keeley (1987) found that the seeds of *Haplopappus squarrosus* (Hook. & Arn.) Greene responded negatively to heat treatments, but charate presence and available light had no associated effect on germination. Prior to this finding, Keeley and Keeley (1984) also found that *H. squarrosus* was capable of vigorously resprouting in the first-year following burns. We propose that seeds of *E. fasciculata* may demonstrate a similar post-fire seedling establishment strategy to *H. squarrosus* by occupying a niche in the low burn intensity environment. In this environment, access to light and charate would be less available due to burial depth and the increased likelihood of unburned surviving aboveground vegetation cover. In low intensity burns, mature *E. fasciculata* were more apt to vigorously crown resprout and flower (Detka 2007), increasing seed availability for dispersal into areas containing little charate and more intact vegetation cover.

Trends in post-burn germination of *Ericameria ericoides* (Less.) Jeps. may also support the observed fire-related germination responses of *E. fasciculata* seed. *Ericameria ericoides* is closely

TABLE 1. MEAN PERCENTAGE GERMINATION OF *E. FASCICULATA* IN RESPONSE TO ORTHOGONALLY GROUPED TREATMENTS OF GA_3, LIGHT OR DARK, HEAT TREATMENTS, AND CHARATE. Each mean value is based on (n = 3) Petri dishes each containing 150 seeds. Temperature treatments sharing the same superscript letter were not significantly different ($P > 0.05$ from Bonferroni post hoc test). Standard error (SE) values are reported in parentheses. In all cases charate and non-charate treatments were significantly different. Significance values for the remaining main effects in the multi-way ANOVA were not significant ($P > 0.05$).

	Light				Dark			
	Control[a]	70°C 1 hr[a]	100°C 5 min[b]	120°C 5 min[b]	Control[a]	70°C 1 hr[a]	100°C 5 min[b]	120°C 5 min[b]
GA_3								
Control	5.78 (1.11)	2.89 (0.59)	—	—	5.33 (1.15)	3.78 (0.44)	—	—
Charate	0.22 (0.22)	0.44 (0.44)	—	—	0.44 (0.44)	0.22 (0.22)	0.22 (0.22)	—
Control (no GA_3)								
Control	4.44 (1.18)	3.78 (0.44)	—	—	8.22 (4.26)	4.44 (1.74)	—	—
Charate	0.22 (0.22)	0.22 (0.22)	—	—	0.67 (0.01)	0.44 (0.44)	—	—

related to *E. fasciculata* (Roberts and Urbatsch 2003) and resides in the same habitat range. Further experimental comparisons between the common *E. ericoides* and the rare *E. fasciculata* may serve to elucidate differences in post-fire recovery performance leading to patterns of rarity in *E. fasciculata*. Holl et al. (2000) observed high rates of germination in *E. ericoides* following surface burn treatments using fresh *Adenostoma fasciculatum* stems, which may initially seem contrary to trends observed in *E. fasciculata*. Observed germination of *E. ericoides* seed following surface burn treatments may have been associated with burial depths that were deep enough to protect seed from high temperature exposure (e.g., >70°C) (Holl et al. 2000). In addition, the proposed associated toxicity of allelopathic chemicals present in *A. fasciculatum* charate may have been volatized as stems were reduced entirely to ash.

The extremely low germination rates are apparently due to the complete absence of embryonic tissues in a large proportion of achenes. The lack of embryonic tissue in otherwise intact achenes has been frequently observed in the Asteraceae (Keeley 1987; Padgett et al. 1999; Meyer and Carlson 2001; Alkio and Grimm 2003; Ransom Seed Laboratory 2006). Previous studies have proposed that the high frequency of empty achenes is the result of increased seed abortion due to self-pollination or pollination among closely related plants (Connor and Hall 1997) or the result of variation in resource availability (Sobrevila 1989). Padgett et al. (1999) has suggested that reduced seed set reflects an adaptive mechanism to deter herbivores by hiding viable achenes among empty ones (Connor and Hall 1997). In this study, we did not test specific mechanisms for the observed low seed, but the implications are significant for restoring this rare species.

Active restoration of *E. fasciculata* into areas of suitable habitat may be required to insure the conservation of the species. We recommend that active restoration efforts include wildland seed collection and viability testing in advance of prescribed burning events. Ripe capitula should be collected for site specific propagation from local populations to increase the likelihood of preserving the genetic integrity of the species and insure that seed stock is representative of healthy individuals best adapted to localized conditions (Pavlik 1996). Wildland seed collection of rare species in this fashion should also set limits on the extent of seed collection from donor plants and adopt measures to reduce the risk of decimating available wildland seed stocks from established populations (Guerrant 1996).

Ericameria fasciculata propagation should include preliminary tetrazolium staining assessments of seed stock viability. Tetrazolium staining techniques can be employed quickly and inexpensively as a means of estimating germination potential and the amount of seed needed to produce the projected number of seedlings for restoration efforts. In this study, estimates of germination potential using tetrazolium staining were slightly higher (9–10%) than the highest observed germination in Petri dish propagation trials (6–8%). Two explanations can account for the overestimation of seed viability using this technique. First, seeds were not rejected if they had any indication of pink to red staining along portions of the embryo. This approach increased the speed of assessment but may have reduced the accuracy by not accounting for those seeds that were experiencing the late stages of gradual tissue die-off (Lakon 1949; Grooms 2006). Secondly, bacteria and fungi can result in a surface staining of seeds. All embryos were inspected by surface scraping and sectioning to insure that staining was complete, but it is still plausible that advanced fungal invasions could have yielded a false positive reading (Lakon 1949; Gutormson 2005).

Caution should be used in interpreting germination potential from Petri dish propagation as several dishes experienced fungal colonization that may have reduced germination. Keeley (1987) proposed that some of the fungal invasion that he observed in similar germination trials of woody chaparral shrubs may be attributed to the lack of fungal resistance by empty or inviable seed. Due to the large number of potentially empty *E. fasciculata* seed, special care should be exercised to visually evaluate and discard seeds that appear to have signs of fungal invasion or physical defects. In addition, further propagation research should be conducted to establish if conventional vermiculite sowing techniques yield higher rates of emergence in *E. fasciculata*.

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DISTRIBUTION AND COMMUNITY ASSOCIATIONS OF CAPE IVY (*DELAIREA ODORATA*) IN CALIFORNIA

RAMONA ROBISON
ICF International, 630 K Street, Suite 400, Sacramento, CA 95814
RROBISON@ICFI.COM

JOSEPH M. DITOMASO
University of California, Davis, CA 95616

ABSTRACT

Cape ivy (*Delairea odorata* Lem.) was found to occur throughout coastal California and southern Oregon. It was most abundant in urbanized coastal areas such as the San Francisco Bay, and Santa Cruz, Monterey, San Luis Obispo, Santa Barbara and Los Angeles counties. Field observations showed Cape ivy to occur in seven different broad community types, including both riparian and non-riparian areas. Of the two morphological forms, the exstipulate type occurred more frequently at the northern and southern ends of the distribution, and the stipulate type was more common in the middle of the distribution range, from southern Humboldt County to Los Angeles County. Only 21 locations were found that supported both stipulate and exstipulate plants, and they were most often located in urbanized coastal areas. Analysis with GIS determined the elevation, temperature and precipitation ranges that Cape ivy occupies in California. The analysis indicated that Cape ivy occurs at elevations between 0 and 891 meters, annual mean temperatures between 10.5 and 17.7°C, and in areas with annual precipitation ranging between 232 and 2270 mm. An overlay analysis of Cape ivy locations using GIS was also compared with the California Natural Diversity Database sensitive species location information to determine which species might be threatened by Cape ivy expansion. Three sensitive animals and five sensitive plants were expected to have >40% of their occurrences with a 500 m buffer to Cape ivy infestations.

Key Words: German Ivy, invasive, *Senecio mikanioides*, South Africa, weed.

INTRODUCTION

Cape ivy (*Delairea odorata* Lem., syn. *Senecio mikanioides* Walp.) (Asteraceae) is native to South Africa, but has escaped cultivation to invade wildlands in Europe, Australia, New Zealand, Hawaii, and South America, as well as coastal regions of western North America (Parodi 1959; Abrams and Ferris 1960; Palhinha 1974; Zangheri 1976; Pignatti 1982; Haselwood and Motter 1983; Hirano 1983; Webb et al. 1988; Fagg 1989; Jacobi and Warshauer 1992; Scott and Delfosse 1992; Hickman 1993; Gallo 2000; DiTomaso and Healy 2007). It was first collected in California in 1892 (*F.T. Bioletti s.n.* UC36003), and since that time has spread to all coastal counties and many adjacent inland sites (Jepson 1951; Abrams and Ferris 1960; Thomas 1961; Hoover 1970; Howell 1970; Munz 1974; Smith 1976; Beauchamp 1986; Smith and Wheeler 1992; Hickman 1993; Best et al. 1996; Junak et al. 1995; Matthews 1997). Cape ivy's spread into undisturbed wildland areas of California is of great concern, particularly because effective control is difficult (Alvarez 1997; Bossard et al. 2000). Although Cape ivy has firmly established itself along California's coast, the question remains as to whether it has occupied the full extent of its potential range.

To date, only one California study has documented the community types invaded by Cape ivy within the state (Alvarez and Cushman 2002). Alvarez and Cushman (2002) compared the effect of Cape ivy on invaded and un-invaded coastal scrub, and willow and alder riparian communities. Their results showed that plots invaded by Cape ivy had a 31, 88, and 92% decrease in species diversity, abundance of native seedlings, and non-native seedlings, respectively, compared to uninvaded sites of the same community types.

In this study, we provide a map of the current distribution, as well as more detailed information on the plant community types occupied by Cape ivy. In addition, we identify the distribution of the two morphological forms of Cape ivy in California, a stipulate and exstipulate type. The results provided here will help identify threatened community types or sensitive species in proximity to Cape ivy infestations.

MATERIALS AND METHODS

Mapping

The California Exotic Pest Plant Council (now known as the California Invasive Plant Council, Cal-IPC) Cape Ivy Working Group began

collecting Cape ivy distribution data in 1995. In May 1995, the distribution of Cape ivy was mapped along streams and hillsides in the coastal region of California, south of Monterey Co. (no vouchers taken). Additionally, appropriate habitats such as lakes, campgrounds, and parks along the coast were also surveyed. All populations that were reported by Cal-IPC members, California Native Plant Society (CNPS) members, park rangers, and other concerned citizens were visited, confirmed, and described for future analysis. The boundaries of the populations were estimated and drawn on maps. The data collected were then digitized as point data on 1:100,000 topographic base maps using MapInfo Professional 5.0 (LizardTech, Seattle, WA).

Additional areas were surveyed in 1999, including coastal counties north of Monterey and the San Francisco Bay Area. In addition to collecting maps from field experts, data were collected using a hand-held Trimble GeoExplorer II GPS (Global Positioning System) unit (Trimble, Sunnyvale, CA) with an overall corrected accuracy of 1 to 3 m. A series of sites originally mapped in 1995 by Cal-IPC were re-surveyed for Cape ivy in 2000, with 95% of these locations still supporting the invasive species.

Several individuals and organizations provided large Cape ivy distribution data sets that were incorporated into our database. Most notable was an extensive set of maps provided by Golden Gate National Recreation Area (GGNRA) employees. These maps included data from Marin, San Francisco and San Mateo counties in the form of ArcView shapefiles. Electronic data were also provided for Pt. Reyes National Seashore by the National Park Service, Catalina Island by the Catalina Island Conservancy, and Contra Costa Co. by the Contra Costa Watershed Forum. The other data collected were on paper maps obtained from 12 sources ranging from Oregon to San Luis Obispo. These were digitized onto 1:100,000 scale topographic maps. Other mapping points were provided for a number of counties from Del Norte to San Diego.

All the spatial data were brought into a GIS (Geographic Information System). In 1999, MapInfo Professional 5.0 was used to create maps, as well as store and edit the data. The GPS data was exported from Pathfinder to a MapInfo format, and ArcView shapefiles were converted to MapInfo format and included in the GIS. Some of the data provided by GGNRA were polygon or line data and these were converted to point data for the final analysis.

In 2000, vegetative community types and stipulate or exstipulate morphological forms of Cape ivy were also recorded using GPS. The California Natural Diversity Data Base (CNDDB) (California Fish and Game, http://www.dfg.ca.gov/biogeodata/cnddb/) community type which classifies vegetation using a five-number land cover code was chosen for the mapping analysis, and field data was collected using the number code. From 2001 to 2004, data were collected with a Garmin eTrex Vista GPS (Garmin Ltd., Olathe, KS) with accuracy of 15 meters alone and <3 m with the Wide Area Augmentation System (WAAS) enabled. Waypoints collected with the eTrex Vista were converted to ArcView shapefiles with Waypoint+ version 1.8.03. After conversion, the data files were edited to contain attribute fields listed in Table 1. Maps presented here are in the Teale-Albers projection, geographic coordinate system NAD 1927.

GIS Analysis

BIOCLIM Raster Extraction. GIS analysis was performed with ArcView version 9.0 (ESRI, Redlands, CA) and the Spatial Analyst extension (version 9.0). Polygon data collected in the distribution mapping phase were converted to points and 1465 Cape ivy location points were used as the basis for GIS analysis. In order to determine the elevation and climate parameters associated with the distribution data set in California, the point data was joined with BIOCLIM raster datasets. The bioclimatic variables (BIOCLIM) raster layers were derived from WorldClim interpolated climate layers (http://www.worldclim.org/methods). The WorldClim climate layers contain precipitation records for 47,554 locations, mean temperature from 24,542 locations, and minimum and maximum temperature for 14,835 locations (Hijmans et al. 2004). WorldClim altitude was obtained from the Shuttle Radar Topography Mission (SRTM) Digital Elevation Models (http://www2.jpl.nasa.gov/srtm/). Grids used in the analysis were at 30 seconds (1 km). A spatial join of Cape ivy point data and BIOCLIM rasters was accomplished with the ArcView Spatial Analyst "extract values to points" tool. For example, when the BIOCLIM annual precipitation raster data set was spatially joined to the Cape ivy point data a column with annual precipitation was generated in the attribute table. This was repeated for all the raster layers. Excel (Microsoft Corp., Redmond, WA) and JMP IN (version 5.1) (SAS Institute Inc., Cary, NC) were then used to determine the range and mean values for the raster layers.

CNDDB Sensitive Species Overlay. Overlay analysis was performed with the Cape ivy point data and the California Natural Diversity Database (CNDDB) sensitive species location data. The data are available within an application called RareFind, a Windows based program developed by the California Department of Fish

TABLE 1. ATTRIBUTES OF CAPE IVY USED IN FINAL MAPPING SHAPEFILES.

Field name	Description
SHAPE	all points
SITECODE	map identification point, using county abbreviation and number
COUNTY	county
GPS	true or false
VISITED	date of GPS
SURVEYOR	surveyor or source of data
ENTEREDBY	person digitized by
DATAFILE	name of rover file for GPS data or original shapefile name
SCI_NAME	*Delairea odorata*
COMMENT	source of data, location, directions, etc.
GPS CODE	waypoint code for eTrex Vista data
VEGTYPE	Holland (1986) numerical code used by CAGAP (Davis et al. 1998)
ST_NS	either stipulate (ST), exstipulate (ES) or both (STES)
VIABLE	viable seeds present, Yes or No
LAT	generated with the "add XY" tool in ArcView 9.0
LONG	generated with the "add XY" tool in ArcView 9.0

and Game, Sacramento, CA (http://www.dfg.ca.gov/biogeodata/cnddb/rarefind.asp) and designed to perform queries and produce reports. RareFind comes with GIS layers, which were used for this analysis (RareFind version 3.0.5 dated September 2, 2005). The CNDDB data consists of locations for sensitive plants, animals and natural communities as well as population data voluntarily submitted by field biologists. Sensitive species are defined as federally and state listed plants and animals, all species that are candidates for listing, all species of special concern and those species that are considered sensitive by government agencies and conservation organizations (http://www.dfg.ca.gov/whdab/pdfs/cnddbfaq.pdf). The data were then reviewed for accuracy and mapped by CNDDB personnel as "occurrences" at various levels of precision, from specific points to non-specific buffered polygons.

For the CNDDB GIS analysis, Cape ivy points were buffered out 100 m to represent the current extent of their direct or indirect influence on sensitive species locations. The "select by location" feature in ArcView was used to select the sensitive species occurrences, which overlapped with the 100 m buffered points. The selected polygons from the CNDDB data were then saved into a separate shapefile. Another file was created with Cape ivy points buffered out to 500 m, representing an estimate of future spread, while another shapefile with sensitive species occurrences was generated for comparison.

RESULTS AND DISCUSSION

Mapping

California and Oregon Cape Ivy Distribution. Cape ivy has been known to occur in California since 1892, yet many of the historic floras only mention it in passing and do not indicate it as a widespread weed (Munz 1974; Smith 1976; Beauchamp 1986; Junak et al. 1995). In the 1970's it was noted as "climbing on trees, mostly willows, along coastal streams," and "forming dense tangles in shaded canyons or on moist open slopes" (Hoover 1970; Howell 1970). Floras from the 1990's noted that it is common or invasive in coastal areas (Best et al. 1996; Matthews 1997). Surprisingly, as late as 1992 the Mendocino Flora states that it is "occasional but seldom collected" (Smith and Wheeler 1992). In fact, there are no voucher records of Cape ivy in Mendocino County in the Consortium of California Herbaria (http://ucjeps.berkeley.edu/consortium/) prior to 2001, despite it widespread occurrence there today.

Based on the field survey, Cape ivy occurs throughout all coastal counties of California, as well as the Channel Islands (Santa Rosa, Santa Cruz and Santa Catalina) and Curry Co., Oregon (Figs. 1–3). Furthermore, it was also found in most of the major river systems along the coast. Although the vast majority of Cape ivy infestations were found within a few kilometers of the coast, populations occurred 60 to 70 km inland in Contra Costa and Los Angeles counties.

Interestingly, in its native range in South Africa, nearly all collections of Cape ivy have been reported to be the stipulate form (Balciunas and Smith 2006; Robinson 2006). In California, however, the exstipulate form is far more commonly encountered than in its native range (Fig. 4). Although the exstipulate form is found throughout California, it is the primary morphological type in the northern extent of its range, including Curry Co., Oregon, and northern Humboldt Co., as well as the southern range of its distribution in Los Angeles and San Diego counties.

The stipulate forms were most widespread throughout the center of the range of the species, from Mendocino Co. to Santa Barbara Co.

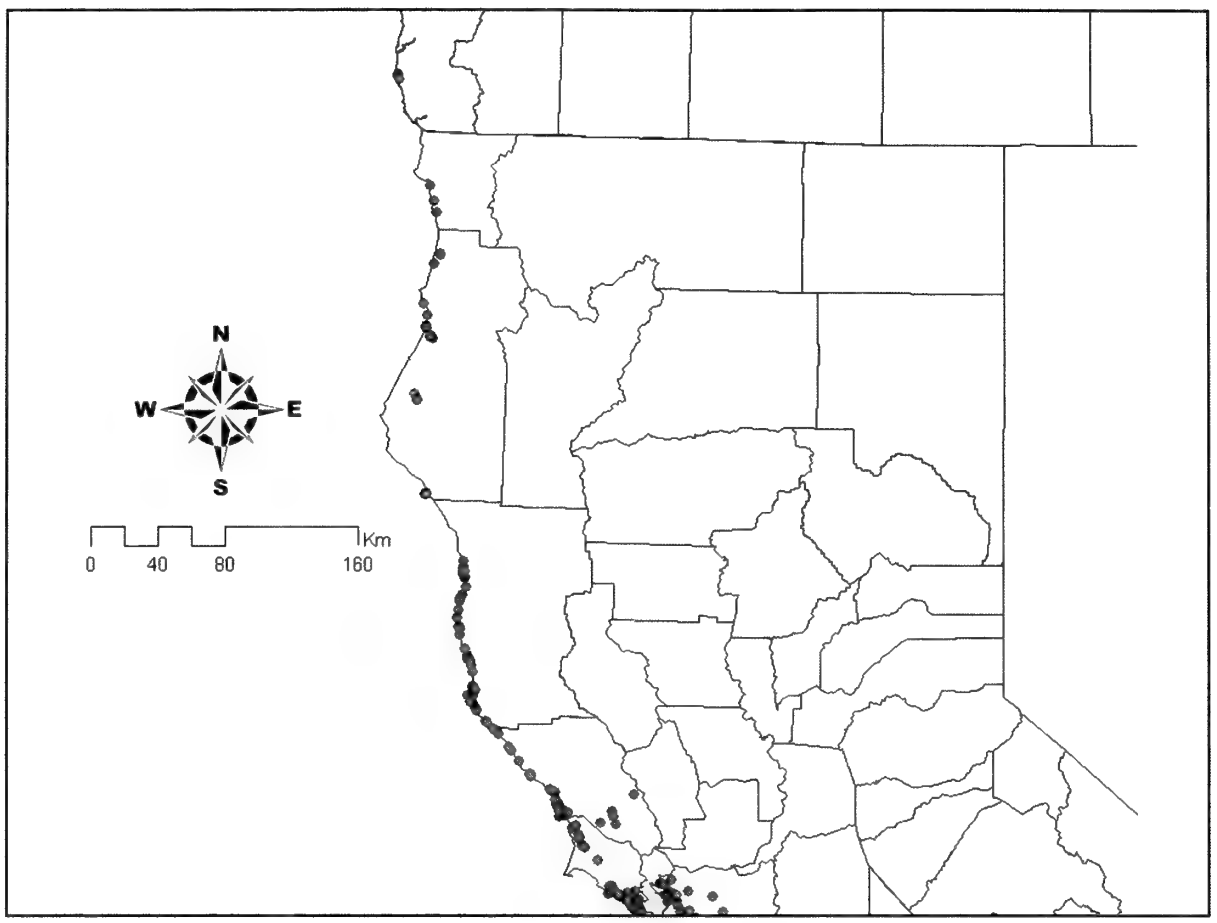

FIG. 1. Cape ivy locations in northern California and southern Oregon.

(Fig. 4). A combination of the two morphological forms was most common in heavily populated areas, particularly the San Francisco Bay region and San Luis Obispo Co.

GIS Analysis

The climate where Cape ivy grows in California can be broadly described as Mediterranean. Mediterranean climates are characterized by dry summers and an average of 25 to 100 cm annual rainfall concentrated during the mild winter months (Dallman 1998). Snow is infrequent except at higher elevations, and the amount of winter rain is highly variable from year to year.

BIOCLIM Raster Extraction. BIOCLIM Raster Extraction analysis indicates that Cape ivy in

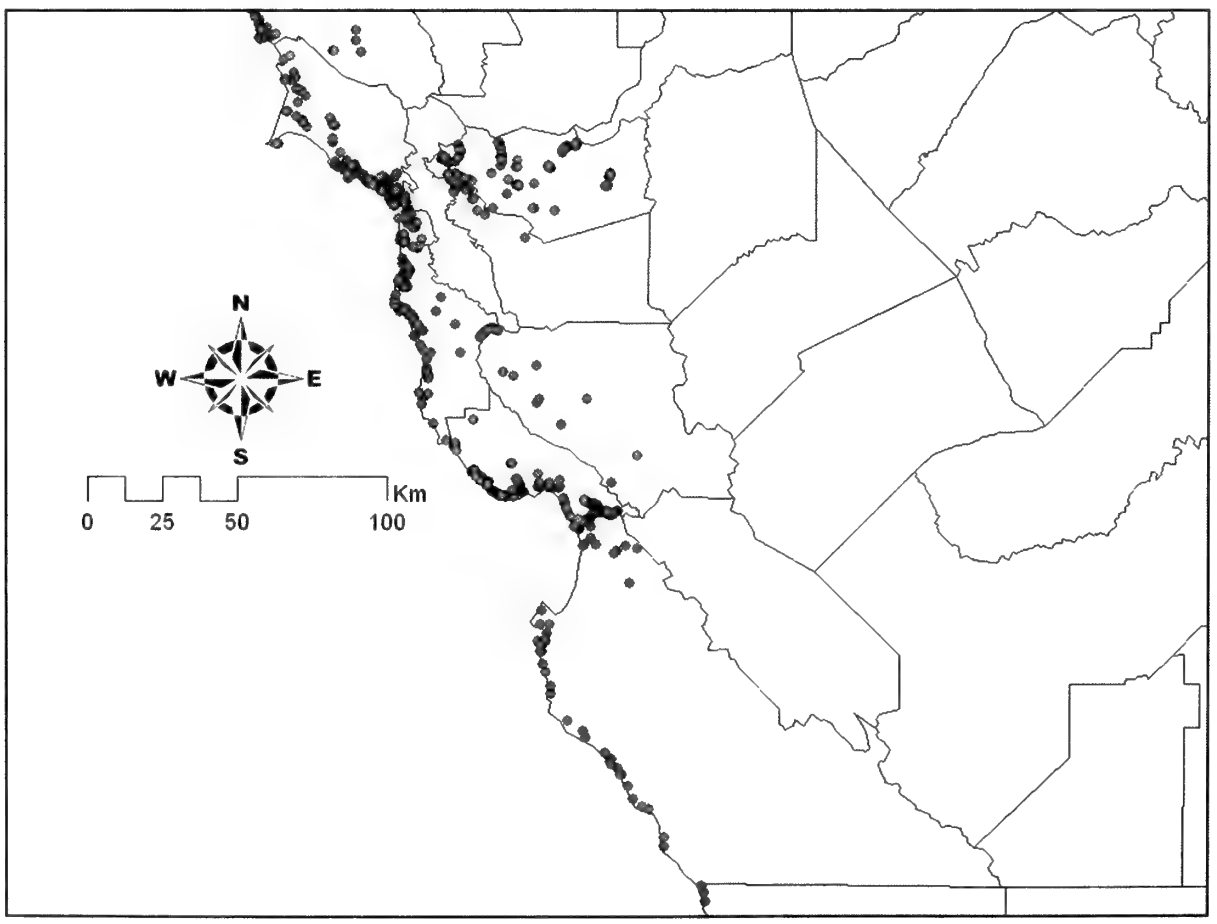

FIG. 2. Cape ivy locations in the San Francisco Bay Area and central California.

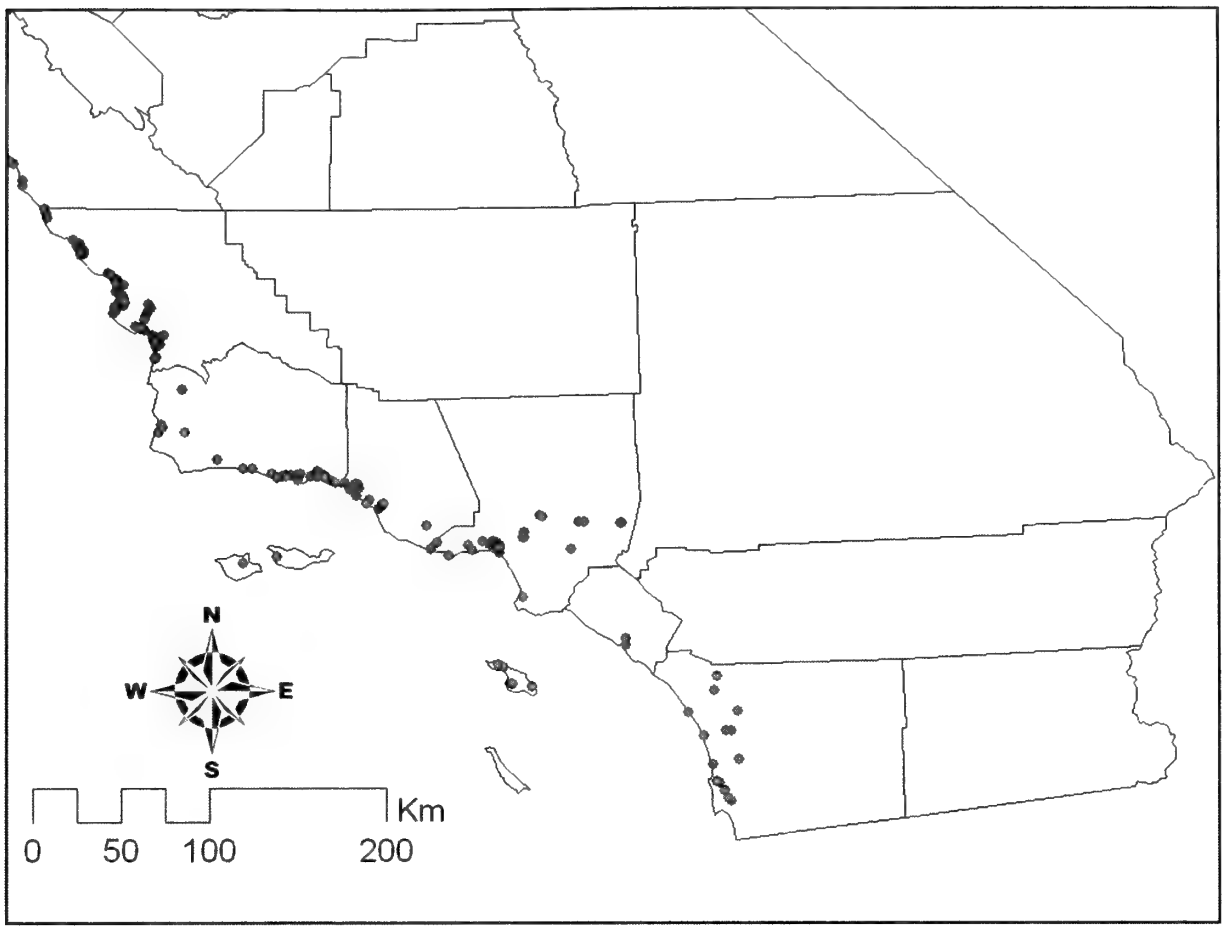

FIG. 3. Cape ivy locations in southern California.

California occurs at elevations between 0 and 891 meters, annual mean temperatures between 10.5 and 17.7°C, and in areas with annual precipitation ranging between 232 and 2270 mm (Table 2). Examining the maximum temperature of the warmest month and the minimum temperature of the coldest month, the results suggest that Cape ivy can tolerate temperatures between 1.8 and 31.8°C.

Vegetation community types. From the field GPS data, Cape ivy was most often observed in urban or agricultural areas (Table 3). This was expected, as Cape ivy was introduced as a horticultural plant and many of the surveys were conducted in easily accessible urban areas. Invasive populations were also common in riparian and non-native *Eucalyptus* forests, oak woodlands, and coastal scrub communities. In contrast, only two Cape ivy populations were observed in coniferous forests and only one occurred in a salt marsh.

CNDDB sensitive species overlay. Using either a 100 or 500 m buffer, we determined the number of CNDDB sensitive species overlapping with community types known to be invaded by (Table 4). For example, 163 sensitive vascular plants were expected to overlap with predicted Cape ivy sites using a 100 m buffer around the infested location, whereas 211 sensitive species overlapped the expected Cape ivy infested areas as predicted by the 500 m buffer. Each Cape ivy infestation was predicted to overlap with a mean of 2.2 sensitive vascular plant species at a 100 m buffer, and 2.8 sensitive plants at a 500 m buffer.

The number of predicted sensitive species occurrences per infestation was relatively small using the 100 and 500 m buffer areas. In all cases, except non-vascular plants, the number of overlapped occurrences and the mean number of sensitive species occurrences in Cape ivy sites increased as the buffer size increased. Although most groups only had a few predicted overlaps between sensitive species and Cape ivy infestations, some species within these groups frequently overlapped in their predicted occurrences. Species that had a significant overlap in occurrences using a 100 or 500 m buffer are listed in Table 5. With the 100 m buffer, only animals overlapped with Cape ivy infestations, while the 500 m buffer overlapped both animals and plants. The percent potential overlap between each sensitive species and Cape ivy was calculated by dividing the number of predicted overlapping occurrences (100 and 500 m buffer areas) by the number of total sensitive plant occurrences. Among the sensitive vascular plant species, several showed >40% potential overlap, including the San Francisco Bay spineflower (*Chorizanthe cuspidata* S. Watson var. *cuspidata*), Franciscan thistle (*Cirsium andrewsii* (A. Gray) Jeps.), San Francisco gumplant (*Grindelia hirsutula* Hook & Arn. var. *maritima* (Greene) M. A. Lane), perennial goldfields (*Lasthenia macrantha* (A. Gray) Greene ssp. *macrantha*) and marsh microseris (*Microseris paludosa* (Greene) J. T. Howell). These species are expected to be greatly impacted by the expansion of Cape ivy infestations.

There was also a considerable overlap between predicted Cape ivy infestations and steelhead

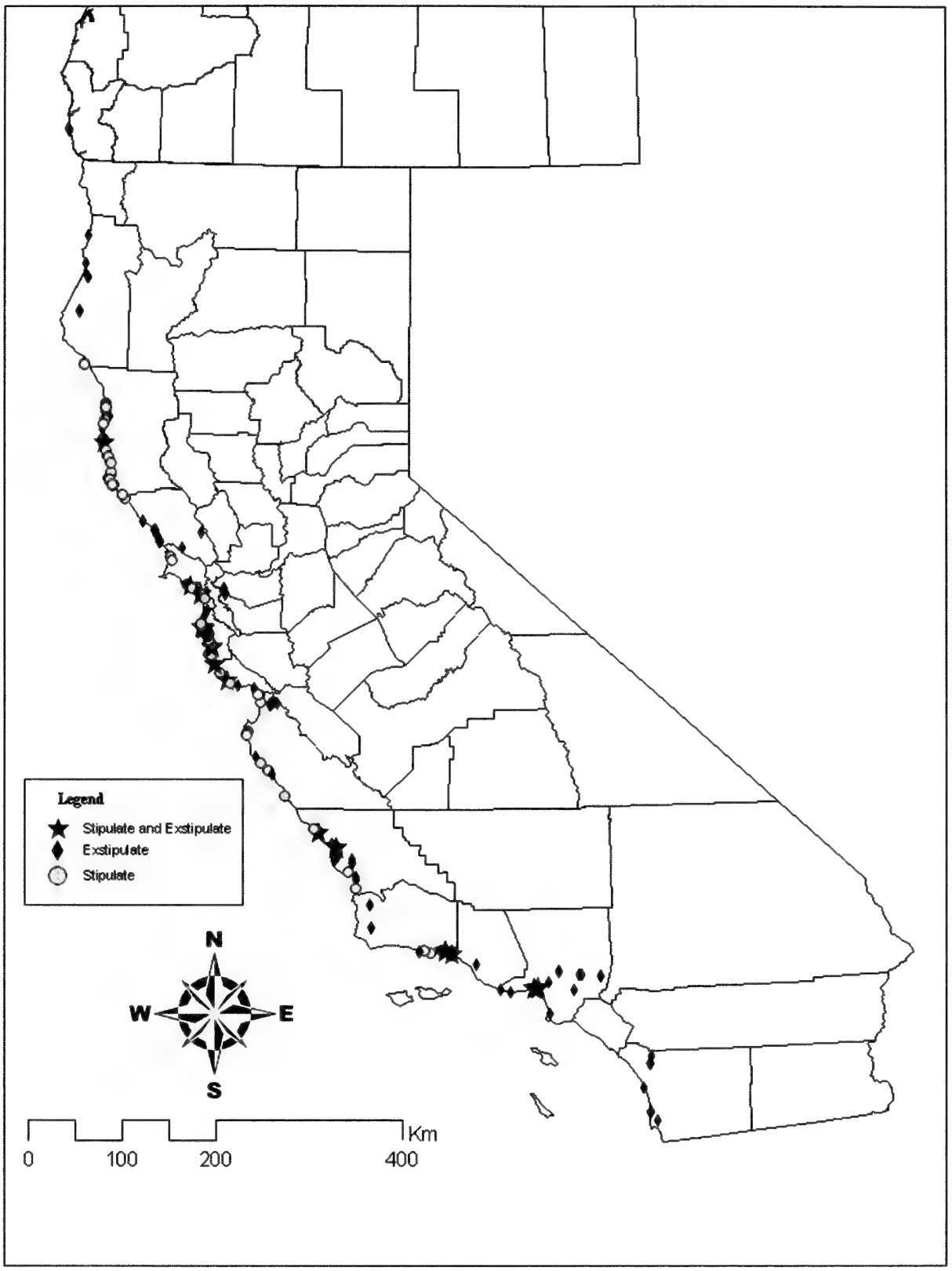

FIG. 4. Distribution of stipulate and exstipulate forms of Cape ivy, including locations where both forms co-occur.

salmon (*Oncorhynchus mykiss*) populations (Table 5). Using a 500 m buffer, the percentage overlap between Cape ivy and streams supporting steelhead ranged between 42 and 50%. Although no published studies have been reported on the toxicity of Cape ivy to fish, some evidence (C. Bossard unpublished data) suggests that Cape ivy is toxic to the golden shiner (*Notemigonus*

TABLE 2. CAPE IVY DISTRIBUTION ATTRIBUTES EXTRACTED FROM BIOCLIM RASTER DATA (n = 932 EXCEPT WHERE NOTED). BIOCLIM link: http://www.worldclim.org/methods. [1] Quarter = three consecutive months.

BIOCLIM variable	Mean ± SE	Minimum value	Maximum value
Elevation (m) n =1057	66.7	0	891
Annual mean temperature (°C)	13.3 ± 0.04	10.5	17.7
Maximum temperature of warmest month (°C)	23.2 ± 0.08	19.5	31.8
Minimum temperature of coldest month (°C)	4.6 ± 0.03	1.8	7.9
Mean annual precipitation (mm)	826 ± 11	232	2270
Precipitation in wettest quarter[1] (mm)	369 ± 4.5	101	950
Precipitation in driest quarter (mm)	8.2 ± 0.3	0	72

TABLE 3. RECORDED COMMUNITY TYPES WHERE CAPE IVY WAS OBSERVED, BASED ON GPS DATA. [1] Community codes from Holland (1986).

CNDDB community type code[1]	General type	Specific type	Number of observations from field data
11100	urban or agriculture	urban or built-up land	33
11300	non-native forest	*Eucalyptus*	11
21310	coastal scrub	northern dune scrub	1
31100	coastal scrub	northern coastal bluff scrub	12
32100	coastal scrub	northern (Franciscan) coastal scrub	12
32200	coastal scrub	central (Lucian) coastal scrub	5
32300	coastal scrub	venturan coastal sage scrub	3
52120	salt marsh	southern coastal salt marsh	1
61110	riparian forest	northern coast black cottonwood riparian forest	1
61130	riparian forest	red alder riparian forest	23
61210	riparian forest	central coast cottonwood-sycamore riparian forest	2
61220	riparian forest	central coast live oak riparian forest	1
61230	riparian forest	central coast arroyo willow riparian forest	28
61310	riparian forest	southern coast live oak riparian forest	2
61320	riparian forest	southern arroyo willow riparian forest	6
62100	riparian forest	sycamore alluvial woodland	1
62400	riparian forest	southern sycamore-alder riparian woodland	4
63100	riparian forest	northern coast riparian scrub	21
63320	riparian scrub	southern willow scrub	1
71160	oak woodland	coast live oak woodland	13
82320	conifer forest	upland redwood forest	1
83120	conifer forest	Bishop pine forest	1

crysoleucus) and crushed Cape ivy leaves caused mortality in mosquito fish (*Gambusia affinis*) within three days (J. Balciunas unpublished data). However, the latter study used crushed leaves of Cape ivy which may not represent exposure typically found in nature. Because other related species (i.e., *Senecio*) are known to contain pyrrolizidine alkaloids (Manske 1936; Adams and Gianturco 1956; Stelljes et al. 1991; Catalano et al. 1996), which can cause liver damage in humans, animals, and fish (Hendricks et al. 1981), the potential toxic effect of Cape ivy on steelhead populations is of concern because of its close proximity to water and its high density in many infested areas.

Of the invertebrates co-occurring within predicted Cape ivy populations, only one species, Monarch butterfly (*Danus plexippus*), showed any significant overlap, with 13 and 25% of its occurrences within the 100 and 500 m buffers, respectively (Table 5). The potential for Cape ivy alkaloids to affect the Monarch butterfly has been studied indirectly, but with no conclusions as to the potential impact. Monarch butterflies were found to have accumulated pyrrolizidine alkaloid after over-wintering in Cape ivy infested areas (Stelljes and Seiber 1990). The butterflies accumulate pyrrolizidine alkaloids after using Cape ivy as a nectar source. Although this was postulated to provide a chemical defense mech-

TABLE 4. CNDDB SENSITIVE SPECIES OVERLAP WITH CAPE IVY SUMMARIZED BY GROUP CLASSIFICATION.

Group classification	Species overlapping with Cape ivy at 100 m buffer	Mean number of species per occurrence at 100 m buffer ± SE	Species overlapping with Cape ivy at 500 m buffer	Mean number of species per occurrence at 500 m buffer ± SE
Natural communities	24	2.1 ± 0.3	35	2.4 ± 0.4
Non-vascular plants	8	1 ?	8	1.5 ± 0.3
Vascular plants	163	2.2 ± 0.1	211	2.8 ± 0.2
Invertebrates	32	3.5 ± 1.3	37	5.1 ± 2.2
Fish	7	9.3 ± 4.6	9	10.0 ± 5.0
Reptiles	7	3.4 ± 1.1	9	4.8 ± 1.5
Amphibians	4	4.8 ± 2.8	8	6.9 ± 4.6
Birds	20	2.6 ± 0.6	28	3.3 ± 0.8
Mammals	13	2.2 ± 0.5	18	2.8 ± 0.6

TABLE 5. CNDDB SENSITIVE SPECIES LOCATIONS AND PREDICTED OVERLAP OF CAPE IVY AND SENSITIVE SPECIES AT EITHER 100 OR 500 M BUFFERS. [1]ESU = evolutionarily significant unit.

Scientific name	Common name	Number of occurrences tracked by CNDDB	Number (percent) of occurrences overlapping with predicted Cape ivy populations	
			Using 100 m buffer	Using 500 m buffer
Animals				
Rana draytonii	California red-legged frog	831	13 (2)	39 (5)
Charadrius alexandrinus nivosus	western snowy plover	109	13 (12)	22 (20)
Eucyclogobius newberryi	tidewater goby	112	28 (25)	41 (37)
Oncorhynchus mykiss irideus	steelhead—central California coast ESU[1]	28	13 (46)	14 (50)
Oncorhynchus mykiss irideus	steelhead—south/central California coast ESU	27	9 (33)	12 (44)
Oncorhynchus mykiss irideus	southern steelhead—southern California ESU	12	4 (33)	5 (42)
Danaus plexippus	monarch butterfly	335	43 (13)	83 (25)
Arborimus pomo	Sonoma tree vole	208	—	10 (5)
Actinemys marmorata	western pond turtle	302	—	11 (4)
Actinemys marmorata pallida	southwestern pond turtle	308	—	13 (4)
Vascular plants				
Campanula californica (Kellogg) A. Heller	swamp harebell	100	—	10 (10)
Castilleja mendocinensis (Eastw.) Pennell	Mendocino coast indian paintbrush	42	—	12 (29)
Chorizanthe cuspidata S. Watson var. *cuspidata*	San Francisco Bay spineflower	20	—	10 (50)
Cirsium andrewsii (A. Gray) Jeps.	Franciscan thistle	27	—	11 (41)
Grindelia hirsutula Hook & Arn. var. *maritima* (Greene) M. A. Lane	San Francisco gumplant	15	—	11 (73)
Lasthenia californica subsp. DC. ex Lindl. *macrantha* (A. Gray) R. Chan	perennial goldfields	32	—	13 (41)
Microseris paludosa (Greene) J. T. Howell	marsh microseris	22	—	10 (46)

anism against potential predators, it is also possible that these alkaloids may have a direct negative affect on the butterflies.

CONCLUSIONS

This updated state-wide mapping of Cape ivy populations should aid in regional weed planning and in identifying areas of greatest potential invasion. Cape ivy was present in seven different broad plant community types. This is contrary to the common assumption that Cape ivy is primarily or even exclusively a riparian invasive (Hoover 1970; Smith 1976; Beauchamp 1986; Barbour and Billings 2000). State-wide trends in distribution of the two morphological forms indicate that exstipulate types occur more frequently at the northern and southern range of its distribution, while stipulate types are more frequent in the center of its distribution range, extending from southern Humboldt Co. to Los Angeles Co. Only 21 locations were found that supported both stipulate and exstipulate plants and these were most often in urbanized coastal areas.

Another important aspect of this study was to evaluate the potential threat of Cape ivy on CNDDB sensitive species known to occur in or around invaded plant community types. Although the threat to biodiversity was not measured directly, the CNDDB dataset served as a surrogate for native species biodiversity. This analysis suggests that six plants of limited distribution and nearly 50% of steelhead streams are threatened by the potential expansion of Cape ivy populations. This is of great concern to ecosystem integrity of these sensitive sites and should result in prioritization of effective Cape ivy management programs in California and southern Oregon.

Acknowledgments

The Cape ivy mapping work, which began in 1995 by Cal-IPC, was an inspiration as well as a starting point for this research. We thank Eva Grotkopp, Alfred Kuo and Mike Pitcairn for collecting and digitizing information, which they made available in the state-wide database. Numerous other individuals and organizations, especially CNPS and Cal-IPC, provided useful data sets and Rosie Yacoub and Pat Akers from the California Department of Food and Agriculture assisted with the GPS and GIS technology. We also wish to thank Guy Kyser for providing invaluable review and advice. Finally, we thank the UC IPM Exotic Pest and Disease Program for their financial support.

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SPECIES BOUNDARIES IN *PYRROCOMA LIATRIFORMIS* AND *PYRROCOMA SCABERULA* (ASTERACEAE) BASED ON AFLP DATA

JAMES F. SMITH AND DUSTY N. PERKINS
Department of Biological Sciences, MS1515, Boise State University, 1910 University Drive, Boise, ID, 83725 USA
jfsmith@boisestate.edu

CURTIS R. BJÖRK
Box 131 Clearwater, B.C., V0E 1N0, Canada

GINA GLENNE
Western Colorado Field Office, 764 Horizon Drive, Building B, Grand Junction, CO, 81506-3946 USA

ABSTRACT

Previous investigations into the morphology of *Pyrrocoma liatriformis sensu lato* in northern Idaho and adjacent Washington have revealed two distinct morphologies that correspond to their geographical ranges. These same populations and individuals have been analyzed using AFLP data. Over 400 loci were identified among all individuals using two sets of AFLP adaptors. The data are in agreement with the morphological data and separate the populations from the Snake River Canyon/ Camas Prairie from those of the Palouse grasslands. Data clustering methodologies using both presence/absence data for all individuals and allele frequencies for each population produced similar results. We suggest the name *P. scaberula* be resurrected to encompass the populations from the Snake River Canyon and Camas Prairie.

Key Words: AFLP, Asteraceae, Idaho, *Pyrrocoma*, species boundaries, Washington.

Numerous species concepts have been proposed to unambiguously determine species boundaries (see Niklas 1997; Howard and Berlocher 1998; Wilson 1999; Coyne and Orr 2004; Sites and Marshall 2003, 2004, for more detailed summaries). Selecting a concept poses a challenge to biologists, particularly botanists, where relatively common gene flow among morphologically distinct populations seems to preclude the widespread adoption of the biological species concept (Burger 1975; Donoghue 1985; Mishler and Brandon 1987; Ellstrand et al. 1996).

Over the past 20 yr, molecular methods have provided systematists an additional means of assessing species concepts beyond morphological variability (Miller and Spooner 1999; Lopez et al. 1999; Duim et al. 2001; Parsons and Shaw 2001; Dawood et al. 2002; Wiens and Penkrot 2002; Richardson et al. 2003; Martínez-Ortega et al. 2004; Sites and Marshall 2004; Whittall et al. 2004; Garzón et al. 2005; Irwin et al. 2005; Pons et al. 2006; Suatoni et al. 2006; Roe and Sperling 2007; Manoko et al. 2007; Meudt and Clarke 2007; Guo et al. 2008). Molecular data allow a means to determine if populations are genetically distinct from each other, obtain estimates of gene flow between populations, and resolve if they are mutually monophyletic.

Assessing population variation to resolve taxonomic status by molecular genetic means can be done by a variety of methods including simple sequence repeats (SSRs or microsatellites, Akkaya et al. 1995), inter-simple sequence repeats (ISSRs; Smith and Bateman 2002), randomly amplified polymorphic DNA (RAPDs; Smith and Pham 1996), amplified fragment length polymorphisms (AFLPs; Vos et al. 1995) and randomly amplified fingerprinting (RAFs; Waldron et al. 2002). Many studies have employed AFLPs to provide evidence that a single species should be divided into multiple species where morphological data were limiting or conflicting (Kardolus et al. 1998; Lopez et al. 1999; Mueller and Wolfenbarger 1999; Duim et al. 2001; Hedrén et al. 2001; Koopman et al. 2001; Bottini et al. 2002; Parsons and Shaw 2001; Richardson et al. 2003; Martínez-Ortega et al. 2004; Whittall et al. 2004; Garzón et al. 2005; Irwin et al. 2005; Manoko et al. 2007; Meudt and Clarke 2007; Roe and Sperling 2007; Travis et al. 2008). According to Becker et al. (1995) AFLP fragments are derived from throughout the genome and bands of identical size (co-migrating) are predominantly homologous in closely related organisms (Waugh et al. 1997; Rademaker et al. 2000). Herein, we examine the AFLP variation among populations of *Pyrrocoma liatriformis sensu lato*.

Pyrrocoma liatriformis E. Greene (syn. *Haplopappus liatriformis* (Greene) St. John) is an herbaceous perennial found in northern Idaho and adjacent Washington (Fig. 1). This taxon has

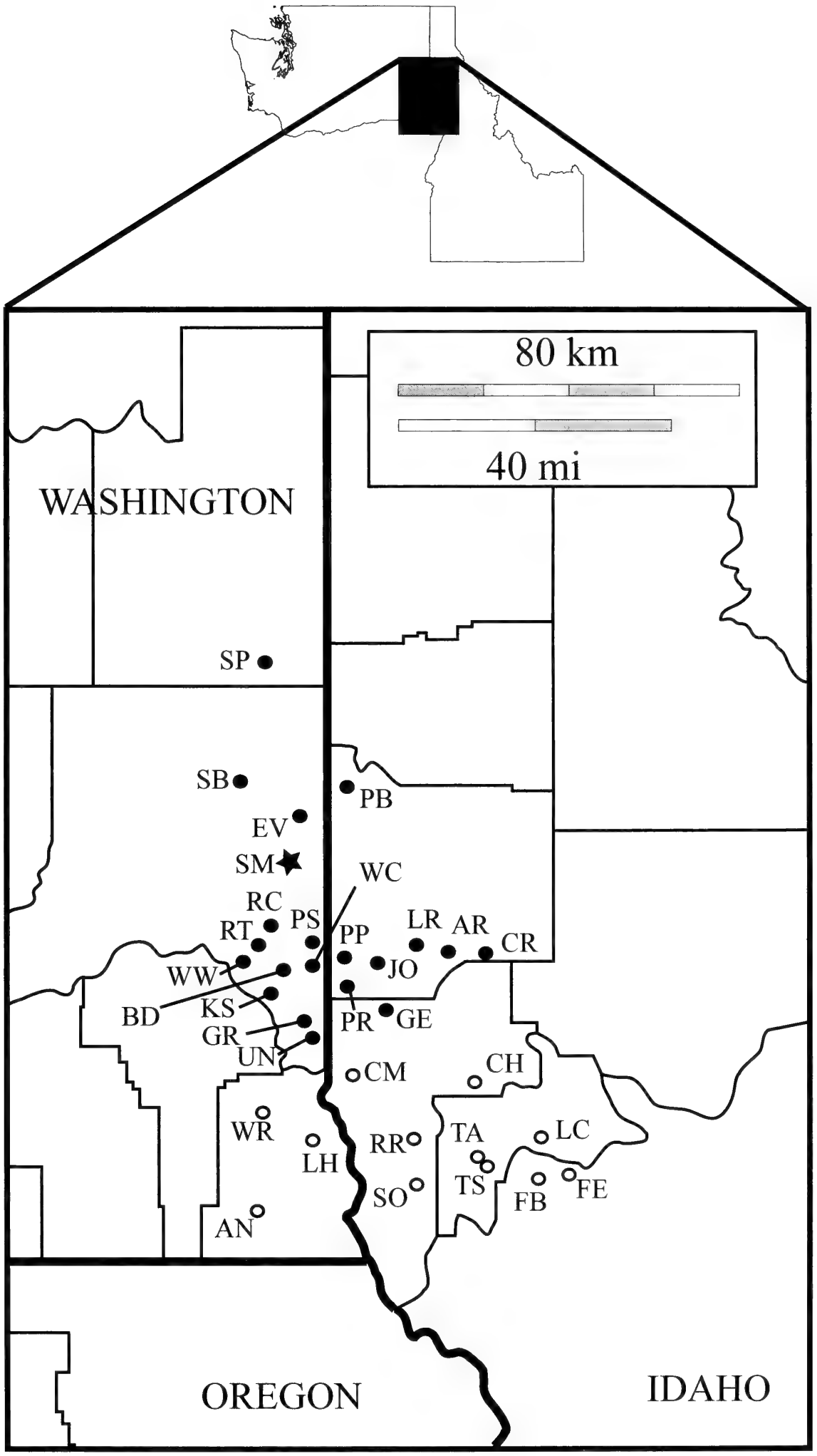

FIG. 1. Map showing the distribution of populations sampled in this analysis. Abbreviations follow Table 1. Open circles represent *P. scaberula* and closed circles represent *P. liatriformis*, the star represents *P. carthamoides*.

generally been viewed with a broad species concept (Hitchcock et al. 1955) that may encompass two distinct morphological species. Recent work on the morphology of *P. liatriformis sensu lato* has discovered that neither qualitative nor quantitative morphological characters are uniform across *P. liatriformis sensu lato* (Björk and Darrach 2009). Instead, the morphological variation is correlated with geographical range; plants from the Palouse grasslands are mostly

heavily tomentose throughout, resinous-punctate glands are absent and flower heads tend to be smaller (10–13.8 mm) whereas those of the canyon/Camas Prairie regions are hispid, with conspicuous resinous-punctate glands and flower heads are larger (13.8–15.1 mm). Taxonomic investigations of the type specimens of *Pyrrocoma* have revealed that these morphological entities have been described in the past as *P. scaberula* E. Greene (canyon/Camas Prairie plants) and *P. liatriformis* (Palouse grasslands); the former species largely has been considered a synonym of the latter in most treatments.

Björk and Darrach (2009) studied the morphological variation that separates *P. liatriformis* from *P. scaberula* by examining eight continuously variable morphological characters for 325 plants, 201 individuals in 18 populations from the Palouse and 124 individuals in 13 populations from the canyon/Camas Prairie region. The characters showed clear non-uniformity and although there is overlap in the ranges of the characters, the means of six characters (lateral branches, number of heads, head length, head width, phyllary width, and leaf width) are statistically significant for each group of populations. Furthermore, principle component analyses clearly separated the populations into two distinct groups. Although some populations in the Palouse grasslands share the lack of tomentum and strong glandularity of the canyon/Camas Prairie plants, they were clearly attributable to the morphology of *P. liatriformis sensu stricto* based on quantitative characters.

Despite the ability of the characters to separate the Palouse and canyon/Camas Prairie populations into distinct clusters, there is a strong overlap among the ranges of the morphological characters. These data suggest the two species are either closely related, represent a progenitor-derivative species pair (Gottlieb 1973, 1974; Gottlieb and Pilz 1976; Crawford and Smith 1982; Ranker and Schnabel 1986; Perron et al. 2000), undergo hybridization, or are a combination of these.

It is the goal of this study to resolve whether the morphological species as defined by Björk and Darrach (2009) are congruent with patterns shown by molecular data.

Materials and Methods

To obtain an estimate of molecular genetic variability, we sampled 32 populations of *Pyrrocoma liatriformis sensu lato* from both Palouse grassland and canyon/Camas Prairie populations (Fig. 1). One additional population of *P. carthamoides* was used as an outgroup for comparison. Populations, their abbreviations, and tentative species identifications are presented in Table 1 and plotted onto a map in Fig. 1. These are the same populations and individuals sampled for morphological data by Björk and Darrach (2009). At the time sampling was conducted, exceptionally dry conditions in the region resulted in few populations that were flowering, which minimized the number of individuals that were sampled per population (Björk personal observation). Ideally 25–35 individuals were to have been sampled, but a total of 25 or more individuals was possible for only five of 33 populations (Table 1). Leaves were collected on silica gel and were the source of DNA extraction using DNeasy kits (Qiagen, Valencia, CA). One fertile stem per plant from populations of larger size was collected as a voucher. Plants from smaller populations were vouchered nondestructively with photographs taken with a ruler for scale. All vouchers, including photographic ones, are deposited at the University of Idaho Stillinger Herbarium (ID).

Approximately 500 ng of DNA from each individual was digested with *Mse*I and *Eco*RI while simultaneously annealing the *Mse*I and *Eco*RI adaptors at room temperature overnight. Annealing of the adaptors to the ends of the fragments alters the restriction site and precludes further digestion or need to perform reactions separately. To reduce the overall number of fragments amplified, and thus improve detection of homologous amplified fragments, a pre-selective amplification was performed using the primers *Eco*RI + A (5′-GACTGCGTACCAATTCA-3′) and *Mse*I + C (5′-GATGAGTCCTGAGTAAC-3′) and 1 μL of digested/ligated DNA. Pre-selective amplification was run with 20 cycles of denaturing at 94°C for 30 sec, annealing at 56°C for 1 min and extension at 72°C for 1 min. Products were diluted 1:20 with TE buffer and 3 μL of the dilution were used in selective amplification using either the A primer set, M-CAC (5′-GATGAGTCCTGAGTAACAC-3′) and labeled (with dye for Li-Cor system) E-ACT (5′-GACTGCGTACCAATTCACT-3′) or the T primer set, M-CTC (5′-GATGAGTCCTGAGTAACTC-3′) and labeled E-ACC (5′-GACTGCGTACCAATTCACC-3′). Final selection amplification used an initial denaturation at 94°C for 2 min followed by 10 cycles of denaturation at 94°C for 20 sec, annealing at 66°C for 30 sec and extension at 72°C for 2 min. This was followed with 25 more cycles that differed only in reducing the annealing temperature to 56°C. Lastly there was a 30 min extension period.

Final AFLP products were separated on 6.5% polyacrylamide gels and visualized on a Li-Cor LongreadIR automated sequencer (Li-Cor Biotechnology Division, Lincoln, Nebraska). Molecular weight size standards were run on each end of each gel. Digital images of the gels were analyzed using Gene ImagIR (Li-Cor Biotech-

TABLE 1. LOCATIONS AND ABBREVIATIONS OF POPULATIONS SAMPLED IN THIS ANALYSIS, THEIR SPECIES DESIGNATION BASED ON MORPHOLOGICAL DATA, AND AMPLIFICATION SUCCESS FOR EACH SET OF AFLP ADAPTORS (A AND T). Vouchers are deposited at the University of Idaho Stillinger Herbarium (ID).

Species designation	Population abbreviation (number of individuals sampled)	Number of individuals amplified: (A/T)
P. scaberula—Anatone, Asotin Co., WA	AN (12)	12/3
P. scaberula—Chesley Railroad, Lewis Co., ID	CH (25)	21/24
P. scaberula—Craig Mountain, Nez Perce Co., ID	CM (24)	24/23
P. scaberula—Ferdinand Butte, Idaho Co., ID	FB (25)	22/24
P. scaberula—Ferdinand east/Meadow Creek, Idaho Co., ID	FE (16)	13/13
P. scaberula—Lawyer Canyon, Lewis Co., ID	LC (12)	11/12
P. scaberula—Lime Hill, Asotin Co., WA	LH (25)	22/25
P. scaberula—Redbird Road, Nez Perce Co., ID	RR (25)	23/25
P. scaberula—Soldiers Meadow, Lewis Co., ID	SO (1)	1/1
P. scaberula—Talmacks North, Lewis Co., ID	TA (12)	0/12
P. scaberula—Upper Cold Spring Creek, Lewis Co. ID	TS (5)	5/5
P. scaberula—Weissenfels Ridge, Asotin Co., WA	WR (8)	7/7
P. liatriformis—American Ridge, Latah Co., ID	AR (16)	16/15
P. liatriformis—Barking Dog, Whitman Co., WA	BD (16)	16/16
P. liatriformis—Cedar Ridge, Latah Co., ID	CR (10)	10/10
P. liatriformis—Eden Valley, Whitman Co., WA	EV (7)	6/7
P. liatriformis—Genesee South, Nez Perce Co., ID	GE (4)	3/3
P. liatriformis—Gross Road, Whitman Co., WA	GR (11)	10/10
P. liatriformis—Joel, Latah Co., ID	JO (5)	5/5
P. liatriformis—Kramer Prairie, Whitman Co., WA	KS (19)	19/18
P. liatriformis—Lenville Road, Latah Co., ID	LR (11)	10/11
P. liatriformis—Mix Road, Latah Co., ID	PP (5)	5/4
P. liatriformis—Palmer Butte, Latah Co., ID	PB (5)	5/5
P. liatriformis—South end of Paradise Ridge, Latah Co., ID	PR (15)	15/15
P. liatriformis—Palouse Prairie Strip,Whitman Co. WA	PS (9)	8/8
P. liatriformis—Rose Creek, Whitman Co., WA	RC (5)	5/5
P. liatriformis—Armstrong Road, Whitman Co., WA	RT (6)	3/2
P. liatriformis—Steptoe Butte, Whitman Co., WA	SB (7)	7/7
P. liatriformis—Spaulding Road, Spokane Co., WA	SP (3)	2/2
P. liatriformis—Uniontown, Whitman Co., WA	UN (3)	2/2
P. liatriformis—Whelan Cemetery, Whitman Co., WA	WC (25)	20/23
P. liatriformis—Wawawai Grade, Whitman Co., WA	WW (21)	20/20
P. carthamoides—Smoot Hill, Whitman Co., WA	SM (23)	23/22

nology Division, Lincoln, NE) to determine molecular weight designations for each fragment. Gel images were edited to ensure that fragments of identical size were correctly assigned the same weights. These data were exported using Gene Profiler (Scanalytics, Inc., Fairfax, VA) and fragments were assigned an allele designation based on which set of adaptors was used (herein designated as either the A or T set of alleles) and their molecular weight. Each individual was then scored for presence or absence of each allele.

Fragments greater than 600 bp and less than 49 bp were excluded from the AFLP analyses. Large fragments may be amplified with lower frequency during the process, as a result their consistency may be less reproducible and reliable. Similarly, homology of larger fragments becomes less probable since there are greater opportunities for insertions and deletions of DNA to alter fragment sizes between individuals. Smaller fragments were excluded due to potential difficulties in resolving fragment sizes.

Data were entered into MacClade (Maddison and Maddison 2000) and exported as a simple table. The table was modified using AFLPDAT (Ehrich 2006) to convert the files to formats usable in STRUCTURE (Pritchard et al. 2000; Falush et al. 2007) as well as to generate allele frequency data for each population.

There are many alternative approaches to analyze AFLP data to detect structure within the data set (Bonin et al. 2007). In general, these break down into two analyses: 1) analysis of bands directly (presence/absence) or 2) converting the band data into allele frequency data for each population. Both data types can then be used in an array of methodologies to detect diversity and structure within and among the sampled populations.

Here we opt to make use of both band data and allele frequency data to generate tree-based representations of the variation (Bonin et al. 2007). We make use of two methods to analyze the data for both band and allele frequency data:

Neighbor-joining (NJ) using Jaccard's genetic similarities (bands) or Nei's (1972) genetic distance and UPGMA. These analyses are among the most widely used methodologies for resolving species boundaries using AFLP data (Miller and Spooner 1999; Duim et al. 2001; Koopman et al. 2001; Parsons and Shaw 2001; Coulibaly et al. 2003; Jacoby et al. 2003; Richardson et al. 2003; Dehmer and Hammer 2004; Martínez-Ortega et al. 2004; Whittall et al. 2004; Garzón et al. 2005; Manoko et al. 2007; Guo et al. 2008).

A data matrix of presence/absence for all bands was directly imported into PAUP* (Swofford 2002) for NJ and UPGMA analyses. Allele frequencies were used to calculate population genetic distances that were then used to generate NJ and UPGMA trees in PHYLIP (version 3.67; Felsenstein 2007).

We used the Bayesian clustering method implemented in STRUCTURE 2.2 (Falush et al. 2007) to determine the optimal number of groups indicated by the data. Assuming all populations are in Hardy-Weinberg and linkage equilibria, this method assigns individuals to one of the pre-specified numbers of genetic clusters, K, using multi-locus genotypes and Markov Chain Monte Carlo sampling. We ran separate clustering simulations for all populations over a range of one to 40 clusters (i.e., K = one to 40). Individuals with data missing for either the A or T set of alleles were removed from the analyses. This reduced some populations to few samples (only three individuals for AN) and resulted in TA being removed completely. Simulations were run assuming an ancestry model that incorporates admixture and correlated allele frequencies across loci for one million generations with a burn-in of 25,000 generations, sampling every 100 generations.

We compared posterior probabilities of K from one to 40 clusters using the ad hoc statistic, ΔK (Evanno et al. 2005). This statistic has been shown to be a better estimator of structure in some data sets, especially those where homogeneous dispersal among populations cannot be assumed (Evanno et al. 2005; Travis et al. 2008). In such cases, a common pattern is for STRUCTURE to plateau near the true value of K, and then to continue increasing gradually. Evanno et al. (2005) showed that ΔK consistently returns a clear peak at the true value of K under a variety of migration models. We chose a maximum K of 40 because this exceeded the number of sampled populations.

Results

Amplification of DNA was successful for nearly all individuals for both sets of AFLP adaptors, A and T (Table 1). A few individuals did not amplify well which is perhaps the effect of plant resin that inhibits the reactions. No individuals from the TA population were amplified for the A alleles and only three from AN were amplified fully for the T alleles. Some individuals were not amplified for either allele. Resin was detected in the precipitation stage of the DNA extraction for some individuals of these populations.

For several of the gels, bands that were distinctly different in size below 50 bp were classified as the same size by the software. Therefore the limitations of the software for resolving bands at this stage required us to eliminate these bands from the analyses. Scanning gels visually indicated that these fragment sizes tended to be consistent across nearly all individuals, therefore their exclusion was unlikely to affect the results whereas their inclusion may have indicated erroneous relationships among individuals and populations.

For the A set of adaptors, 245 alleles were scored for 373 individuals ranging from 569 to 50 bp in size. For the T set of adaptors, 177 alleles were scored for 387 individuals ranging from 581 to 50 bp in size. The complete data matrix had 407 individuals from 33 populations and 422 alleles.

The analyses that used presence/absence data for all individuals were sensitive to missing data. Individuals with missing data for either allele were either 1) clustered together regardless of population designation, or 2) in disparate parts of the tree (often included in populations that were neither morphologically or geographically similar, data not shown). Therefore we removed these samples (AN1-9, AR8, CH2, 11, 12, CM11, EV5, FB9, 12, KS8, LC12, LH6,7,18, LR11, MIX1, RCA5, RR4, 7, RT4, TAO1-12, WC3,6,19,20, 22,23,25, WW6, and 12) and re-ran the analyses. We also ran analyses of the A and T alleles separately to confirm the placement of the individuals excluded above in their respective population (data not shown).

The analyses based on allele frequencies divided all populations into two distinct groups corresponding to *Pyrrocoma scaberula* and *P. liatriformis* based on a priori designations by Björk and Darrach (2009; Fig. 2). *Pyrrocoma carthamoides* clustered within *P. liatriformis* with the UPGMA analysis (data not shown) because this analysis does not allow the outgroup to be specifically designated as such. The NJ tree in contrast (Fig. 2), results in two distinct and monophyletic groups each for *P. liatriformis* and *P. scaberula*.

There are clear clusters within each of these groups. Populations that cluster together in both the NJ and UPGMA trees within *P. scaberula* are AN/FB/SO/TS, FE/WR, and LC/CH/LH/RR/CM. Only population TA changes position between the analyses and is found close to CH/

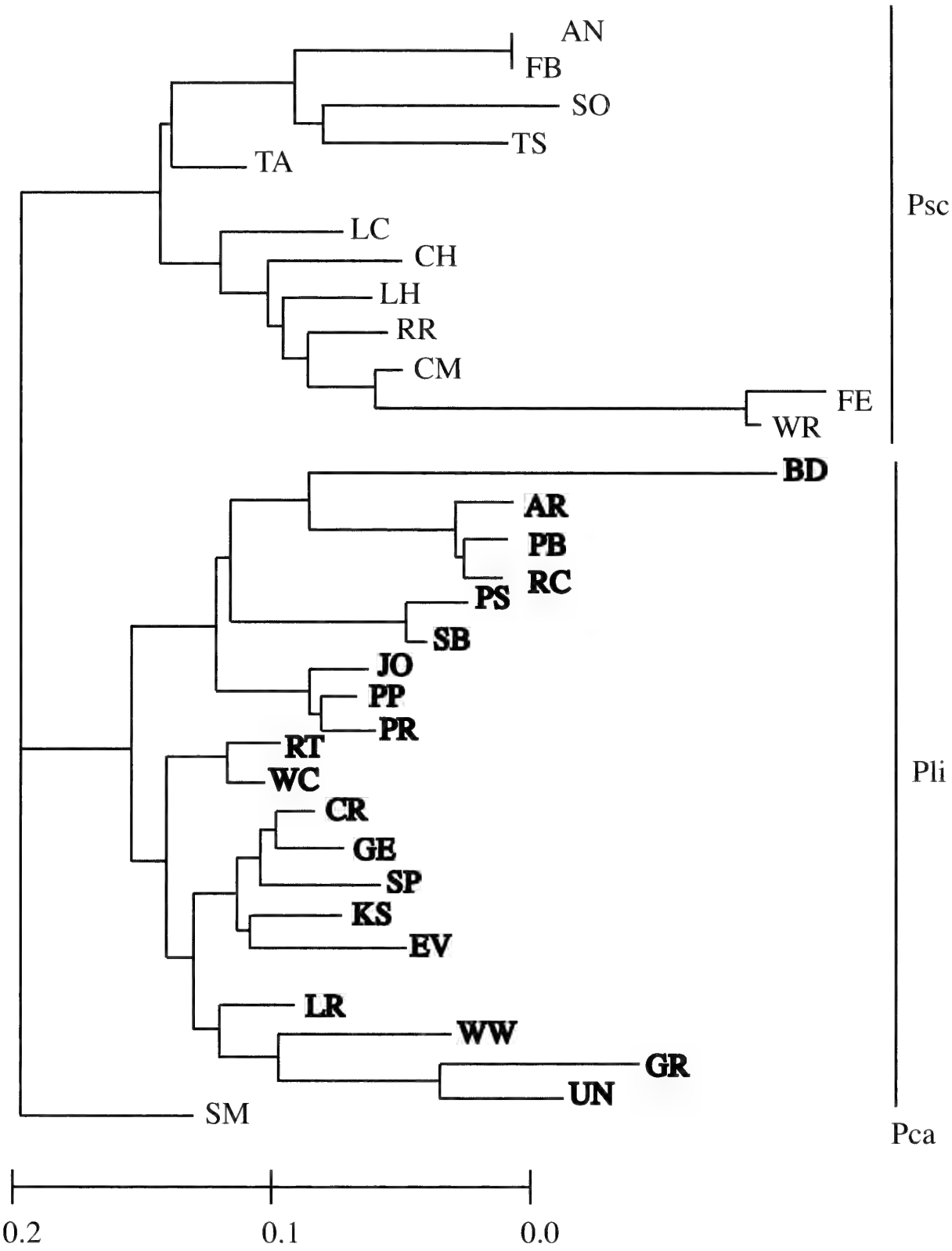

FIG. 2. Neighbor-joining based tree derived from AFLP allele frequency data. Population names follow abbreviations of Table 1. Bars to the right of the tree mark species boundaries, Psc—*Pyrrocoma scaberula*, Pli—*Pyrrocoma liatriformis*, Pca—*Pyrrocoma carthamoides*. Population names are abbreviated following Table 1 and are designated as normal font (*P. scaberula*) or bold (*P. liatriformis*).

CM/RR/LH/LC in the UPGMA analysis (data not shown). It should be noted that TA is lacking data for over half of the alleles. Within *P. liatriformis* there are similar clusters of populations that are consistent between analyses. These are AR/PB/RC, PS/SB, JO/PP/PR, RT/WC, GR/UN, and CR/GE/SP/KS/EV. The grouping of populations BD, LR and WW differ between the two analyses. With UPGMA, BD is close to all other populations of *P. liatriformis*, LR is close to RT/WC, and WW is close to CR/GE/SPKS/EVLR/RT/WC (data not shown).

The NJ analyses based on bands produced trees that were nearly identical to those based on allele frequencies (Figs. 2, 3) both in terms of clustering populations into species groups, and relationships of populations within each cluster. The greatest differences are that 1) not all populations were recovered as a single monophyletic group (Fig. 3), 2) the NJ tree did not result in a monophyletic *P. scaberula* due to the position of population LH, and 3) *P. carthamoides* is clustered within *P. scaberula* instead of *P. liatriformis* with the UPGMA tree (data not shown).

Groupings within each species are also similar to the frequency-based methods. The major differences within *P. scaberula* are the position of population LH in the NJ tree (Fig. 3) and that individual AN5 was more closely associated to

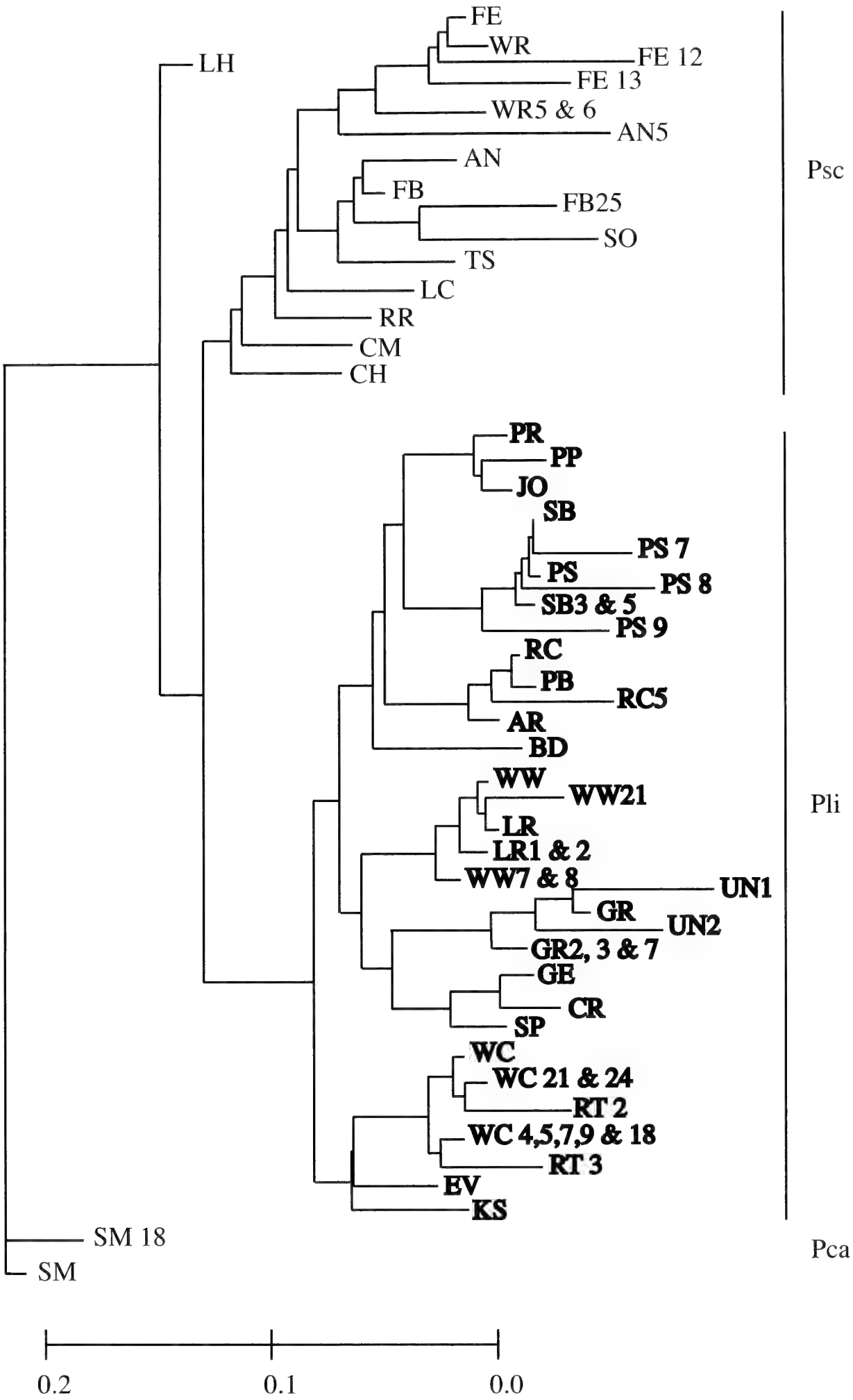

FIG. 3. Neighbor-joining based tree derived from AFLP band presence/absence. Where the majority of sampled individuals formed a single cluster only the population name abbreviation is used. In instances where individuals fell outside of their respective population cluster, they are designated with a population name and number for the individual. Population names are abbreviated following Table 1 and are designated as normal font (*P. scaberula*) or bold (*P. liatriformis*). Bars to the right of the tree mark species boundaries, Psc—*Pyrrocoma scaberula*, Pli—*Pyrrocoma liatriformis*, Pca—*Pyrrocoma carthamoides*.

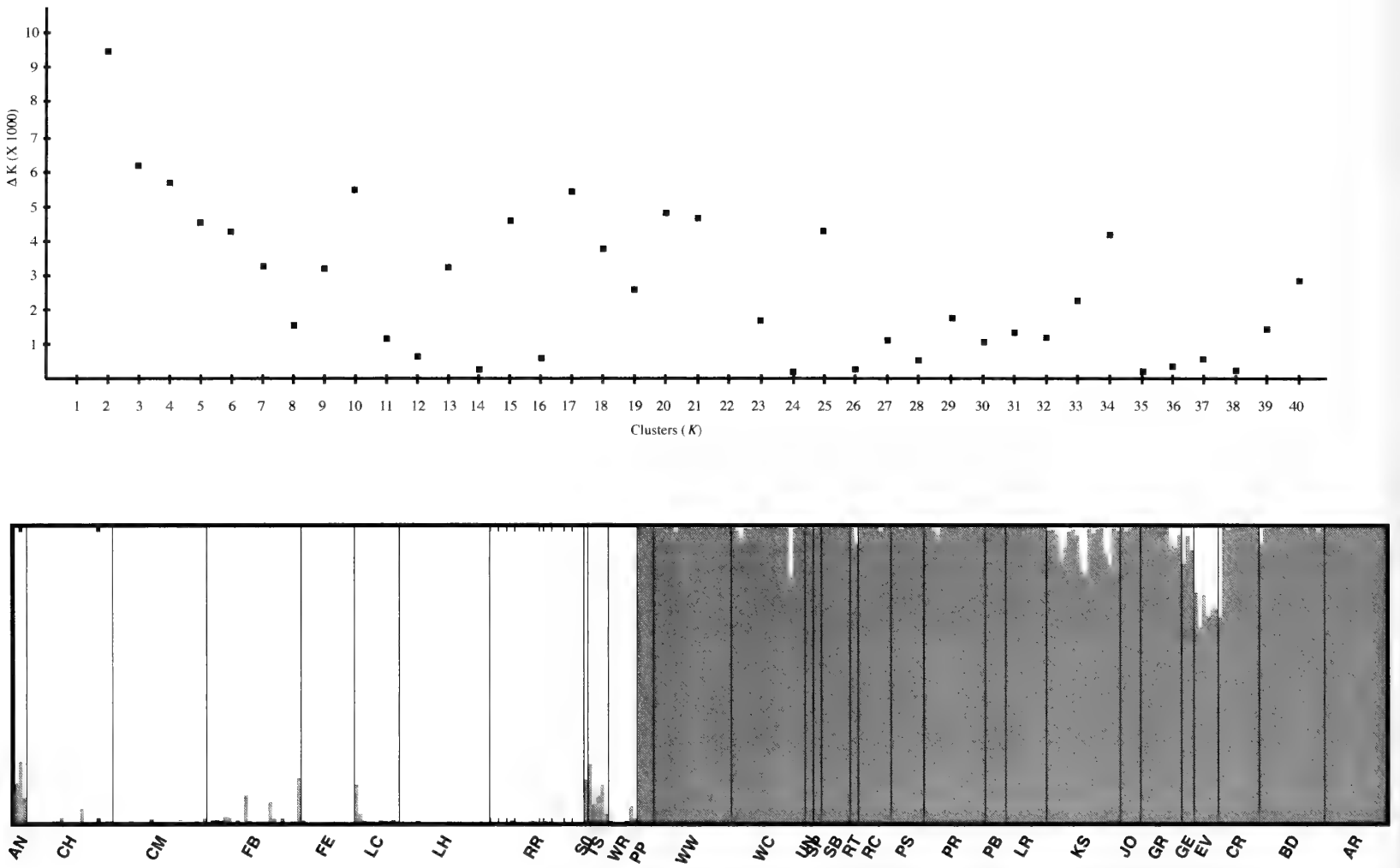

FIG. 4. Structure analysis of all populations of *Pyrrocoma liatriformis sensu lato* sampled in this analysis. A. Plot showing values of ΔK for each value of K. B. Bayesian assignment of individuals to two clusters. The bars represent the estimated posterior probabilities of each individual belonging to each of the two inferred clusters.

WR/FE than to FB/SO/TS. The lack of unity of population AN is likely due to the fact that only approximately half of the T alleles were scorable for AN5. Population TA was excluded from these analyses due to a complete lack of A alleles. Based on analysis that utilized only the T alleles, TA was close to AN/FB/SO/TS as it is in the NJ tree based on frequencies (Fig. 2). Likewise, groupings were similar within *P. liatriformis*, the main exceptions being populations KS and EV which showed a closer affinity to WC/RT based on bands (Fig. 3).

Plotting the actual values of K from one to 40 indicated that there was a plateau after $K = 2$ with small increases in probability for each subsequent value of K. With all populations except the outgroup *P. carthamoides* included, the greatest ΔK was at 2 distinct clusters (Fig. 4). These results agree with the clustering results that divide the populations into two species. Only individuals of population EV show any significant probability of being assigned to the other species (Fig. 4).

DISCUSSION

All analyses of AFLP data presented here, regardless of whether bands or frequencies were used, separate the populations into *Pyrrocoma liatriformis* and *P. scaberula* as determined by Björk and Darrach (2009) using morphological data (Figs. 2–4). The congruence of different methodologies is largely considered a means of overcoming potential problems of homology with AFLP data (Koopman et al. 2001) and the results of these analyses are congruent with previous work on morphology.

Relationships Between Species

Pyrrocoma scaberula is paraphyletic based on NJ analysis of bands (Fig. 3). This raises the question whether these two species may or may not represent a progenitor-derivative pair (Gottlieb 1973, 1974; Gottlieb and Pilz 1976). The progenitor species would be expected to be paraphyletic since the derivative species would have resulted from a subset of populations. However, a second important criterion for a progenitor-derivative species is that the derivative species should contain a subset of the total diversity found in the progenitor. A summary of the presence/absence data shows that 73.2% of the alleles are shared between the two species, 15.7% are unique to *P. liatriformis* and 11.1% are unique to *P. scaberula*. These results indicate that a large portion of the data is shared among the individuals and populations rather than being unique to either the putative progenitor species (*P. scaberula*) or the putative derivative (*P.

liatriformis) and thus argues against a progenitor-derivative pair. Likewise, the results of the Bayesian simulations in STRUCTURE do not indicate any overlap of populations between the two species, but instead the optimal data partition is equivalent to two groups (Fig. 4). It seems more likely that the paraphyly of *P. scaberula* is a result of recent common ancestry with shared alleles between the populations.

Relationships of Populations within Species

The AFLP results clearly show population genetic structure within each species (Figs. 2, 3). Within *P. scaberula*, groups that consistently hold together following the tree-based methods include AN/FB/SO/TS/TA, FE/WR, and perhaps LC/LH/CH/RR/CM. Within *P. liatriformis*, AR/PB/RC, SB/PS, JO/PP/PR, GR/UN, CR/GE/SP, and RT/WC are commonly recovered. Populations BD, KS, WW, LR, and EV sometimes show relationships with other groups but not always. Population BD has the longest branches showing the greatest genetic distance from other populations. The results from STRUCTURE indicate that the populations are best treated as two clusters, corresponding to the two species. The one exception is population EV where individuals show the greatest probability of being assigned to *P. liatriformis*, but also have a noticeable probability of being assigned to *P. scaberula* (Fig. 4). These data may indicate some recent hybridization within this population, be the retention of a large number of alleles that are ancestral to both species, or reflect incomplete lineage sorting.

Acknowledgments

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ONE TAXON OR TWO: ARE *FRASERA UMPQUAENSIS* AND *F. FASTIGIATA* (GENTIANACEAE) DISTINCT SPECIES?

BARBARA L. WILSON
Carex Working Group, 1377 NW Alta Vista, Corvallis, OR 97330
bwilson@peak.org

VALERIE HIPKINS
USDA Forest Service, NFGEL, 2480 Carson Road, Placerville, CA 95667

TOM N. KAYE
Institute for Applied Ecology, 563 SW Jefferson, Corvallis, OR 97333

ABSTRACT

Frasera fastigiata and *F. umpquaensis* are large, long-lived, perennial herbs with hollow stems, whorled leaves, large nectaries hidden by fringed hoods, and synchronized flowering. They differ in flower color and their ranges are disjunct. Some authors have treated them as conspecific due to their overall morphological similarity. The taxa can be distinguished by isozyme band patterns and by morphological traits including corolla color, relative lengths of corolla and calyx, and calyx lobe shape. Both isozyme differences and morphological differences are not completely fixed, but plants with one atypical feature can be identified by their combination of traits. The taxa should be recognized as distinct species.

Key Words: Conservation, *Frasera umpquaensis*, isozymes, rare plants.

Frasera umpquaensis M. Peck & Applegate (Peck and Applegate 1941) is a rare plant with a discontinuous range entirely west of the Cascade-Sierra axis from Lane Co., Oregon, to Trinity Co., California (Fig. 1). Young plants produce a rosette of slightly fleshy leaves, surprisingly lush in their upland habitat. After four to ten or more years, the long, thick rhizome puts up a flowering stalk that may exceed 1.7 m in height, bearing dozens of 1.2 cm-long, white to clear light green flowers that may have a purple tinge. Each of the four petal lobes bears a single large nectary surrounded and partly concealed by a fringed hood. Additional hairs arise below the nectaries, between the filaments. Flowering tends to be synchronized, with almost no plants over a large area flowering in some years and many flowering in other years. After fruiting, the plant returns to the rosette stage for four or more years. An individual plant may live for decades (Kaye 2001).

Frasera umpquaensis belongs to a group of four species characterized by this unusual life history, including synchronized flowering, and by tall, hollow stems and whorled leaves (Post 1958). The other three species are *F. caroliniensis* Walter of eastern North America, and the western species *F. speciosa* Douglas ex Griseb. and *F. fastigiata* (Pursh) A. Heller. *Frasera caroliniensis* and *F. speciosa* have more open inflorescences with larger, rotate, white or greenish corollas speckled with purple or brown. *Frasera speciosa* is unique in this group in having two nectaries on each petal and large, fringed scales (the corona) that originate below the nectaries and partially cover them (Beattie et al. 1973). *Frasera umpquaensis* and *F. fastigiata* have denser inflorescences with smaller, usually solid-colored flowers that do not open flat, and their corona is represented by a row of long hairs below the nectary, originating between the bases of the filaments (Table 1). *Frasera fastigiata* grows in Idaho and southeast Washington, while *F. umpquaensis* lives in southwest Oregon and northwest California (Fig. 1).

Frasera umpquaensis was said to differ from *F. fastigiata* in the corona hairs, the length and shape of the calyx lobes, and the corolla lobe width and apex shape (Card 1931; Peck and Applegate 1941; St. John 1941). Pringle (1990) stated that *F. fastigiata* can have corona hairs and implied that earlier illustrations omitting them (Card 1931) were in error, attributed the supposed calyx lobe differences to diverse interpretations of the words "lanceolate" and "linear" by different botanists, and dismissed supposed differences in corolla apex shape as variable within *Frasera* species. He summarized, "comparison of specimens from California identified as ... *F. umpquaensis* with specimens from the Blue Mountains of Oregon and from Idaho identified as ... *F. fastigiata* disclosed no differences by which the two taxa could be distinguished" (Pringle 1990, p. 186).

Pringle's (1990) rejection of *F. umpquaensis* species status had an air of finality, but botanists working with the plants were not satisfied. The color difference, the difference in average inflorescence size, and the 500-km disjunction between their ranges suggested that they were genetically

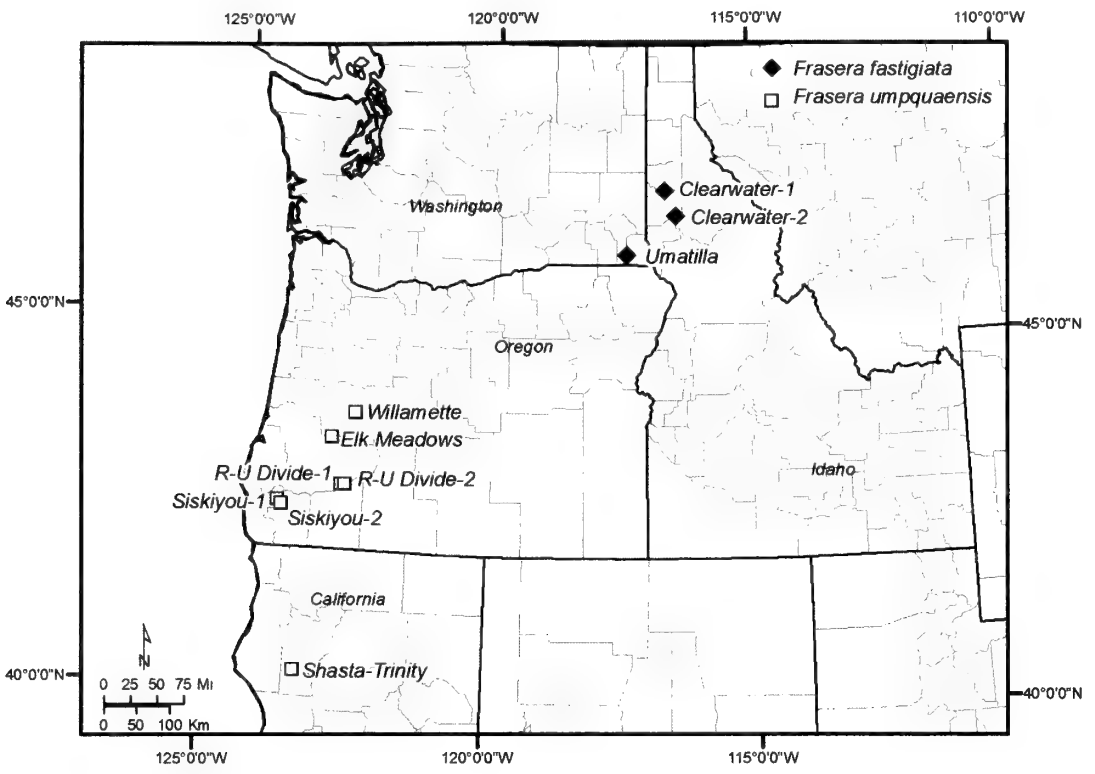

FIG. 1. Locations of populations of *Frasera fastigiata* and *F. umpquaensis* sampled for the isozyme study.

distinct and therefore should be treated as two species.

We report results of isozyme analysis and morphological studies of *F. umpquaensis* and *F. fastigiata* with the goal of clarifying their taxonomic status. We consistently use the name *F. umpquaensis* for the white to green-flowered plants of southwest Oregon and northern California, and *F. fastigiata* for the blue-flowered plants of Idaho and southeast Washington.

MATERIALS AND METHODS

Chromosomes

In October 2008, the somatic chromosome number of *Frasera umpquaensis* was determined by observation of mitosis in root tips of plants that had been grown at the Oregon State University greenhouse from seed collected in 2007 near the Elk Camp shelter, near Sourgrass Mountain in the Willamette National Forest, Lane Co., Oregon, at 4500 ft elevation. Excised root tips were pretreated in a saturated aqueous solution of p-dichlorobenzene at 4°C for 5 hr prior to fixation in Carnoy's fluid (95% ethyl alcohol and glacial acetic acid, 3:1 v:v). The root tips were hydrolyzed in a mixture of concentrated HCl and 95% ethanol (1:1 v:v) for 5–15 min, macerated in acetocarmine, and mounted with a small amount of Hoyer's solution (Beeks 1955). A Zeiss Photoscope III microscope equipped with phase-contrast optics was used to determine chromosome numbers in cells undergoing mitosis.

Isozymes

In the summer of 1996, two to five leaves per plant were collected from 75 individuals in three populations of *Frasera fastigiata* and 178 individuals in seven populations of *F. umpquaensis* (Table 2; Fig. 1). Leaf samples were shipped on ice to the National Forest Genetic Electrophoresis Laboratory (NFGEL). For each individual, three 7 mm diam. leaf discs were placed in a Tris buffer pH 7.5 (Gottlieb 1981) and stored at −70°C. On the morning of the run, samples were thawed, macerated, and absorbed onto 3 mm wide wicks prepared from Whatman 3MM chromatography paper.

Methods of electrophoresis follow the general methodology of Conkle et al. (1982) except that most enzyme stains are somewhat modified as outlined in USDA Forest Service (1995). A lithium borate electrode buffer (pH 8.3) was used with a Tris citrate gel buffer, pH 8.3 (Conkle et al. 1982), to resolve alcohol dehydrogynase (ADH), leucine aminopeptidase (LAP), phosphoglucomutase (PGM), and phosphoglucose isomerase (PGI). A sodium borate electrode buffer (pH 8.0) was used with a Tris citrate gel buffer, pH 8.8 (Conkle et al. 1982), to resolve catalase (CAT), glutamate-oxaloacetate transaminase (GOT), triosephosphate isomerase (TPI), and uridine diphosphoglucose pyrophosphorylase (UGPP).A morpholine citrate electrode and gel buffer, pH 8.0 (USDA Forest Service 1995), was used to resolve diaphorase (DIA), florescent esterate (FEST), isocitrate dehydrogenase (IDH), and malate dehydrogenase (MDH). A Tris citrate electrode and gel buffer, pH 7.2 (USDA Forest Service 1995), was used to resolve phosphogluconate dehydrogenase (6PGD). All enzymes were resolved on 11% starch gels. Enzyme stain recipes follow USDA Forest Service (1995) except that GOT was stained using the recipe from Wendel and Weeden (1989). Two people independently scored each gel. When they disagreed, a third

TABLE 1. SELECTED MORPHOLOGICAL TRAITS OF THE FOUR SPECIES OF *FRASERA* WITH HOLLOW STEMS AND WHORLED LEAVES.

Trait	*F. umpquaensis*	*F. fastigiata*	*F. speciosa*	*F. caroliniensis*
Calyx lobes, at and after anthesis	longer than corolla lobes	shorter than corolla lobes	longer than corolla lobes	longer than corolla lobs
Calyx lobes, shape	usually linear or widest above the base, occasionally subulate	usually subulate (at least some calyx lobes on all plants subulate)	usually linear, or broadly subulate	lanceolate (sometimes to subulate)
Corolla color	white or light green, sometimes purple-tinged	deep blue to light purple, rarely white, occasionally spotted	white or light green with purple spots	white or light green with purple or brown spots
Corolla lobes, at anthesis	angled; corolla nearly campanulate	angled; corolla nearly campanulate	flat; corolla rotate	flat; corolla rotate
Corona	several long hairs	several, few, or no long hairs	scale(s) with several long hairs	short hairs
Nectaries	1 per corolla lobe	1 per corolla lobe	2 per corolla lobe	1 per corolla lobe
Filament length	3–4 mm	2.3–3.3 mm	6.5–8.5 mm	7–8 mm

person resolved the conflict. For quality control, 10% of the individuals were run and scored twice.

Two zones, designated 1 (faster) and 2 (slower), were resolved for each of the enzymes DIA, GOT, MDH, PGI, and TPI for a total of 18 enzyme systems.

Because the limited information available (Post 1958) suggested that these species might be hexaploid and there are no crossing studies to determine the inheritance of isozymes in *Frasera*, we were unable to provide a genetic interpretation for the complicated band patterns observed on the isozyme gels. Gels were therefore scored for (1) banding pattern, and (2) band presence/absence. This type of data results in a phenotypic (band pattern and presence) instead of genotypic (alleles and loci) analysis. The band pattern data were used to calculate the average phenotypic identities between pairs of populations using Hedrick's measure of phenotypic identity (Hedrick 1971).

Diversity measures within populations were calculated by several methods (after Chung et. al. 1991): (1) the number of bands found in each population, (2) percentage of the enzymes that yield more than one band pattern among individuals in a population, (3) the average number of band patterns per stain in a population, (4) the polymorphic index (PI), based on the frequency of occurrence of each band, and (5) the Shannon-Weaver Diversity Index (Shannon 1948) based on band pattern frequency.

Ordination was performed in the R statistical environment (R Development Core Team 2008). A simple pair-wise distance matrix was constructed from isozyme phenotypes using the function dist.gene from the R package 'ape' (Paradis et al. 2004). Kruskal's non-metric multidimensional scaling (NMS) was performed on the matrix of pair-wise distances using the R function isoMDS. In order to facilitate NMDS, zeros in the pair-wise distance matrix were replaced with the arbitrarily small value of 0.0001.

Morphology

We examined 27 sheets of *F. umpquaensis* representing 16 distinct collections and 137 sheets of *F. fastigiata* representing 105 distinct collections, from HSC, ID, ORE, OSC, UC, WILLU, and WTU (Appendix 1). Selected flowers were soaked in water and opened to observe the corona. Other traits we examined included flower color (when it could be determined from the label or dried material), number of leaves in each whorl, inflorescence width, relative length of calyx and corolla, lengths of filaments and petals, and length, width, and shape of calyx lobes. To examine the relationship between the two taxa with morphometric information, we used NMS as implemented in PC-ORD (McCune and

TABLE 2. *FRASERA UMPQUAENSIS* AND *F. FASTIGIATA* POPULATIONS USED IN THE ISOZYME STUDY. The *F. umpquaensis* populations are listed from north to south. NF = National Forest; BLM = Bureau of Land Management; RNA = Research Natural Area. TRS (township, range, and section) for Oregon and Washington are based on the Willamette Meridian; for Idaho, the Boise Meridian; and for California, the Humboldt Meridian. n = sample size.

Population	State	Location	T	R	S	n
F. fastigiata						
Clearwater-1	ID	Clearwater NF; Giant White Pine Campground	42N	3W	2	25
Clearwater-2	ID	Clearwater NF; Little Boulder Creek	39N	1W	33	25
Umatilla	WA	Umatilla NF; Asotin Creek	08N	43E	28	25
F. umpquaensis						
Willamette	OR	Willamette NF; Nevergo Creek	19S	3E	31	25
Elk Meadows	OR	Eugene District, BLM; Upper Elk Meadows RNA	23S	2W	35	25
R-U Divide-1	OR	Umpqua NF; Rogue-Umpqua divide	31S	1E	10	25
R-U Divide-2	OR	Rogue River NF; Rogue-Umpqua divide	31S	2E	8	27
Siskiyou-1	OR	Siskiyou NF; Bear Camp, Galice Ranger District	34S	10W	12	26
Siskiyou-2	OR	Medford BLM; Hobsen Horn Gravel Pit	34S	9W	34	25
Shasta-Trinity	CA	Shasta-Trinity NF; Fern Campground	1S	7E	36	25

Mefford 2006). Non-metric multidimensional scaling searches iteratively for an ordination with low stress, a measure of the relationship between ranked distances in multidimensional space to the ranked distances in the reduced ordination (Peterson and McCune 2001). The following quantitative traits were used: inflorescence width, filament length, length and width of the longer calyx lobe, difference in length between two adjacent sepals, petal length, and difference in length between petal and longer sepal. In addition, the qualitative trait of flower color was scored 1 = blue, 0 = non-blue (white or green). We used a random seed with 250 runs of real data to ensure the ordination had low stress. Monte Carlo simulations with 250 iterations were used to assess the probability that final stress could have been obtained by chance. A stability criterion of 0.0001 was used. Student's t-tests were performed in Microsoft Excel (Microsoft Corportation 2003) to test for significance of differences between the two taxa in sepal length and width, petal length, inflorescence width, and the difference between petal length and sepal length.

RESULTS

Chromosomes

In 2008, counts of 78 chromosomes in each of 4 root tip cells undergoing mitosis confirmed that *F. umpquaensis* was polyploid and, because the base chromosome number in *Frasera* is 13 (Rork 1949), presumably hexaploid.

Isozymes

Frasera isozymes produced the variable, often complicated band patterns expected of polyploids. Tentative genetic interpretations could be developed only for the simpler band patterns, biasing genetic analysis against the more variable enzymes (MDH-2, PGI-1, TPI-1, and UGPP; Appendix 2) that most clearly distinguished the two taxa. Therefore, analyzing the isozyme patterns phenotypically, as patterns and bands, was more appropriate than genetic analysis for these *Frasera* species (Chung et al. 1991).

Most populations of *Frasera fastigiata* and *F. umpquaensis* were moderately to highly variable with 40–60% polymorphic loci (Table 3). The Willamette and Shasta-Trinity populations, isolated at the northern and southern ends of the *F. umpquaensis* range, respectively, were the least variable. In the Shasta-Trinity population all but two stains were monomorphic.

Although no fixed isozyme differences distinguished *F. fastigiata* from *F. umpquaensis*, overlap was slight in certain enzymes (e.g., MDH and PGI-1) and restricted to the Shasta-Trinity population in one enzyme (TPI-1). In general, Hedrick's measure of phenotypic similarity had high values for within-taxon comparisons and low values for between-taxon comparisons (Table 4). The NMS ordination based on the band patterns in individual plants resulted in two clusters corresponding to the two species (Fig. 2).

The geographically isolated Shasta-Trinity population of *F. umpquaensis* was relatively dissimilar to other *F. umpquaensis* populations (Table 4). When *F. umpquaensis* and *F. fastigiata* differed at an enzyme for which the Shasta-Trinity population was monomorphic, the Shasta-Trinity population shared its band pattern with other *F. umpquaensis* populations (e.g., for MDH, PGI-1, and PGM; Appendix 2). However, at one of its two variable enzymes (TPI-1), the Shasta-Trinity population had band patterns

TABLE 3. MEASURES OF PHENOTYPIC VARIATION IN ISOZYMES FOR TEN POPULATIONS OF TWO SPECIES OF *FRASERA*. The *F. umpquaensis* populations are listed from north to south.

Population	Sample size per stain	Bands	% polymorphic stains	Band patterns/stain (mean)	Polymorphic index	Shannon-Weaver diversity index
F. fastigiata	73.8					
F. fastigiata mean (SE)	24.6 (0.04)	44 (0.70)	53.7 (0.49)	2.13 (0.13)	3.87 (0.136)	0.4403 (0.008)
Clearwater-1	24.4	44	44.4	2.06 (0.38)	4.8932	0.4386
Clearwater-2	24.4	38	55.6	1.94 (0.25)	2.5920	0.3709
Umatilla	25.0	50	61.1	2.39 (0.39)	4.1248	0.5115
F. umpquaensis	177.6					
F. umpquaensis mean (SE)	25.4 (0.06)	41.3 (0.412)	39.7 (0.62)	1.83 (0.12	2.9207 (0.11)	0.3448 (0.012)
Willamette	25.0	39	27.8	1.39 (0.16)	1.9488	0.2184
Elk Meadows	24.9	47	55.6	2.33 (0.48)	4.6252	0.4978
R-U Divide-1	24.9	45	50.0	2.06 (0.33)	3.8592	0.4438
R-U Divide-2	27.0	47	55.6	2.28 (0.42)	4.7107	0.5415
Siskiyou-1	25.9	38	44.4	1.67 (0.20)	1.5136	0.2414
Siskiyou-2	24.9	41	33.3	1.89 (0.34)	2.7252	0.3513
Shasta-Trinity	25.0	32	11.1	1.22 (0.15)	1.0624	0.1195

otherwise observed only in the *F. fastigiata* populations. At the other (CAT), 60% of the individuals had a unique band pattern that seemed attributable to a unique allele. Therefore, Hedrick's distances indicate that the Shasta-Trinity population was as different from the northern *F. umpquaensis* population as from *F. fastigiata* populations (Table 4).

Morphology

Some traits reported to distinguish *F. umpquaensis* from *F. fastigiata* failed to separate the taxa consistently, but others were effective (Tables 5 and 6). Corona hairs were sometimes difficult to assess on herbarium specimens because chipping into the dried flowers often broke them, while soaking the flowers rendered them nearly transparent. The hairs often stuck to the fringed membrane that surrounds the nectary, and freeing them intact was difficult. Corona hairs were numerous and easy to see in all 19 *F. umpquaensis* flowers examined for them (Table 5). These hairs were also numerous in 18 of the 20 *F. fastigiata* specimens examined for them, but were often hard to see even when they were numerous. They were absent or sparse in flowers of two *F. fastigiata* specimens, varying among flowers in one inflorescence in one specimen (*Sondenaa 327*).

In *F. umpquaensis*, calyx lobes were longer than the mature corolla lobes (Table 5); in *F. fastigiata* they were shorter, and the difference was statistically significant (Table 6). However, in one of the 93 *F. fastigiata* specimens examined for this trait, *Bjork 7727*, the calyx lobes were clearly longer than the corolla on many of the mature flowers. Relative calyx length was often difficult to assess because the calyx lobes were longer than the corolla in bud, in both species. After anthesis the corolla lobes withered and folded, making comparisons of length misleading unless the flower was soaked and the corolla lobes unfolded.

In general, the two species were differentiated by calyx lobe shape (Table 5) and length (Tables 6). In *F. umpquaensis*, calyx lobes were usually linear (uniform in width) at least in the proximal half or lanceolate (widest above the base), though some were subulate (widest at the base and tapering uniformly to the tip). In *F. fastigiata*, all calyx lobes in most inflorescences and some calyx lobes in all inflorescences were clearly subulate. In both species, the two pairs of calyx lobes were sometimes found to differ in shape and/or length. Calyx lobes were significantly longer in *F. umpquaensis* than in *F. fastigiata* and corolla lobes were signficantly longer in *F. fastigiata*, although the the range of variation in these traits overlapped between the two species (Table 6).

TABLE 4. HEDRICK'S MEASURE OF SIMILARITY AMONG ISOZYME BAND PATTERN FREQUENCIES IN TEN POPULATIONS OF *FRASERA*. A value of 1.0 indicates identical variation in a population pair.

Species	Population	Clearwater-1	Clearwater-2	Umatilla	Willamette	Elk Meadow	R-U Divide-1	R-U Divide-2	Siskiyou-1	Siskiyou-2
F. fastigata	Clearwater-1									
F. fastigata	Clearwater-2	0.894								
F. fastigata	Umatilla	0.723	0.747							
F. umpquaensis	Willamette	0.492	0.455	0.480						
F. umpquaensis	Elk Meadow	0.450	0.402	0.431	0.672					
F. umpquaensis	R-U Divide-1	0.573	0.533	0.497	0.767	0.781				
F. umpquaensis	R-U Divide-2	0.399	0.367	0.374	0.629	0.688	0.771			
F. umpquaensis	Siskiyou-1	0.621	0.673	0.576	0.766	0.702	0.774	0.680		
F. umpquaensis	Siskiyou-2	0.575	0.522	0.521	0.657	0.722	0.872	0.724	0.773	
F. umpquaensis	Shasta-Trinity	0.644	0.606	0.584	0.685	0.574	0.712	0.553	0.700	0.870

Flower color was difficult to assess on herbarium specimens because corollas in many older specimens of both species faded to tan. Flower color was not reported on the labels of the 16 *F. umpquaensis* specimens examined. Field workers report that the flowers are white to greenish, often lightly tinged with purple (Thomas Kaye personal observation; Jennifer Lippert, Willamette Natl. Forest, personal communication). Flower color was pale and greenish on the more recently collected herbarium specimens. Labels of the 21 *F. fastigiata* specimens that mentioned flower color reported it to be blue (including pale blue and "fairly deep blue"), purple, or lavender. Corollas of the more recent dried herbarium specimens varied from deep gentian blue to light purplish blue, with few exceptions (Table 5). One sheet (*Richards 116*) consisted of a shoot with blue corollas speckled with darker blue, and two shoots with pale flowers that may have been white in life. Flowers on several *F. fastigiata* specimens had inconspicuous darker speckles on blue corollas, and on a few sheets the speckles were relatively conspicuous (e.g., *Constance 1771*, *Williams & Goff 16*, and *Wilson 241*).

Nonmetric multidimensional scaling based on multiple morphometric traits produced a final stress of 6.188 and usually separated *Frasera fastigiata* from *F. umpquaensis* (Fig. 3). The two *F. fastigiata* that overlapped the cluster of *F. umpquaensis* were *Bjork 7727* which had sepals up to 2.3 mm longer than the petals, and the pale-flowered individual in *Richards 116*. Despite these anomalies, *Bjork 7727* could easily be assigned to *F. fastigiata* because of its blue flowers, subulate calyx lobes, and two cauline leaves per whorl. The pale-flowered plants in *Richards 116* had blue speckles and mostly subulate calyx lobes that were shorter than or barely longer than the corollas. Those traits, plus its occurrence in a population with blue flowered-plants, would lead to its correct identification.

DISCUSSION

Frasera umpquaensis and *F. fastigiata* are more similar to each other morphologically than they are to any other species, but they are not the same. Isozymes consistently distinguished the two taxa, although differences were not completely fixed (Fig. 2). The two taxa could be distinguished morphologically as well (Fig. 3), but as was true for isozymes, the differences were not completely fixed. Despite these occasional inconsistencies, all specimens could be easily identified to taxon when all traits were taken together. For example, a specimen that had an unexpected calyx length was typical of its taxon for other traits.

The past confusion over the differences between these taxa results in part from using

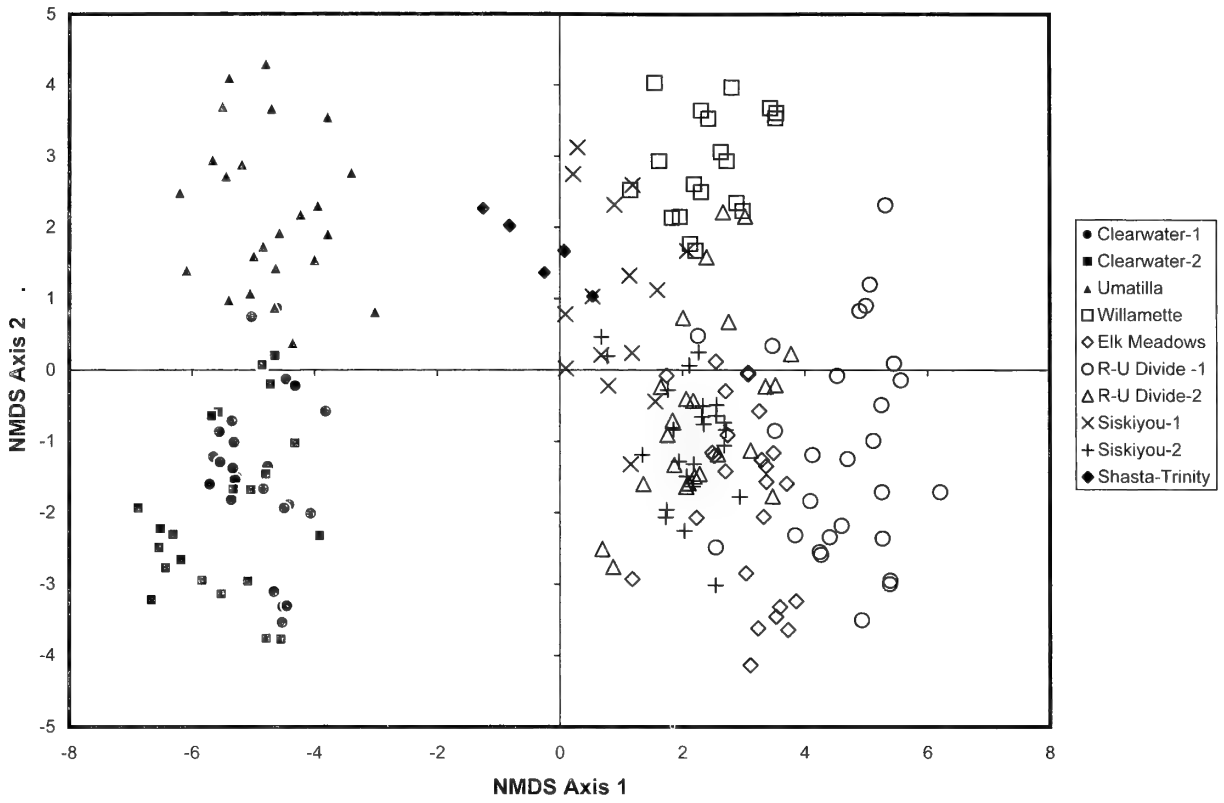

FIG. 2. Non-metric multidimentional scaling of isozyme band patterns in individual samples of *Frasera fastigiata* (dark symbols) and *F. umpquaensis* (open and shaded symbols).

unreliable traits (Peck and Applegate 1941; St. John 1941; Pringle 1990). Corona hairs do help differentiate the *umpquaensis-fastigiata* species pair from *F. speciosa*, which has fringed, membranous corona scales, and from *F. caroliniensis*, which has very short hairs (Table 1), but corona hairs are variable within *F. fastigiata* and hard to assess in both that species and *F. umpquaensis*. Corolla tip shape, too, is variable and hard to assess. Although calyx lobe shape tends to differentiate *F. fastigiata* from *F. umpquaensis*, it may vary even within an inflorescence.

TABLE 5. COMPARISON OF QUALITATIVE TRAITS OBSERVED IN *FRASERA UMPQUAENSIS* AND *F. FASTIGIATA*.

Trait	*F. fastigiata*	*F. umpquaensis*
Corolla color		
Sample size	n = 62	n = 15
Blue	62	0
Pale	1	15
Corolla lobes		
Sample size	n = 96	n = 16
>sepals	95	0
=sepals	5	0
<sepals	1	16
Calyx lobes widest		
Sample size	n = 44	n = 9
Base	44	2
Above	0	9
Mixed	4	2
Calyx lobe shape		
Sample Size	n = 21	n = 15
Lanceolate & linear	7	15
Subulate	19	1
Corona Hairs		
Sample size	n = 19	n = 9
0 to few	18	9
Many	1	0

When the appropriate traits are evaluated, *F. umpquaensis* and *F. fastigiata* are readily distinguished (Tables 5 and 6). *Frasera umpquaensis* has white to green flowers, calyx lobes that surpass the corolla, and usually longer, linear to lanceolate calyx lobes. In general, *F. fastigiata* has blue flowers, calyx lobes that are exceeded by the corolla (at anthesis), and shorter, subulate calyx lobes. Some *F. fastigiata* plants resemble *F. umpquaensis* in one of these traits, but not in all of them. In both species, calyx lobe shape may vary within the inflorescence.

Both isozyme variation and morphology indicate that these two taxa are genetically distinct. Their geographic separation suggests that the differences between the two will only become greater due to reproductive isolation. Because of this evidence, *Frasera umpquaensis* and *F. fastigiata* should be recognized as separate species.

Key to the Four Species of *Frasera* with Whorled Leaves and Wide, Hollow Stems

1. Corolla rotate, opening flat; filaments 6.5–8.5 mm long; petals white or green with purple-brown spots
   2. Nectary pit 1 on each corolla lobe; corona consisting of short hairs between the filament bases; range in eastern North America. . . . . *F. caroliniensis*
   2′ Nectary pits 2 on each corolla lobe; corona consisting of 1+ scale(s) with several long

TABLE 6. COMPARISON OF QUANITATIVE TRAITS OBSERVED IN *FRASERA UMPQUAENSIS* AND *F. FASTIGIATA*. For each species, trait values for the mean, standard deviation, standard error and range are provided. Values from the t-test of differences between means and the P-value are provided in the final two columns.

	Frasera fastigiata					*Frasera umpquaensis*						
	n	mean	SD	SE	range	n	mean	SD	SE	range	t	P
Leaves/whorl												
Top whorl	45	2.89	0.53	0.08	2–4	15	3.4	0.51	0.13	3–4	−3.34	0.00264
2nd whorl	40	2.85	0.48	0.08	2–4	10	3.3	0.48	0.15	3–4	−2.63	0.01960
Inflorescence width (cm)	46	5.27	1.12	0.16	3.5–9	15	3.6	1.04	0.27	2.1–5.5	5.11	0.00000
Filament length (mm)	22	2.91	0.53	0.11	1.8–4	15	2.86	0.57	0.15	1.7–3.9	0.28	0.77906
Calyx length (mm)	42	8.44	1.84	0.28	4.4–12.8	30	10.08	1.57	0.29	7.3–14.2	−3.97	0.00017
Calyx width (mm)	42	1.35	0.40	0.06	0.7–2.2	30	1.53	0.40	0.07	0.9–2.3	−1.92	0.05860
Corolla length (mm)	21	9.94	1.46	0.32	6.7–13.4	15	8.29	1.37	0.35	5.3–10.8	3.43	0.00160
Calyx L–Corolla L (mm)	21	−1.50	1.30	0.28	−4.8–0.95	15	1.79	1.10	0.28	0.55–4.9	−7.97	0.00000

hairs between the filament bases; range in western North America . . . . . *F. speciosa*

1′ Corolla +/− campanulate, not opening flat; filaments 1.7–4 mm long; petals blue, white, or green, usually unspotted or inconspicuously spotted
- 3. Corollas usually pale to dark blue or purple; calyx lobes usually subulate, usually shorter than the corolla lobes (when corolla lobes fully expanded); inflorescence width 3.5–9 cm wide; range in Idaho and SE Washington . . . . . *F. fastigiata*
- 3′ Corollas white to green, sometimes purple-tinged; calyx lobes usually linear to lanceolate, longer than the corolla lobes; inflorescence 2.1–5.5 cm wide; range SW Oregon to NW California . . . . *F. umpquaensis*

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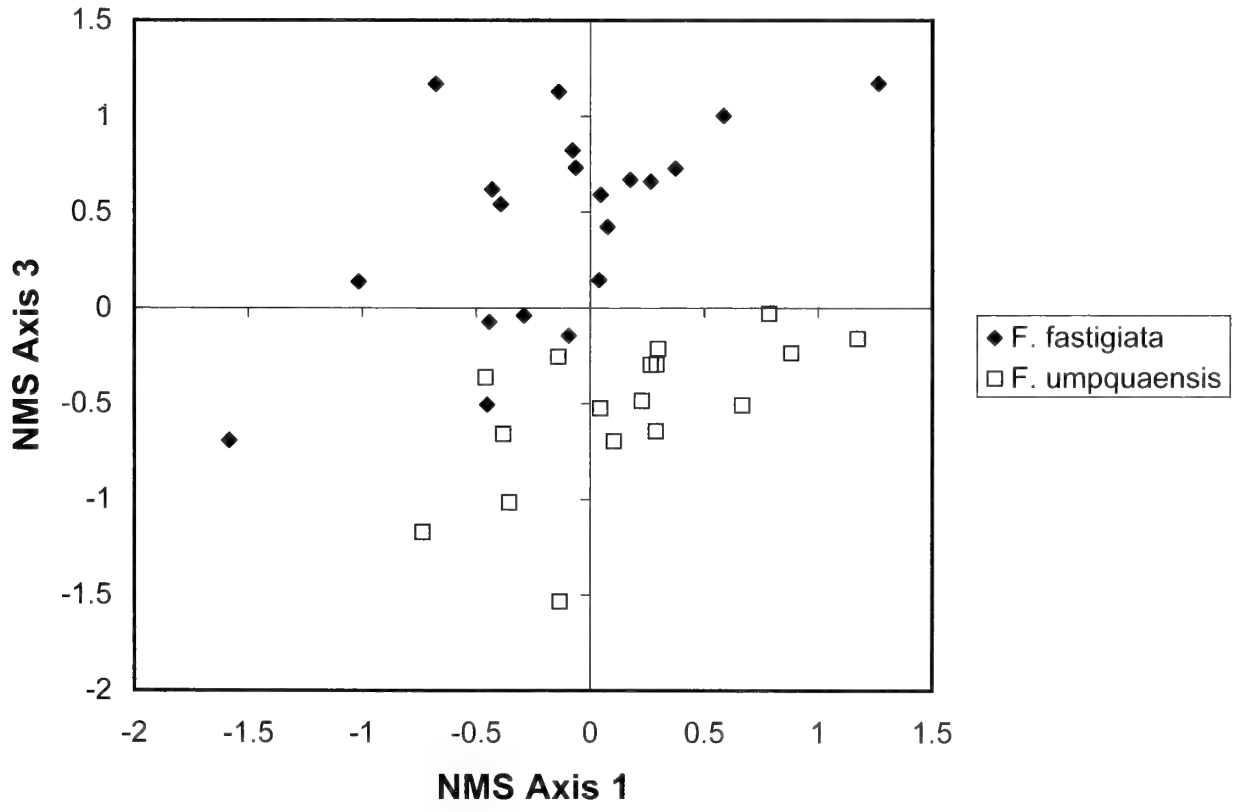

FIG. 3. Non-metric Multidimensional Scaling (NMS) of selected morphological traits in 17 specimens of *Frasera umpquaensis* and 20 specimens of *F. fastigiata*. Axes 1 and 3 explained the most variation in morphological traits.

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Appendix 1

Specimens Examined

Specimens are from the herbaria of Humboldt State University (HSC), Oregon State University (OSC), Shasta-Trinity National Forest (S-T NF), University of California (UC), University of Idaho (ID), University of Oregon (ORE, at OSC), University of Washington (WTU), Willamette University (WILLU, at OSC). * = measured for morphological study.

Frasera fastigiata: IDAHO. **Benewah Co.**: slopes of Plummer Butte southeast of Plummer, 17 May 1961, *Baker 16101* (ID); Lolo Creek Road, 47°13.345′ 116°50.734′, 10 June 2003, *Brunsfeld 2659* (ID); Mary Minerva McCroskey Memorial State Park, near park entrance, T43N R4W S30, 47°3′31.18″ 116°51′57.46″, 1 June 2004, *Brunsfeld 2974* (ID); Mary Minerva McCroskey Memorial State Park, ca. 1.5 mi W of park entrance, 47°3′37.06″ 117°59′36.960″, 1 June 2004, *Brunsfeld 3023* (ID); St. Joe National Forest, upper slopes of St. Joe Baldy in vicinity of saddle between St. Joe Baldy and Reed's Baldy, on ridge running to top of St. Joe Baldy from the saddle., 30 June 1996, *Fox 656* (ID); summit of Moose Mtns., 19 June 1927, *Jones 607* (WTU); near Chatcolet, 12 May 1928, *Warren 893* (WTU). **Clearwater Co.**: Elk Butte Summit and Lookout, 11 July 2002, *Brunsfeld 2191* (ID); Elk Butte south face, near summit, 27 July 1976, *Cavanaugh 20* (ID); Freemon Creek, Brown's Creek meadows, 28 July 1929, *Cook s.n.* (WTU); Weipe, 22 June 1937, *Davis 271-37* (WTU); 8 mi NE of Dent, along the north fork of the Clearwater River, 3 May 1941, *Weber 2115* (WTU). **Idaho Co.**: 9 mi W of Grangeville, 22 June 1937, *Christ 7715* (ID); 10.0 mi S of Grangerville on Nez Perce NF Road 4649, headwaters of N Fk White Bird Creek, W side of road at edge of forest near base of road embankment, T29N R3E S34, 29 July 1989, *Ertter 8822* (UC); Nezperce National Forest, Wild Horse Corral, 25 July 1952, *Evanko ABE-19* (ID); ca. 10 mi S of Sweet Water, along highway, 1 July 1949, *Hitchcock 19162* (UC, WTU); ½ mi SW of Whitebird Summit, T29N R2E S5, 19 June 1950, *Jones 79* (WTU); Nez Perce National Forest, 12 mi S of Grangeville along the Grangeville-Salmon Road #221, near Cayuse Meadows, T29N R3E S34 SE 1/4, 16 June 1995, *Sondenaa 135* (ID); Nez Perce Naitonal Forest, Pinnacle Ridge. 14.8 mi S of Grangeville on FS Rd 221, then 4.4 mi W of FS Rd 1870, T28N R3E S7 SW 1/4, 21 June 1996, *Sondenaa 327* (ID*). **Kootenai Co.**: Mica Peak area, Clearwater Bioregion, about 5.5 mi N of Setters, lower south slopes of Mica Peak, occasional along Bozard Creek Road, 47°33′ 117°2′, 4 June 2003, *Bjork 7727* (ID*); Coer d'Alene, 30 May 1928, *Christ 92* (ID*); (no location), July 1891, *Leiberg 213* (UC); (nono location), 1890, *Leiberg 217* (ORE); (no location), July 1891, *Leiberg s.n.* (ORE). **Latah Co.**: Paradise Hills, April 1900, *Abrams 611* (UC); 8 mi E of Moscow on Troy road, 2 June 1960, *Aller s.n.* (ID*); on slope Near Paradise Creek, Moscow Mtns., about 6 mi NE of Moscow, 16 June 1951, *Bacon s.n.* (WTU); NE of Moscow, 22 June 1939, *Baker 1235* (ID); Thatana Hills, Moscow Mountain, 24 June 1939, *Baker 1301* (ID);

Moscow Mountain, 2 July 1939, *Baker 1372* (ID); along the Troy Road 4 mi E of Moscow, 14 May 1949, *Baker 5847* (ID); slope above Gnat Creek, about 4 mi NE of Moscow, 13 May 1952, *Baker 8875* (ID*, UC, WTU); east slope of Tomer's Butte, 5 mi SE of Moscow, 15 May 1952, *Baker 8885* (ID, WTU); along Skyline Drive, 2 mi W of Highway 95 junction, T43N R4W S13, 9 June 1960, *Baker 15881* (ID*); Moscow Mt., 5 mi NE of Moscow, 22 June 1960, *Baker 15954* (ID); Moscow Mts., 12 May 1906, *Botany Class s.n.* (ID); St. Joe National Forest, north side of Moscow Mountain between Rock Creek and Hatter Creek, T47N R4W S21, 30 May 2002, *Brunsfeld 2002* (ID); south slope of Moscow Mountain, 5 mi NE of Moscow, 19 June 1950, *Chichester 149* (ID); Crumarine Creek, 5 mi NE of Moscow, 23 June 1950, *Chichester 288* (ID); north slope of Moscow Mountain, 5 mi NE of Moscow, 26 June 1950, *Chichester 476* (ID); south slope and banks of Crumarine Creek, 13 May 1954, *Chichester 880* (ID*); Crumarin Ridge, S of East Twin, Palouse Range, 18 June 1954, *Chichester 984* (ID); Crumarine creek Drainage at base of Moscow Mountain (Palouse Range), 17 June 1954, *Chichester 1111* (ID); Shatuna [?] Ridge, 1 June 1937, *Daubenmire 37285* (WTU); north slope NW of The Twins, 12 June 1936, *Dillon 587* (UC, WTU); Cedar Mt., 20 May 1916, *F. L. P. 411* (WTU); Moscow, July 1915, *Gail s.n.* (ID*); Moscow Mt., Moscow, 1916, *Gail s.n.* (ID); Moscow, 1930, *Gail s.n.* (ID); ca. 1 mi S of Deary on Highway 3 (2290 State Hwy 3), farm on east side of highway, west side of patch of small woods ca. 1/4 mi from residence and buildings, 46°47.030′ 116°33.5′, 20 May 2003, *Hill 286* (ID); 3 mi S of Thorp, 27 May 1944, *Hitchcock 8395* (WTU); southern base of Moscow Mountain, 7 May 1954, *Johnson 34* (ID); intersection of Randall Flat Rd. and Beulah Rd. approx. 2 mi N of the junction of Randall Flat Rd. and Hwy. 8., *Jonassen 19* (WTU); on Gold Hill N of Potlatch; USGS Potlatch Quadrangle 15 min series, T42N R4W S21 SE 1/4 SE 1/4 SE 1/4, 10 May 1976, *McMahon 31* (WTU); along northeastern slopes of Tomer's Butte, 2.5 mi SE of Moscow, T38N R5W S23, 23 July 1955, *Nisbet 86* (ID); 1/4 mi E of Robinson Lake, T39N R4W S6, 5 May 1958, Moscow Mt., 3 May 1930, *Nyberg s.n.* (WTU); *Oppe 68* (ID); above Big Meadow and Big Meadow Creek, N of Troy (3.4 mi up road from picnic area, 1.7 mi up from holding pond), T40N R4W S14, 3 July 2002, *Parks 49* (ID*); (no location), 16 July 1893, *Piper 1618* (ORE, UC, WTU); (no location), 16 July 1893, *Piper s.n.* (ORE); Moscow, 26 April 1895, *Ransom s.n.* (ID); summit of Bald Mountain, 17 July 1955, *Richards 116* (ID*); Moscow Mt., 6 June 1925, *Ridout s.n.* (WTU); Moscow Mts., 28 April 1906, *Simpson s.n.* (ID); Cedar Mt., 5 June 1921, *St. John 6036* (UC, WTU); Lunch Bucket Ridge, T40N R1W S36 SW 1/4, 13 June 1972, *Wagner 48* (ORE); near Viola, Moscow Mts., 1 June 1924, *Warren 874* (WTU); along trail to ridgetop to Three Tree Butte, 9 June 1974, *Wellner 167* (ID). **Lewis Co.**: S of Grangerville, 18 June 1939, *Baker 1188* (ID*); 1 mi S of Forest, IDD, on Merek Rd., 6 June 2002, *Brunsfeld 2031* (ID); along Forest Road, 1.1 mi N of Forest, T33N R3W S36 NW corner, 16 August 2002, *Parks 135* (ID). **Nezperce Co.**: 2 mi S of Zaza, 9 October 1927, *Hardin 377* (WTU); about Lake Waha, 24 June 1896, *Heller 3285* (UC); Benton Meadows, Craig Mountain, ca. 6.5 mi S of Waha, T32N R4W S15 NE 1/4, 5 June 1993, *Mancuso 900* (ID); top of Winchester grade, 6 June 1951, *Torrell 78* (ID). **ShoshoneCo.**: north slope, Freezeout Saddle, 14 mi E of Clarkia, 20 July 1958, *Baker 15359* (ID); Freezout Saddle, St. Joe National Forest, 14 mi E of Clarkia, 5 July 1963, *Baker 16429* (ID*); 12 mi W of Clarkia, 1 July 1961, *Daubenmire 6114* (WTU); on Freezout Rd ca. 1 mi beyond Freezout Saddle at junction with first rd. to right, along ridgeline to N & E, along E-W portion of ridgeline, T42N R3E S1 NE 1/4, 8 July 1996, *Fox 702* (ID); Squaw Springs, 17 June 1969, *Swalley s.n.* (ID); vicinity of Windy Peak, 18 July 1941, *Wilson 241* (UC, WTU). **Undetermined Co.**: Elk River, 19 June 1927, *Gail s.n.* (ID); Ingham's Mt., 12 June 1892, *Lake s.n.* (WTU); Strohm Canyon, 26 May 1957, *Laughlin 168* (ID); Carder Ranch, River Hill, 23 May 1912, *Rust 42* (ID); Viola Grade, 18 May 1927, *Williams s.n.* (ID). WASHINGTON. **Asotin Co.**: S of Anatone, 2 June 1950, *Baker 6738* (ID, WTU); along summit of Blue Mountains, overlooking Indian Tom Creek, 30 mi SW of Asotin, T7N R43E S1, 25 June 1949, *Cronquist 5902* (ID, UC*, WTU); Blue Mountains, S of Smyth Rd., W of Washington 129, about 2 mi (air) SW of Anatone, 46°6′ 117°9′, 30 May 1998, *Fishbein 3392* (ID,WTU*); 2 mi W of Anatone, 15 May 1926, *Gissell s.n.* (WTU); Petty Ridge, 15 mi SW of Asotin, T8N R44E S20, 10 June 1959, *Hitchcock 21832* (WTU); near Anatone, 21 May 1922, *St. John 9757* (WTU). **Columbia Co.**: foothills of Blue Mountains between Godman Guard Station and Dayton, 2 August 1950, *Kruckeberg 2537* (WTU); Stockade Springs, 7/6 1927, *St. John 8288* (WTU). **Garfield Co.**: Powell Camp, near Clearwater Ranger Stateion, Blue Mountains, T9N R42E S, 14 June 1936, *Constance 1771* (WTU*); Umatilla National Forest, intersection of Forest Road 40 and Forest Road 4022, 27 June 1997, *Williams 16* (OSC, WTU*). **Spokane Co.**: (no location), 5 June 1889, *Suksdorf 938* (UC); Latah Creek, 28 July 1916, *Suksdorf 8965* (WTU). **Whitman Co.**: Kamiack Butte, June 1897, *Elmer 802* (UC, WTU); Kamiack Butte, 12 June 1952, *King 52-100* (WTU*); near top of Kamiak Butte in dense woods, 28 May 1922, *Parker 406* (OSC, WTU); along ridge, Kamiak Butte, 21 May 1921, *St. John 5887* (WTU). **Undetermined Co.**: Palouse, 15 July 1892, *Henderson s.n.* (WTU); Palouse country, July 1880, *Marsh s.n.* (WTU), UNDETERMINED STATE. Santianne Creek Bottom, 27 July 1895, *Leiberg 1064* (ORE, UC).

***Frasera umpquaensis*:** CALIFORNIA. **Trinity Co.**: near Pickett Peak, close to the town of Mad River, 40°20′52.39″ 123°22′2.7″, 16 July 1979, *Clifton 7842* (HSC*); near Pickett Peak, 40°20′58″ 123°22′8″, 28 June 1980, *Copeland 293* (HSC*); near Pickett Peak, 40°20′58″ 123°22′8″, 28 June 1980, *Copeland 294* (HSC*); Hayfork Ranger District, Picket Peak Lookout Road, T1S R7E S7 (Humboldt Meridian), 19 July 2001, *Erwin 1099* (S-T NF*); north slopes of Pickett Peak, South Fork Mountain, T1S R7E S27 (Humboldt Meridian), 9 July 1971, *Sawyer 2416* (HSC*). OREGON: **Curry Co.**: Bear Camp summit, T34S R10W S12, 16 July 1979, *Hess, R. s.n.* (OSC*). **Douglas Co.**: Upper Elk Meadows, 32 km SSE of Cottage Grove, T23S R2W S35 SE 1/4, 25 July 1979, *Christy, J. 2529* (ORE*); Umpqua National Forest, Rogue-Umpqua Divide; below Bald Ridge, T31S R1E S2, 30 June 1979, *Fosback, J. s.n.* (OSC*); Huckleberry Gap Glades, 5 August 1924, *Ingram, D. 1507* (ORE*,OSC); Rogue-Umpqua Divide 22 mi W of Crater Lake, 31 July 1916, *Peck, M. 4497* (WILLU*); alpine slopes of Abbott Butte, Rogue River Nat. Forest, 2 July 1936, *Thompson, J. 13067* (WILLU*, WTU*). **Jackson Co.**: near

Anderson Camp, Rogue-Umpqua Divide, upper waters of Rogue River, northeastern Jackson County, 11 July 1929, *Applegate, E. 5930* (UC*, WILLU); mainly on the Umpqua drainage (W) side of the Rogue-Umpqua Divide, 1 mi NE of Butler Butte, Rogue River Nat'l Forest, 28 June 1950, *Kruckeberg 2010* (UC,WTU); Woodruff Meadows, 5 July 1925, *Pendleton, R. s.n.* (OSC*). **Undetermined Co.**: along drainage of the east fork of Abbot Creek, ca. 20 mi W of Crater Lake, near Abbott Butte; to the SE of Elephant Head at 5100 ft, 6 July 1972, *Mitchell 215* (OSC*); along drainages of the E fork of Abbott Creek, ca. 20 mi W of Crater Lake, near Abbott Butte, 42°56′ 122°31′, 27 July 1972, *Mitchell 299* (OSC*).

APPENDIX 2. ISOZYME BAND PATTERN FREQUENCIES IN POPULATIONS OF *FRASERA UMPQUAENSIS and F. FASTIGIATA*. See Methods for enzyme abbreviations.

		F. fastigiata			*F. umpquaensis*						
Enzyme	Pattern	Clearwater-1	Clearwater-2	Umatilla	Willamette	Elk Meadows	R-U Divide-1	R-U Divide-2	Siskiyou-1	Siskiyou-2	Shasta-Trinity
6PGD	A	1.000	0.960	0.960	1.000	0.240	1.000	1.000	1.000	0.960	1.000
6PGD	B		0.040								
6PGD	C					0.480					
6PGD	D			0.040		0.280					
6PGD	F									0.040	
ADH	A	1.000	1.000	0.840	1.000	0.875	0.840	1.000	1.000	1.000	1.000
ADH	C					0.125	0.160				
ADH	D			0.160							
CAT	A	0.360	0.240	0.120	1.000	1.000	0.760	0.778	0.846	1.000	0.080
CAT	B						0.120				0.320
CAT	C	0.240	0.120	0.240			0.120	0.222	0.115		
CAT	D			0.120					0.039		
CAT	E	0.320	0.480	0.480							
CAT	F	0.080	0.160	0.040							
CAT	J										0.600
DIA-1	A	1.000	1.000	1.000	0.400	1.000	1.000	0.111	1.000	1.000	1.000
DIA-1	B				0.080						
DIA-1	C							0.519			
DIA-1	D							0.370			
DIA-1	E				0.520						
DIA-2	A	0.889	1.000	0.680	1.000	0.261	1.000	0.593	0.391	1.000	1.000
DIA-2	B			0.120		0.739		0.407	0.609		
DIA-2	C	0.111		0.200							
FEST	A	1.000	0.920	0.720	0.400	1.000	1.000	0.667	0.731	1.000	1.000
FEST	B			0.200	0.600			0.333	0.269		
FEST	C		0.040	0.040							
FEST	D		0.040	0.040							
GOT-1		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GOT-2	A				1.000	0.360	0.480	0.482		0.042	
GOT-2	B	0.680	0.960	0.040			0.480	0.222	0.846	0.500	1.000
GOT-2	C	0.160		0.880			0.040	0.148	0.077	0.291	
GOT-2	D	0.160	0.040	0.080		0.280			0.077	0.083	
GOT-2	E					0.080				0.042	
GOT-2	F					0.280		0.148			
GOT-2	G									0.042	
IDH	A	1.000	1.000	0.760	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IDH	B			0.240							
LAP	A					0.960	0.880	1.000		0.920	
LAP	B	1.000	0.880	0.960	0.280	0.040			0.923	0.040	1.000
LAP	C		0.120	0.040	0.720		0.120		0.077	0.040	
MDH-1		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

APPENDIX 2. CONTINUED.

Enzyme	Pattern	*F. fastigiata*			*F. umpquaensis*						
		Clearwater-1	Clearwater-2	Umatilla	Willamette	Elk Meadows	R-U Divide-1	R-U Divide-2	Siskiyou-1	Siskiyou-2	Shasta-Trinity
MDH-2	A				1.000	1.000	0.680	0.963	1.000	0.400	1.000
MDH-2	B	0.120	0.200	1.000							
MDH-2	C	0.680	0.760							0.040	
MDH-2	D	0.080									
MDH-2	E						0.240	0.037		0.440	
MDH-2	F						0.040			0.120	
MDH-2	G	0.120	0.040				0.040				
PGI-1	A				0.800	0.880	1.000	1.000	1.000	1.000	1.000
PGI-1	B	0.120	0.280	0.440							
PGI-1	C	0.800	0.240	0.200							
PGI-1	D	0.080	0.360	0.360							
PGI-1	E		0.120			0.120					
PGI-1	G				0.200						
PGI-2	A	1.000	1.000	1.000	1.000	1.000	0.800	0.037	0.885	0.600	1.000
PGI-2	B							0.704	0.038	0.040	
PGI-2	C						0.200	0.259	0.077	0.360	
PGM	A				1.000		0.417	0.222	0.923		1.000
PGM	B	1.000	0.920	0.040				0.074			
PGM	C		0.080								
PGM	D			0.520							
PGM	E			0.160							
PGM	F			0.120		0.040					
PGM	G			0.040		0.120	0.083	0.037		1.000	
PGM	H					0.360		0.037			
PGM	I			0.040							
PGM	J							0.037			
PGM	N			0.080							
PGM	O					0.440		0.074			
PGM	P						0.500				
PGM	S					0.040		0.482	0.077		
PGM	T							0.037			
TPI-1	A	0.120	0.600	1.000							0.200
TPI-1	B	0.160	0.360								
TPI-1	C	0.040									
TPI-1	D	0.040									
TPI-1	E	0.200									
TPI-1	F	0.200	0.040								0.480
TPI-1	G	0.240									
TPI-1	H				1.000	0.960	1.000	1.000	1.000	1.000	0.320
TPI-1	I					0.040					
TPI-2	A	0.720	0.280	1.000	1.000	0.640	0.440	0.222	1.000	0.600	1.000

APPENDIX 2. CONTINUED.

		F. fastigiata			*F. umpquaensis*						
Enzyme	Pattern	Clearwater-1	Clearwater-2	Umatilla	Willamette	Elk Meadows	R-U Divide-1	R-U Divide-2	Siskiyou-1	Siskiyou-2	Shasta-Trinity
TPI-2	B					0.320	0.440	0.630		0.120	
TPI-2	C					0.040	0.120	0.148		0.280	
TPI-2	D	0.280	0.720								
UGPP	A	0.760	1.000	0.640			0.160				
UGPP	B				0.400	0.040					
UGPP	C				0.560	0.320	0.320		0.039	0.120	
UGPP	D				0.040	0.120	0.080				
UGPP	E			0.200		0.040	0.040	0.296	0.807	0.440	1.000
UGPP	F					0.080	0.360	0.371	0.154	0.440	
UGPP	G							0.074			
UGPP	H					0.080		0.259			
UGPP	I						0.040				
UGPP	J	0.240									
UGPP	K					0.160					
UGPP	L			0.160		0.120					
UGPP	M					0.040					

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THE EFFECTS OF LONG-TERM DROUGHT ON HOST PLANT CANOPY CONDITION AND SURVIVAL OF THE ENDANGERED *ASTRAGALUS JAEGERIANUS* (FABACEAE)

T. R. HUGGINS, B. A. PRIGGE, M. R. SHARIFI, AND P. W. RUNDEL
Deptartment of Ecology and Evolutionary Biology, University of California, Los Angeles, CA 90095-1606
huggins@ucla.edu

ABSTRACT

Astragalus jaegerianus Munz (the Lane Mountain milkvetch) is a federally endangered species that exists in only four fragmented populations within and adjacent to the U.S. Army's National Training Center, Fort Irwin, CA. Since 1999, our monitored *A. jaegerianus* populations have consistently declined, and are now 12% of their previous size. A number of subpopulations are in danger of local extinction. The decline of *A. jaegerianus* has occurred simultaneously with severe drought in the Mojave Desert. These drought conditions began in 1999 and are predicted to continue for decades, or may continue indefinitely under warmer temperature conditions projected by global climate change-type drought. Our results suggest that drought has direct and indirect affects on *A. jaegerianus* by killing or degrading its host shrubs. *Astragalus jaegerianus* host shrubs have decreased in shrub volume and cover by roughly 10 percent since the onset of drought, and shrub mortality has been high. Our results show that canopy condition has a profound affect on the microclimate within host shrubs. Furthermore, our results show a significant increase in survival of *A. jaegerianus* among host plants with more intact canopies. These results support our study hypothesis that drought-related changes to host plant canopies affect *A. jaegerianus* survival, and represent an indirect negative effect of long-term drought on *A. jaegerianus* populations.

Key Words: Endangered species, Lane Mountain milkvetch, Mojave Desert, plant-plant interactions, precipitation.

The Lane Mountain milkvetch, *Astragalus jaegerianus* Munz (Fabaceae), is a narrowly endemic plant that exists in small fragmented populations restricted to granite outcrops in the central Mojave Desert. Approximately two-thirds of all known *A. jaegerianus* populations occur within the boundaries of the U.S. Army's National Training Center at Fort Irwin, approximately 50 km NE of Barstow, CA (Charis Professional Services Corp. 2002). Because of its limited distribution and potential threat from military training, the U.S. Fish and Wildlife Service (USFWS 1998) listed *A. jaegerianus* as a federally endangered species in 1998.

A weak-stemmed herbaceous perennial, *A. jaegerianus* grows within the canopy of common desert shrubs such as *Thamnosma montana* Torr. & Frèm., *Ambrosia dumosa* (A. Gray) Payne, and *Eriogonum fasciculatum* Benth. The relationship between *A. jaegerianus* and its host shrubs is not clear. It is likely to be complicated because positive and negative effects of host plants on protégé occur simultaneously (Holmgren et al. 1997), and may change with host or protégé life stage (Shumway 2000; Miriti 2006; Reisman-Berman 2007). While adult *A. jaegerianus* are certainly dependent on host shrubs for structural support, host shrub canopies may provide *A. jaegerianus* with protection from herbivores (Gibson et al. 1998), as well as a modified microclimate conducive to growth and recruitment of seedlings (Charis Professional Services Corp. 2002; Sharifi et al. 2009). Because of their proximity, *A. jaegerianus* and its host shrubs share water resources, but the degree to which they compete when water resources are limited is unknown. Although the relationship between *A. jaegerianus* and its host plants is likely to be antagonistic in some respects (e.g., competition for water or nutrients), in other respects host plants may benefit from the increased soil nitrogen associated with *A. jaegerianus* nitrogen fixation (Gibson et al. 1998).

For the past eleven years, this historically rare plant has undergone alarming population contractions. Since 1999, monitored *A. jaegerianus* populations have consistently declined, and in 2009 were less than 12% of their size in 1999 (Rundel et al. 2009). A number of subpopulations have dropped to critically low levels, and are in danger of local extinction (Rundel et al. 2009). This population decline has occurred simultaneously with recent severe drought conditions in the Mojave Desert (Hamerlynck and McAuliffe 2008) caused by regional climate patterns as well as global climate change processes (Hoerling and Kumar 2003). Drought in the Mojave began in 1999 and is predicted to continue for decades (Hereford et al. 2006), or may continue indefinitely under warmer temperature conditions

projected by global climate change-type drought (Cook et al. 2004; Breshears et al. 2005).

Drought conditions may have direct and indirect negative affects on *A. jaegerianus*. Laboratory studies suggest that frequent, above average winter precipitation is critical for seedling establishment and survival of *A. jaegerianus* (Rundel et al. 2005, 2006). These findings are supported by field studies that showed a small increase in *A. jaegerianus* seedling survival in 2005, an unusually wet year in the Mojave Desert. Drought conditions may also indirectly affect *A. jaegerianus*, by killing or degrading its host shrub. Recent drought conditions have led to unusually high shrub mortality and canopy dieback in the Mojave Desert and other parts of the arid southwest U.S. (Bowers 2005; Miriti et al. 2007; Hamerlynck and McAuliffe 2008; Hamerlynck and Huxman 2009). The deterioration of a host shrub's canopy due to drought should negatively affect *A. jaegerianus*, because shrub canopies provide shade, which affects the microclimate and water availability within and below shrubs (Valiente-Banuet et al. 1991; Nolasco et al. 1997; Shumway 2000; Flores et al. 2004; Barchuk et al. 2005) where *A. jaegerianus* carries out the majority of its early development as a seedling, and resprouts every year.

In this study we document the effects of severe long-term drought on the population dynamics of *A. jaegerianus*, evaluate the condition of *A. jaegerianus* host shrub canopies and their effect on sub-canopy microclimate, and investigate the effect of host shrub canopy condition on survival of *A. jaegerianus*.

Methods

The *A. jaegerianus* populations monitored in this study are located in two adjacent geographic areas, the Gemini Conservation Area (GCA, previously referred to as Goldstone) and Brinkman Wash, previously established as discrete areas of *A. jaegerianus* distribution (Prigge et al. 2000; Charis Professional Services Corp. 2002; Walker and Metcalf 2008). Each area contains two study populations. The soils at these population sites are composed of shallow granite alluvium and rocky, granitic outcrops, within the transition zone between Mojavean creosote bush scrub and Joshua tree woodland communities.

Each *A. jaegerianus* study population is located on a 1 ha permanent plot that has either been surveyed since 1999 (Brinkman Wash) or 2003 (GCA). Each year several site visits are conducted to each of these permanent survey plots. During these visits, old, previously tagged plants and their host shrubs are located and searches are conducted for new plants under and around host shrubs and previously tagged *A. jaegerianus* plants. Tagged *A. jaegerianus* plants are assessed to determine if they resprouted (alive) or not (dead or dormant). Seedlings found are measured for height and number of leaves and are revisited on subsequent surveys to monitor their development and survival. For all *A. jaegerianus* plants found, UTM coordinates are recorded using a GPS unit and readings from previous years are updated for accuracy.

During drought, living shrubs may shed foliage as a result of water stress, and to reduce carbon allocation and increase water-use efficiency (Hereford et al. 2006). This process creates defoliated gaps in shrub canopies, reducing their capacity to shade sub-canopy microhabitats. In 2009, the dimensions of host shrubs with live or dead *A. jaegerianus* were recorded, and the foliation level of each *A. jaegerianus* host shrub canopy was estimated as a percentage of its total canopy minus dead, defoliated canopy.

In addition to host shrub measurements made during *A. jaegerianus* surveys, shrubs were resurveyed along vegetation transects established in 2000 (Prigge et al. 2000). These shrub transects were located within high-density *A. jaegerianus* populations, but no *A. jaegerianus* occurred within the transects. Measurement of shrub density, frequency, and cover was done by using 2 belt-transects (Mueller-Dombois and Ellenberg 1974). Transects were 24 m long and 2 m wide. Cover was determined by measuring the maximum diameter (d_1) and the diameter (d_2) perpendicular to the maximum and calculating the area for an ellipse (cover = $[d_1/2][d_2/2]\pi$). Shrub volume was determined using an additional height (h) measurement and calculating the volume of an ellipsoid (volume = $4/3\pi d_1 d_2 h$).

To determine the effect of canopy condition on the microclimate within host shrubs, three shrubs with canopies and three recently dead shrubs without leaves but with branches intact were selected for measurement. Light intensity (PFD) was measured using a solar monitor (Licor L1-1776) attached to a quantum sensor (Licor L1-190SB) placed in a horizontal position close to the interior base of the host shrub. Soil surface temperature was measured using a thermometer (Omega HH21) attached to thermocouples placed no more than 1 mm beneath the soil surface at the base of the shrub. Temperature and light measurement were taken on the hour from 6:00 a.m. to 8:00 p.m.

Precipitation data were obtained from the remote automated weather station at Opal Mountain CA (35°09′15″E; 117°10′32″W; 988 m). This weather station is approximately 30 km from monitored Milkvetch sites at a similar elevation. The Opal Mountain data were in near perfect agreement with data collected closer to *A. jaegerianus* populations (Rundel et al. 2006), but has the advantage of being continuous

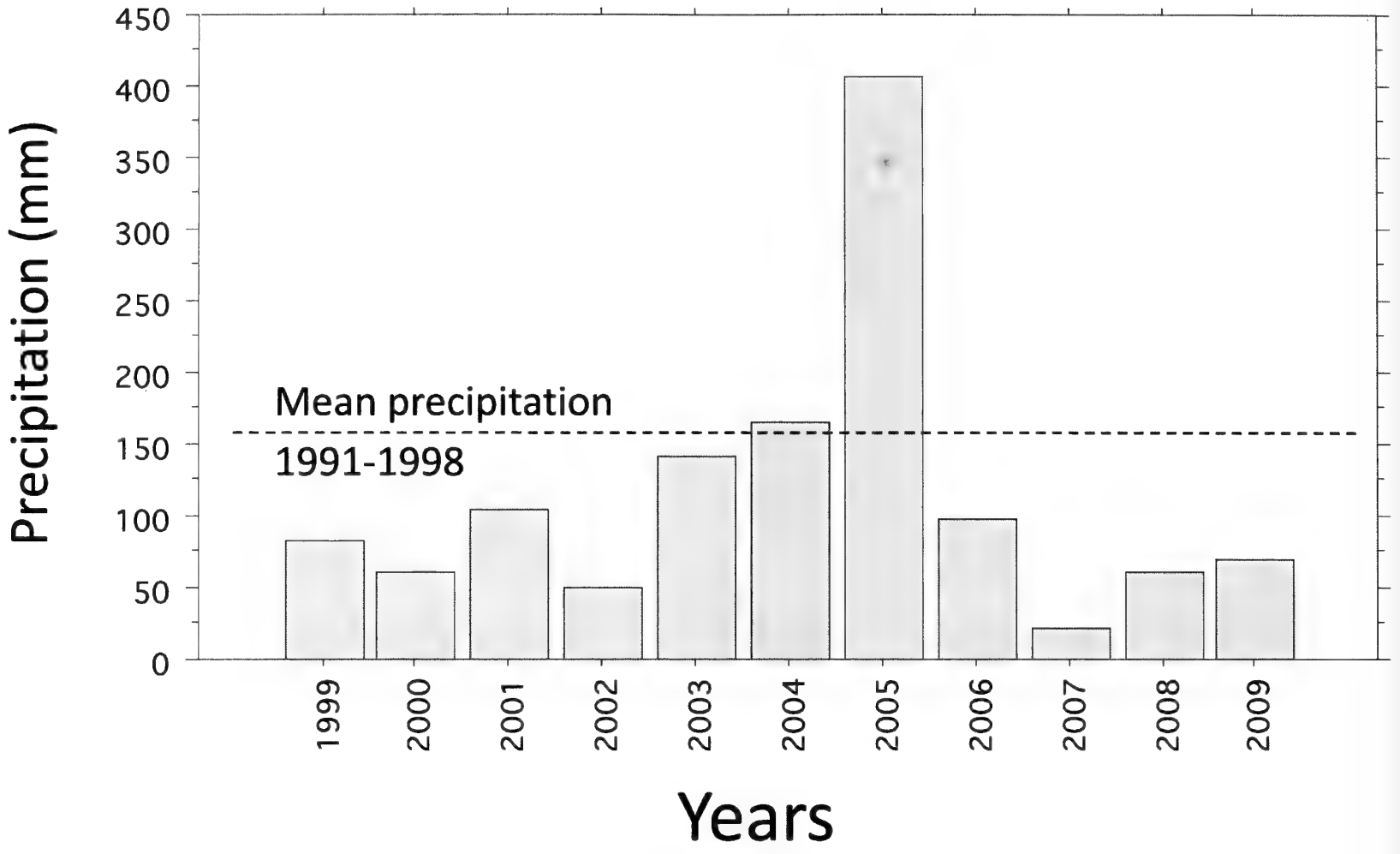

FIG. 1. Annual precipitation (OCT-SEP) from 1999 to 2009 at the remote automated weather station at Opel Mountain, CA (35°09′15″E; 117°10′32″W; 3240 ft). Years refer to the season in which *Astragalus jaegerianus* is reproductive (for example, 1999/2000 is denoted as 2000). This weather station is approximately 18 mi from monitored *A. jaegerianus* sites and at a similar elevation. The dash line is mean precipitation (160.4 mm·yr^{-1}) from 1991 to 1998 at the same location. The mean precipitation during the current drought from 1999 to 2009 was 114.4 mm·yr^{-1}. Weather data archived by the Western Regional Climate Center.

from 1992 to the present. Precipitation from October through September was used because it includes winter and spring rainfall that affects *A. jaegerianus* growth and reproduction. Thus, annual precipitation includes October through December precipitation of the previous year.

Shrub data were analyzed using Statview (SAS Institute Inc., Cary, NC). Nonparametric statistics were used because some shrub data was not normally distributed and resistant to transformation to normality (SAS 1999). Paired sign tests were used to analyze changes in shrub volume and cover. An unpaired t-test was used to compare shrub canopy condition in shrubs that supported live *A. jaegerianus* with shrubs in which *A. jaegerianus* had died since monitoring began in 1999 (Brinkman Wash) and 2003 (GCA).

RESULTS

The current drought began in the fall of 1998 (Hereford et al. 2006) and is in its eleventh year (Fig. 1). Despite an unusually wet 2005 (407 mm), this drought period has a mean precipitation of 114.4 mm·yr^{-1} compared to the relatively wet years preceding it from 1991 to 1998, in which mean precipitation was 160.4 mm·yr^{-1}. These wet years represent the tail end of a wet period from 1976 to 1998 (Hereford et al. 2006) that presumably generated the high *A. jaegerianus* population numbers recorded in 1999. While the difference in precipitation between these wet and dry periods is considerable (46.1 mm·yr^{-1}), the severity of the drought and its impact on *A. jaegerianus* is better appreciated by considering the years before and after 2005; the six-year period between 1999 and 2004 had a mean precipitation of 100.5 mm·yr^{-1}, and the four year period from 2006 to the 2009 had a mean precipitation of 61.9 mm·yr^{-1}. The year 2007 had the lowest precipitation in the 1991 to 2009 Opel Mountain data set (22 mm).

While *A. jaegerianus* continues to decrease in density at our long-term study sites, its decline has slowed and appears to be reaching a plateau (Fig. 2). No *A. jaegerianus* mortality was observed in Brinkman Wash populations in 2009, and GCA populations lost only two *A. jaegerianus*, the lowest absolute decline in seven years of observation. Despite these decreases in mortality, *A. jaegerianus* numbers remain dangerously low. Of the 161 original plants at the four study sites, only 20 remain alive, with zero recruitment of new *A. jaegerianus* plants and 100% seedling mortality since surveys began in 1999 (Brinkman

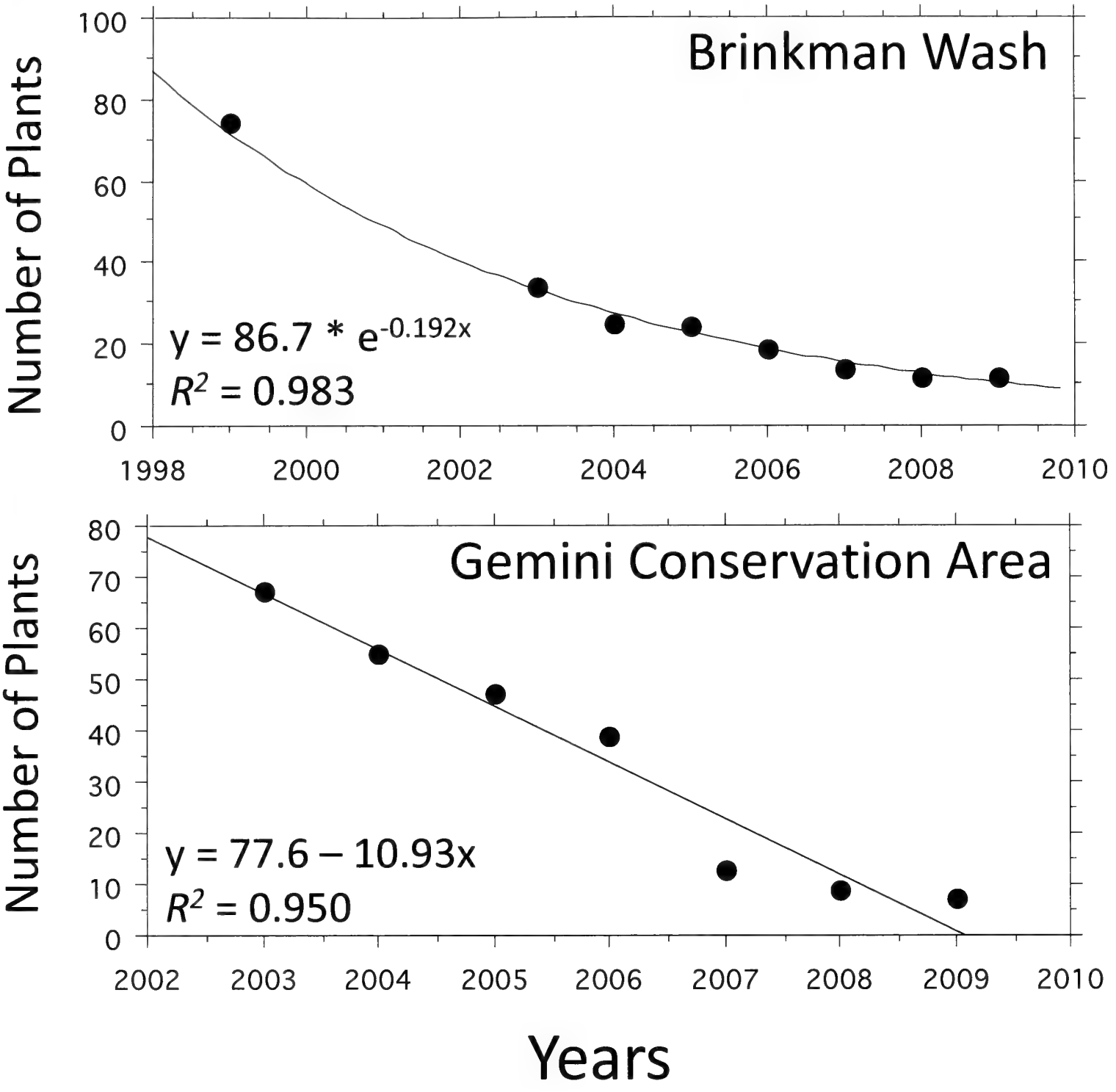

FIG. 2. Population declines of *Astragalus jaegerianus* at two study areas, Brinkman Wash (1999, 2003 through 2009) and Gemini Conservation Area (2003 through 2009). Each study area contains multiple monitored *A. jaegerianus* populations.

Wash) and 2003 (GCA). All monitored populations have dropped to critical levels and are at risk of local extinction. Assuming that the *A. jaegerianus* mortality observed at our long-term study sites is characteristic of the species across its range, the 5723 mature *A. jaegerianus* plants which constituted the plants found in 2001 (Charis Professional Services Corp. 2002) would now number approximately 686 individuals.

One of these populations precariously close to extinction is M2 at Brinkman Wash (Fig. 3). Since 1999, M2 has declined from 23 plants to one remaining plant. However, mortality has not been constant; population decreases were relatively slow between 1999 and 2003, but accelerated between 2003 and 2006 to a loss of 6 plants per year in 2005 and 2006 (Fig. 3). Although 2005 was an unusually wet year with more than twice mean annual precipitation, it appears that even this unusually high rainfall could not diminish the momentum of *A. jaegerianus* mortality. By 2007, population M2 had fallen to one plant that has managed to survive the last three years of intense drought.

Host shrubs populations have declined simultaneously with the decline of *A. jaegerianus* populations. In our shrub transects within *A. jaegerianus* sites, while some shrubs increased in size, total shrub cover and volume have decreased significantly by roughly 10% between 2000 and 2009 (Fig. 4; paired sign test: $P < 0.001$, $n = 75$, for both shrub cover and volume). Mortality of these long-lived shrubs has been high (48%), and the recruitment of new shrubs (5%) has been too low to maintain their populations at previous levels. Among *A. jaegerianus* host shrubs, shrubs

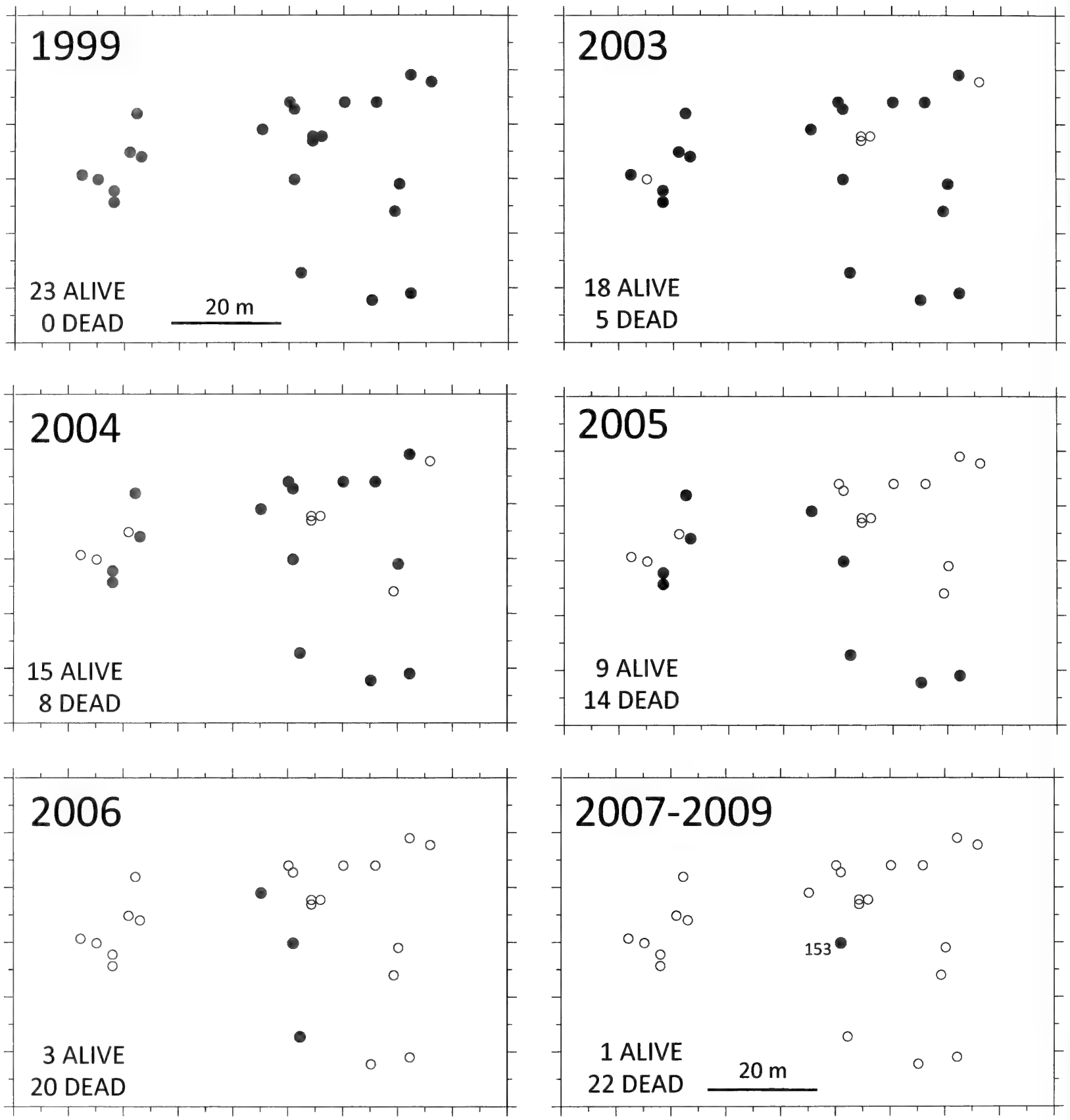

FIG. 3. Aerial view of *Astragalus jaegerianus* population M2 at the Montana Mine site (1999, 2003, 2004, 2005, 2006, 2009). Solid dots are live *A. jaegerianus* plants, and empty dots are dead *A. jaegerianus*. Population M2 decreased from 23 *A. jaegerianus* plants in 1999 to one plant in 2009. No recruitment has been observed at this site during this period. The position of *A. jaegerianus* plants was determined using each plant's UTM coordinates.

with live *A. jaegerianus* have more intact canopies than host shrubs that once supported *A. jaegerianus*, which are now dead (Fig. 5; unpaired t-test: $F_{1,118} = 11.48$; $P = 0.0010$).

Soil surface temperature and light intensity beneath shrubs were dependent on the condition of the shrub's canopy; shrubs with open canopies had light levels five times higher than shrubs with closed canopies, and soil surface temperature beneath shrubs with open canopies were as much as 20°C higher than shrubs with closed canopies (Fig. 6). While most shrubs do not have completely open canopies, among LMMV host shrubs originally surveyed in 1999 and 2003, the average host shrub had only 45 percent of its canopy intact in 2009.

DISCUSSION

Studies in arid and semi-arid environments demonstrate that the shade produced by host plant canopies mitigate severe abiotic conditions

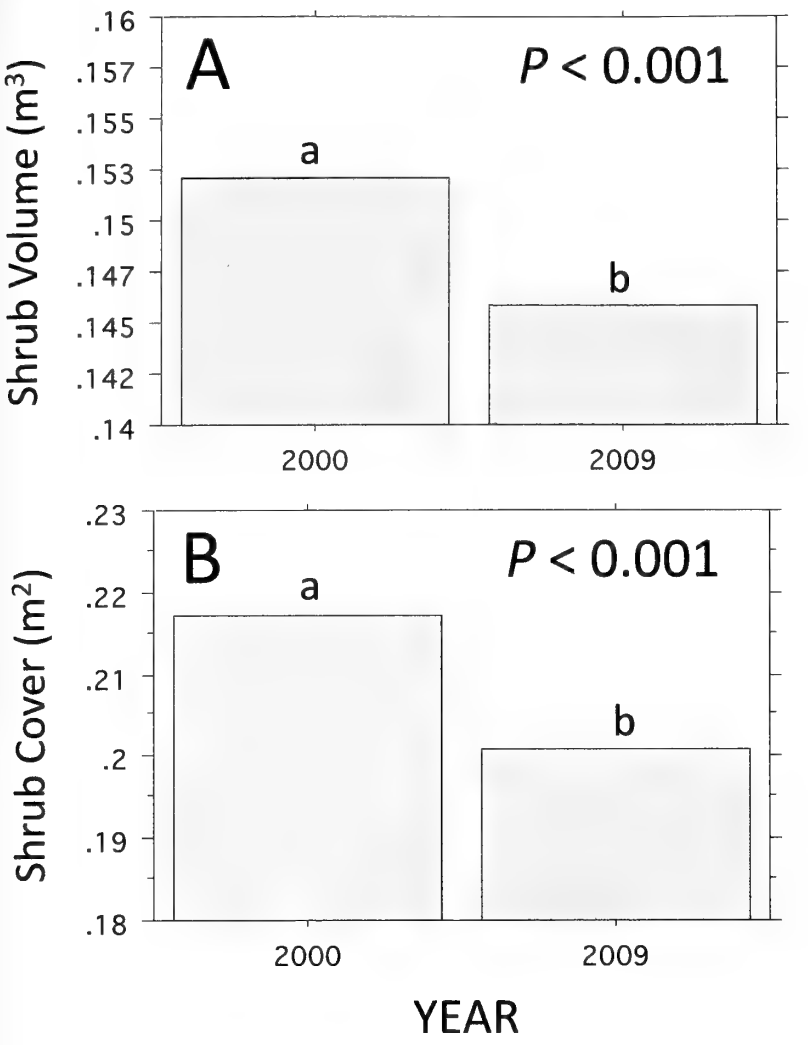

FIG. 4. Changes in shrub size between 2000 and 2009. Both shrub volume and shrub cover deceased significantly in the nine years between censuses. Shrubs were censused along a transect adjacent to *A. jaegerianus* population M1. A. Mean shrub cover measured as an ellipsoid (paired sign test: $P < 0.001$, $n = 75$). B. Mean shrub cover measured as an ellipse (paired sign test: $P < 0.001$, $n = 75$).

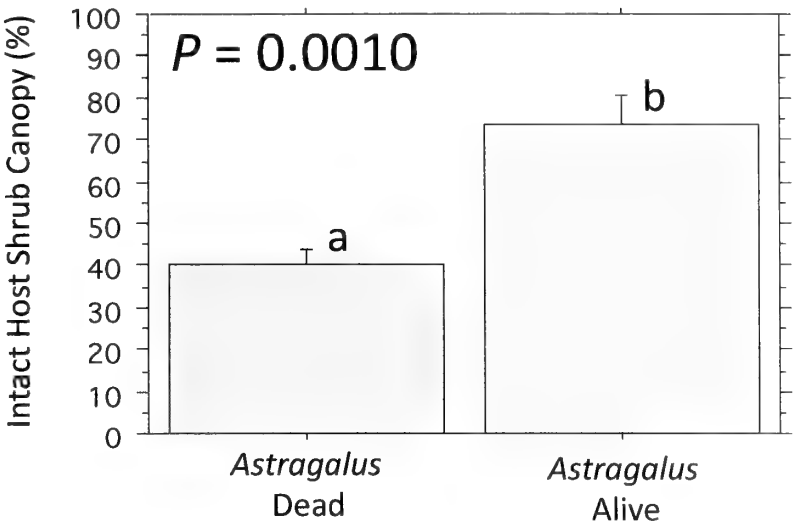

FIG. 5. Percent live host plant canopy and *Astragalus jaegerianus* status, GCA and Brinkman Wash site combined, 2009. *Astragalus jaegerianus* was found in host shrubs with more intact canopies (unpaired t-test: $F_{1,118} = 11.48$; $P = 0.0010$). Intact host shrub canopy condition was estimated as a percentage of total canopy (live plus dead canopy).

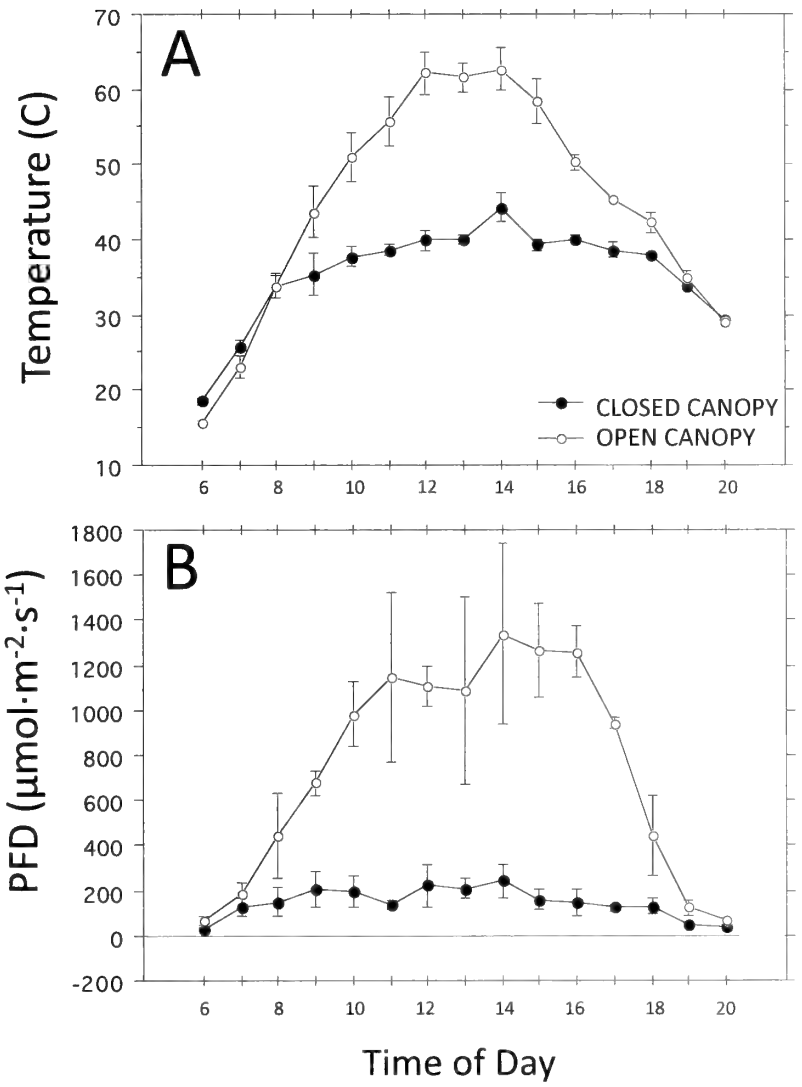

FIG. 6. The effect of *Astragalus jaegerianus* host shrub canopy condition on shrub micro-climate in June 2009 at *A. jaegerianus* population M1, Montana Mine site, Brinkman Wash. A. Soil surface temperature beneath open and closed canopy shrubs (6:00 to 20:00). B. Light intensity (photon flux density, $\mu mol \cdot m^{-2} \cdot s^{-1}$) beneath open and closed canopy shrubs (6:00 to 20:00). Closed circles are measurements recorded under shrubs with closed canopies. Open circles are values recorded under shrubs with open canopies. Points are means with standard errors ($n = 3$).

by reducing air and soil temperature (Franco and Nobel 1989; Valient-Banuet et al. 1991; Paez and Marco 2000; Flores et al. 2004), and increasing soil moisture availability (Nolasco et al. 1997; Shumway 2000; Warnock et al. 2007). Facilitation occurs when microclimate effects such as these increase the establishment and survival of protégé plants growing under host shrub canopies (Cody 1993). Because the facilitative effect of host plants depends on the capacity of its canopy to modify the environment beneath it, changes in canopy structure can affect the facilitative effect of the host plant (Reisman-Berman 2007).

In this study we have documented the drought-induced mortality and canopy deterioration of *A. jaegerianus* host plants, and have demonstrated the effect of host plant canopy foliation on soil temperature and light intensity in sub-canopy, *A. jaegerianus* microhabitat. We have also demonstrated a significant increase in survival of *A. jaegerianus* among host plants with more intact canopies. These results support our study hypothesis that drought-related changes to host plant canopies affect *A. jaegerianus* survival, and represent an indirect negative effect of long-term

drought on *A. jaegerianus* populations. Theory suggests that positive and negative interactions should change along gradients in abiotic stress, with positive interactions dominating under harsh physical conditions where host plants ameliorate abiotic stress (Bertness and Callaway 1994). While a number of studies have demonstrated the positive, facilitative effect of host plants in stressful arid environments (Valiente-Banuet and Ezcurra 1991; Paez and Marco 2000; Pugnaire and Luque 2001; Barchuk et al. 2005; Miriti 2006; Reisman-Berman 2007), to our knowledge, the idea that severe stress associated with long-term drought may diminish host plant facilitation through negative effects on host plant canopies has not been previously documented.

The negative effects of long-term drought on Sonoran, Great Basin, and Mojave Desert perennial plants are well documented (Goldberg and Turner 1986; Turner 1990; Bowers 2005; Hereford et al. 2006; Miriti 2006; Hamerlynck and McAuliffe 2008; Hamerlynck and Huxman 2009; Ralphs and Banks 2009), and are similar to drought effects described in this study for *A. jaegerianus* host shrubs: high shrub mortality, shrub canopy deterioration, and low recruitment. Increases and decreases in mortality associated with fluctuation in interannual precipitation have been reported in other herbaceous desert perennials (*Cryptantha flava* (A. Nelson) Payson, Casper 1996), and the population declines of *A. jaegerianus* fit this general pattern, with the exception of 2005, when adult *A. jaegerianus* mortality continued more or less unaffected by unusually high precipitation (e.g., M2 at Brinkman Wash experienced its highest recorded adult mortality in 2005 and 2006). Seedling establishment also responded weakly to the increase in precipitation in 2005; nine seedlings were established, went dormant through the summer of 2005, and resprouted in 2006. While this was the only observed case of seedling establishment since 1999, 2006 was again a drought year, and these resprouted, second-season plants did not achieve reproductive maturity, and failed to resprout in 2007.

The reason for this insensitivity to increased precipitation in 2005 is unclear, but could be the result of the accumulated damage to host shrub canopies inflicted by long-term drought. The effects of drought on *A. jaegerianus* host plants may proceed rapidly because of positive feedback within the canopy/micro-climate interaction; as shrub canopies deteriorate, evapotranspiration beneath shrubs increases, which increases shrub water stress leading to further canopy deterioration. This positive feedback between shrub canopy and microclimate, and the slow growing nature of desert shrubs may explain why the momentum of *A. jaegerianus* population declines could not be slowed by a single year of high rainfall in 2005.

Episodic recruitment associated with high precipitation has been observed or inferred from demographic analysis in a number of desert perennials (Shreve 1917; Barbour 1969; Sheps 1973; Jordan and Nobel 1979, 1982; Goldberg and Turner 1986; Turner 1990; Parker 1993; Bowers 1995; McDaniel et al. 2000; Godinez-Alvarez et al. 2003). We have previously hypothesized that pulses in high annual precipitation, such as those associated with ENSO events, drive *A. jaegerianus* recruitment, and between high recruitment years mortality occurs in a more or less constant manor (Sharifi et al. 2009). Consistent with this pulse model is the expectation that *A. jaegerianus* recruitment and mortality should be sensitive to years with high rainfall, and recruitment should increase during high rainfall years; but continued adult mortality and low recruitment through an unusually wet year like 2005 suggests that *A. jaegerianus* recruitment is likely to be gradual, and may occur during long-term wet periods. Long-term wet periods in the Mojave Desert occur more or less regularly, and are associated with the Pacific Decadal Oscillation that causes decadal-scale variability such as prolonged dry and wet episodes (Hereford et al. 2006). Prolonged wet periods in the Mojave Desert, such as 23 yr wet period between 1976 and 1998, may positively affect *A. jaegerianus* population growth factors that are relatively insensitive to short-term precipitation such as the condition of slow-growing host shrub canopies. Similarly, dry periods result in the deterioration of host plant canopies, which diminishes *A. jaegerianus* recruitment even during years of high precipitation such as 2005. An expectation of this climate-period model is that the sensitivity of recruitment to precipitation is dependent on the climatic context in which precipitation occurs: recruitment sensitivity is high during prolonged wet periods and low during dry periods. Given these hypothetical circumstances, *A. jaegerianus* populations would tend to oscillate between multi-decadal, high and low population states that are determined by long-term precipitation patterns characteristic of climate-periods.

Although adult *A. jaegerianus* mortality has occurred each year since observations began in 1999, mortality has slowed and stopped in some populations. In population M2 (Fig. 3), a single remaining LMMV has survived alone for three years despite the intense drought (mean precipitation 50 $mm \cdot yr^{-1}$, 2007–2009, Fig. 1). *Astragalus jaegerianus* it thought to be deep-rooted, and this drought-resistant plant may have access to deeper or more reliable sources of water in its fractured granite substrate, and thus better water relations, than *A. jaegerianus* that died earlier in the drought. This idea assumes that soil water resources are heterogeneous, and that only the most consistent water resources are able to

maintain *A. jaegerianus* after prolonged drought. Reduced but persistent populations of *A. jaegerianus* are consistent with expectations of the climate-period model described above.

Our previous studies have shown that *A. jaegerianus* seed density is low to extremely low in the soil seed band compared to other desert shrubs (Rundel et al. 2009; Rundel and Gibson 1996), and seed dispersal beyond host shrub canopies is rare (Rundel et al. 2009). For *A. jaegerianus*, these are grim ecological circumstances: as its host shrubs deteriorate and die, and without the ability to disperse to other host shrubs, its recovery to 1999 populations levels in the immediate future is unlikely. If our climate-period model is correct, and surviving, drought resistant *A. jaegerianus* have access to deep, reliable water sources, drought-reduced populations could persist until the current drought is over, and then expand under wetter climatic conditions. However, if drought conditions continue, it is equally possible that *A. jaegerianus* numbers may erode further, leaving most, if not all populations in eminent danger of local extinction. Unfortunately, regional climate indicators suggest that the Mojave Desert may remain dry for 1 to 2 decades or longer (Breshears et al. 2005; Hereford et al. 2006). In anticipation of prolonged drought, efforts should be made to preserve the *A. jaegerianus* as a unique and rare component of the Mojave Desert flora. These efforts should focus on habitat preservation, experimental repopulation of endangered or extinct subpopulations, and further investigation into the effects of drought on facilitative interactions between *A. jaegerianus* and its host shrubs.

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A RESURRECTION FOR SISKIYOU BELLS, *PROSARTES PARVIFOLIA* (LILIACEAE), A RARE SISKIYOU MOUNTAINS ENDEMIC

MICHAEL MESLER, ROBIN BENCIE, AND BIANCA HAYASHI
Department of Biological Sciences, Humboldt State University, Arcata, CA 95521
mrm1@humboldt.edu

ABSTRACT

We conducted a study of *Prosartes parvifolia* S. Watson, a rare Siskiyou Mountains endemic, currently known from only 15 sites in Del Norte Co., California, and Curry and Josephine counties, Oregon. We found that *P. parvifolia* is (a) fertile, (b) probably not of hybrid origin, and (c) distinct and worthy of recognition as a species. Unlike congeners, its flowers produce ovaries with a single locule, and are pollinated by bees that buzz pollen from connivent anthers. Nectar is not produced. We provide an expanded description, illustrations, and distribution map for *P. parvifolia* as well as a key to the *Prosartes* of northwestern California and southwestern Oregon.

Key Words: Liliaceae, *Prosartes parvifolia*, rare plant, Siskiyou Mountains.

Prosartes D. Don is small genus of North American reticulate-veined Liliaceae formerly treated as part of the Asian genus *Disporum* (Jones 1951; Utech et al. 1995). Sereno Watson (1880) described *P. parvifolia* S. Watson, a species with small campanulate flowers from the Siskiyou Mountains. Although Howell (1903), Jepson (1909) and Peck (1961) accepted *P. parvifolia* as distinct from *P. hookeri* Torr. and *P. smithii* (Hook.) Utech, Shinwari & Kawano, other authors have regarded it as either a sterile hybrid between these two more widely distributed taxa (Jones 1951; Munz 1959) or as a minor variant of *P. hookeri* (McNeal 1993; Utech 2002). A recent discovery of a small population near Bear Basin Butte in Del Norte Co., California, prompted a re-examination of the taxonomic status of *P. parviflora*. Here we provide an expanded description of the species, and show that it is fertile, clearly distinct from the other five members of the genus, and probably quite rare.

TAXONOMIC STATUS

Jones (1951) regarded *Prosartes parvifolia* as a probable hybrid between *P. hookeri* and *P. smithii* because "it occurs in an area where these overlap, it is morphologically intermediate between them, and it is sterile". In contrast, Utech (2002) argued that the "the known variation in *P. hookeri* unquestionably encompasses the morphology described for *P. parvifolia*." However, *P. parvifolia* is not intermediate between *P. hookeri* and *P. smithii*, nor does it combine the traits of these two species in the mosaic-like fashion expected of a later-generation recombinant (Figs. 1, 2; Table 1; see next section). Moreover, flowers produce well-formed, apparently viable pollen, and although the ovaries of some flowers are abortive, we have observed fruits with fully filled seeds as well as seedlings at several sites. Thus, a hybrid origin seems unlikely. Although *P. parvifolia* resembles *P. hookeri* vegetatively, the two species differ consistently for several qualitative characters (Fig. 2, Table 1). In addition, they are sympatric at several sites without intergradation.

FLORAL MORPHOLOGY, POLLINATION BIOLOGY, AND RELATIONSHIPS

Prosartes parvifolia differs from other *Prosartes* in floral morphology and pollinator reward. Species in the genus form three groups based on floral plan. (1) *P. hookeri*, *P. languinosa* (Michx.) D. Don, *P. maculata* (Buckley) A. Gray, and *P. trachycarpa* S. Watson have more-or-less spreading tepals comprising a turbinate to open perianth and long filaments with exposed anthers well separated from the style and stigma (Fig. 2D). The base of each tepal is nectariferous and deeply concave. (2) The tepals of *P. smithii* likewise produce nectar at their concave bases, but the tepals are erect and reflexed only at the tip, forming a more-or-less cylindrical perianth with a narrow opening. The erect filaments position the anthers inside the perianth tube just below the stigmas (Fig. 2G). (3) The tepals of *P. parvifolia* form a campanulate perianth (Fig. 2A). In contrast to the other five species, the tepals are not strongly concave at the base, and do not produce nectar. The bases are flat or shallowly concave with a lustrous green patch contrasting sharply with the white perianth. The filaments are short and erect, and the anthers form a loose cone around the base of the style, well below the stigma (Fig. 2B). The anthers are introrse.

In northern California, flowers of *P. hookeri* and *P. smithii* are pollinated by bees (mainly *Bombus*) which probe tepal bases for nectar and

FIG. 1. Habit of *Prosartes parvifolia*. French Hill Road, Del Norte Co., California.

collect pollen either passively or actively (Mesler personal observations). Although the shiny green tepal patches of *P. parvifolia* resemble nectaries, the flowers offer only pollen as reward. On three separate occasions (in different populations), we witnessed bumblebees buzz pollen from the anther cone as in sympatric Ericaceae (*Gaultheria shallon* Pursh, *G. ovatifolia* A. Gray, *Vaccinium ovatum* Pursh) (Mesler personal observations).

Prosartes parvifolia differs most strongly from other *Prosartes* species in gynoecial morphology. The ovary has a single locule (not three) that produces 1 to 4 ovules (usually 2), which are attached near the base of a parietal placenta. The ovules are held erect so that the raphe lies next to the placenta and the micropyle faces down (hypotropous-ventral; see Simpson 2006, Fig. 11.14). In contrast the ovules of *P. hookeri* and *P. smithii* are pendent from the top of an axile placenta; the raphe faces the placenta but the micropyle points up (epitropous-ventral). The ovules of *P. lanuginosa* appear to be likewise epitropous-ventral. The ovules of *P. trachycarpa* and *P. maculata* have been described as horizontal (Jones 1951; Utech 2002), with the micropyle below the funiculus (Jones 1951). An alteration in orientation (horizontal to erect) could convert such a pleurotropous-dorsal organization to the hypotropous-ventral plan seen in *P. parvifolia* (Simpson 2006, Fig. 11.14). The fact that the

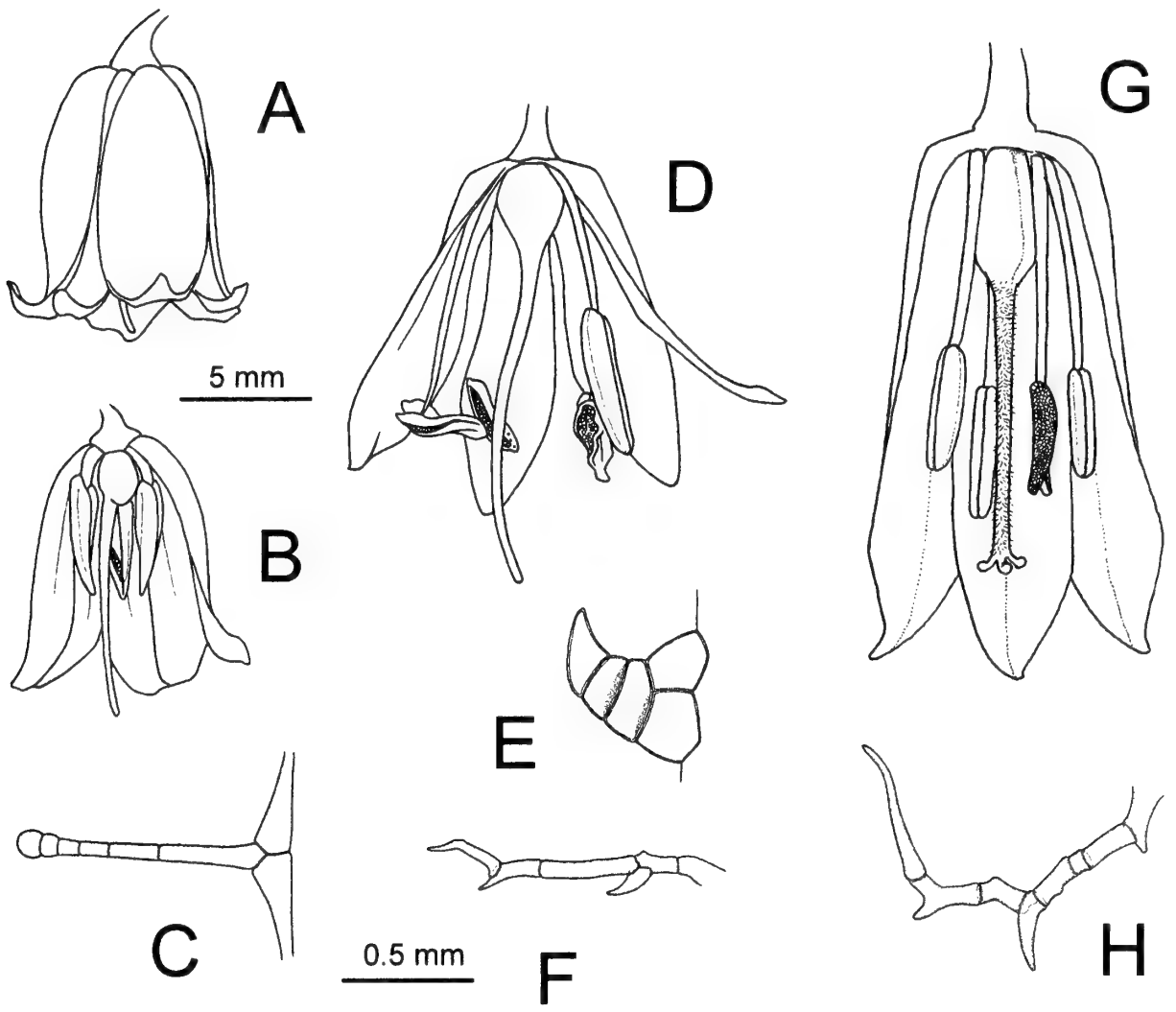

FIG. 2. Comparison of *Prosartes parvifolia* (A–C), *P. hookeri* (D–F), and *P. smithii* (G and H). A, B: intact flower and flower dissection. C: glandular hair from leaf margin. D: flower dissection. E: hair from leaf margin. F: hair from upper stem. G: flower dissection. H: hair from upper stem. Hairs and flowers are shown at the same scale.

ovaries of *P. parvifolia* are relatively small and even abortive in some flowers, coupled with routine fruit abortion, probably contributed to the conclusion of some authors (e.g., Abrams 1923; Jones 1951; Munz 1959) that the species is completely sterile.

The fleshy fruits of *P. parvifolia* typically produce two seeds, unlike other species in genus, which (except for *P. lanuginosa*) usually produce at least one seed per ovary locule (Jones 1951; Utech 2002). Developing fruits are strongly asymmetrical and remain slightly so at maturity, with the stylar scar offset from the tip. This asymmetry in conjunction with parietal (vs. central or basal) placentation suggests that the unilocular condition of *P. parvifolia* has resulted from suppression of two of the three original locules as opposed to the loss of septa to form a common chamber. Detailed anatomical and developmental studies will be needed to verify this interpretation.

The strongly divergent floral traits of *P. parvifolia* make it difficult to assess its relationships to other members of the genus, but there is currently no reason to suspect a close affinity with *P. hookeri*, the prior taxonomic connection between the two taxa notwithstanding. The orientation of ovules of *P. maculata* and *P. trachycarpa* and their glandular trichomes provide some hint of relationship with *P. parvifolia*, but resolution of the issue awaits molecular phylogenetic study and determination of chromosome number.

KEY TO PROSARTES OF NORTHWESTERN CALIFORNIA AND SOUTHWESTERN OREGON

The following key allows reliable separation of *Prosartes hookeri*, *P. parvifolia*, and *P. smithii* in northern California and southwestern Oregon. Vestiture traits are especially useful in the field because the diagnostic glandular hairs of *P. parvifolia* are seen easily on both juveniles and adults, and they persist throughout the season. However, these hairs shrink and twist upon drying, making their glandular character obscure on herbarium specimens.

1a. Leaf margins, stems, and pedicels with glandular hairs; filaments <1 mm, much shorter than dehisced anthers; fruits generally with 2 seeds, style scar offset from the apex . . . . . *Prosartes parvifolia*

1b. Leaf margins, stems, and pedicels hairy or not, but lacking glandular hairs; filaments >3 mm, longer than dehisced anthers; fruits generally with >3 seeds, style scar centered at the apex

2a. Leaf margins with numerous short, sharp, forward-pointing hairs; lower blade sur-

TABLE 1. COMPARISON OF *PROSARTES HOOKERI*, *P. PARVIFOLIA*, AND *P. SMITHII*. Based partly on Jones (1951), McNeal (1993), and Utech (2002).

	P. hookeri	*P. parvifolia*	*P. smithii*
Vestiture			
Stem	simple or branched sharp hairs (Fig. 2F)	mix of slender simple glandular hairs + shorter, eglandular clavate hairs	branched sharp hairs (Fig. 2H)
Leaf surfaces	both surfaces scabrous, with numerous short, simple sharp hairs	both surfaces smooth, with simple glandular hairs	both surfaces smooth, upper surface glabrous, lower surface glabrous or with short simple or branched hairs
Leaf margin	short, forward-pointing hairs (Fig. 2E)	slender, spreading, glandular hairs (Fig. 2C)	glabrous or with slender, spreading, simple or branched sharp hairs
Perianth shape	turbinate, tepals spreading from the middle, base narrowed, obtuse	campanulate to narrowly campanulate, tepals recurved at tip, base tapered, acute to obtuse	cylindrical, tepals closely appressed, spreading slightly at tip to form narrow opening, base truncate
Tepals			
Color	pale-green, yellow-green, or white	bright white	cream-white to white
Shape	oblanceolate to elliptical, lower 1/3 to 1/2 deeply folded along midvein	elliptical, base weakly gibbous	oblong-lanceolate, lower 1/5 to 1/4 deeply folded along midvein
Androecium			
Filament orientation and anther position (post-dehiscence)	spreading, anthers held away from style, gen exserted or +/− equal to tepals	erect, anthers included, loosely connivent around lower half of style	erect, anthers included, surrounding upper part of style, immediately below stigma lobes
Filament length	>5 mm, longer than anthers, generally unequal at dehiscence	<1 mm, much shorter than anthers, equal	>5 mm, longer than anthers, equal
Anther shape	oblong to lanceolate, apex tapered with a short, blunt mucro	lanceolate, apex narrowly acute	oblong, apex blunt or notched
Dehiscence	latrorse, anther walls folded back at maturity, often twisted	introrse, anther walls not folded back	latrorse, anther walls folded back
Gynoecium			
Style	exserted or +/− equal to tepals, 3 minute lobes surrounding central depression at apex	exserted or +/− equal to tepals, unlobed, obscurely cleft on one side at apex	included, 3-lobed, each lobe with an obscure adaxial cleft
Ovary x.s.	weakly triangular, vertices rounded	+/− terete to slightly flattened	triangular, vertices acute
Locule number	3	1	3
Ovule number	2/locule	2 (3, 4) [total]	gen >2/locule
Ovule orientation	pendent from top of placenta, micropyle facing up	erect from base of placenta, micropyle facing down	pendent from top half of placenta, micropyle facing up
Pollinator reward	nectar and pollen	pollen only	nectar and pollen
Fruits			
Color	red to orange-red	orange-red	orange to orange-red
x.s. shape	+/− terete	slightly flattened	+/− terete
Position of stylar scar	at tip	offset from tip	at tip
Chromosome number	2n = 18	not known	2n = 16
Geography	widely distributed in the mountains of the Pacific Northwest to the Rockies (disjunct in Michigan), 100–2000 m	probably rare, limited to the Siskiyou Mountains of Del Norte Co., California, and Curry and Josephine Cos., Oregon, 600 to 1525 m	common near the coast, from the San Francisco Bay area to British Columbia, 0–1500 m

face scabrous; stigma unlobed . . . . . *Prosartes hookeri*

2b. Leaf margins glabrous or with slender spreading hairs; lower blade surface smooth; stigma three-lobed . . . *Prosartes smithii*

Revised Description

Prosartes parvifolia S. Watson, Botany of California 2:179. 1880. *Disporum parvifolium* (S. Watson) Torrey. 1888. Bull. Torrey Bot. Club 15: 188.—Type: USA, California, Del Norte Co., between Happy Camp and Waldo, 16 June 1879, *V. Rattan s.n.* (holotype: GH 30030!; isotype: DS 49627!).

Plants 10–75 cm tall, sometimes clumped, from deeply buried, often vertically oriented rhizomes; flowering individuals with 1 to several aerial shoots, each with 1 to 9 spreading (shade) to strongly ascending (sun) main branches, these branched 0 to 4 times. **Stems** densely glandular pubescent, with slender multicellular glandular hairs and shorter, eglandular, clavate hairs; base of stem with 2–4 densely pubescent cataphyll bracts, these sometimes subtending the first or second main branch. Foliage **leaves** sessile; blade broadly ovate to lance-ovate, less often lance-oblong or elliptic, flat (shade) or strongly folded (sun), 1.8–5.3 cm long, 0.6–3.4 cm wide; apex acute to acuminate, base rounded to cordate, symmetrical to slightly oblique, often +/− clasping (especially when subtending major branches), both surfaces with slender, erect multicellular glandular hairs especially along veins; margins flat or undulate (sun), with slender, spreading, multicellular, glandular hairs. **Inflorescences** with 1–4 flowers; pedicels 4–10 mm long, densely pubescent, with multicellular glandular hairs. **Flowers** 8–10 mm long, 6–8 mm wide, pendent, bright white, campanulate to narrowly campanulate; base of perianth tapered; tepals elliptical, broadly concave, apex recurved, acute, base weakly gibbous, shallowly concave abaxially or +/− flat, with a rounded or quadrate shiny dark green patch; outer tepals 9–10 mm long, 3–5 mm wide; inner tepals 9–10 mm long, 2–4 mm wide; stamens subsessile, +/− erect, forming a loose cone around the style; filaments short and broad, 0.4–0.8 mm long, 0.4 mm wide; anthers lanceolate, introrse, basifixed, 3.6–4.3 mm long, 0.8 mm wide, apex narrowly acute, base sagittate; pollen white; style 8–10 mm long, sparsely pubescent or glabrous at base, not centered on apex of ovary, slightly curved, exserted ≤1 mm or slightly included, tip very obscurely cleft on one side; ovary small (sometimes abortive), pubescent to sparsely pubescent at top, 0.9–1.0 mm long, 0.7–1.0 mm wide, weakly angled, asymmetrical (flat or grooved on one side, convex on the other), with one locule and 2 (3) ovules; placenta parietal; ovules erect, hypotropous-ventral. **Fruits** fleshy, orange to orange-red, slightly flattened, not expanded equally around pedicel-style axis; stylar scar displaced to one side of apex, 10–13 mm long, 8–10 mm wide; seeds (1) 2 (3), white, 5.5–6.5 mm long when fresh.

Specimens Examined

CALIFORNIA. **Del Norte Co.**: E base of Hazelview Summit grade, 19 May 1929, *D. Kildale 7874* (CAS); Hazelview Summit, 25 May 1929, *D. Kildale 9176* (CAS); French Hill Road, 21.6 mi from intersection with Hwy 199, cut-over Douglas Fir forest, 30 June 1970, *J. P. Smith and S. Silva 4254* (HSC); Near Bear Basin, herb layer of evergreen conifer forest, 41°48′21.7″, 123°44′11.1″, 22 June 1979, *G. L. Clifton and T. Griswold 5670* (HSC); T17N, R3E, sec 25, FS road 17N05, 1.5 mi N of road 17N04, UTM 435423 E, 4632830 N (NAD 27), elev. 1005 m (3300 ft), steep roadside below *Lithocarpus* and *Pseudotsuga*, 11 June 2006, *M. R. Mesler 615* (HSC); T17N R4E, sec 32, UTM 437032E, 4629798N (NAD 27), along unlabeled logging spur of FS road 17N05, 1.0 mi from intersection with FS road 17N04, about 100 m from the main road, elev. 1067 m (4000 ft), on edge of road and under *Pseudotsuga* and *Chrysolepis*, 10 June 2007, *M. R. Mesler 762* (HSC); T16N, R4E, sec 4, UTM 437109 E, 4629757 N (NAD 27), FS road 16N02, 40 paces beyond spur to Bear Basin Lookout, below road, elev. 1524 m (5000 ft), understory of *Abies concolor/Abies magnifica* forest, 29 June 2006, *M. R. Mesler 623* (HSC); T18N, R3E, sec 1, UTM 434606 E, 4648843 N (NAD 27), elev. 945 m (3100 ft), logging spur running S from FS 4402, 0.1 mi E of county road 316, exposed roadside, 9 September 2006, *M. R. Mesler 633* (HSC); UTM 434606 E, 4648843 N (NAD 27), elev. 945 m (3100 ft), logging road running S of FS 4402, 0.1 mile E of intersection with road 316. 1 September 2006, *Mesler 634* (HSC); T18N, R4E, sec. 9, UTM 438620 E, 4646536 N (NAD 27), elev. 700 m (2300 ft), county road 324, 4.4 mi from its western intersection with Hwy 199, E of Hazelview Summit, 17 May 2007, *Mesler 755* (HSC); 41.826°N, 123.929°W, elev. 733 m (2404 ft), French Hill Road, 7.4 mi from Hwy 199, 29 May 2008, *M. Simpson 3028* (HSC). OREGON. **Curry Co.**: Coast Mountains, 42nd parallel, 13 June 1884, *T. J. Howell* (OSU); Bear Wallow Lookout, 4 June 1932, *L. Leach 3548* (OSU); T40S, R11W, sec 9, UTM 416824 E, 4660822 N (NAD 27), elev. 580 m (1900 ft), at the end of road 330, running S from FS road 1107, 0.8 mi SE of intersection with road 334, 1 September 2006, *M. Mesler 636* (HSC); T40S, R10W, sec. 24, UTM 431559 E, 4657932 N (NAD 27), elev. 1100 m (3600 ft), Buckskin Peak trail, 14 September 2007, *M. Mesler 789* (HSC). **Josephine**

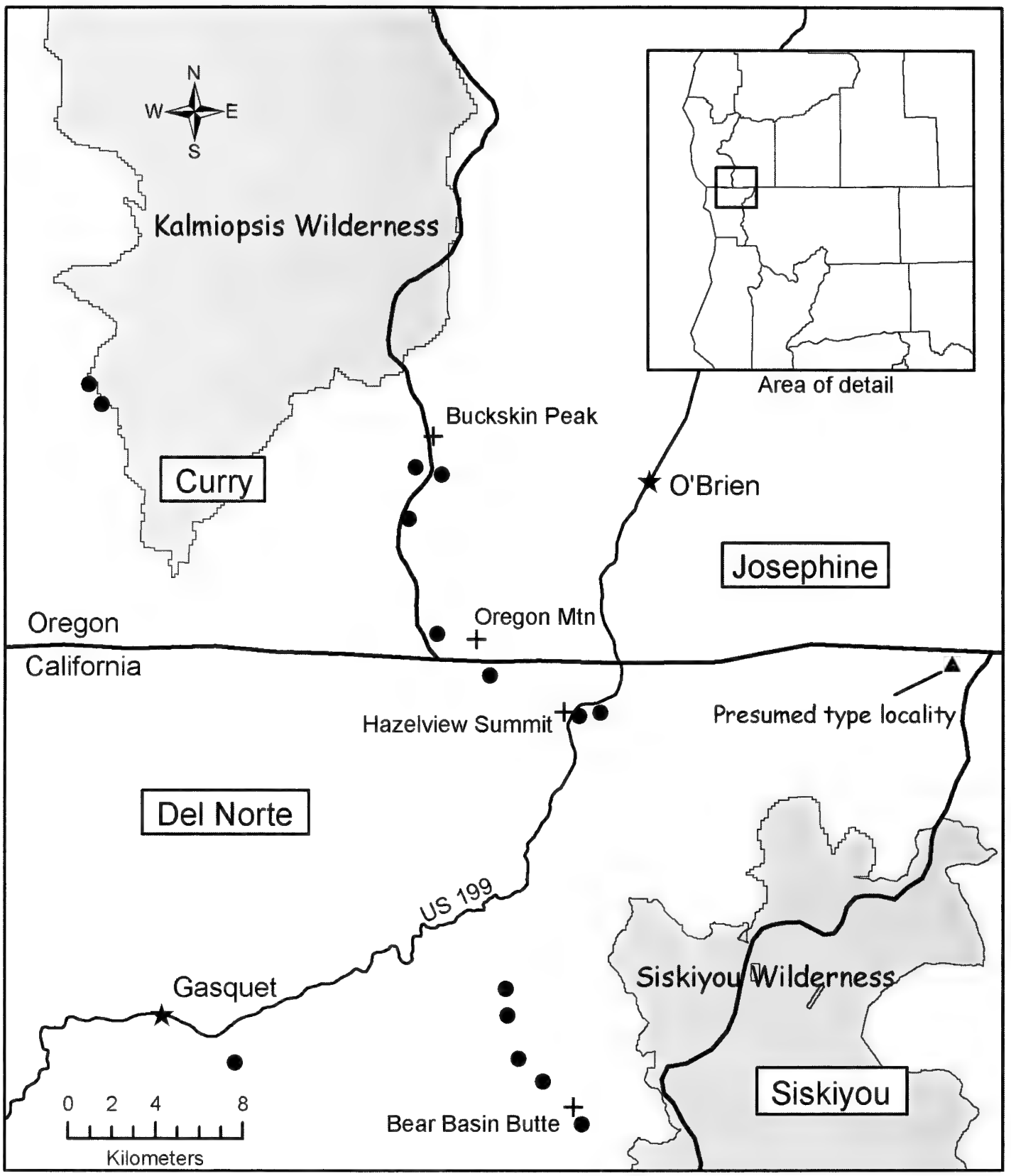

FIG. 3. Distribution of *Prosartes parvifolia*. Points are currently known populations; the diamond shows the estimated position of the type locality.

Co.: Hunter's Camp, between Chetco Ridge trail and Rough and Ready trail, 26 June 1950, *A. Kruckeberg 1972* (OSU); T41S, R10W, sec. 13, UTM 431469 E, 4650295 N (NAD 27), elev. 1070 m (3500 ft), Wimer Rd (FS 4402), 1.0 mi E of intersection of 4402 and 4402.112, 14 September 2007, *M. Mesler 790* (HSC).

DISTRIBUTION AND HABITAT

Prosartes parvifolia is confined almost entirely to the Smith River watershed of the Siskiyou Mountains of northwestern California and southwestern Oregon (Del Norte, Curry, and Josephine counties; Fig. 3). Exceptions are two populations east of the Kalmiopsis Wilderness (Coast Ranges) and one near Buckskin Peak (Illinois River watershed). A putative population, identified by G. J. Muth in 1978 (Flora of Klamath Mountains, unpublished computer-generated checklist, Pacific Union College, Angwin, CA) and located near El Capitan in Siskiyou Co. (*Butler 00026* [PUA]) is *P. hookeri*.

Plants grow on various metamorphic substrates (not ultramafic soils) in shaded forest understories and forest edges as well as on adjacent exposed roadside slopes and at logged and burned sites, at elevations from 600 to

1525 m. The most common tree associate is *Pseudotsuga menziesii* (Mirb.) Franco, occurring in combination with *Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon, & S. Oh at lower elevations and *Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr. var. *lowiana* (Gordon & Glend.) Lemmon and *A. magnifica* A. Murray at higher elevations. Other common associates are *Chrysolepis chrysophylla* (Douglas ex Hook.) Hjelmq., *Gaultheria shallon* Pursh, *G. ovatifolia* A. Gray, *Mahonia repens* (Lindl.) G. Don, *Quercus sadleriana* R. Br. ter, and *Rhododendron macrophyllum* D. Don ex G. Don.

History of Collection and Rarity

Prior to our study, *Prosartes parvifolia* had been collected only eight times. The type collection was made by Volney Rattan in 1879 along the road connecting Happy Camp, California, and Waldo, Oregon. The species was collected five years later by Thomas Howell, probably in the same general area, and then again by Lilla Leach and Doris Kildale Niles in 1929 and 1932, respectively. Each of these inveterate explorers of the Siskiyou Mountains collected the species from just a single locality or pair of closely spaced localities. The most recent collection was made near Oregon Mountain in 1998 by Veva Stansell, who reports having encountered it only once over many years of exploration (local botanist, personal communication).

Prosartes parvifolia qualifies as rare, at least by virtue of its very narrow geographical distribution. Currently it is known from only 15 locations spread over an area of about 525 km^2; the most distant pair of sites is separated by only 40 km (Fig. 3). We have re-discovered all of the historical collection areas with the exception of the type locality and a site visited by Kruckeberg in 1950 (see Specimens Examined, Josephine Co., OR) on the east side of the Kalmiopsis Wilderness that probably lies slightly north of populations we found near Buckskin Peak. Based on field reconnaissance in 2006–2009, we estimate fewer than 500 reproductive-age individuals across the 15 known sites. Our estimates may be conservative since a good deal of the roadless, rugged terrain in the Siskyou/Klamath region remains poorly explored botanically (J. Sawyer, Humboldt State Univ., personal communication). Nevertheless, if such a distinctive taxon were truly abundant, we believe the many avid botanists who have worked in the area would have encountered it much more commonly.

The factors responsible for the apparent rarity of *P. parvifolia* are unknown. The species is not a strong habitat specialist. It occurs across a wide range of elevations on a variety of relatively productive substrates in association with varying mixes of trees, in both shade and sun. The same habitat settings are common throughout the Klamath and adjoining Coast Range Mountains. Logging and road construction may have contributed to population declines, but the paucity of early collections suggests that the species may have been rare historically. The largest, most floriferous plants grow on otherwise bare mineral substrate along road cuts, and the largest known population occupies a recently cut and burned Douglas fir forest. Pollination deficits might be expected given small population sizes, but we have found isolated individuals with heavy fruit crops. The major threats facing *P. parvifolia* appear to be its limited distribution and small population sizes.

Acknowledgments

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SEDUM VALENS (CRASSULACEAE), A NEW SPECIES FROM THE SALMON RIVER CANYON OF IDAHO

CURTIS R. BJÖRK
Stillinger Herbarium, University of Idaho, Moscow, ID 83843
crbjork@gmail.com

ABSTRACT

Sedum valens (Crassulaceae) is described from the Salmon River Canyon of central Idaho. Though it shares numerous morphological traits with *Sedum borschii* and *S. leibergii*, the species differs strikingly in having myriad leaves packed into rosettes as wide as 1 dm. The leaves are ciliate, a characteristic otherwise unknown in temperate North American *Sedum*, except in *Sedum radiatum*, a highly dissimilar species. Further distinguishing characteristics are found in leaf shape, phenology, fruit characteristics and in habitat.

Key Words: Crassulaceae, Idaho, Salmon River Canyon, *Sedum*.

In the northwestern United States, the genus *Sedum* L. (Crassulaceae) includes 20 native taxa as circumscribed by Clausen (1975), including 5 taxa endemic to the region: *S. borschii* (R. T. Clausen) R. T. Clausen, *S. lanceolatum* Torr. var. *nesioticum* (Jones) Hitchc., *S. leibergii* Britt., *S. moranii* R. T. Clausen, and *S. rupicolum* Jones. Clausen (1975) indentified a distinct evolutionary lineage involving *S. borschii* and *S. leibergii*, along with the California and Oregon endemic *S. radiatum* S. Wats. and the more widespread *S. stenopetalum* Pursh. This group is characterized by open, obpyramidal, cymose inflorescences of yellow flowers, widely divergent fruit follicles and observed patterns of interspecies hybrid fertility. A group of populations in the Salmon River Canyon system (hereby referred to as **Sedum valens**) appears to belong to this lineage, sharing its morphological distinctions while at the same time bearing consistent differences from all other species. Within this group, **S. valens** appears to be closest to *S. borschii* and *S. leibergii*, sharing their papillate leaves, variable numbers of flower parts, and glandular-punctate follicles.

TAXONOMY

Sedum valens Björk, sp. nov. (Fig. 1).—Type: UNITED STATES, Idaho, Idaho Co., Salmon River Canyon, 16.5 air km E of Riggins, 900 m W of the junction of Elkhorn Creek and the Salmon River, elev. 609 m, on granite and granitic sand on steep canyon walls, growing with *Pinus ponderosa* Dougl., *Pseudotsuga menziesii* (Mirbel) Franco, *Holodiscus discolor* (Pursh) Maxim., *Philadelphus lewisii* Pursh, *Selaginella douglasii* (Hook. & Grey) Spring, *Micranthes occidentalis* (S. Wats.) Small, *Glossopetalon spinescens* A. Gray, *Heuchera grossulariifolia* Rydb., and *Cystopteris fragilis* (L.) Bernh. 45°24′N, 116°6′W, 3 December 2003, *C. R. Björk 8008* (holotype: ID, isotype: WS).

Paratypes: USA. IDAHO. **Idaho Co.**: Salmon River Canyon, 200 m E of the mouth of French Creek, 45°25′N 116°1′W, elev. 616 m, *C. R. Björk 8007* (ID); Salmon River Canyon, 2.5 km NNW of the Salmon River on west slopes above the Wind River, 45°28′N 115°56′W, elev. 840 m, *C. R. Björk 8006* (ID); Salmon River Canyon, 45°26′N 115°57′W, elev. 614 m, *C. R. Björk 8005* (ID); Salmon River Canyon, 45°25′N 115°59′W, elev. 624 m, *C. R. Björk 8004* (ID).

Herba biennis, folliis plurimus et ciliaris, prolificae vegitativa praecox maturescens, floribus multus aureus, inflorescentia ramosa, foliiculo glandulosi divergens.

Biennial, light green or yellowish green herb. Basal rosettes 3–10 cm wide, with leaves numerous (87–188). Rosette offshoots maturing and detaching by the time of anthesis of the flowering rosettes. Leaves narrowly oblanceolate, (8–) 16–38 × 2–4.4 mm (measurements of largest rosette leaves on dried, pressed specimens of flowering rosettes), strongly flattened dorsiventrally, the blade weakly differentiated, strongly papillate with marginal papillae on the proximal 2/3 of the leaf conspicuously lengthened, forming unicellular cilia up to 1.3 mm long. Inflorescences erect, much-branched, the peduncle 70–115 mm tall. Flowers numerous per inflorescence (36–139). Petals yellow, 3.8–6.3 × 1.2–2.2 mm (measurements from dried, pressed petals). Follicles widely divergent, 4.0–7.7 mm long, 1.5–2.8 mm tall, glandular-punctate. Flowering in April to May.

No other temperate North American *Sedum* taxon is known to produce cilia except *S. radiatum*, a highly dissimilar non-rosettiform species, and the papillate condition is found in only a few species (Clausen 1975). While rosette width varies greatly in *S. valens*, the only other

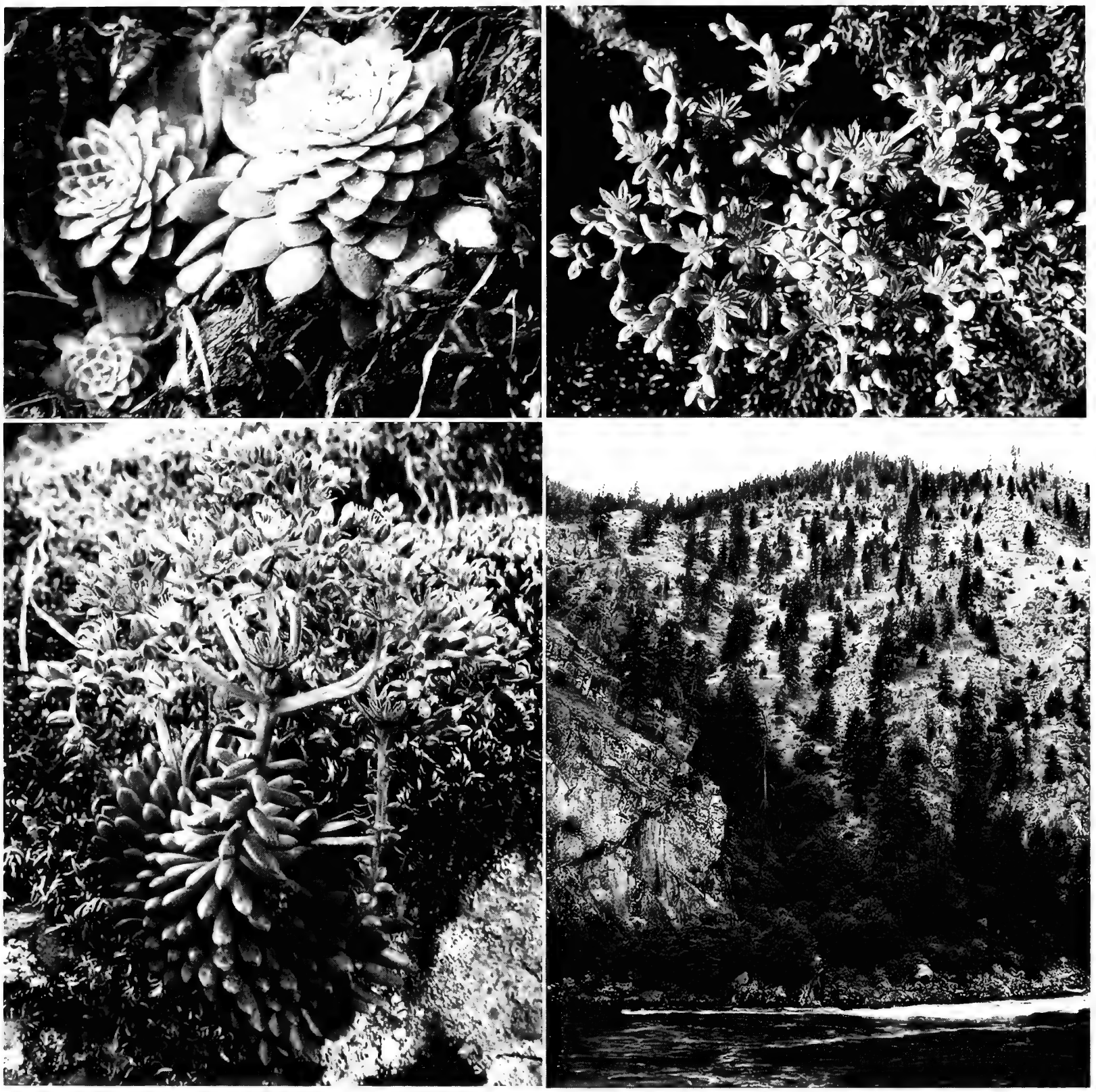

FIG. 1. *Sedum valens*. Upper left: young rosettes in June; upper right: inflorescence seen from above; lower left: habit in flower, the rosette is approximately 1 dm wide; lower right: habitat in the Snake River Canyon, showing the cliffy and woodland habitats of *S. valens*.

western North American *Sedum* capable of producing rosettes as large as its maximum width are *S. albomarginatum* R. T. Clausen, which is endemic to the Feather River Canyon of California (Denton 1993), and *S. oregonense* (S. Watson) Peck of western Oregon and northwestern California. Both of these species differ greatly from *S. valens* in reproductive and vegetative morphology. Additionally, no other North American *Sedum* produces rosettes bearing a number of leaves approaching that found in *S. valens*.

Leaf and rosette characters of *S. valens* are the most striking morphological distinctions from *S. leibergii*, *S. borschii* and all other *Sedum*. Individual plants of *S. valens* are remarkable for their large rosettes (to 10 cm across), formed by a maximum of nearly 200 narrowly oblanceolate leaves. Rosettes of *S. leibergiii* are smaller, rarely reaching 5 cm wide, and are formed by no more than 40 leaves. Leaf shape of these two species is unusual among North American *Sedum* in being both several times longer than wide, and widest near the apex. Leaves of *S. valens* are strongly flattened dorsiventrally and lack a distinct blade, while those of *S. leibergii* are broadly elliptical to terete in blade cross-section, and are spatulate with a well defined blade. Leaves of *S. valens* are more strongly papillate, and the marginal papillae on the proximal 2/3 of the leaf are lengthened

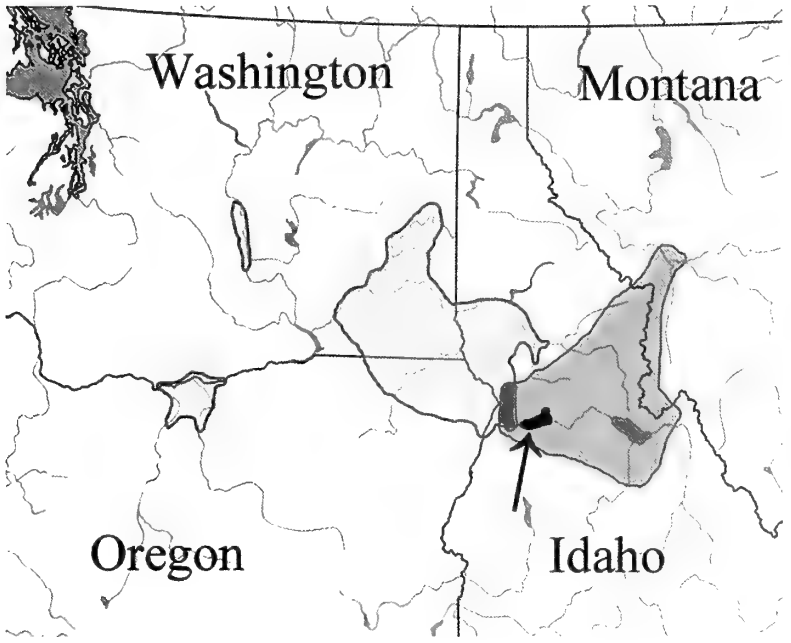

FIG. 2. Map of the ranges of *Sedum leibergii* (light gray with outlines, western), *Sedum borschii* (medium gray with outlines, eastern), and *Sedum valens* (black). The arrow points to the type locality of *S. valens*. The dark gray areas with outlines in the range of *S. borschii* indicate the region of overlap in the ranges of *S. leibergii* and *S. borschii*.

conspicuously, forming unicellular cilia as long as 1.3 mm. Leaves of *S. leibergii* lack cilia. Otherwise among North American *Sedum*, only *S. radiatum* has distinctly ciliate leaves (Ohba 2009), but that species differs from *S. valens* in lacking basal rosettes and non-papillate fruit follicoles. *Sedum borschii* produces rosettes up to only 2 cm wide, formed of no more than 15 leaves, and its leaves are ovate, elliptical or lanceolate. The papillae of *S. borschii* occur mostly on the leaf margins and apex, but they are inconspicuous and never lengthen into cilia.

Sedum valens inflorescences are larger than those of either *S. leibergii* or *S. borschii*. They are taller, though this is due only to branch length, not to stem length, which is roughly the same as those of *S. borschii* and *S. leibergii* (Table 1). Flower number and degrees of division in the cymes are greater than in *S. leibergii* or *S. borschii*. The size and number of flower parts do not differ significantly. Follicle dimensions of *S. valens* are greater than those of *S. leibergii*, and they are longer but equally wide to those of *S. borschii*.

Phenological differences also distinguish *S. valens* from *S. leibergii* and *S. borschii*. *Sedum valens* produces offshoot rosettes that detach around the time of flowering, producing independent clones with fully formed, surficial rosettes that do not contract in the summer. *Sedum leibergii* also produces offshoots prior to flowering, but they remain attached to the parent rosette well after flowering, and often through winter. These offshoot buds do not form mature, leafy rosettes until late winter or early spring of the following year. Prior to that time, they remain pale, turion-like and subsurficial in moss mats. In winter, the old, senesced rosettes and flowering stems of the previous summer almost always bear at least one offshoot, while old stems of *S. valens* never bear attached offshoots. *Sedum borschii* produces mature offshoot rosettes by the time of flowering, but the rosettes remain permanently attached. This gives *S. borschii* a suffruticose growth form.

The known population of *S. valens* occurs on siliceous rock of the Idaho Batholith, but at the western end of its distributional range, it extends onto the batholith margins, on contact-metamorphics with calcareous modification. Most *S. borschii* populations are also on granite, while *S. leibergii* is thus far known only on basalt and calcareous rocks. The range of *S. borschii* reaches to within 10 km of *S. valens*, but its populations occur at least 500 m higher in elevation. *Sedum borschii* grows in montane to subalpine woods and rock outcrops, while *S. valens* occupies drier, warmer *Pinus ponderosa*/*Pseudotsuga menziesii* woodlands and canyon scrub communities. *Sedum leibergii* occurs mostly northwest of the range of *S. valens*, but it also occurs disjunctly eastward in Lemhi Co., Idaho (Fig. 2). Specimens of *S. leibergii* from Montana (MONTU, WTU) are misidentified *S. borschii*. *Sedum leibergii* grows at similar elevations, to within about 10 km of *S. valens*, but no overlap in ranges has been observed. *Sedum leibergii* grows in hotter, drier, usually non-forested habitats and

TABLE 1. MEANS AND RANGES OF QUANTITATIVE CHARACTERS IN *SEDUM VALENS*, *S. LEIBERGII* AND *S. BORSCHII*. Measurements obtained from the type, paratype specimens and specimens of the other species as cited under "other specimens examined".

Character	*S. valens*	*S. leibergii*	*S. borschii*
Rosette leaf length (mm)	27.7 (14–62)	17.0 (13–24)	4.9 (2.3–7.5)
Rosette leaf number	122.4 (87–188)	10.6 (12–36)	9.0 (6–14)
Stem length (mm)	88.3 (70–115)	83.0 (50–143)	75.3 (43–105)
Number of cyme divisions	3.2 ([1] 2–5)	1.9 (1–3)	1.1 (1 [2])
Inflorescence width	72.6 (38–118)	44.9 (18–74)	21.3 (9–44)
Flower number	78.7 (36–139)	19.8 (4–44)	6.5 (2–15)
Follicle length	5.5 (4–7.7)	2.8 (2.2–3.4)	3.9 (2.2–5.1)
Follicle width	2.1 (1.5–2.8)	1.0 (0.7–1.2)	2.3 (1.2–3)
Follicle length/width ratio	2.7 (2.2–3.7)	2.9 (2.3–3.7)	1.7 (1.4–2.2)

almost always in moss mats on ledges and in crevices, never in forest understory. *Sedum valens* also often grows in moss mats, but unlike *S. leibergii*, it frequently occupies soils and humus amid woodland understory vegetation.

Ecology

Sedum valens appears to be limited to lower elevations in the Salmon River Canyon and tributary canyons. About half of the observed individuals of *S. valens* occupy duff over granitic sand in woodland understory with *Pinus ponderosa* Dougl., *Pseudotsuga menziesii* (Mirbel) Franco, *Holodiscus discolor* (Pursh) Maxim., *Philadelphus lewisii* Pursh, *Synthyris missourica* (Raf.) Pennell, *Carex geyeri* Boat, *Poa wheeleri* Vasey in Rothr., and *Cystopteris fragilis* (L.) Bernh. The remainder grow on mossy ledges, crevices and cliff faces with *Glossopetalon spinescens* A. Gray, *Heuchera grossulariifolia* Rydb., *Micranthes idahoensis* (Piper) Brouillet & Gornall, *Sedum stenopetalum* Pursh, *Selaginella douglasii* (Hook. & Grev) Spring., and *Woodsia scopulina* D.C. Eaton. In either case, it grows mostly on north- and east-facing slopes. Few individuals occur on south- or west-facing slopes, suggesting that *S. valens* is best adapted to relatively cool, shaded conditions.

The total range of *S. valens* could not be elucidated due to the extremely rugged terrain and nearly impassible slopes upstream from the easternmost populations encountered. The continuance of suitable habitat eastward into these impassible areas suggests that *S. valens* extends beyond the area searched. No individuals were found in apparently suitable habitat in some tributary canyons however, including French, Elkhorn, or Partridge Creeks. *Sedum valens* has been found no higher than 1300 m elev. So far, fewer than 10,000 individual plants have been encountered in the study area. Despite the wilderness status of the potential habitat upstream, *S. valens* may be a priority for conservation given its limited known range, small populations, and its proximity to a well-traveled recreation road. Since the first discovery of *S. valens*, large portions of the population along the road have been destroyed during a road-widening project (Karen Gray personal communication).

Key To *Sedum* Of Idaho (Excluding *Rhodiola*)

1a. Plants rhizomatous, forming dense to loose mats often >20 cm wide; leaves alternate, bright yellow-green, 3–5 × 3–3.5 mm; growing in disturbed sites, introduced. . . . . *Sedum acre*

1b. Plants not or only weakly rhizomatous, not forming mats, though often clustered; leaves alternate or opposite, color various, but not bright yellow-green, if as small as *S. acre*, then opposite; native species, mostly in undisturbed habitats 2

2a. Leaves opposite . . . . . *Sedum debile*

2b. Leaves alternate 3

3a. Mature follicles erect; inflorescences domed; leaves broadest at the base, lacking buds on the flowering stems 4

4a. Leaves of the flowering stems 4–9 (rarely to 20) mm long, ovoid to elliptical, slightly flattened, curving toward the stem; rare, canyons of central Idaho . . . . . *Sedum rupicolum*

4b. Leaves of the flowering stems 7–20 mm long, linear or narrowly lanceolate, terete, not or scarcely curving toward the stem; common throughout the state . . . . . *Sedum lanceolatum var. lanceolatum*

3b. Mature follicles widely spreading; inflorescences obpyramidal; leaves variously shaped, but if broadest near the base, then buds numerous in leaf axils of the flowering stems 5

5a Flowering stems with sterile buds in the leaf axils; leaves keeled, the midrib persistent after the leaf withers . . . . . *Sedum stenopetalum var. stenopetalum*

5b Flowering stems lacking sterile buds in the leaf axils; leaves not keeled, the midribs withering with the leaves 6

6a. Plants suffrutescent; rosette leaves 2.3–7.5 mm long; follicle length/width ratio 1.4–2.2; growing at elevations >1200 m. . . . . *Sedum borschii*

6b. Plants not suffrutescent; rosette leaves 13 mm long or >; follicle length/width ratio at least 2.2; mostly growing at elevations <1000 m 7

7a. Rosettes with 12–36 leaves, contracted and turion-like through the summer drought; leaves subterete, with a distinct blade, never ciliate; not known from granite, never in forest understory, widespread . . . . . *Sedum leibergii*

7b. Rosettes with 87–188 leaves, growing surficially as mature, leafy rosettes through the summer drought; leaves distinctly flattened, without a distinct blade, ciliate; mostly on granite, often in forest understory, Salmon River Canyon . . . . . *Sedum valens*

Other Specimens Examined

Sedum borschii: USA. IDAHO. **Idaho Co.**: Meadow Creek, above Selway Falls, 31 May 1936, *Rollins 1661* (WS); Seven Devils Mountains, 27 June 1961, *Clausen 61.178.8* (ID); Patrick Butte, 22 August, 1980, *Wellner 2215* (ID). **Custer Co.**: Camas Creek drainage, Salmon

River Mts., 23 July 1982, *Henderson 5312* (ID). **Lemhi Co.**: ca. 32 air mi NW of Challis, 9 June 1982, *J. Civille 286* (ID); Bighorn Crags, 1 August 1990, *Moseley 1931* (ID); Warm Spring Creek, 25 July 1980, *S. P. Brunsfeld 1618* (ID). **Valley Co.**: Salmon River area ca. 9 air mi W of Loon Creek Point, 17 June 1982, *Civille 299* (ID). MONTANA. **Ravalli Co.**: Bitterroot Mts., W above N Kootenai Lake, 26 July 1972, *Lackschewitz 3892* (WTU); Bitterroot Mts., above Bass Creek Falls, 21 August 1976, *Lackschewitz 6879* (WTU). **Missoula Co.**: Rattlesnake Valley, 6 km NE of Missoula, October 1942, *F. Rose C42-31* (WTU). *Sedum leibergii*: USA. IDAHO. **Idaho Co.**: Snake River 0.5 mi N of Willow Creek, 19 May 1976, *Henderson 2947* (ID); Hells Canyon above Wild Sheep Rapids, 23 May 1976, *Henderson 3034* (ID); 3/4 mi S along SR Trail from S end of Pittsburg Landing, 13 May 1990, *Loraine 2048* (ID); cliffs above Salmon River, near Lucille, 16 May 1937, *Christ 7280* (ID); rocky cliff, 2 mi up Race Creek, from the mouth, W of Riggins, 29 May 1965, *Baker 16784* (ID); Hells Canyon, mouth of Bernard Creek, 24 May, 1974, *Wellner 131* (ID); Whitebird, *Vaughn 4581* (WS). **Nez Perce Co.**: rocky banks along the Snake River, 4 mi E of Lewiston, 25 May 1957, *Baker 14794* (ID); Lewiston, 26 May 1900, *Hunter 43* (WS); S side Clearwater, 29 May 1937, *Meyer 870* (WS). OREGON. **Crook Co.**: Ochoco NF, Grids Creek Rd., 9 June 2000, *Goff 00-03* (WS). **Wallowa Co.**: Deep Creek, 15 May 1936, *Moore, W.R. 53* (WS). WASHINGTON. **Whitman Co.**: at the head of Rock Lake, 1904, *Beattie 2398* (WS); Almota, 3 June, 1976, *Old s.n.* (WS); Wawawai, 20 June 1901, *Piper s.n.* (WS); Wawawai, 2 December 2004, *Björk 8130* (ID). **Garfield Co.**: Ilia Grade, 17 June 1913, *Darlington s.n.* (WS). **Klickitat Co.**: Rockland, 5 May 1898, *Suksdorf s.n.* (WS). **Yakima Co.**: Rattlesnake Mts., 16 July 1902, *Colton 703* (WS). **Asotin Co.**: 3 mi S of Asotin, 27 May 1944, *Hitchcock C.L. 8362* (WS).

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ABIES MAGNIFICA VAR. CRITCHFIELDII, A NEW CALIFORNIA RED FIR VARIETY FROM THE SIERRA NEVADA

RONALD M. LANNER[1]
2651 Bedford Ave., Placerville, CA 95667
pinetree30@comcast.net

ABSTRACT

Abies magnifica A. Murray bis var. **critchfieldii** var. nov. Lanner (Critchfield red fir) is described. The new variety comprises the southernmost Sierra Nevada populations of California red fir. It differs from the typical variety in having smaller cones with protruding cone bracts. Because of the protruding bracts, populations of the new variety have been assumed to be disjuncts of the bracted *A. magnifica* var. *shastensis* Lemmon (Shasta red fir), described over a century ago from Mt. Shasta and considered present in NW California and SW Oregon. However, geographic patterns of morphological variation, artificial crossing results, and recent molecular studies indicate that Shasta red fir consists of California red fir introgressed by noble fir (*A. procera* Rehder), and that the new variety is not hybridized with noble fir.

Key Words: *Abies magnifica*, *Abies procera*, California red fir, natural hybridization, Shasta red fir.

Generations of investigators have been confused and intrigued by a complex consisting of California red fir (*Abies magnifica* A. Murray bis), noble fir (*A. procera* Rehder), and morphologically intermediate populations. California red fir, which ranges south down the Sierra Nevada and noble fir, which extends north into Washington are clearly differentiated by leaf, bark, and cone characters (Lamb 1912; Lanner 1999). Between their ranges, however, lies a transition zone that includes the southern Cascades, Klamath Mts., and Coast Ranges of northwestern California and southwestern Oregon. In this region, trees with intermediate morphology occur that resemble California red fir but whose cones have the long protruding (exserted) bracts similar to those of noble fir, as opposed to the hidden (included) bracts of California red fir cones (Figs. 1, 2). These populations with exserted bracts, extending from about Mt. Lassen in California to Crater Lake in Oregon have long been referred to as Shasta red fir, *A. magnifica* var. *shastensis* Lemmon or even *A. shastensis* (Lemmon) Lemmon, the type locality for which is Mt. Shasta, California (Sargent 1898; Little 1979). Lemmon (1890) was apparently infatuated with his new variety, or species as he later discerned it, whose "peculiarity ... is connected entirely with the fact of its cone-bracts becoming long and protruded, a half to a full inch between the scales, rendering the large purple cones, thus decked out with tasseled fringes, a most beautiful object".

Remarkably, protruding bracts are also found in the southernmost Sierra Nevada populations of California red fir, about 480 km. from the nearest Shasta red firs to the north. These too have, historically, often been considered to be Shasta red fir (Sargent 1898; Sudworth 1908; Chase 1911; Jepson 1923; Peattie 1953; Griffin 1993; Stuart and Sawyer 2001), despite their geographic remoteness from the northern Shasta red fir area and the absence of any such intention in Lemmon's varietal description (Lemmon 1890).

The pattern of morphological variation of trees in the northern transition zone, more noble fir-like from south to north, and from east to west within that zone (Griffin and Critchfield 1972) suggests hybridization leading to introgression. Hybridization is further suggested by the ease of artificially crossing these firs, especially when California red fir is the maternal parent but in the reciprocal cross as well (Silen et al. 1965; Critchfield 1988). Liu (1971) found this evidence compelling enough to denote Shasta red fir as *A. X shastensis* Lemmon.

Persuasive evidence of introgression has emerged also from recent molecular studies. Oline (2008) analyzed the distribution of chloroplast haplotypes throughout the range of California red fir and within the transition zone extending into southern Oregon. Sierra Nevada populations, including the southernmost bracted ones, displayed only California red fir haplotypes. But the transition zone populations, including one from the type locality of Shasta red fir, were polymorphic, with haplotypes of both species. Oline (2008) viewed these results as "supporting a broad zone of hybridization". Oline's results undermine the concept of a

[1]The author is Visiting Emeritus Scientist at the Institute of Forest Genetics, USDA Forest Service, Pacific Southwest Research Station, Placerville, CA.

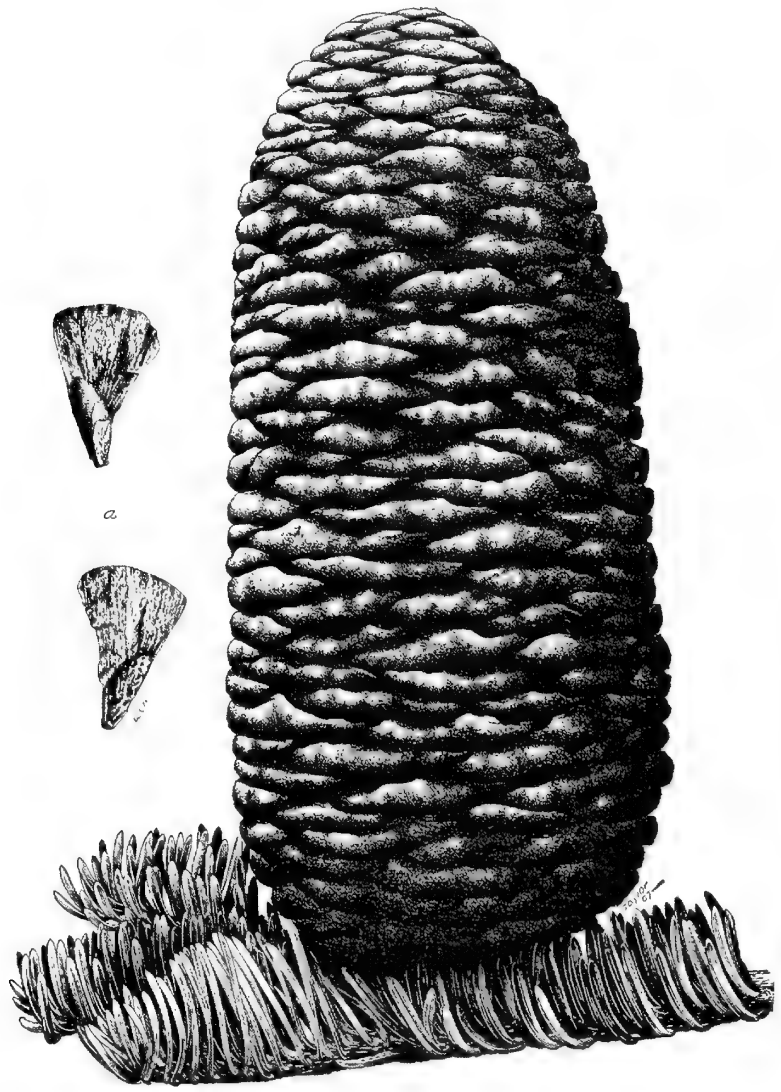

FIG. 53.—*Abies magnifica: a*, seed.

FIG. 1. Mature seed cone of California red fir with hidden (included) bracts. This morphology exemplifies the typical variety. Drawing by Taylor in Sudworth (1908).

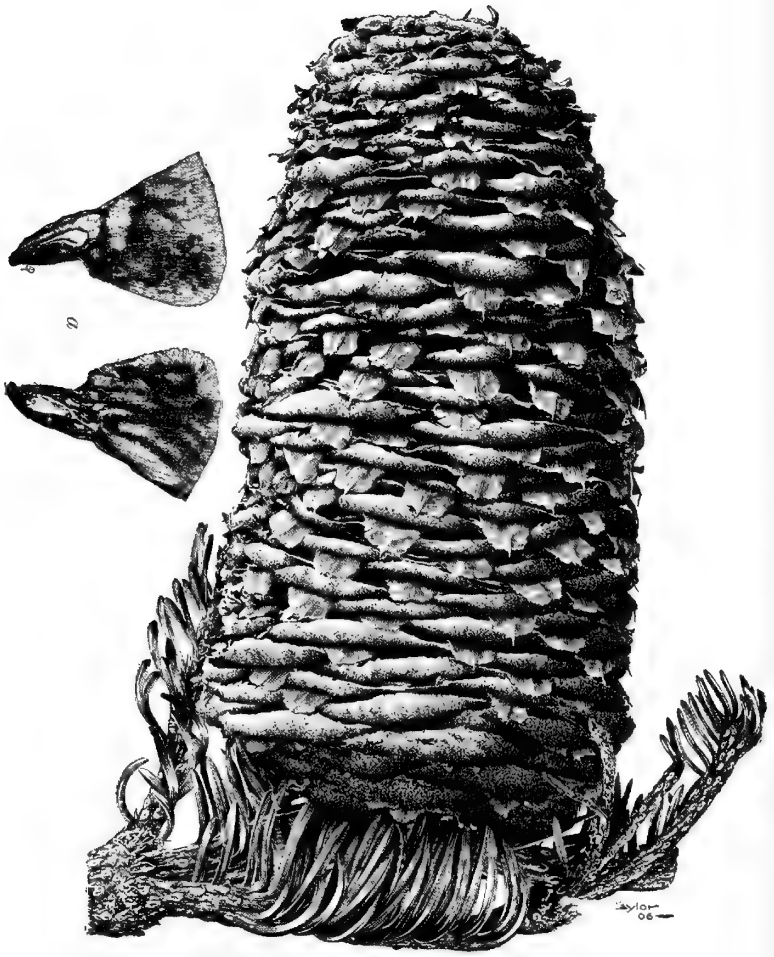

FIG. 54.—*Abies magnifica shastensis: a*, seed.

FIG. 2. Mature seed cone of California red fir with protruding (exserted) bracts. This morphology exemplifies the new southern Sierra Nevada variety (Critchfield red fir) as well as hybrid segregates with noble fir in NW California and SW Oregon (Shasta red fir). Drawing by Taylor in Sudworth (1908).

distinctive Shasta red fir variety and strongly support viewing it as a series of hybridized and introgressed California red fir and noble fir populations—in effect a geographically widespread mature hybrid swarm.

What then of the southern "Shasta red fir" whose protruding bracts are "identical in their shape with those of the north" (Sargent 1898)? Ustin (1976) reported that California red fir cones from eight locations south of the Kings River watershed (Panoramic Point, Rabbit Meadow, Montecito, Little Baldy, Mineral King, Holby Meadow, Sherman Peak, and Mule Peak) had protruding bracts. I have examined cones or cone parts from ten additional locations south of the Kings (Alta Peak, Panther Peak, Tar Gap, Kaweah River, Mountain Home State Forest, Greenhorn Mts. (presumably Sunday Peak), Siretta Ridge, Bald Mountain, Mineral King Valley 1, and Mineral King Valley 2) and found all bearing protruding bracts. Sudworth (1916) reported finding in 1899 trees bearing cones with all protruding bracts, and trees with all hidden bracts at Alta Meadows, in Sequoia National Park. This location should be further investigated. Jeff Bisbee (personal communication) has observed and photographed protruding bracts at Onion Valley and the Kearsarge Pass trail. These locations fall between 35°47′N (Sunday Peak) and 36°46′N (Onion Valley) and from 2012 m elevation (Mountain Home State Forest) to 2850 m (Sherman Peak).

Ustin (1976) found that cones from twenty Sierra Nevada locations north of the Kings had hidden bracts. Nor have bracted cones been reported from that area in field guides or floras I have consulted, though some show illustrations of bracted cones without explanation or comment (Storer and Usinger 1963; Storer et al. 2004). Sargent (1898), in what was perhaps the first published mention of the bracted southern red firs, pointed out that "in all the central part of the range occupied by this tree its cone bracts are acute and included". The only apparently inconsistent observations on this point are those of cones with "slightly" protruding bracts at Onion Valley campground (Inyo National Forest) where most of the cones had protruding bracts; and at Minaret Summit and Mammoth Lakes, where they occurred north of the Kings in an area of hidden bracts (Bisbee personal communication). Photographs show these cones have only the free tips of their bracts visible. This may be evidence of interbreeding between the new variety and the

typical variety and should be examined in more detail.

Oline's (2008) finding of only California red fir haplotypes in the southern Sierra Nevada populations is not the only evidence uncoupling these populations from northern Shasta red fir. In addition, the monoterpene composition of cortical oleoresins has shown the southern red firs to be chemically much more similar to the typical California red fir than to Shasta red fir of the northern transition zone (Ustin 1976; Zavarin et al. 1978). For these reasons it is appropriate to provide for the southernmost Sierra Nevada populations of California red fir a new variety.

A New Variety of *Abies magnifica*

Abies magnifica var. **critchfieldii** Lanner, var. nov. (Critchfield red fir; Fig. 2).—Type: USA, California, Tulare Co., Mountain Home State Forest, SW 1/4 SE 1/4 Sec. 25, T19S R30E, MDB & M, in mixed conifer forest on well-drained south slope, 6600 ft. (2012 m), 7 October 1947, *L. T. Burcham 260* (holotype: UC-907558 including separately filed cone coll. no. 0335).

Abies magnifica var. *critchfieldii* ex var. *magnifica* differt in strobilus parvis (9–17 cm vs. 14–23 cm) cum squamae bracteae in maturitas siue siccitas reflexae.

California red fir is a large forest tree to over 60 m tall. Young trees are pyramidal and symmetrical, old crowns become ragged from snow breakage. **Leaves** linear, 6–35 mm long and flattened on lower branches (shade leaves), 7–40 mm long and quadrangular on upper branches (sun leaves), with 2 resin ducts, crowded, bent upward, new growth silvery-glaucous turning blue-green (thus local name "silvertip"), with stomates on all surfaces, apex blunt to acute, retained to at least 12 yr. The shortest needles surround terminal buds at their base and remain to mark the annual growth increments. **Twigs** pubescent, turning from yellow-green to light brown to gray. **Winter buds** ovate with acute to rounded apex, 2–8 mm long, light brown, shiny, not resinous. **Bark** thin, silvery gray, smooth with resin blisters on young stems; thick, reddish or purplish brown (thus "red fir"), deeply furrowed between broad ridges on mature trees. **Seed cones** oblong or cylindrical, 14–23 cm long, 6–9 cm wide in the typical variety, 9–17 cm long, 3–9 cm wide in var. *critchfieldii*, purple tinged with brown when mature, bracts hidden in typical variety but protruding conspicuously and reflexing when mature, finally covering much of the cone surface in var. *critchfieldii*.

The variety is named in honor of William B. Critchfield (1923–1989), American forest geneticist, in recognition of his distinguished contributions to the genetics, systematics, biogeography, and evolution of western North American conifers, including the California red fir beneath which he enjoyed hiking in the Sierra Nevada. A native of Fargo, N. D., he earned a bachelor's degree in forestry (1949) and doctorate in botany and genetics (1956) at the University of California at Berkeley. After serving as forest geneticist with the Cabot Foundation for Botanical Research at Harvard University (1956–1959), he was a geneticist at the Institute of Forest Genetics, a unit of the USDA Forest Service's Pacific Southwest Research Station, at Placerville, CA from 1959 to his retirement in 1988.

Critchfield red fir is distributed in the southern Sierra Nevada Mountains in Tulare, Inyo, and Kern (and perhaps Fresno) counties, extending into the Greenhorn Mts. in Kern Co. It is found in Kings Canyon and Sequoia National Parks and Sequoia and Inyo National Forests. It therefore comprises the southern extremity (about 1 degree of latitude) of the range of California red fir (Griffin and Critchfield 1972). Common coniferous associates are white fir, *A. concolor* (Gordon & Glend.) Hildebr. var. *lowiana* (Gordon & Glend.) Lemmon; Jeffrey pine, *Pinus jeffreyi* Balf.; western white pine, *P. monticola* Douglas ex D. Don; lodgepole pine, *P. contorta* Douglas ex Loudon subsp. *murrayana* (Balf.) Critchf.; whitebark pine, *P. albicaulis* Engelm.; and Sierra juniper, *Juniperus occidentalis* Hook. subsp. *australis* Vasek. The type locality, Mountain Home State Forest in Tulare County, supports white fir, sugar pine (*P. lambertiana* D. Douglas) and giant sequoia (*Sequoiadendron giganteum* (Lindl.) J. Buchholz.

Critchfield red fir, as reported to date, is similar in phenotype to the typical variety except for its smaller cones with protruding bracts. However, its marginal location with respect to the species' range may be found upon further study to harbor adaptations to a drier climate than that of the typical variety.

Protruding cone bracts occur in more than twenty firs worldwide, including three North American species in addition to noble fir. They also characterize all *Pseudotsuga* and several *Larix* (Eckenwalder 2009). In bristlecone fir (*A. bracteata* [D. Don] Poit.) very long attenuated bracts characterize the species as a whole (Lanner 1999). Balsam fir (*A. balsamea* [L.] Mill.) has long-bracted populations termed "bracted balsam fir" (var. *phanerolepis* Fernald), which occur sporadically from the Appalachians of Virginia and West Virginia to the Maritimes (Hawley and DeHayes 1985). Hybridization with the long-bracted Fraser fir (*A. fraseri* (Pursh) Poir) has been invoked to explain this occurrence (Liu 1971).

It is not surprising that protruding bracts – a trait widespread in its family and common in its

genus—should appear in a fir with ordinarily hidden bracts. Whether there is some selective advantage to a tree that has papery objects sticking out from between the scales of its seed cones, or if we are merely observing a neutral character occasionally expressed and subject to fixation through random drift in a marginal population, cannot be judged at this time.

Representative Collections

CALIFORNIA. **Tulare Co.**: Alta Peak, Kaweah River Basin, 1901, *Ralph Hopping s.n.* (UC-400343); Panther Peak, Sequoia National Park. October 1934, *P. H. Bailey & W. W. Frost s.n.* (UC-525811); Tar Gap, vicinity of Mineral King, 9000 ft, 5 August 5 1904, *H. M. Hall & H. D. Babcock s.n.* (UC-64470); Kaweah River, ca. 1918, *Ansel Hall s.n.* (JEPS-46605). **Kern Co.**: Greenhorn Mts., 7500 ft, 31 May 1947, *Lyman Benson 1618* (SDSU-01567).

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