

1 Variation in the physiology of *Metrosideros polymorpha* (Myrtaceae)

2 ecotypes along an elevational gradient

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9 Abstract

10 Plant species persist across environmental gradients as a result of local adaptation, but also
11 through phenotypic plasticity of physiological and morphological characters. Ohi‘a lehu‘a,
12 *Metrosideros polymorpha* (Myrtaceae), is remarkable in its capacity to colonize habitats in
13 Hawaii ranging from the alpine to sea-level. Plants have been shown to adjust aspects of their
14 physiology in response to this gradient, and also differ morphologically, existing as trees with
15 glabrous leaves at lower elevations, and as shrubs with small, pubescent leaves at higher
16 elevations. Less is known, however, about how distinct morphological variants (pubescent and
17 glabrous ecotypes) differ in their integrated responses to environmental variables, and what the
18 primary function of the pubescence is. We assessed ecophysiological traits for both pubescent
19 and glabrous ecotypes along an elevational gradient to assess how this species balances plasticity
20 in morphological and physiological characters. Aspects of leaf morphology (specific leaf area)
21 and photosynthesis/gas exchange physiology (including maximum photosynthetic rate and $\delta^{13}\text{C}$)
22 differed significantly along the gradient, as did foliar nitrogen (N) and $\delta^{15}\text{N}$. Leaf physiology
23 appears to respond to different environmental conditions, but there was some indication that the
24 two ecotypes differed in their integrated responses to the gradient. Experimental tests for a
25 specific function of foliar pubescence revealed that pubescence is unlikely to confer protection
26 from increased UV radiation at high elevations, but that it may act to buffer high elevation plants
27 from cooler temperatures. However, its primary role at lower elevations may be to act as a
28 diffusional barrier to transpiration.

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32 Introduction

33 Plant communities in the Hawaiian archipelago assemble along several distinct environmental
34 gradients, including a gradient of soil and nutrient development on lava flows of different ages
35 (successional gradient) and gradients of precipitation and temperature along the islands' montane
36 slopes (elevational gradient). At a broad scale, these two intersecting gradients comprise a
37 matrix of interacting environmental drivers that has become a model for tropical community and
38 ecosystem studies (Vitousek, 2004). For example, along the east (windward) side of Hawaii,
39 both temperature and precipitation decline with increasing elevation; these two factors drive
40 decomposition and nutrient fluxes, although these rates depend on the parent material (lava type)
41 and the age of the flow (Raich *et al.*, 1997; Vitousek *et al.*, 1994). The constancy of the parent
42 material along a single flow of lava from high to low elevation allows controlled tests of the
43 effects of climate, while the range of very young (<100 yr) to very old (>10 000 yr) lava flows at
44 the same elevation allows tests of substrate quality and soil processes independent of climate.

45 The plant communities along Hawaiian environmental gradients differ from those in most
46 tropical ecosystems however, in having a relatively small number of plant species (Pearson and
47 Vitousek 2002; Crews *et al.*, 1995). Communities along extreme elevational and successional
48 gradients are dominated by a few common species, the most prevalent of which is Ohi'a lehu'a,
49 *Metrosideros polymorpha*, which together with *Acacia koa*, is the most common tree and shrub
50 species in Hawaiian forests. The prevalence of *M. polymorpha* along these gradients means that
51 its physiology and morphology must integrate multiple environmental stresses, and it is therefore
52 interesting both as an indicator of ecosystem properties, and as a model for how multiple plant
53 traits adapt or acclimate to local conditions (Cordell *et al.*, 1998; Cordell *et al.*, 2001). For
54 example, *M. polymorpha* shows a remarkable degree of phenotypic variation along the slopes of

55 Hawaii's two major volcanoes, Mauna Kea and Mauna Loa. At high elevations, and also on
56 younger substrates, plants are small shrubs tall, with small, thick leaves that bear a dense coat of
57 pubescence, while at the lowest elevations, and on generally older lava flows, plants are
58 overstory dominant trees with large, thin, and primarily glabrous leaves (Cordell *et al.*, 1998).
59 At middling elevations, and across a range of substrate ages, both of these glabrous and
60 pubescent ecotypes coexist, with the proportion of 'glabrous' plants declining with increasing
61 elevation (Alyokhin *et al.*, 2004). This morphological variation (pubescence and SLA) has
62 recently been shown to have a genetic basis (Martin *et al.*, 2007), and covaries with a suite of
63 leaf physiological traits such as leaf pigment concentrations and chlorophyll fluorescence
64 parameters (Martin *et al.* 2007). Variation in pubescence, leaf thickness and leaf
65 ecophysiological characters has been shown to have a genetic component but is also
66 environmentally induced (Cordell *et al.*, 1998).

67 In a common garden, these physiological traits showed greater plasticity in their
68 individual responses to local environmental conditions than did SLA and the degree of
69 pubescence (Cordell *et al.*, 1998). In other words, plants may be able to adjust their physiology
70 in response to climatic factors such as precipitation, but may be constrained by aspects of their
71 leaf morphology that are less plastic. For example, changes in stomatal behaviour (g_s) in
72 response to water availability or carbon demand may differ if a plant has an additional
73 diffusional resistance in the form of pubescence. This would suggest that different ecotypes at
74 the same microsite should demonstrate different physiological (photosynthetic, stomatal)
75 responses to their environments. Conversely, leaf morphology may not only represent a
76 limitation or adaptation to gas exchange, but may also have an additional or alternative function.
77 Documented, alternative functions for leaf pubescence in other species include protection from

78 oxidative damage caused by excess ultraviolet (UV) radiation, regulation of leaf temperature
79 (Press, 1999), and protection from chewing herbivores (Alyokhin *et al.*, 2004). All of these are
80 potential explanations for the variation observed in *M. polymorpha*, which together with water
81 and CO₂ regulation could act to determine the success (and hence the frequency) of different
82 ecotypes over the elevational and substrate age matrix. While pubescence has been shown to
83 reduce herbivory by an invasive insect on *M. polymorpha* (Alyokhin *et al.*, 2004),
84 photoprotective and thermoregulatory functions have not, to our knowledge, been tested.

85 Our goal was to better elucidate the integrated physiological responses of *M. polymorpha*
86 along Hawaii's montane climatic gradient, particularly the role of leaf morphology and
87 pubescence ecotypes. We asked three questions: (1) Does leaf pubescence provide protection
88 from stress caused by UV? (2) Do different leaf ecotypes differ in their thermoregulatory
89 capacity at different elevations? (3) Does morphological variation (i.e., ecotypes) explain
90 phenotypic variation in gas exchange physiology for plants at different elevations?

91

92 Methods

93 *Study Sites and sampling protocol*

94 This study was conducted in January 2007 along the eastern slope of the Mauna Loa volcano
95 along the 1855 'a' flow on the Big Island of Hawaii. Site A was located 314.25 m above sea
96 level at 19°41.172'N 155°07.863'W. Site B was located 743.41 m above sea level at
97 19°41.540'N 155°12.132'W. Site C was located 1,188.42 m above sea level 19°41.283'N
98 155°16.139'W. Site D was located 2465.53 m above sea level at 19°34.960'N 155°26.317'W
99 (Fig. 1).

100 At sites B and C, paired samples of *M. polymorpha* were collected. We chose plants

101 growing in close proximity (< 2m apart) in which one plant was pubescent and one glabrous.
102 Twenty-centimeter long branches, with both past (old) and current (young) years growth, were
103 collected from comparable heights on the plants using a pole pruner. Unpaired samples were
104 collected at sites A and D since there were only pubescent plants present. These branches were
105 wrapped in moist paper towel, placed in water in a cooler for transport back to the lab. Relative
106 abundance and height measurements of glabrous and pubescent *Metrosideros polymorpha* plants
107 were taken along a single, randomly oriented 50 x 10 m transect at each of sites A, B, and C.

108

109 *(1) Does leaf pubescence provide protection from stress caused by UV?*

110 The collected branches from sites A, B, C, and D were split into a treatment and control group.

111 All branches were re-cut, placed in water, and were protected from direct sunlight outside.

112 Treatment plants received a supplemental 12-hour treatment of UV light (3 UV-A bulbs:

113 Bulbtronics, 365 nm; 1 UVB bulb: National Biological, 311 nm) at an intensity of 5 W m^{-2} over

114 6 days. To evaluate the effect of this treatment on the quantum efficiency of photosystem II (a

115 measure of photo-oxidative stress), we measured the dark-adapted ratio of variable to maximal

116 chlorophyll fluorescence (Fv/Fm) with a Li-6400 (LiCOR, Inc., Lincoln, NE) between 20:00 and

117 00:00 on leaves of both treatment and control branches. These values were compared with initial

118 dark-adapted Fv/Fm values taken on-site in the field.

119

120 *(2) Do different leaf ecotypes differ in their thermoregulatory capacity at different elevations? At*

121 sites A, B and D, paired pubescent and glabrous plants ca. 1-2 m apart and of approximately

122 equal size were selected to determine whether the two leaf types differed in how closely they

123 tracked ambient temperatures over 24 hours. At site D, there were no glabrous *M. polymorpha*,

124 however *Vaccinium reticulatum* (Ericaceae) occurring at this site has glabrous leaves of
125 approximately the same area as *M. polymorpha*, and thus this species was used as the ‘glabrous’
126 comparison. Fine-wire thermocouples were embedded in the mesophyll of 2-3 leaves of each
127 plant and connected to a data-logger. Leaf temperatures were compared with measurements
128 from paired thermocouples positioned to record ambient air temperature. In the leaf
129 thermocouples, a small amount of wire was unavoidably exposed to air surrounding the leaf,
130 making estimates of differential leaf and air temperatures conservative in this experiment.

131

132 (3) Does morphological variation explain phenotypic variation in physiology?

133 Using the control plants in the UV experiment, we compared pubescent and glabrous leaves
134 among sites for the maximum velocity of rubisco carboxylation (V_{cmax}) and the CO_2 -saturated
135 photosynthetic rate (A_{max}) based on the response of the net photosynthesis (A) to variation in c_i
136 (A - c_i curve) (Jacob *et al.*, 1995; Reid and Fiscus, 1998) using the Li6400. As an integrated
137 measure of gas exchange (e.g., diffusional resistances) over the life of the leaves, we analysed
138 dried and finely ground leaves for %C, %N, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ using isotope ratio mass
139 spectrometry in the Cornell Stable Isotope Laboratory (www.cobsil.com). For replicate leaves
140 from each branch, we measured leaf area (AM-200 flat-bed scanner, ADC Bioscientific,
141 Hoddesdon, UK) and dry mass to determine specific leaf area (SLA).

142

143 *Statistics*

144 Data were analyzed in SAS (v. 9) or JMP (v.4) using general linear models (ANOVA or least
145 squares regression), followed by Tukey’s test for multiple comparisons among sites, ecotypes,
146 and treatments.

147

148 Results

149 The efficiency of PSII in the field varied slightly ($P < 0.05$) among elevations (Fig. 2, 3), with
150 Fv/Fm values being lowest at the highest elevation site (Site D). However, there was no
151 indication that Fv/Fm varied between pubescent and glabrous ecotypes, nor as a result of our UV
152 treatment. In fact, the time spent in the lab experiment appeared to minimise the differences we
153 saw in the field (Fig. 4), suggesting that the lab conditions ameliorated elevation-induced
154 environmental stress on PSII. Thus, while higher UV stress at site D may have contributed to the
155 observed field values, there was no experimental evidence that high intensity UV radiation has
156 different effects on pubescent and glabrous plants.

157 Ambient and leaf temperatures varied among elevations. At Site A (low elevation), the
158 mildly pubescent leaves were only slightly (0.1 to 0.4°C) above ambient during daylight hours
159 (Fig. 5). This difference was greater at site B, where both pubescent and glabrous leaves were
160 0.3-0.6°C warmer than ambient during the hours between sunrise and noon (with little indication
161 that the two ecotypes differed); in the late afternoon, however, neither pubescent or glabrous
162 leaves appeared to deviate from ambient (<0.1°C), although there was a small, but consistent
163 difference between the two ecotypes, with pubescent plants being kept cooler (closer to ambient)
164 than the glabrous. At Site D (high elevation), ambient temperature was lower on average (7.29-
165 7.59°C over 24 hours), and yet relative to ambient, *M. polymorpha* leaves were kept up to 1°C
166 warmer in the morning, and up to 2.5°C warmer in the late afternoon prior to sunset (Fig. 5).
167 *Metrosideros polymorpha* leaves (pubescent) were consistently warmer than the glabrous
168 *Vaccinium reticulatum* leaves at this site. In the late afternoon, *M. polymorpha* leaves were 0.5-
169 2.0°C warmer than the *V. reticulatum*, and in the early morning, *M. polymorpha* leaves were
170 close to ambient when those of *V. reticulatum* were 1°C ambient.

171 In terms of elevational variation in gas exchange and photosynthesis, we observed a
172 linear increase in $\delta^{13}\text{C}$ values with increasing elevation (Fig. 6), in accord with previous studies
173 of carbon isotope discrimination with elevation in both *M. polymorpha* (Cordell *et al.*, 1999) and
174 other species. In contrast to other studies, however, we also observed ecotypic variation at one of
175 our sites (Site B), with glabrous plants at that site having strikingly lower $\delta^{13}\text{C}$ values (Fig. 6).
176 This difference was not found at the other site with both ecotypes (Site C). As well as varying
177 in pubescence (Aplet and Vitousek, 1994; this study), leaves from different elevations appeared
178 to differ slightly in specific leaf area (SLA), with leaves from Site D having the lowest SLA
179 (thickest leaves for a given area). SLA was the same, however, between the two ecotypes (Fig.
180 7). The slopes and maxima for the $A:C_i$ curves also varied among elevations, (Fig. 8). High
181 elevation (Site D) plants, and the pubescent individuals from Site B had the highest light-
182 saturated photosynthetic rate (A_{max}) as estimated directly from the $A:C_i$ curves (Fig. 8). This
183 variation in gas exchange parameters may be in part related to leaf morphology (see Discussion).
184 Foliar % N was generally very low ($\leq 1\%$), and differed among sites, being lowest at Site D
185 (high elevation) and highest at Site B (Fig. 9). $\delta^{15}\text{N}$ values ranged from near zero for Site D, to
186 near -8‰ for Sites A and C; Site B plants had dramatically higher $\delta^{15}\text{N}$ (Fig. 10).

187

188 Discussion

189 Although previous studies in other species have documented that leaf pubescence provides
190 protection from oxidative damage caused by excess ultraviolet (UV) radiation (Press, 1999),
191 there was no experimental evidence that high intensity UV radiation has different effects on
192 pubescent and glabrous plants of *Metrosideros polymorpha*. However, field measurements
193 revealed that high elevation plants had significantly lower F_v/F_m values, indicating lower

194 photosynthetic efficiency. This could have been due to higher oxidative stress, and it is possible
195 that our UV treatment was not strong enough to mimic these conditions. Given the strength of
196 our UV source (and the greater proportion of damaging UVB) however, it seems more plausible
197 that the lower F_v/F_m value in the field was due to an unknown source of stress, which was
198 alleviated upon placing the plants in a more benign environment (e.g., sufficient water, and out
199 of direct sunlight). F_v/F_m did not vary between pubescent and glabrous plants, suggesting that
200 the efficiency of light use is not affected by morphological variation between these plant types.

201 Pubescence on leaves of *M. polymorpha* may act as insulation against both warm and
202 cool temperatures. At the different elevations, the pubescence varied in either keeping the leaf
203 warmer than ambient temperatures, or cooler, in comparison to their glabrous counterparts.
204 While there were no glabrous leaves at Site A for comparison, the pubescent leaves were close to
205 ambient (only 0.1-0.2°C warmer than the ambient temperature). At the warm, mid-elevation site
206 (B), there was a small, but consistent difference between ecotypes, with the pubescent leaves
207 being kept slightly cooler than the glabrous leaves. At the high elevation site, however,
208 pubescent leaves were consistently warmer than ambient, and were warmer than the glabrous
209 leaves of an adjacent *V. reticulatum* used as a control. While there are likely leaf thickness
210 differences between these two species that might also contribute to temperature differences, we
211 suggest that at colder, high elevation sites, pubescence may have a thermoregulatory function
212 that would complement an observed supercooling capacity of high elevation *M. polymorpha*
213 (Melcher *et al.*, 2000).

214 Our most striking result was the observed linear increase in $\delta^{13}\text{C}$ values with increasing
215 elevation (Fig. 6). For the pubescent plants (which occurred along the entire elevational transect)
216 this relationship was highly linear ($R^2 = 0.94$, $P < 0.05$). This is in agreement with previous

217 studies on covariation of carbon isotope discrimination with elevation in both *M. polymorpha*
218 (Vitousek *et al.*, 1990; Cordell *et al.*, 1999) and other species. This linear relationship may reflect
219 variation in the CO₂ pressure gradient (ratio of internal to external CO₂ concentration, c_i / c_a)
220 created by elevational variation in morphologically-based diffusional resistance. For example,
221 in this study, SLA declined with increasing elevation, and was lowest at high elevations (leaves
222 were thicker), and these plants had the highest $\delta^{13}\text{C}$ values. Increased leaf thickness should act
223 to increase the diffusional resistance and alter the observed $\delta^{13}\text{C}$, and lead to a negative
224 correlation between SLA and $\delta^{13}\text{C}$, as suggested by our data (also see Schulze *et al.*, 2006). An
225 additional, and complementary interpretation of these isotope data is that in high elevation
226 plants, the ratio of C assimilation to transpirational water loss (water use efficiency, WUE) was
227 lower, perhaps through increased stomatal conductance to water, g_s (data not shown).

228 In contrast to other studies, we also observed $\delta^{13}\text{C}$ variation between pubescence
229 ecotypes at one of our sites (Site B), with glabrous plants at that site having significantly lower
230 $\delta^{13}\text{C}$ values (Fig. 6). A predicted consequence of having pubescence is that it should act
231 (together with mesophyll thickness and stomatal behaviour) as a component of the diffusional
232 resistance to gas exchange between the leaf and atmosphere. However, this difference was not
233 found at the other site with both ecotypes (Site C), suggesting that any putative differences in
234 isotope discrimination due to leaf morphology may be dependent on local environment (e.g.,
235 precipitation, nutrient supply; see further). SLA was the same for both ecotypes at Site B,
236 suggesting that it was pubescence (rather than a spurious correlation between SLA and
237 pubescence) that may explain the $\delta^{13}\text{C}$ variation.

238 High elevation (Site D) plants, and the pubescent individuals from Site B had the highest
239 light-saturated photosynthetic rate (A_{max}), and this variation in gas exchange may be in part

240 related to leaf morphology, and perhaps to the availability of nitrogen along the elevational
241 gradient. Foliar % N was low (but in the normal range for this species [Vitousek et al. 1989]),
242 lowest at Site D (high elevation) but highest at Site B (Fig. 9). The $\delta^{15}\text{N}$ values for Site D
243 conform to expectations for minimal soil development and a rainwater/mineral N source (as
244 found there), while values near -8‰ for Sites A and C suggest similar microbial soil
245 development at these sites. Site B plants had dramatically higher $\delta^{15}\text{N}$ (in addition to having
246 higher total foliar N), suggesting that these plants accessed a different N pool, or that a different
247 soil microbial community led to different N discrimination. It is possible that this is driven by
248 differences in precipitation, which varies rapidly with elevation (particularly near Site B, Applet
249 and Vitousek, 1994), and which can drive soil development and nutrient accumulation (Vitousek
250 *et al.*, 1994). High A_{max} at the high elevation site may reflect the greater allocation of the
251 available N to photosynthesis compared to the low elevation plants, as found by Martin *et al.*
252 (2007).

253 In addition, higher A_{max} for the site B pubescent plants might have contributed to the
254 variation in foliar $\delta^{13}\text{C}$. Higher photosynthetic demand can prompt increased stomatal opening,
255 which can in turn drive variation in $\delta^{13}\text{C}$; environmental constraints on photosynthesis could
256 thereby affect $\delta^{13}\text{C}$ values. At Site B, higher N availability may have revealed differences
257 between ecotypes in foliar $\delta^{13}\text{C}$. At lower N sites (particularly given the low foliar N values at
258 these sites), the primary constraint on photosynthesis may be nitrogen (enzyme) based, rather
259 than CO_2 -based, while at Site B, higher foliar N may have contributed to higher photosynthetic
260 demand for C. Given a sufficiently high water potential, this demand could have negated the
261 effect of stomatal variation between ecotypes (both ecotypes may have had their stomata
262 completely open), but accentuated the contribution of pubescence to the overall leaf diffusional

263 resistance, and therefore ecotypic variation in carbon isotope discrimination. The hypothesis that
264 N enrichment could increase A_{\max} in this N-limited system is supported by the results of Cordell
265 et al. (2001), and by our finding that carboxylation capacity and electron transport rate (two
266 parameters expected to be strongly N-limited) were higher in plants at site B (data not shown).
267 Future research could aim to solidify putative linkages between soil N availability,
268 photosynthetic capacity, and stomatal behaviour for these two ecotypes.

269 In conclusion, we have shown that *M. polymorpha* exhibits strong ecophysiological and
270 morphological variation along an elevational gradient. Our results are consistent overall with
271 previous research (e.g., Cordell *et al.*, 1998). We show that temperature regulation may be a
272 function of leaf pubescence in this species. Finally, our results suggest that genetic and plastic
273 variation in morphological traits (e.g., pubescence, SLA) may act to constrain physiological
274 acclimation (e.g., gas exchange) to diverse environments.

275

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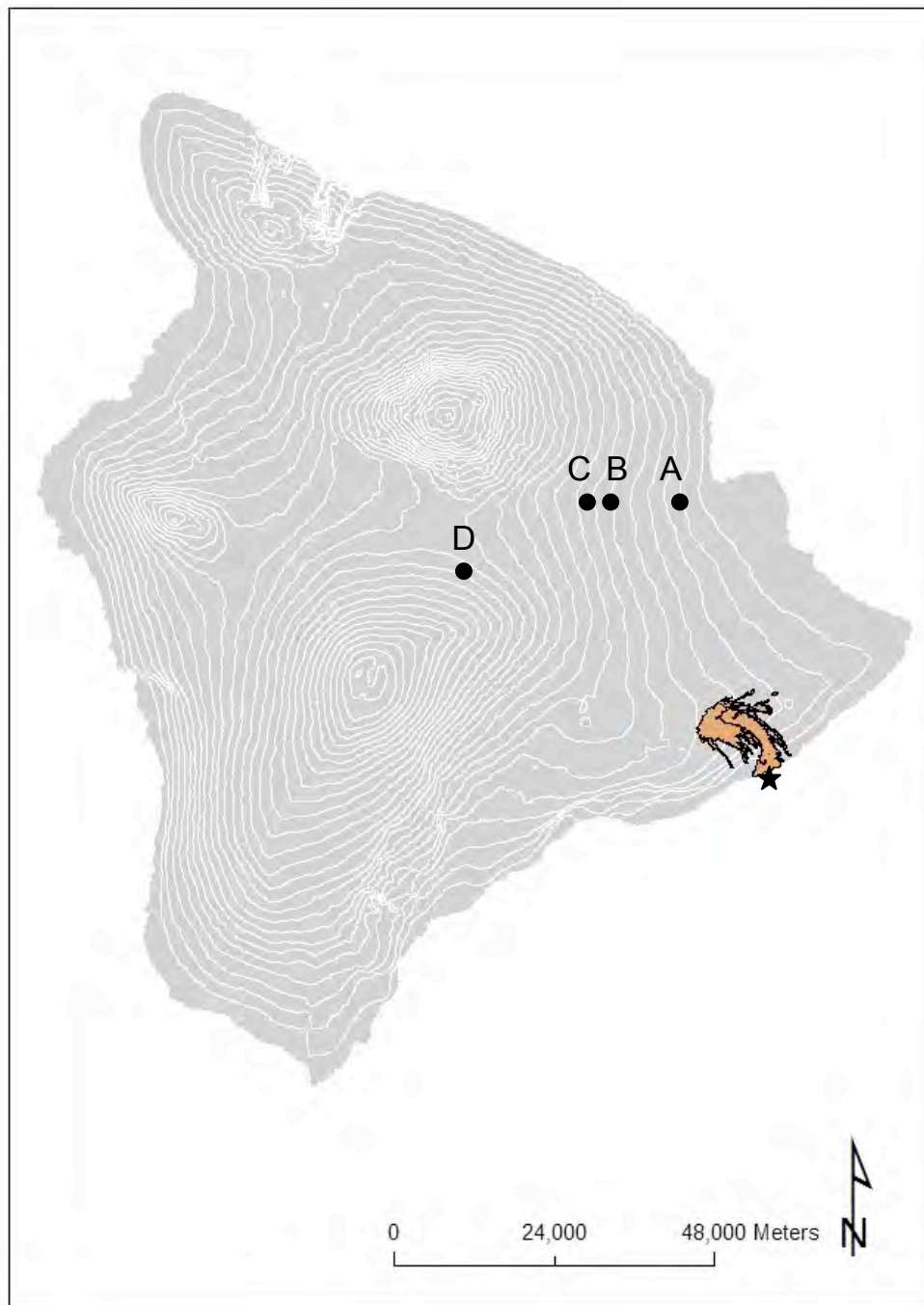
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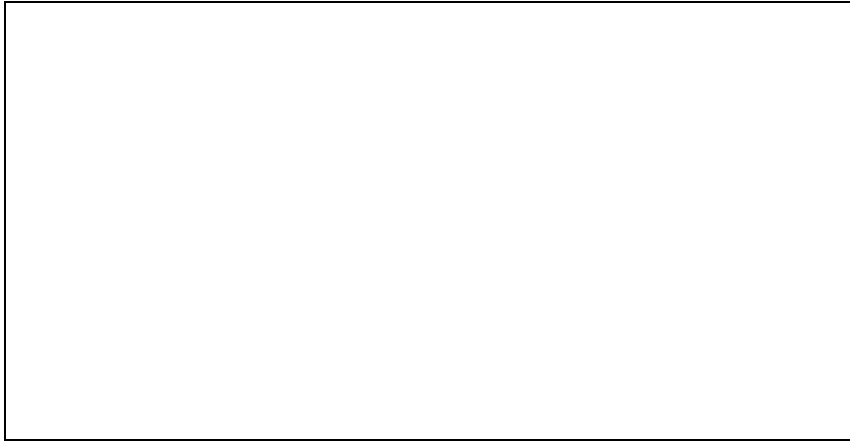
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340 Figure 1. Topographic map of Hawai'i Island with 150 m elevational isolines and the location of
341 the field sites. *active lava flow

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344 Figure 2. Fv/Fm field measurements at four sites along elevational gradient. Site D differs
345 significantly from other sites (Tukey's test: $P < 0.05$).

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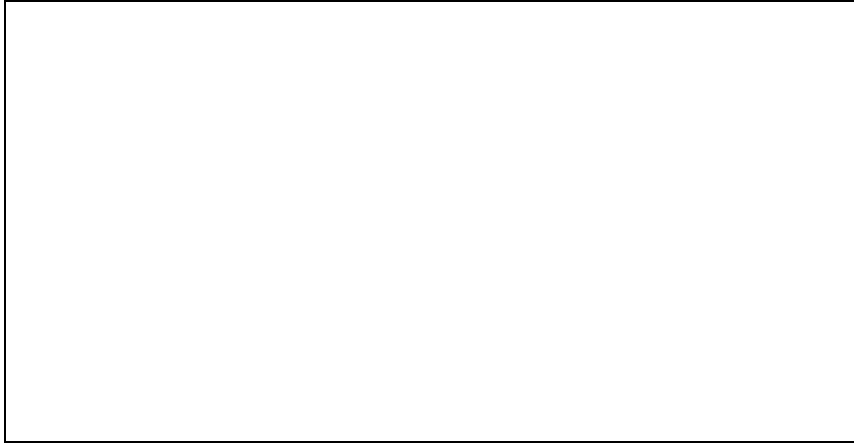
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361 Figure 3. Fv/Fm field measurements at four sites along elevational gradient with glabrous (g) and
362 pubescent (p) ecotypes separated at sites B and D. Columns with similar letters are not
363 significantly different (Tukey's test: $P > 0.05$).

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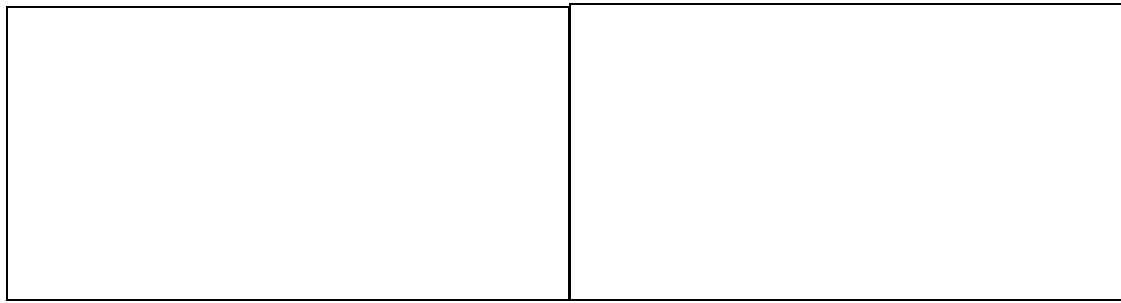
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366 Figure 4. Fv/Fm measurements over the course of UV treatment experiment. Letters indicate
367 different sites, and numbers indicate UV treatments (1= UV, 2 = control). Day 0 corresponds to
368 field measurements. After Day 1, there were no significant differences in Fv/Fm (ANOVA: $P >$
369 0.05).

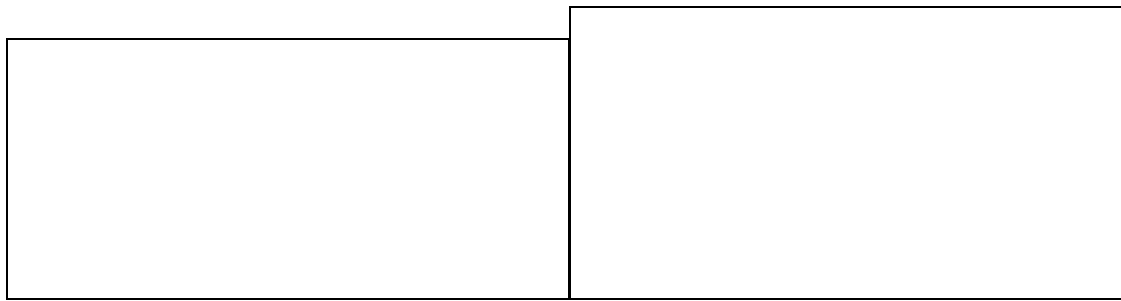
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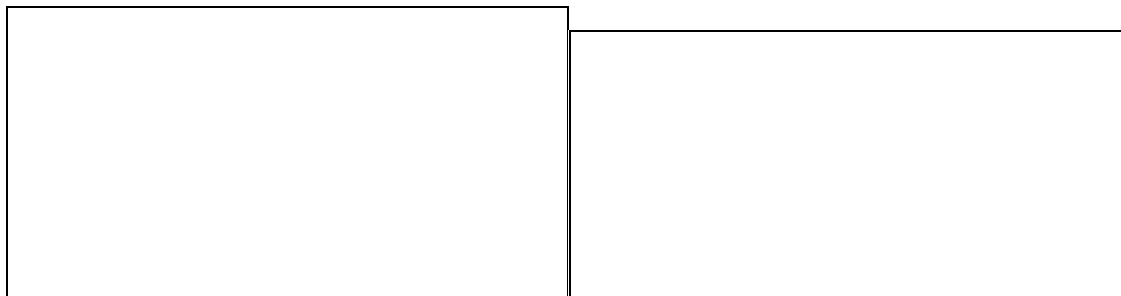
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376 Figure 5. Average leaf temperature (Site A: pubescent only; Site B: pubescent and glabrous
377 compared; Site D: pubescent and *Vaccinium reticulatum* compared) relative to ambient
378 temperature during dawn (left panels) and dusk (right panels).

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Elevation (Feet)



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389 Figure 6. $\delta^{13}\text{C}$ values of pubescent and glabrous *M. polymorpha* ecotypes along an elevational
390 gradient. Points are the mean \pm the standard error. Regression line is plotted for pubescent plants
391 ($P < 0.05$). There was only one glabrous sample at the low elevation site.

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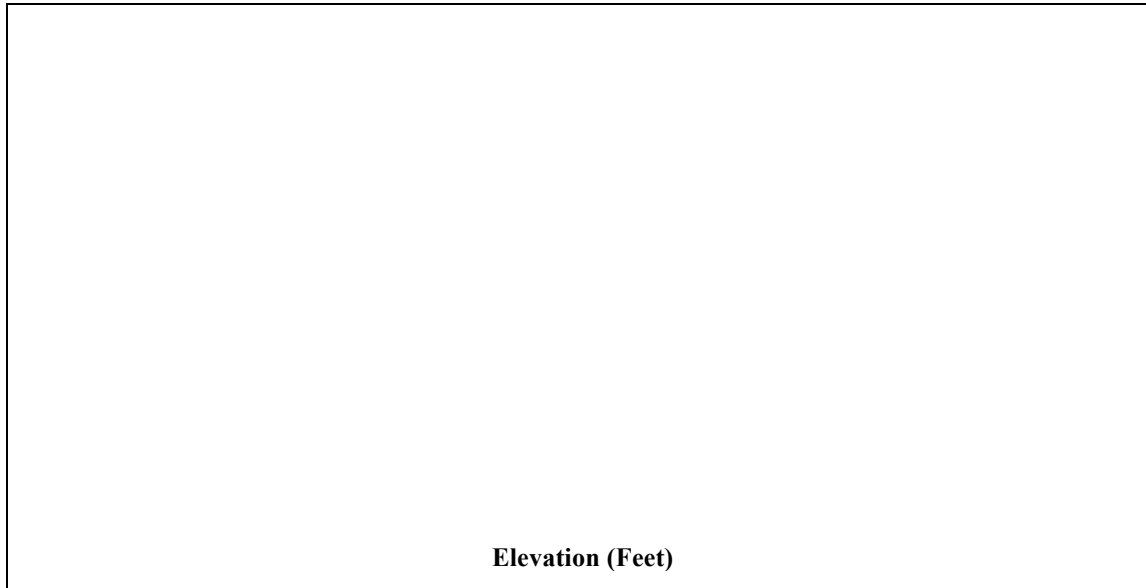
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405 Figure 7. Variation of specific leaf area of pubescent and glabrous *M. polymorpha* ecotypes
406 along an elevational gradient. Regression line is plotted ($P < 0.05$). Black or grey data points
407 denote pubescent leaves; white points denote glabrous leaves at the two mid elevation sites.

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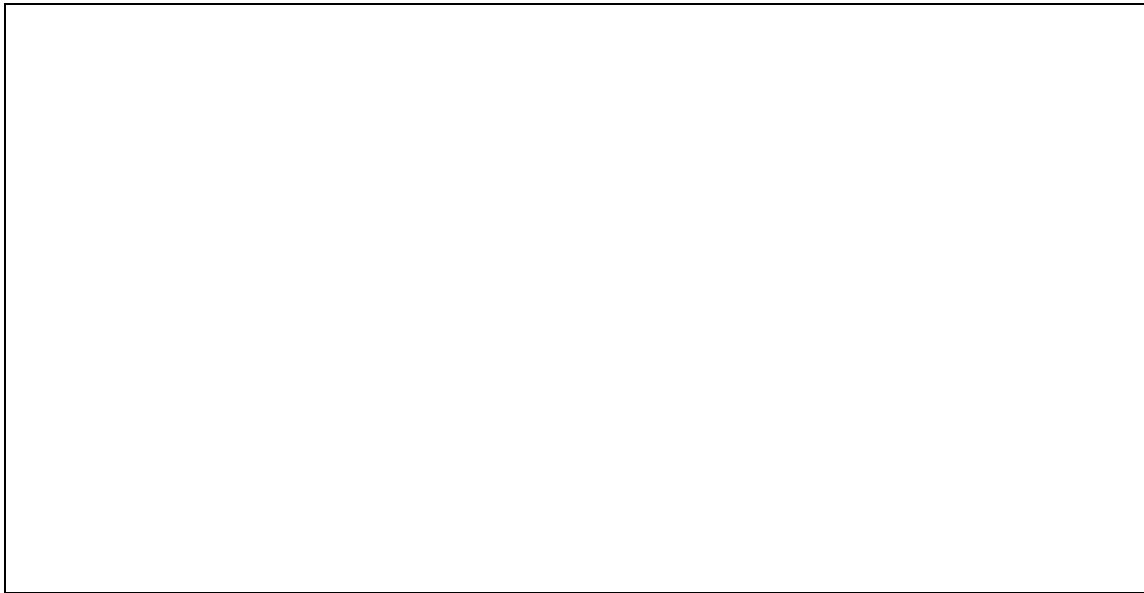
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422 Figure 8. A:*c*_i curves. Plot of light-saturated photosynthesis (Photo) as a function of internal
423 CO₂ concentration (*c*_i) for glabrous (g) and pubescent (p) leaves at sites along an elevational
424 gradient (A-D). Bars denote the standard error.

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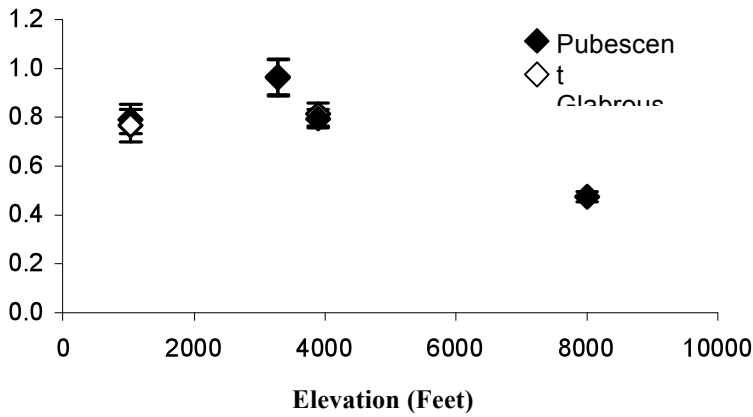
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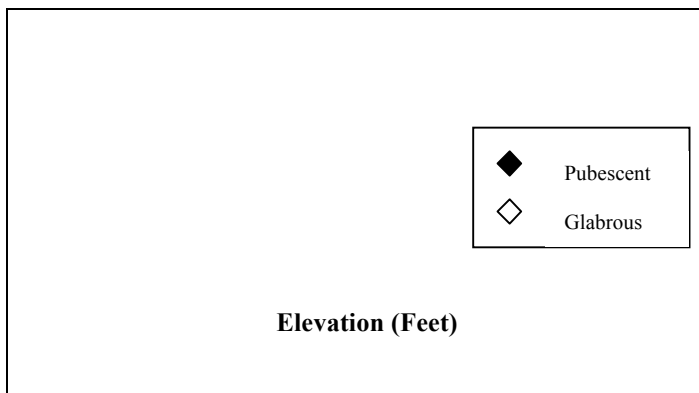


436

437 Figure 9. Variation in foliar percent nitrogen along an elevational gradient.

438 Points are the mean \pm the standard error.

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440

441 Figure 10. $\delta^{15}\text{N}$ values of pubescent and glabrous *M. polymorpha* ecotypes along an elevational

442 gradient. Points are the mean \pm the standard error.