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## Original Article

# Leaf trichomes in *Metrosideros polymorpha* can contribute to avoiding extra water stress by impeding gall formations

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- **Background and Aims** Plants inhabiting arid environments tend to have leaf trichomes, but their adaptive significances remain unclear. Leaf trichomes are known to play a role in plant defence against herbivores including gall makers. Because gall formations can increase water loss partly through increased surface area, we tested the novel hypothesis that leaf trichomes could contribute to avoiding extra water stress by impeding gall formations, which would have adaptive advantages in arid environments.
- **Methods** We focused on *Metrosideros polymorpha*, an endemic tree species in the Hawaiian Islands, whose leaves often suffer from galls formed by the specialist insects, Hawaiian psyllids (*Pariaconus* spp.). There is a large variation in the amount of leaf trichomes (0 to 40 % of leaf mass) in *M. polymorpha*. Three gall types were found on the island of Hawaii: largest one is “cone” type, followed by “flat” and “pit” types. We conducted laboratory experiments to quantify to what extent gall formations are associated with leaf water relations. We also conducted a field census of 1,779 individuals from 48 populations across the entire range of habitats of *M. polymorpha* on the island of Hawaii to evaluate associations between gall formations (presence and abundance) and the amount of leaf trichomes.
- **Key results** Our lab experiment showed that the leaf minimum conductance was significantly higher in leaves with a greater number of cone- or flat-type galls but not pit-type galls. Our field census suggested that the amount of trichomes was negatively associated with the presence probabilities of cone- or flat-type galls but not with the pit-type galls, irrespective of environmental factors.
- **Conclusion** Our results suggest that leaf trichomes in *Metrosideros polymorpha* can contribute to the avoidance of extra water stress through the interactions with

some gall making species, and potentially increase the fitness of plants under arid conditions.

**Key words:** leaf trichome, water limitation, plant–insect interaction, defence, gall, Hawaiian psyllid, *Pariaconus*, *Metrosideros polymorpha*.

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## INTRODUCTION

Plants under water–stress conditions, such as arid environments, sun–exposed environments, and during dry seasons, tend to have greater density or amount of leaf trichomes compared to those under mesic conditions (e.g., Aronne and De Micco, 2001; Agrawal *et al.*, 2009; Ichie *et al.*, 2016). These patterns may be attributable to one or multiple functions of leaf trichomes including reflecting sunlight (e.g., Ehleringer, 1984), saving water by increasing the boundary layer (e.g., Wuenscher, 1970), and promoting foliar water uptake (e.g., Benzing *et al.*, 1976). On the other hand, defence against herbivores, which is one of the major functions of leaf trichomes for a wide range of taxa, is not typically considered as a key function in water–stress conditions. Leaf trichomes play a role of physical and chemical resistance against insect oviposition and/or feeding (Johnson, 1975; Levin, 1978; Dalin *et al.*, 2008), which appears to be independent from drought tolerance. However, we consider that leaf trichomes could indirectly contribute to the plant adaptation to arid environments through plant–herbivore interactions, especially for plants suffering from gall makers. The gall formations are likely to increase water stress in host plants (Florentine *et al.*, 2005; Nability *et al.*, 2012) because the gall formations increase surface area of leaves and in theory increase the evaporative water loss. In line with this hypothesis, Bailey *et al.* (2015) reported that genes associated with drought tolerance were notably expressed in galled leaves compared to ungalled leaves. Based on these studies, we propose the novel hypothesis that leaf trichomes have adaptive significance under arid conditions by impeding the gall formations that otherwise increase water stress.

*Metrosideros polymorpha*, an endemic and dominant tree species in the Hawaiian Islands, shows extreme phenotypic variations across a wide range of habitat conditions: mean annual temperature (MAT) from 8 to 23 °C, mean annual precipitation (MAP) from <400 to >10,000 mm yr<sup>-1</sup>, and soil age (SA) from a few decades to over 4,000,000 years

(Kitayama and Mueller–Dombois, 1995; Cordell *et al.*, 1998; Cornwell *et al.*, 2007). The variation in the amount of trichomes (non–glandular) on *lower* leaf surface is remarkable, ranging from 0 to ca 150 g m<sup>-2</sup> and accounting for up to 40 % of total leaf mass (Joel *et al.*, 1994; Tsujii *et al.*, 2016; Amada *et al.*, 2017). Such large phenotypic diversity is mostly genetically determined rather than acclimation to habitat conditions (Tsujii *et al.*, 2016) and associated with three major varieties on the island of Hawaii: glabrous variety (*var. glaberrima*) and pubescent varieties (*var. polymorpha* and *var. incana*). It should be noted that there are many intermediate forms due to a lack of reproductive isolation mechanisms among the varieties (Stacy *et al.*, 2014; Stacy *et al.*, 2016). Glabrous individuals are often abundant in moderately wet areas whereas pubescent individuals are more abundant in dryland, high–elevation, or bog areas where plants may suffer from drought, low–temperature, or anaerobic conditions (Vitousek *et al.*, 1992; Stacy *et al.*, 2014; Tsujii *et al.*, 2016). While previous studies have repeatedly considered that the leaf trichomes have adaptive significances in arid environments (Joel *et al.*, 1994; Hoof *et al.*, 2008; Tsujii *et al.*, 2016), Amada *et al.* (2017) showed that the increased boundary–layer resistance due to the leaf trichomes of *M. polymorpha* had negligible effects on water–use efficiency, which is calculated as the ratio of the assimilation rate to the transpiration rate. Therefore, the leaf trichomes should have ecological functions, other than boundary–layer resistance, that can contribute to higher fitness under water stress.

In the present study, we focus on plant–insect interactions in relation to water stress. Leaves of *M. polymorpha* often suffer from galls of various morphological types made by endemic Hawaiian psyllids (*Pariaconus* spp.; Fig. 1; Nishida *et al.*, 1980; Lee, 1981; Gruner *et al.*, 2004; Percy, 2017). Thirty–six *Pariaconus* species, which all inhabit only on *M. polymorpha*, have been diversified from a single ancestor probably in response to diverse phenotypes of the host tree species across Hawaiian Islands (Percy, 2017). This *M.*

*polymorpha*–*Pariaconus* relationship offers an ideal model system to understand how plant–insect interactions are formed and how they are related to plant adaptation under water stress. The morphology of galls differs depending on psyllid species (but not all psyllid species make galls as some of them are free–living species; Percy, 2017). On the island of Hawaii, three morphological types of galls are commonly observed on leaves of *M. polymorpha*: cone–, flat–, and pit–type galls, which are induced by *P. pyramidalis*, *P. pele*, and *P. minutus*, respectively (Fig. 1; Nishida *et al.*, 1980; Percy, 2017). *Pariaconus pyramidalis* makes enclosed cone–type galls that largely extend from the leaf lower surface, and the galls open on the leaf upper surface by circular fissure resembling a trap door (Fig. 1 a–c; Percy, 2017). *Pariaconus pele* makes enclosed flat–type galls that moderately extend from both sides of leaves, and the galls open on the leaf lower surface by irregular fissures (Fig. 1 d–f; Percy, 2017). *Pariaconus minutus* makes open pit–type galls that slightly extend only on the leaf upper surface, and the immatures develop on the galls which do not dehisce (Fig. 1 g–j; Percy, 2017). *Pariaconus pyramidalis* and *P. pele* attack leaves from the leaf lower surface while *P. minutus* attacks from the leaf upper surface (Percy, 2017; leaf trichomes are located on leaf lower side).

Previous studies showed that the number of psyllid galls was smaller on pubescent individuals than on glabrous ones in some sites but not in other sites on the island of Hawaii without classifying the morphological types of galls (Lee, 1981; Gruner *et al.*, 2005). Recently, Percy (2017) reported that the cone– and flat–type galls were found in both glabrous and pubescent individuals, but the pit–type galls were typically found in pubescent individuals. Nishida *et al.* (1980) found that the distributions of these psyllid species were different along elevation at least on a south–east slope of Mauna Loa, implying that psyllid species may have distinct preference for environmental conditions (Price *et al.*, 1987; Stone and Schönrogge, 2003) such as temperature (Henson, 1958), aridity (Fernandes and Price,

1992; Price *et al.*, 1998), or soil fertility (Blanche and Westoby, 1995). Therefore, the impact of leaf traits on the abundance of the Hawaiian psyllids needs to be examined for each species with considerations of these environmental conditions.

In order to test the above-mentioned novel hypothesis “leaf trichomes have adaptive significance under arid conditions by impeding the gall formations”, first we conducted a laboratory experiment to examine whether water loss is higher in leaves with a greater number of galls. Second, across 48 populations on the island of Hawaii (1,799 individuals), we examined to what extent the *in-situ* variation in leaf trichomes is associated with the presence/absence or abundance of each type of galls, while considering other leaf traits and environmental conditions. Based on these examinations, we discuss the ecological significance of the large variation of leaf trichome amounts in *M. polymorpha*.

## MATERIALS AND METHODS

### *Study species*

*Metrosideros polymorpha* (Myrtaceae) is an endemic and dominant tree species in the Hawaiian Islands. This species is distributed across a wide range of habitat conditions as described later and shows a large phenotypic polymorphism (Stemmermann, 1983; Joel *et al.*, 1994; Tsujii *et al.*, 2016). While the variation in phenotypic traits is continuous (Tsujii *et al.*, 2016), *M. polymorpha* on the island of Hawaii has been classified into three major varieties, namely *var. polymorpha*, *var. glaberrima*, and *var. incana*. In this study, however, we did not discriminate our samples into these varieties as there were many intermediate forms. Instead we measured some key traits, such as the amount of leaf trichomes, in a quantitative manner as described later. In the following sections, we describe (1) the experiments that quantify to what extent gall abundance is associated with leaf water relations, and (2) the field

investigations that evaluate associations between gall formations (presence and abundance) and leaf traits across 48 study sites covering almost the full range of habitat conditions of *M. polymorpha* on the island of Hawaii.

#### *Leaf physiological functions with and without galls*

To evaluate the associations between galls and leaf water relations, we conducted physiological and morphological measurements on the leaf samples that were collected from one site where all three types of galls were found (ML-E-700-Y; altitude: 750 m, MAT: 17.1 °C, MAP: 5,934 mm yr<sup>-1</sup>; Supplementary data Table S1, Fig. S1). To determine the size of galls, we measured thicknesses of the galled parts and the intact parts of mature leaves using a thickness gauge (7173, Mitsutoyo, Japan) (11, 28, and 7 pairs were measured for cone-, flat-, and pit-type galls, respectively).

To examine whether the presence of galls is associated with foliar specific mass and water content in leaves, we compared mass per area and water content per area between the intact and the galled parts of mature leaves for each gall type separately. Prior to this measurement, leaves were fully hydrated with a wet paper towel sealed in Ziploc plastic bags overnight. We punched out three 6-mm-diameter disks for galled parts and intact parts respectively using a hole punch. We measured the weights of these disks before and after drying in an oven (70 °C) for more than two days. Mass per area (g m<sup>-2</sup>) and water content per area (g m<sup>-2</sup>) were defined as dry mass and water mass per disk area, respectively (10, 10, and 8 leaves were measured for cone-, flat-, and pit-type galls, respectively). Because all diameters of galls were smaller than 6 mm (i.e., the size of a leaf disk), the excised disks included intact leaf as well as galled part; therefore, the increases in mass per area and water content per area due to gall formation should be considered as conservative estimates.

To examine whether the abundances of galls are associated with water loss in leaves, we measured leaf minimum conductance ( $g_{min}$ ), which is defined as water–vapor conductance when stomata are assumed to be closed (Duursma *et al.*, 2019). Leaf minimum conductance was measured from the rate of water loss from a leaf in a dark condition (photosynthetic photon flux density  $< 10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; Sack *et al.*, 2003). We selected a pair of mature leaves: one with galls and another without galls for each individual (1–28 galls per leaf). We included six pairs of leaves that had no galls to quantify natural variations between pairs of leaves. After the leaves were fully hydrated with a wet paper towel in Ziploc plastic bags overnight, we dried leaves in a dark condition, at room temperature  $21.0 \pm 1.0 \text{ }^\circ\text{C}$  (Mean  $\pm$  SE), and relative humidity  $73.0 \pm 5.0 \%$ , and then measured changes in the weights of leaves for more than eight times at intervals of 30 min. Across the measurements, the leaf weight and drying time showed a linear relationship ( $R^2 > 0.99$ ), which suggested that stomata were closed (Sack *et al.*, 2003). After the mass measurement, leaf areas were measured from the scanned leaf images (GT–S630, EPSON, Japan) using ImageJ (National Institutes of Health, USA). We calculated  $g_{min}$  ( $\text{mmol m}^{-2} \text{s}^{-1}$ ) from the rate of decrease in leaf weight per area (13, 21, and 10 paired leaves were measured for cone, flat, and pit types, respectively).

#### *Associations among galls, leaf traits and environmental conditions*

During Jul. and Aug. 2016 and 2017, we established 48 study sites which covered almost the full range of habitat conditions of *M. polymorpha* on the island of Hawaii; the altitude ranges from 10 to 2,400 m a.s.l. (from coast to treeline), the mean annual temperature (MAT) from 8.9 to 23.3  $^\circ\text{C}$ , the mean annual precipitation (MAP) from 470 to 6,390  $\text{mm yr}^{-1}$ , aridity index (AI) from 0.29 to 3.95, and soil age (SA) from 45 to over 260,000 years old (Supplementary data Table S1, Fig. S1; Sherrod *et al.*, 2007; Giambelluca *et al.*, 2014). The

aridity index was calculated as mean annual precipitation/mean annual potential evapotranspiration and can be used as an index of dryness: more arid when AI is smaller (AI < 0.03: hyper arid zone, 0.03 < AI < 0.20: arid zone, 0.20 < AI < 0.50: semi-arid zone, 0.50 < AI < 0.75: dry sub-humid zone, AI > 0.75: humid zone; UNESCO 1979). At each site, 40 shoots were collected from the outer crown of different individuals (1,779 individuals in total) to analyse the associations among galls, leaf traits and environmental conditions.

For each individual, we counted the numbers of cone-, flat-, and pit-type galls on 6–14 leaves of the second-newest shoot, which is more stable and ideal for counting the number of galls than the newest shoot because psyllids typically attack the newly developing shoots. We identified each type of galls according to Percy (2017) (see Introduction and Fig. 1). Although the number of galls is not equal to the abundance of adult psyllids, we assumed that the presence of galls reflects the presence of adult psyllids to some extent; thus we can explore distribution patterns of three psyllid species in relation to environmental conditions.

We used fully matured young leaves to measure leaf traits in order to facilitate the comparison of leaf traits among individuals or among populations. We divided leaf mass into lamina mass and trichome mass by a shaving technique (Tsuji *et al.*, 2016). We defined leaf mass per area (LMA) associated with lamina and trichome as  $LMA_L$  and  $LMA_T$ , respectively, according to Tsuji *et al.* (2016) (i.e.,  $LMA = LMA_L + LMA_T$ ). On one half side of a mature leaf (separated by the midrib), we shaved leaf trichomes using a rubber thimble, and punched out two 10-mm-diameter disks from both sides of the leaf. These leaf disks were dried in silica gel and used for weighing with a digital scale (0.01 mg precision).  $LMA_T$  was calculated from the differences in dry weight between the disks with and without trichomes.



## Statistical analyses

To evaluate to what extent gall formation influenced leaf physiological functions, we calculated the response ratios ( $R_r$ ) of thickness, mass per area, water content per area, water content per fresh mass, and  $g_{min}$  for gall formation as follows:

$$R_{rX} = \frac{X_G}{X_I}, \quad (1)$$

where  $R_{rX}$  is the ratio of a trait  $X$  with gall formation ( $X_G$ ) to that without galls ( $X_I$ ). We calculated  $R_r$  based on leaf disks except for  $g_{min}$  where *whole* leaves were used. We separately considered  $R_r$  for cone-, flat-, and pit-type galls. As ratios do have a log-normal distribution by nature (Sokal and Rohlf, 1995),  $R_r$  was natural log-transformed before all statistical analyses.

Student's  $t$ -test was used to test the differences between galled and intact samples. One-way analysis of variance (ANOVA) with *post-hoc* Turkey HSD multiple comparisons was used to test the differences in traits among the three gall types. A standardized major axis (SMA) slope was used to fit bivariate relationships between the natural log-transformed  $R_r$  of  $g_{min}$  and the natural log-transformed number of galls per leaf area. Mean values and standard deviation of each trait in the galled and intact leaves are shown in Supplementary data Fig. S2.

To examine whether leaf traits and environmental conditions were associated with gall formations (presence and abundance), we used a zero-altered negative-binomial generalized linear mixed model (*glmmADMB* package of R; Skaug *et al.*, 2012). This model was used because many shoots had no galls, and the frequency of the number of galls except for non-galling shoots was strongly over-dispersed from Poisson distribution (Supplementary data Fig. S3). The zero-altered negative-binomial model is based both on



the binomial–distribution model for presence/absence data, and on the negative–binomial–distribution model for count data except for zero (Martin *et al.*, 2005; Zuur *et al.*, 2009).

Thus, we constructed two models to test the associations of environmental conditions and leaf traits with presence or absence of galls (Model I) and with the number of galls (Model II).

This is the standard analytical approach for this type of dataset (Zuur *et al.*, 2009). In terms of the choices of leaves by psyllids, we assumed that Model I is more relevant because the presence/absence data may be related to the process whether psyllids decide to lay eggs or not. On the other hand, Model II may be more related to the behaviour or capacity of psyllids after they decide to lay eggs. We assumed that the associations between leaf traits and presence/absence of galls (Model I) or the number of galls (Model II) irrespective of environmental factors reflect the influences of leaf traits on gall formation. In addition, we assumed that the associations between environmental conditions and presence/absence of galls (Model I) reflect the habitat preferences of each psyllid species.

Because we intended to test whether the amount of leaf trichomes has consistent negative effects on the gall formations, a linear term was assumed for this factor in the model. On the other hand, because the psyllids may have a unimodal distribution across environmental gradients (e.g., elevational gradients; Nishida *et al.*, 1980), both linear and quadratic terms were assumed for the environmental factors in the model. A significant negative quadratic term can be selected if psyllids have a unimodal distribution across the environmental gradient. We employed annual mean temperature, aridity index, and soil age (as indicator of soil fertility; Vitousek *et al.*, 1988) as explanatory variables in the models because the abundance of gall makers may be related to temperature (Henson, 1958), aridity (Fernandes and Price, 1992; Price *et al.*, 1998), and soil fertility (Blanche and Westoby, 1995). We examined the estimated effects of  $LMA_T$ ,  $LMA_L$ , the annual mean temperature (MAT,  $MAT^2$ ), aridity index (AI,  $AI^2$ ), and soil age (SA,  $SA^2$ ) as fixed effects, and site as a

random effect, on the numbers of each type of galls per leaf area. Although leaf traits and environmental conditions were not independent from each other on the island of Hawaii (Joel *et al.*, 1994; Tsujii *et al.*, 2016), we treated these parameters as independent fixed effects due to the small variance inflation factor (VIF; <1.2). Each fixed effect was standardized (zero mean and standard deviation equal to one). Because the number of galls should increase with leaf area per shoot, total leaf area of a shoot was used as an offset term in Model II. Soil age was used as the median of soil–age ranges available from Sherrod *et al.* (2007) for the analysis (Supplementary data Table S1). Soil ages were natural log–transformed before the standardization. Akaike’s Information Criterion (AIC) was used to examine which explanatory variables were important for the abundances of each type of galls (Supplementary data Table S2).

All analyses were carried out with the R statistical package (version 3.5.0; R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

### *Leaf water relations and gall formations*

The leaf segments with gall formations had significantly higher thickness, mass per area, water content per area, and water content per fresh mass irrespective of gall types ( $p < 0.05$ ; Fig. 2 a–d). These increases in thickness, mass per area, and water content per area were largest in the cone type, followed by flat and pit types (Fig. 2 a–c). The increase in water content per fresh mass was larger in the pit type than in the cone and flat types (Fig. 2 d). Our conservative estimates (see MATERIALS AND METHODS) showed that mean response ratio of mass per area was 197, 149, and 115 %, and mean response ratio of water content per area 211, 158, and 142 % for the cone–, flat– and pit–type galls, respectively (Fig. 2 b, c).

Response ratio ( $R_r$ ) of leaf minimum conductance per area ( $g_{min}$ ) was significantly positively associated with the number of galls per leaf area in the cone or flat types ( $R^2 = 0.31$  and  $0.30$ , respectively) but not in the pit type (Fig. 3). The slopes of regression lines (log–log scale) were  $0.52$  and  $0.47$  for the cone and flat types, respectively, suggesting that the leaf minimum conductance becomes more than double compared to the intact leaves when the densities of these galls exceed one per square centimeter.

#### *Associations among galls, leaf traits and environmental conditions*

In line with previous studies (Joel *et al.*, 1994; Tsujii *et al.*, 2016), pubescent individuals were dominant in dry conditions and glabrous individuals were dominant in wet conditions (Supplementary data Fig. S4). The correlation between the amount of leaf trichomes and aridity index was negative as expected ( $r = -0.42$ ,  $p < 0.01$ ). In the present study, we tested whether leaf traits and environmental conditions were associated with presence/absence of galls (Model I) and with the abundance of galls (Model II; see MATERIALS AND METHODS). Table 1 shows standardized coefficients of the best fit model based on AIC. These coefficients reflect relative importance of each factor on the presence or abundance of galls (Model I or Model II). Interpretation of linear coefficient is straightforward when only linear term is selected (higher values indicate stronger effects) but may be more complicated when a quadratic term is selected because the linear coefficient is subjected to the quadratic coefficient. Negative or positive values in the quadratic term indicates an upward– or downward–convex relationship within the range of each factor.

The binomial–distribution model for presence/absence data (Model I) showed that the quadratic terms of temperature ( $MAT^2$ ) were significantly negative in each gall type, suggesting that there was a peak presence of each gall type across the temperature gradient

(Table 1; Fig. 4 a–c). The peak temperature for the presence was different among the gall types; cone, flat, and pit types at around 20, 16, and 12 °C, respectively (Fig. 4 a–c). In particular, the cone-type galls were rarely observed at the sites below 14 °C and pit-type galls were rarely observed above 20 °C (Fig. 4 a, c). The negative quadratic term of aridity index ( $AI^2$ ) was selected for the presence of pit type, and the peak aridity index was 2.4 (Table 1; Fig. 4 f). Similarly, the quadratic terms were negative in soil age ( $SA^2$ ) in the cone and flat types; the peak soil ages were roughly 2,000 years for both large gall types (Table 1; Fig. 4 g, h). For the pit type, soil age was negatively associated with the presence (Table 1; Fig. 4 i). In relation to leaf traits, the presence of cone and flat types was negatively associated with trichome mass ( $LMA_T$ ) as well as lamina mass ( $LMA_L$ ) (Table 1; Fig. 4 j, k, m, n) while the presence of pit type was neither associated with these leaf traits (Table 1; Fig. 4 l, o). The standardized coefficients of  $LMA_T$  were larger on the cone type than on the flat type (Table 1) reflecting a stronger negative association between  $LMA_T$  and the presence of galls for the cone than for the flat types (Fig. 4 j, k).

The negative–binomial–distribution model for the count data (Model II) showed that the numbers of cone- and pit-type galls were negatively associated with annual mean temperature (Table 1; Fig. 5 a, c). Unlike the results of Model I analysis, negative quadratic terms were not selected in these relationships partly because zero count data (absence data) were excluded in Model II analysis. For the flat type, its number of galls was best fitted by an upward–convex curve with the peak at around 15 °C (negative quadratic term of temperature ( $MAT^2$ ); Fig. 5 b). Across aridity index, the numbers of cone- and flat-type galls were best fitted by a downward–convex curve with the bottoms at 3.0 and 1.2, respectively (positive quadratic terms of aridity index ( $AI^2$ ); Fig. 5 d, e). The number of pit-type galls was weakly negatively associated with aridity index (Table 1; Fig. 5 f). All types of galls were not associated with soil age (Table 1). In relation to leaf traits, the numbers of galls were

negatively and positively associated with  $LMA_T$  in the cone and flat types respectively although these associations were weak (Table 1; Fig. 5 j, k). The number of pit-type galls was weakly positively associated with  $LMA_L$  but not associated with  $LMA_T$  (Table 1; Fig. 5 l, o).

## DISCUSSION

### *Leaf traits, physiological functions, and galls*

Leaf minimum conductance, which is associated with uncontrolled water loss from plant surface, should be minimized, especially for plants inhabiting dry areas (Irvine *et al.*, 1998; Medrano *et al.*, 2002). The leaf minimum conductance sharply increased with the number of galls in the cone and flat types while such trend was not found in the pit type (Fig. 3). When the densities of cone- and flat-type galls exceeded one per square centimeter, the leaf minimum conductance became more than double compared to the intact leaves. In addition, our field census suggested that the densities of cone- and flat-type galls were greater than that of pit-type galls (Supplementary data Fig. S3). These results suggest that formations of larger galls (i.e., cone and flat types) can bring a strong water stress on *M. polymorpha* probably due to the increased surface area (Fig. 1, 2 a), impaired stomata (Jiang *et al.*, 2018), and the fissures when the psyllids mature (Fig. 1). This finding is also supported by a genome study which found drought tolerance genes were significantly upregulated in galled leaves in *M. polymorpha* (Bailey *et al.*, 2015). While we focused only on leaf minimum conductance, some studies focusing on other gall makers reported that gall formation also increased stomatal conductance (Fay *et al.*, 1996; Larson, 1998; Huang *et al.*, 2014) whereas other studies reported the opposite trend (Larson, 1998; Florentine *et al.*, 2005; Patankar *et al.*, 2011; Jiang *et al.*, 2018). Although the effects on gall formations on stomatal conductance

remains inconclusive, our study clearly showed that the uncontrolled loss of leaf water was increased by gall formations in *M. polymorpha*.

The negative trends between leaf trichomes and the presences of the cone- and flat-type galls but not that of the pit-type galls (Table 1; Fig. 4 j, k) suggest that the leaf trichomes of *M. polymorpha* may contribute to impede the cone- and flat-type gall makers but not pit-type gall makers. These different patterns could be explained by the direction of attacks of each psyllid species. The cone- and flat-type gall makers (*P. pyramidalis* and *P. pele*, respectively) typically attack from *lower* surface where leaf trichomes are present. Thickness of leaf trichome layer ranged from 0 to ca. 0.80 mm (Supplementary data Fig. S5), which is often longer than ovipositor lengths of the *P. pyramidalis* and *P. pele* (ca. 0.09 and 0.11 mm, respectively, from Percy, 2017). On the other hand, as the pit-type gall maker (*P. minutus*) attacks from the *upper* surface (Percy, 2017), the *lower* leaf trichomes did not hinder the attack of this species. Therefore, the leaf trichomes of *M. polymorpha* could effectively deter the egg deposition of *P. pyramidalis* and *P. pele* but not *P. minutus*. Previous studies that did not taxonomically discriminate these psyllid species found inconsistent relationships between leaf trichomes and gall formations (Lee, 1981; Gruner *et al.*, 2005). We speculate that such inconsistency might be partly due to different ways of egg deposition among psyllid species. On the other hand, the specific mass of lamina ( $LMA_L$ ) itself also was negatively associated with the presence of cone- and flat-type galls (Table 1; Fig. 4 m, n). While leaf lamina is the tissue of photosynthesis, a large fraction of lamina mass is allocated to structural tissues including cell walls and cuticles (>40 % for evergreen woody species), which is important for leaf physical strength (Onoda *et al.*, 2011) and resistance against herbivores and other physical stresses (Read and Stokes, 2006). Thus, it may be natural to observe negative associations of  $LMA_L$  with the presence of the galls irrespective of the amount of leaf trichomes.

Contrary to the results of Model I,  $LMA_T$  and  $LMA_L$  had the mixed associations with the number of galls in Model II analysis albeit rather small effects (Table 1; Fig. 5 j, k, o). While trichomes are typically considered as barriers for herbivores, some previous studies reported that some insects can utilize trichomes (e.g., Nishijima, 1960). It might be the case that some psyllid species such as *P. pele* can utilize trichomes as a foothold to oviposit and/or as a refuge to escape from environmental stresses, which may result into a weak but positive association between leaf trichomes and abundance of galls. We also speculate that lamina with a higher  $LMA_L$  may accommodate a greater number of galls particularly smaller galls which induce a lower stress because such leaves are often more durable and long-lived (Wright *et al.*, 2004). Moreover, while we used the fully-matured young leaves for the leaf-trait measurement, the psyllid species might choose young developing leaves for oviposition. To clarify such mixed results may require more detailed observations and experiments on behaviours in relation to leaf traits, on associations between gall formations and leaf longevity, and on timing of oviposition for each psyllid species (e.g., cafeteria experiments).

#### *Environmental conditions and galls*

The three psyllid species may differ in their habitat temperature as suggested by different distributions of the three gall types across temperature (Table 1; Fig. 4 a–c). Because these three psyllid species have been diversified from a single ancestor on the same host tree species (Percy, 2017), they are likely to have partitioned their niches along a temperature gradient (i.e., an elevational gradient) over evolutionary time scales. This niche partitioning could be related to the size of galls; small galls (i.e., pit type) are more common in the colder conditions while the intermediate (i.e., flat type) and larger galls (i.e., cone type) are more common in the intermediate and warmer areas respectively (Table 1, Fig. 4 a–c).

This may be in line with the temperature limitation of physiological activities known for gall makers (Henson, 1958). Because a formation of larger galls generally requires higher energy as compared to smaller galls, the psyllids that form larger galls may not be adaptive to low-temperature conditions. Other environmental factors such as air pressure and UV radiation may also be related to the elevational distribution of the three psyllid species (e.g., Hodkinson, 2005).

Among the three psyllid species, only the pit-type galls showed an upward-convex curve for probability of presence across the aridity index (Table 1; Fig 4 f). Since the pit-type galls have an open structure while other two types have enclosed structures, the immatures of *P. minutus* may be more susceptible to external environmental stresses. For example, in arid conditions, the pit-type galls cannot protect the immatures from dry air, as indicated by the particularly low abundance of the pit-type galls in the arid areas (aridity index < 0.5; Fig. 4 f, Fig. 5 f). On the other hand, in heavy-rainfall conditions, the immatures seated in pit-type galls on upper surfaces are often exposed to raindrops, which may increase the risks of being washed away. Such less protected habitat for *P. minutus* may exclude this species from both extreme ends of the aridity gradient. In contrast, enclosed galls, i.e., cone- and flat-type galls, can protect immatures from drought and physical stresses (Fernandes and Price, 1992; Stone and Schönrogge, 2003); thus, the distributions of *P. pyramidalis* and *P. pele* may be less susceptible to aridity index. Other possibilities such as light intensity might also contribute to these patterns; therefore, our interpretation should be considered as a hypothesis and subject to more robust tests.



## CONCLUSIONS

This study demonstrates that formations of galls, in particular cone and flat types, greatly increase leaf water loss possibly through the increased leaf surface area and the fissures on the leaves in *M. polymorpha*. Our field observation suggests that leaf trichomes can be effective to impede the colonization of psyllid species that form cone-type galls, and possibly that form flat-type galls. These findings support our hypothesis that leaf trichomes can contribute to the avoidance of extra water stress through impeding gall formations.

It should be noted that present results do not rule out the possibility of other functions of leaf trichomes: such as saving water against evaporative demands, capturing dew, repelling dust and water, and maintaining leaf temperature (Johnson, 1975; Haworth and McElwain, 2008; Bickford, 2016; Amada et al., 2017). Moreover, it remains uncertain whether the large allocation of leaf mass to trichomes up to 40 % can be explained just from the defence against gall formations because the thickness of trichome layer often seem to be too long for the ovipositor lengths of psyllids (up to several-fold). That leaves with the largest amount of trichomes are common in the alpine zone where psyllids are not so abundant also suggests that other selective forces are involved for the adaptive development of leaf trichomes (Fig. 4). More comprehensive knowledge of those other functions is required to fully understand the ecological significances of the large diversity of leaf trichomes in *M. polymorpha*.

The diversity of gall morphology and the pattern of gall abundance elucidated in our study may reflect the adaptive radiation of *Pariaconus* species as a consequence of the coevolution with various phenotypes of *M. polymorpha*. Leaf trichomes of *M. polymorpha* must have exerted a strong selective pressure on *Pariaconus* species, which in turn favoured *M. polymorpha* phenotype in terms of avoiding water stress from leaf surfaces. However, the

associations between leaf trichomes and the abundance of galls were not strict because leaf trichomes seem to have evolved also in response to various other abiotic and biotic environmental factors on the island of Hawaii. Our study is so far the most extensive description about the relationships between leaf trichomes and the abundance of galls over the whole island of Hawaii. The knowledge obtained in this study would be also useful to unravel the genetic basis of the history of coevolution between *M. polymorpha* and *Pariaconus* species on this unique ecosystem (Stacy *et al.*, 2014; Izuno *et al.*, 2016; Percy, 2017)

#### SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Table S1: site information. Table S2: coefficients of selected explanatory variables and AIC values in Model I and Model II for each type of galls. Figure S1: locations of study sites on the island of Hawaii. Figure S2: Mean and standard error of thickness, mass per area, water content per area, water content per fresh mass in intact and galled part of leaves, and  $g_{min}$  in intact and galled leaves. Figure S3: frequencies of the numbers of each type of galls per shoot. Figure S4: Associations between trichome mass and environmental factors. Figure S5: the relationship between trichome thickness and trichome mass, and the associations between trichome thickness and the abundance of each type of galls.

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## LITERATURE CITED

- Agrawal AA, Fishbein M, Jetter R, et al. 2009.** Phylogenetic ecology of leaf surface traits in the milkweeds (*Asclepias* spp.): chemistry, ecophysiology, and insect behavior. *New Phytologist* **183**: 848–867.
- Amada G, Onoda Y, Ichie T, Kitayama K. 2017.** Influence of leaf trichomes on boundary layer conductance and gas-exchange characteristics in *Metrosideros polymorpha* (Myrtaceae). *Biotropica* **49**: 482–492.
- Aronne G, De Micco V. 2001.** Seasonal dimorphism in the Mediterranean *Cistus incanus* L. subspecies *incanus*. *Annals of Botany* **87**: 789–794.
- Bailey S, Percy DM, Hefer CA, Cronk QCB. 2015.** The transcriptional landscape of insect galls: psyllid (Hemiptera) gall formation in Hawaiian *Metrosideros polymorpha* (Myrtaceae). *BMC Genomics* **16**: 943. doi: 10.1186/s12864-015-2109-9.
- Benzing DH, Henderson K, Kessel B, Sulak J. 1976.** The absorptive capacities of bromeliad trichomes. *American Journal of Botany* **1009–1014**.
- Bickford CP. 2016.** Ecophysiology of leaf trichomes. *Functional Plant Biology* **43**: 807–814.
- Blanche KR, Westoby M. 1995.** Gall-forming insect diversity is linked to soil fertility via host-plant taxon. *Ecology* **76**: 2334–2338.
- Cordell S, Goldstein G, Mueller-Dombois D, Webb D, Vitousek PM. 1998.** Physiological and morphological variation in *Metrosideros polymorpha*, a dominant Hawaiian tree species, along an altitudinal gradient: The role of phenotypic plasticity. *Oecologia* **113**: 188–196.
- Cornwell WK, Bhaskar R, Sack L, Cordell S, Lurch CK. 2007.** Adjustment of structure and function of Hawaiian *Metrosideros polymorpha* at high vs. low precipitation. *Functional Ecology* **21**: 1063–1071.
- Dalin P, Ågren J, Björkman C, Huttunen P, Kärkkäinen K. 2008.** Leaf trichome formation and plant resistance to herbivory. In: Schaller A, ed. *Induced plant resistance to herbivory*. New York: Springer, 89–105.
- Duursma RA, Blackman CJ, López R, Martin-StPaul NK, Cochard H, Medlyn BE. 2019.** On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. *New Phytologist* **221**: 693–705.
- Ehleringer J. 1984.** Ecology and ecophysiology of leaf pubescence in North American desert plants. In: Rodriguez E, Healey PL, Mehta I, eds. *Biology and chemistry of plant trichomes*. New York: Plenum Press, 113–132.
- Fay PA, Hartnett DC, Knapp AK. 1996.** Plant tolerance of gall-insect attack and gall-insect performance. *Ecology* **77**: 521–534.

- Fernandes GW, Price PW. 1992.** The adaptive significance of insect gall distribution: survivorship of species in xeric and mesic habitats. *Oecologia* **90**: 14–20.
- Florentine SK, Raman A, Dhileepan K. 2005.** Effects of gall induction by *Epiblema strenuana* on gas exchange, nutrients, and energetics in *Parthenium hysterophorus*. *BioControl* **50**: 787–801.
- Giambelluca TW, Shuai X, Barnes ML, et al. 2014.** Evapotranspiration of Hawai‘i. Final report submitted to the U.S. Army Corps of Engineers–Honolulu District, and the Commission on Water Resource Management, State of Hawai‘i.
- Gruner DS. 2004.** Arthropods from ‘ōhi‘a lehua (Myrtaceae: *Metrosideros polymorpha*), with new records for the Hawaiian Islands. *Bishop Museum Occasional Papers* **78**: 33–52.
- Gruner DS, Taylor AD, Forkner RE. 2005.** The effects of foliar pubescence and nutrient enrichment on arthropod communities of *Metrosideros polymorpha* (Myrtaceae). *Ecological Entomology* **30**: 428–443.
- Haworth M, McElwain J. 2008.** Hot, dry, wet, cold or toxic? Revisiting the ecological significance of leaf and cuticular micromorphology. *Palaeogeography, Palaeoclimatology, Palaeoecology* **262**: 79–90.
- Henson WR. 1958.** The effects of radiation on the habitat temperatures of some poplar-inhabiting insects. *Canadian Journal of Zoology* **36**: 463–478.
- Hodkinson ID. 2005.** Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews* **80**: 489–513.
- Hoof J, Sack L, Webb DT, Nilsen ET. 2008.** Contrasting structure and function of pubescent and glabrous varieties of Hawaiian *Metrosideros polymorpha* (Myrtaceae) at high elevation. *Biotropica* **40**: 113–118.
- Huang MY, Huang WD, Chou HM, et al. 2014.** Leaf-derived cecidomyiid galls are sinks in *Machilus thunbergii* (Lauraceae) leaves. *Physiologia plantarum* **152**: 475–485.
- Ichie T, Inoue Y, Takahashi N, Kamiya K, Kenzo T. 2016.** Ecological distribution of leaf stomata and trichomes among tree species in a Malaysian lowland tropical rain forest. *Journal of plant research* **129**: 625–635.
- Irvine J, Perks MP, Magnani F, Grace J. 1998.** The response of *Pinus sylvestris* to drought: stomatal control of transpiration and hydraulic conductance. *Tree Physiology* **18**: 393–402.
- Izuno A, Hatakeyama M, Nishiyama T, et al. 2016.** Genome sequencing of *Metrosideros polymorpha* (Myrtaceae), a dominant species in various habitats in the Hawaiian Islands with remarkable phenotypic variations. *Journal of Plant Research* **129**: 727–736.

- Joel G, Aplet G, Vitousek PM. 1994.** Leaf morphology along environmental gradients in Hawaiian *Metrosideros polymorpha*. *Biotropica* **26**: 17–22.
- Jiang Y, Veromann–Jürgenson LL, Ye J, Niinemets Ü. 2018.** Oak gall wasp infections of *Quercus robur* leaves lead to profound modifications in foliage photosynthetic and volatile emission characteristics. *Plant, cell & environment* **41**: 160–175.
- Johnson HB. 1975.** Plant pubescence: An ecological perspective. *The Botanical Review* **41**: 233–258.
- Kitayama K, Mueller–Dombois D. 1995.** Vegetation changes along gradients of long–term soil development in the Hawaiian montane rainforest zone. *Vegetation* **120**: 1–20.
- Larson KC. 1998.** The impact of two gall–forming arthropods on the photosynthetic rates of their hosts. *Oecologia* **115**: 161–166.
- Lee M. 1981.** Insect damage to leaves of two varieties of *Metrosideros collina* subsp. *polymorpha*. *Pacific Science* **35**: 89–92.
- Levin DA. 1973.** The role of trichomes in plant defense. *The Quarterly Review of Biology* **48**: 3–15.
- Martin TG, Wintle BA, Rhodes JR, et al. 2005.** Zero tolerance ecology: Improving ecological inference by modelling the source of zero observations. *Ecology Letters* **8**: 1235–1246.
- Medrano H, Escalona JM, Bota J, Gulias J, Flexas J. 2002.** Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Annals of Botany* **89**: 895–905.
- Nabity PD, Hillstrom ML, Lindroth RL, DeLucia EH. 2012.** Elevated CO<sub>2</sub> interacts with herbivory to alter chlorophyll fluorescence and leaf temperature in *Betula papyrifera* and *Populus tremuloides*. *Oecologia* **169**: 905–913.
- Nishida T, Haramoto FH, Nakahara LM. 1980.** Altitudinal distribution of endemic psyllids (Homoptera: Psyllidae) in the *Metrosideros* ecosystem. *Proceeding Hawaiian Entomological Society* **23**: 255–262.
- Nishijima Y. 1960.** Host plant preference of the soybean pod borer, *Grapholitha glycinivorella* Matsumura (Lep., Eucosmidae) 1. Oviposition site. *Entomologia Experimentalis et Applicata* **3**: 38–47.
- Onoda Y, Westoby M, Adler PB, et al. 2011.** Global patterns of leaf mechanical properties. *Ecology Letters* **14**: 301–312.
- Patankar R, Thomas SC, Smith SM. 2011.** A gall–inducing arthropod drives declines in canopy tree photosynthesis. *Oecologia* **167**: 701–709.

- Percy DM. 2017.** Making the most of your host: The *Metrosideros*-feeding psyllids (Hemiptera, Psylloidea) of the Hawaiian Islands. *ZooKeys* **649**: 1–163.
- Price PW, Fernandes GW, Waring GL. 1987.** Adaptive nature of insect galls. *Environmental Entomology* **16**: 15–24.
- Price PW, Fernandes GW, Lara ACF, et al. 1998.** Global patterns in local number of insect galling species. *Journal of Biogeography* **25**: 581–591.
- Read J, Stokes A. 2006.** Plant biomechanics in an ecological context. *American Journal of Botany* **93**: 1546–1565.
- Sack L, Cowan PD, Jaikumar N, Holbrook NM. 2003.** The “hydrology” of leaves: coordination of structure and function in temperate woody species. *Plant, Cell and Environment* **26**: 1343–1356.
- Sherrod DR, Sinton JM, Watkins SE, Brunt KM. 2007.** Geologic map of the state of Hawaii. US Geological Survey Open-File Report, 1089, 83.
- Skaug H, Fournier D, Nielsen A, Magnusson A, Bolker BM. 2015.** glmmADMB: generalized linear mixed models using AD Model Builder. R package version 0.7. 2.1. 2012.
- Sokal RR, Rohlf FJ. 1995.** *Biometry: principles and practices of statistics in biological research*, 3rd edn. New York: W. H. Freeman.
- Stacy EA, Johansen JB, Sakishima T, Price DK, Pillon Y. 2014.** Incipient radiation within the dominant Hawaiian tree *Metrosideros polymorpha*. *Heredity* **113**: 1–9.
- Stacy EA, Johansen JB, Sakishima T, Price DK. 2016.** Genetic analysis of an ephemeral intraspecific hybrid zone in the hypervariable tree, *Metrosideros polymorpha*, on Hawai‘i Island. *Heredity* **117**: 173–183.
- Stemmermann L. 1983.** Ecological studies of Hawaiian *Metrosideros* in a successional context. *Pacific Science* **37**: 361–373.
- Stone GN, Schönrogge K. 2003.** The adaptive significance of insect gall morphology. *Trends in Ecology and Evolution* **18**: 512–522.
- Tsujii Y, Onoda Y, Izuno A, Isagi Y, Kitayama K. 2016.** A quantitative analysis of phenotypic variations of *Metrosideros polymorpha* within and across populations along environmental gradients on Mauna Loa, Hawaii. *Oecologia* **180**: 1049–1059.
- UNESCO. 1979.** Map of the world distribution of arid regions. Explanatory note. Man and Biosphere (MAB).
- Vitousek PM, Aplet G, Turner D, Lockwood JJ. 1992.** The Mauna Loa environmental matrix: foliar and soil nutrients. *Oecologia* **89**: 372–382.



**Vitousek PM, Matson PA, Turner DR. 1988.** Elevational and age gradients in Hawaiian montane rainforest: foliar and soil nutrients. *Oecologia* **77**: 565–570.

**Wright IJ, Reich PB, Westoby M, et al. 2004.** The worldwide leaf economics spectrum. *Nature* **428**: 821–827.

**Wuenschel JE. 1970.** The effect of leaf hairs of *Verbascum thapsus* on leaf energy exchange. *New Phytologist* **69**: 65–73.

**Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. 2009.** Zero-truncated and zero-inflated models for count data. In Gail M, Krickeberg K, Samet J, Tsiatis A, Wong W, eds. *Mixed effects models and extensions in ecology with R*. New York: Springer, 261–293.

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## Figure legends

Figure 1. Representative images of galls induced by Hawaiian psyllids (*Pariaconus* spp.) on leaves of *Metrosideros polymorpha*. Cone-type galls (large size) from the upper (a) and the lower leaf surface (b), and an immature uncovered by the dissection of the enclosed gall (c). Flat-type galls (intermediate size) from the upper (d) and the lower leaf surface (e), and an immature uncovered by the dissection of the enclosed gall (f). Pit-type galls (small size) from the upper (g) and the lower leaf surface (h), and an immature developed on the hollow of the open gall (i, j). White arrows indicate the immatures of each psyllid (f, g, j).

Figure 2. Response ratio ( $R_r$ ) of thickness (a), mass per area (b), water content per area (c), and water content per fresh mass (d) on a log scale. Different alphabets above the boxes in each figure indicate significant difference at 5 % level (Tukey–Kramer test among the three gall types)

Figure 3. Response ratio ( $R_r$ ) of leaf minimum conductance ( $g_{min}$ ) plotted against the number of galls for cone, flat, and pit types separately on a log–log scale. Filled symbols denote leaves with only closed galls (still immatures were growing in the galls), and open symbols denote leaves with one or more opened galls (some immatures had already left). “No gall” symbols mean the response ratios calculated between two intact leaves (mean  $\pm$  SD). Solid regression lines indicate significant relationship ( $p < 0.05$ ).

Figure 4. The presence/absence of galls are plotted against environmental conditions or leaf traits. For each explanatory variable, when significant association was found in Model I analysis, a partial regression line is calculated and drawn by fixing other explanatory

variables at each mean value. The color of symbol represents mean annual temperature of each study site.

Figure 5. The number of galls more than zero are plotted against environmental conditions or leaf traits. For each explanatory variable, when significant association was found in Model II analysis, a partial regression line is calculated and drawn by fixing other explanatory variables at each mean value. The color of symbol represents mean annual temperature of each study site.

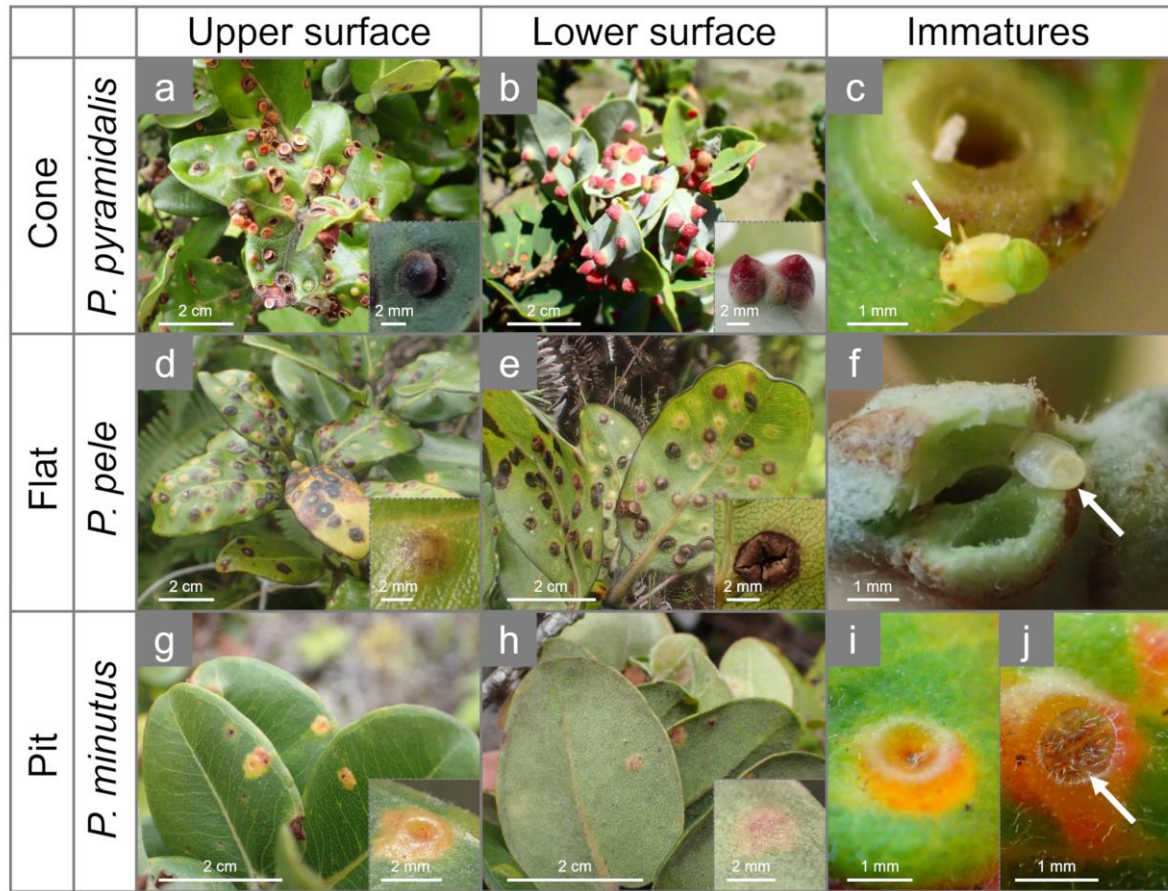
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Table 1. Standardized coefficients for the explanatory variables for presence/absence (binomial–distribution model; Model I) or abundance (negative–binomial–distribution model; Model II) of galls.

Model	Type	MAT <sup>2</sup>	MAT	AI <sup>2</sup>	AI	SA <sup>2</sup>	SA	LMA <sub>T</sub>	LMA <sub>L</sub>
<b>I</b>	<b>Cone</b>	-2.11	2.96	—	—	-0.77	—	-0.73	-0.44
	<b>Flat</b>	-0.56	-0.40	—	—	-0.24	—	-0.26	-0.29
	<b>Pit</b>	-0.48	-1.37	-0.66	1.61	—	-0.37	—	—
<b>II</b>	<b>Cone</b>	—	-0.36	0.29	-1.08	—	—	-0.11	—
	<b>Flat</b>	-0.11	-0.17	0.10	—	—	—	0.20	—
	<b>Pit</b>	—	-0.49	—	-0.25	—	—	—	0.18

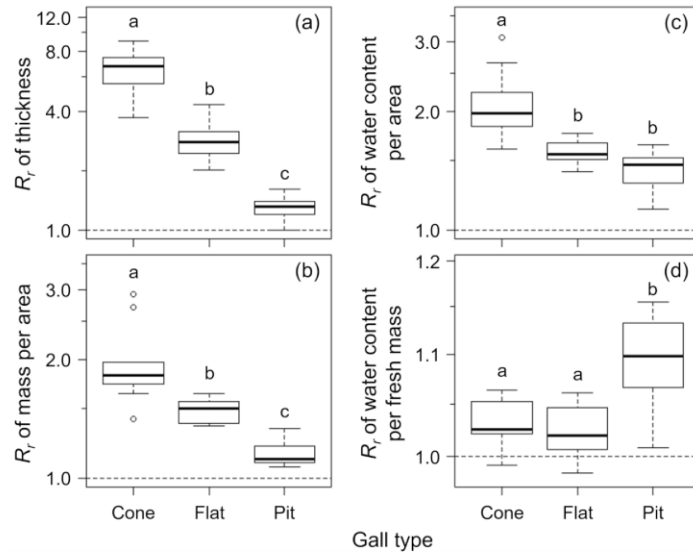
*Notes:* The best fit model was selected based on Akaike’s Information Criterion (AIC) (see Supplementary data Table S2). These coefficients have no unit because each fixed effect was standardized (zero mean and standard deviation equal to one). MAT: mean annual temperature, AI: aridity index, SA: soil age, LMA<sub>T</sub>: LMA associated with trichomes, LMA<sub>L</sub>: LMA associated with lamina. See the main text for more detail.

Figure 1



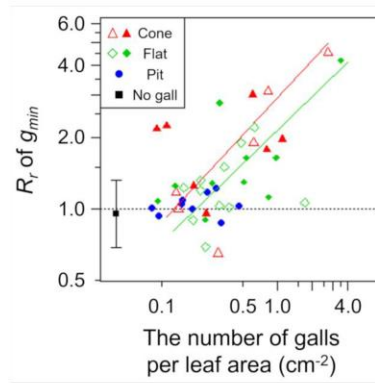
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Figure 2



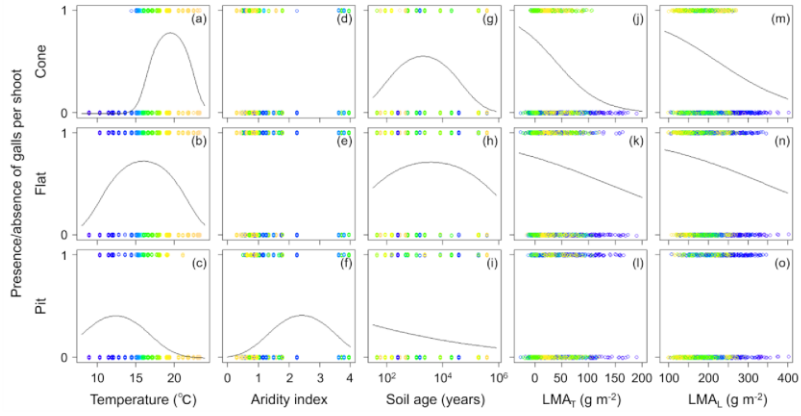
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Figure 3



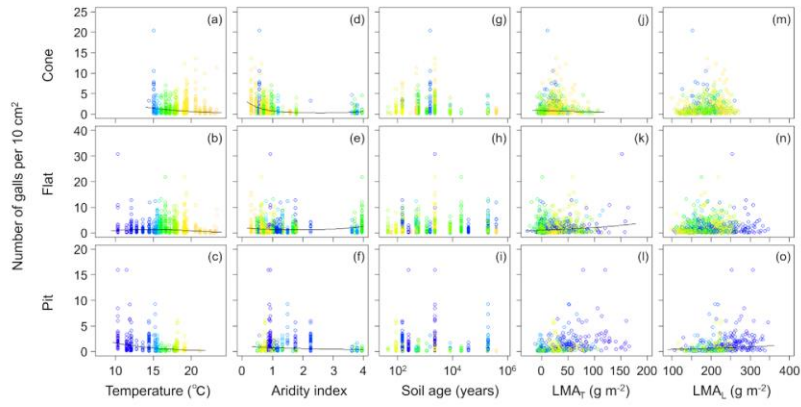
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Figure 4



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Figure 5



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