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Effects of Nitrogen Availability and Climate on Hermes Copper Butterfly (*Lycaena hermes*) Habitat in Southern California

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ABSTRACT

As environmental conditions continue to change, phytophagous insects are under increasing selective pressure when identifying and choosing quality host plants. The Hermes copper butterfly (*Lycaena hermes*) is a rare species endemic to San Diego County and parts of northern Baja California. The small butterfly population is a fascinating topic since the host plant, *Rhamnus crocea*, extends well beyond the range of the insect. Based on what is known about plant herbivore interactions it is possible that variation in plant chemistry is contributing to small population sizes and a limited distribution. Plant tissue chemistry is affected by environmental variations, such as chronic nitrogen deposition, temperature, and precipitation. We hypothesized that plant secondary compounds are likely to vary significantly within and outside of the Hermes copper butterfly range, trends in secondary compounds will be affected by variations in climate, such as temperature and precipitation, and spatial variations in plant secondary compounds will be affected by N availability. Leaf tissue from current and historical Hermes copper habitat sites were analyzed and plant secondary compounds classes were identified. Soil samples were collected in May 2018 to measure extractable N, soil moisture, total N and C, and pH. N availability did not have a significant effect on plant secondary compounds within and outside of the range of the butterfly. However, it was found that climate conditions did have a significant effect on these compounds. The lack of a N effect on plant secondary compounds in the host plant suggests that allocation of resources within the plant may not be significantly influenced by changes in N availability, but may be affected by changes in temperature and precipitation.

INTRODUCTION

Plant Herbivore interactions

As environmental conditions continue to change, phytophagous insects are under increasing selective pressure when identifying and choosing quality host plants (Bruce 2015). Phytophagous insects are known to be the primary consumers in temperate ecosystems and can significantly modify species composition and nutrient cycling (Schowalter et al. 1986; Choudhury 1988; Veblen et al. 1991; Dyer and Shugart 1992). Shifts in interactions between insects and their environment may cause cascading ripple effects that can alter ecosystem processes (Lindroth 1996). Chemical composition is one of the primary factors affecting foliar quality (Schultz 1988; Ehrlich and Murphy 1988) and thus strongly impact insect performance (Slansky and Scriber 1985; Scriber and Ayres 1988).

Lepidopteran herbivores face many challenges as their diet consists entirely of leaves, which have a multitude of chemical and physical defensive qualities (Rosenthal and Berenbaum 1991). Insects have evolved ways of finding quality host plants, whereas plants have evolved defense mechanisms to avoid detection (Bruce 2015). A multitude of interactions, both positive and negative, can occur between an insect and its host plant, as both plants and insects have developed characteristics over time that benefit and aid in their survival. Plant volatile emission responses are induced by interaction with insect feeding and oviposition. Conversely, plant reproductive fitness is improved through increased pollination (Bruce 2015). Often growth and survival traits of caterpillars are strongly correlated with nutritional and defensive traits in their respective host plant (Coley et al. 2006). For example, herbivores have a stronger preference for young, fast

expanding leaves because they have a higher nutritional value and lower toughness due to their high nitrogen and water content (Marquis and Braker 1994; Coley and Barone 1996). Rapid expansion of young leaves is considered a plant defensive trait that decreases the period of vulnerability to herbivores (Feeny 1976; McKey 1979; Aide and Londoño 1989). However, allocation of resources to rapid growth leads to lower chemical defenses, which actually causes the plant to suffer higher rates of herbivory (Coley et al. 2006). In response, specialist insect species often have fewer defensive traits than generalist species resulting from their limited host plant diet. The mechanisms in which a lepidopteran species approaches plant defensive characteristics can affect its growth, fecundity, and overall survivability (Coley et al. 2006). Fast growth during the caterpillar stage, for example, can lead to a larger size at pupation and an increase in quantity and quality of eggs (Haukioja and Neuvonen 1985; Ohmart et al. 1985; Awmack and Leather 2002). Conversely, a longer larval period can lead to an increase in predation as they remain in a vulnerable state for an extended period of time. Therefore, it is possible that certain plant traits contribute to slow larval growth and are considered defense mechanisms as they increase predation on phytophagous herbivorous insect species (Coley et al. 2006).

Rhamnus crocea/Hermes copper

The Hermes copper butterfly (*Lycaena hermes*) is a rare species endemic to San Diego County (Wright 1930; Comstock and Dammers 1935) and parts of northern Baja California (Thorne 1963; Emmel and Emmel 1973). Hermes copper colonies are found in close proximity to its host plant, spiny redberry (*Rhamnus crocea*), and are often found nectaring on California buckwheat (*Eriogonum fasciculatum*) (Marschalek and Deutschman 2008). Over the past few decades the Hermes copper has been petitioned to be listed under

the Endangered Species Act (ESA) with no success (US Fish and Wildlife Service 1993; US Fish and Wildlife Service 1994; Hogan 2004). Due to its limited range there is minimal research on this threatened species, which makes listing very difficult. Researchers have made multiple attempts to observe *in-situ* larvae on spiny redberry, but most have ultimately been unsuccessful (Deutschman and Marschalek 2009). The small butterfly population is a fascinating topic since the host plant extends well beyond the range of the insect (Thorne 1963; Figure 1). Entomologists have been unable to determine the dispersal ability and habitat requirements of the Hermes copper (Harrison and Hastings 1996; Marschalek and Klein 2010), which is vital information for proper management of the remaining populations. The threat of fire and habitat fragmentation make understanding the ecological requirements of this endemic species more imperative for proper conservation and mitigation.

Hermes emerge in either late spring or early summer after overwintering as eggs and spend their short larval stage on their host plant (Marschalek and Deutschman 2008). Emergence from chrysalises remains relatively consistent across different populations and is often dependent on climatic conditions (Deutschman et al. 2011). The preferred conditions for optimal larval performance are not known due to difficulties finding eggs and larvae in the field. Adults become active around 22°C, but typically remain relatively sedentary throughout the entirety of their lives (Thorne 1963; Murphy et al. 1990; Faulkner and Klein 2004). Hermes sedentary nature is considered one of the contributing factors to their dwindling numbers; however, other possible factors affecting population sizes include habitat fragmentation, fire frequency (United States Fish and Wildlife Service 2011), and N deposition (Marschalek and Deutschman 2008; Marschalek and Klein 2010).

Based on what is known about plant herbivore interactions it is possible that variation in plant chemistry is contributing to small population sizes and a limited distribution.

Variable effects on host plant chemistry

Plant tissue chemistry is affected by environmental variations, such as chronic nitrogen deposition, temperature, and precipitation (Kula et al. 2014; Bale et al. 2002; Wilson and Maclean 2011). Increased nitrogen deposition, for example, has the capacity to alter plant secondary chemistry, which can affect insect responses, such as oviposition preference (Chew 1979) further limiting the range of a sensitive species. Chaparral and coastal sage scrub (CSS) are major recipients of N deposition in southern California (Fenn et al. 2003a, 2003b) because they comprise about 70% of the vegetation cover in coastal, interior and montane regions (Westman 1981). Due to favorable conditions and large topographic gradients, Mediterranean ecosystems are considered to be hotspots of biodiversity and harbor many endangered and sensitive species (Dobson et al. 1997). The reason San Diego is a biodiversity hotspot, especially the coastal southern California Floristic Province, is because of its huge variation in climate, soil type, and slope aspect over very small horizontal scales, which causes high beta diversity (Vandergast et al. 2008; Jones et al. 2006; Coblentz and Riitters 2004). Chaparral and CSS are relevant models for semi-arid ecosystems worldwide, and thus, make them ideal study sites for assessing environmental effects on ecosystem mechanisms and their effects on sensitive species (Vourlitis and Pasquini 2009). In particular, San Diego County is an optimal study site because there is still a major gap in N deposition patterns and ecosystem impacts. While N deposition effects on the plant-soil system and the plant community are becoming better

understood, effects of N deposition on plant-herbivore interactions, and specifically the Hermes copper butterfly, are still not well documented.

Anthropogenic Nitrogen deposition is a global-scale threat that intensifies as human populations continue to grow and expand into natural habitat. Effects of increased N deposition on ecosystem dynamics, such as primary productivity and plant species composition (Minnich and Bahre 1995; Pasquini and Vourlitis 2010; Vourlitis 2017), are more widely understood; however, effects of chronic N deposition on plant-insect interactions are less well known. Ecosystem N enrichment is known to increase plant tissue N concentration (Jamieson and Bowers 2012) and reduce tissue C:N and lignin:N ratios (Vourlitis and Fernandez 2012; Vourlitis and Hentz 2016), which should increase the palatability of leaf tissue for herbivores and herbivory (Throop and Lerdau 2004). Results from a variety of terrestrial ecosystems indicate that N enrichment increases defoliation and herbivore performance (Throop and Lerdau 2004; Meloni et al. 2012; Lu et al. 2015); however, changes in leaf tissue chemistry may also alter oviposition preference (Chew 1979) and chemical defenses (Pasteels et al. 1988; Coley et al. 2006; Jamieson and Bowers 2012), which may reduce herbivore performance.

Atmospheric N deposition has the capability to alter plant-insect interactions, including insect herbivory, but there has been minimal research on the effects of N deposition on insect herbivory in regards to the Hermes copper and *Rhamnus crocea*. Changes in herbivory are likely to occur with N deposition because an increase in N can alter the chemical composition of the butterfly host plant (Throop 2004). Kerslake et al. (1998) reported an increase in larval development, growth rate, and pupal weight when exposed to elevated levels of N. Nitrogen addition was shown to increase shoot growth and

decrease the tissue C:N ratio (Vourlitis and Fernandez 2012). Changes in plant chemistry may also affect oviposition preference of the adult Hermes copper butterfly. Insects may use certain chemicals in the host plant to identify an ideal site for its eggs (Bruce 2015). Alterations in these chemicals may influence a female's oviposition preference (Chew 1979; Bruce 2015). It also follows that changes in a plant's chemical defense may correlate to changes in insect chemical defense (Pasteels et al. 1988; Coley et al. 2006; Jamieson and Bowers 2012). Nitrogen deposition does have some potentially positive effects on insect species, such as increased growth rate and palatability (Kula et al. 2014), but global-scale changes due to elevated levels of N input may ultimately have negative impacts on the insect.

Insects are highly affected by climate change and will have more rapid responses to subtle environmental changes due to the fact that they are ectotherms and have short life cycles (Bale et al. 2002). Climate change is now considered by many biologists to be one of the primary threats to biodiversity. As the most diverse taxonomic group, insects provide important insights on the effects of climate change on ecosystem functioning, and therefore, are likely to be of wider ecological significance (Wilson and Maclean 2011). Climatic conditions and their effect on Hermes copper performance, and lepidopteran species in general, remains incomplete. Deutschman et al. (2011) conducted surveys in 2006 and 2010 and documented delayed emergence periods in both surveys despite contrasting climatic conditions. Human-induced global climate changes are contributing to a gradual increase in surface air temperatures (Stocker et al. IPCC, 2013), which can have drastic effects on Hermes performance as they rely on very stable conditions. Changes in precipitation are not as easy to predict, however, most models agree that winter

precipitation will increase in northern latitudes (Stocker et al. IPCC, 2013). Restricted Hermes distribution, and little understanding as to the cause, makes further research a vital component to population mitigation. Hermes copper butterflies interact with host and nectar plants on a daily basis and rely on a delicate balance of certain plant characteristics remaining unchanged. As ecosystem dynamics begin to shift, sensitive species, such as the Hermes copper, are in danger of complete extirpation. This study aims to provide more information on a poorly understood species.

Hypotheses

Given the uncertainty associated with the leaf tissue chemistry of spiny redberry, the objectives of this study were to determine a) if plant secondary compounds varied across the range of the spiny redberry, b) the effects of variation in temperature and precipitation on plant secondary compounds, and c) if there were any notable differences in plant secondary compounds between sites that occur within the butterfly's habitat range and outside of the range based on N availability. Based on known effects of N deposition and climate change on semi-arid shrubs, it was hypothesized that, a) plant secondary compounds are likely to vary significantly within and outside of the Hermes copper butterfly range~~,~~ b) trends in secondary compounds are affected by variations in climate, such as temperature and precipitation, and c) spatial variations in plant secondary compounds are affected by N availability.

METHODS

Site descriptions

Research was conducted at five main sites distributed throughout San Diego County, California, USA including: Elfin Forest Recreational Reserve, Meadowbrook, Mission Trails,

Black Mountain, and McGinty Peak. The five main sites were selected from the Deutschman and Marschalek (2011) two-year evaluation survey of Hermes copper butterfly populations in San Diego County. An additional 25 sites distributed throughout San Diego County and Riverside County, California, USA were added using the CalFlora site (https://www.calflora.org/) to compare sites within Hermes Copper butterfly habitat to sites outside of the butterfly's habitat range (Table 1; Figure 2). Each site is located in a semi-arid Mediterranean climate with varying levels in elevation and dry-season N deposition. These sites were chosen based on the presence of the butterfly host plant, spiny redberry (*Rhamnus crocea*), and in the case of the five main sites, current or historic habitat for the Hermes copper butterfly. San Diego County has an average precipitation of 300 mm annually, most of which is rainfall that falls in the winter and spring months. Vegetation consists of primarily chaparral and coastal sage scrub (CSS) shrublands with patches of native and nonnative grasslands.

Experimental Design

Soil Samples

Soil samples were collected in May 2018 to measure extractable N, soil moisture, total N and C, and pH. Sites were visited in the Spring to collect soil samples from underneath the shrub canopy. During field sampling events, soil samples were obtained from the surface (0-10 cm) mineral layer using a 4.7 cm diameter X 10 cm deep bucket auger immediately after collection of the surface litter pool. Samples were placed in polyethylene bags, transferred to the lab, and stored at 4°C until analysis. A portion of the fresh sample was analyzed for pH within 4 days of collection, where 15 g of soil was added to 30 mL DI-water and pH was measured after 30 minutes using a pH meter (MP 220, Mettler-Toledo, Columbus, OH, USA). Another portion of the fresh soil (10 g) was extracted

in 40 mL of 2M KCl or 0.5M K_2SO_4 to quantify soil extractable NH_4 and NO_3 (extraction efficiencies were similar for each extractant; G. Vourlitis, pers. observ.). Extractable N was measured using a spectrophotometer. N-mineralization was measured by incubating soil at room temperature for one week and measuring the change in extractable N between the beginning and end of the 7 day incubation period. Net N mineralization (gN m^{-2} d^{-1}) was calculated as the change in total inorganic N (TIN = NH_4 + NO_3) over the incubation period divided by the elapsed time. Another portion of the soil sample was dried at 105°C and measured for total N and C concentration using dry combustion.

Leaf Carbon Chemistry

Leaf C chemistry was initially assessed using the Moorhead and Reynolds (1993) method to partition leaf carbon chemistry into three fractions including: lignin, holocellulose, and solubles. This method was used to determine the relative amounts of each fraction as a percent of the total organic carbon content. About 0.5 g of oven-dried, finely ground leaf tissue was placed into a pre-weighed 50 mL centrifuge tube. Approximately 25 mL of distilled water was added to the sample and then the centrifuge tube was placed in a sonicating water bath at 60°C for 30 minutes. Next, the tube was centrifuged at 10,000 rpm for 15 minutes. The supernatant liquid was poured off and the process was repeated 5 times with distilled water and then an additional 5 times with ethanol. The samples were then placed in a drying oven at 60°C for 24 hours and the residual sample was weighed. The soluble content of the leaf tissue was calculated as the difference between the original and remaining sample weights.

About 0.20 g of the remaining dry weight after solubles were extracted was transferred to a 15 mL glass test tube. Exactly 2 mL of 72% sulfuric acid was added to the sample, which acted to degrade the hydrophobic, holocellulose content. The sample was

incubated for 1 hour at 30°C and 56 mL of distilled water was used to transfer the sample to a 125 mL Erlenmeyer flask. The flasks were then placed in an autoclave for 1 hour at 120°C. The sample was suctioned onto a pre-weighed 10 μm Millipore filter paper and oven-dried for 24 hours at 60°C. Extreme heat from the autoclave facilitated further breakdown of the holocellulose content by the sulfuric acid. The holocellulose content was calculated as the difference between the pre- and post-acid digested sample weights. The remaining residue is considered to be comprised primarily of lignin (Moorhead and Reynolds 1993).

The remaining sample residue was placed in a pre-weighed crucible and into a muffle furnace for 24 hours at 500°C. Crucibles were then weighed and the difference was calculated as the ash mass.

Identification and Quantification of Leaf Secondary Compounds

To quantify leaf secondary compounds, we first partitioned samples into polar and nonpolar fractions for detailed evaluation. *Rhamnus crocea* leaves were oven-dried and then crushed by hand and placed in a pre-weighed Erlenmeyer flask. A solvent solution of 75% methanol and 25% dichloromethane was added to the flask, which was then covered with foil and left to extract for 24 hours. The supernatant was vacuum filtered into a pre-weighed tear drop flask and the solvent was removed *in vacuo*. The oily substance was then analyzed by proton nuclear magnetic resonance (^{1}H-NMR) in order to find something to link to chemical communication, such as terpenes, as well as to guide purification. Samples were analyzed by proton NMR in deuterated chloroform ($CDCl_3$) then in deuterated methanol (MeOD) for material that did not dissolve in $CDCl_3$. Using ethyl acetate and methanol, the remaining sample was dissolved from the tear drop flask and placed in a

labeled amber bottle and stored in the freezer for future use. Proton NMR application for this study was used to identify structure determinations of unknown samples (Günther, 2013).

Liquid-Liquid Separation

Liquid-liquid separation was used to separate each sample into the polar and non-polar fractions using liquid and gas chromatography for a more detailed analysis, respectively. About 100 mL of methanol and 100 mL of iso-octane were added to a large separatory funnel. Stored sample from amber bottles was added to the funnel. A cap was placed on the flask, which was then shaken for exactly 1 minute while intermittently releasing pressure. The separation of liquid into polar and nonpolar fractions was allowed to occur and then the liquid was emptied into two separate roto-vap flasks. Solvent was removed from both the methanol (polar) and iso-octane (nonpolar) portions. The remaining sample was transferred into two separate pre-weighed vials, solvent was removed and weights were determined. Samples were stored in the freezer for future use.

GC Mass Spectrometry

Nonpolar fractionated samples were run on the GC/MS to assess molecular ions and identify a match to a known compound. GC/MS vials were prepared with nonpolar fraction from the liquid-liquid separation step. Each GC/MS vial was labeled and prepared with a 5 mg/mL concentration or as close as possible in hexanes. The samples were run on the GC/MS using the Plant1 method. Samples were eluded with a linear oven ramp rate starting at 100°C and held for 5 minutes. Over the next 20 minutes the temperature ramped up to 300°C and was held for 5 minutes at the end. After each sample was run, the mass spectrum was analyzed using the references database, Wiley138 library with known

compounds. Each molecular ion was interpreted for quality match to a known ion and subsequent identification.

The Hewlett Packard 5973 Mass Selective Detector with an Agilent 6890 N Network GC system was used to complete this method. This method ran using helium gas and a standard filament voltage of 70 eV. Each sample was injected at a volume of 2.0 μL. The column used for this method was an Agilent, HP-5MS with a length of 30 meters and an inside diameter of 0.25 mm.

LC Mass Spectrometry

Polar fractionated samples were run on the LC/MS to identify the class of compounds through structure elucidation. LC/MS vials were prepared using the polar fraction from the liquid-liquid separation step. Each LC/MS vial was labeled and prepared with a 0.5 mg/mL concentration or as close as possible. The samples were then run on the LC/MS using the Plant1 method. Each sample was eluded with a linear gradient beginning with a ratio of 60% H_2O with 0.1% formic acid and 40% Acetonitrile (ACN) with 0.1% formic acid and ending with 100% ACN with 0.1% formic acid. Each sample was run separately and resulted in a series of peaks relating to various unknown compounds found in the sample.

Each sample was analyzed in Excel in regard to normalized peak area, which was then run through statistical analysis to determine which peaks were statistically different within and outside of the Hermes habitat range. Total peak area for each unknown compound was calculated and significance was determined between sites within the habitat and outside of the habitat range using a two-sample t-test assuming equal

variances. Peaks that were statistically different within and outside of the hermes habitat range were analyzed further through structure elucidation.

Peaks identified as significantly different were analyzed on the LC/MS using product ion runs to fragment the compounds into their structural components. Samples were run using the original plant 1 method mentioned previously, edited to account for a product ion run with varying levels of collision energy to fragment the compounds. Collision energy is the force at which nitrogen gas hits the sample and fragments the compounds. A more stable compound requires a higher percentage of collision energy to fragment the molecule into structural components, whereas weak bonds are broken easily (Addona and Clauser 2002; Bodnar et al. 2003). Once fragmentation of the compounds was completed the structural components could be identified based on molecular mass and visual representation on the MS spectrum. Structural components were then pieced together using a list of potential compounds found in redberry shrubs to identify the class of compounds.

The Agilent 6410 LC mass spec with an Agilent 1260 HPLC system was used to complete this method. Each sample was run with a flow rate of 0.3mL/min, a gas temperature of 300°C, a cell accelerator voltage of 4, and a positive polarity. Each sample was injected at a volume of 3μL. The Agilent HPLC column used for this method was a Poroshell 120 EC-C18 with a length of 4.6 meters and a width of 50 mm and an inside diameter of 2.7 microns.

Statistical Analysis

Differences in climate, N constituents, and chemical compound classes were analyzed using a two sample t-test when normalized data and homogeneous variances

were maintained. If the assumptions were violated a nonparametric Mann-Whitney U test was used to assess significant ($p<0.05$) differences within and outside the hermes range in the given chemical constituent. Sites that showed significantly different means within and outside the range were graphed in a normalized bar chart, while the subsequent nonsignificant sites were ignored and considered nonessential for further analysis. For all data shown, normalization was conducted by taking the individual peak area of the compound to be identified and dividing by the sum of all peaks in each sample run through LC/MS. Bar charts were also included for climate variables, graphed showing in vs. out of the hermes habitat range.

Average monthly temperature, maximum and minimum average monthly temperature, and total precipitation were calculated for all sites by an inverse-weighting function which interpolates climate variables from 2-4 adjacent weather stations (Table 2). The inverse-distance weighting function makes the weight for each sample inversely proportional to its distance from the point being estimated, and thus, gives more weight to the closest samples and less to those samples that are farthest away (Isaaks and Srivastava 1989).

Canonical correspondence analysis (CCA) is a direct gradient analysis technique that is introduced as a multivariate extension of weighted averaging ordination, which is a method for arranging species along environmental variables (Ter Braak 1987; Palmer 1993). Direct gradient analysis relates species abundance to environmental variables based on species and environment data from the same set of sample plots (Gauch 1982). CCA has been shown to perform well with skewed species distributions, with quantitative noise in species abundance data, with highly intercorrelated environmental variables, and with

situations where not all of the factors determining species composition are known (Palmer 1993). CCA analysis was used to analyze the effects of environmental variables on plant compound constituents. Applications of this method demonstrate that CCA plots can be used to identify species-environment relationships, and for investigating specific questions about the response of species to environmental variables (Ter Braak 1987). The response variables used to analyze chemical compound classes were (1) climate levels: average monthly temperature, maximum and minimum average monthly temperature, and total precipitation; (2) foliage chemistry: leaf N, leaf C, leaf C:N, soluble carbon, lignin, and holocellulose; and (3) soil chemistry: total N, total C, total inorganic N, soil C:N, N mineralization, pH, and estimated N deposition. In addition, a correlation matrix was used to determine if any significant correlations existed between the environmental variables and the chemical compound classes. In this study, understanding the effects of temperature and precipitation on plant secondary compounds is difficult since the specific compound is not known, but a general explanation of climatic effects on these compounds is given to loosely interpret the impact on the Hermes copper butterfly. Data were reduced using MS Excel and all statistical analysis were conducted using R64 statistical package.

RESULTS

Compound Significance Within and Outside Hermes Range

Ten out of the 48 compounds analyzed via GC and LC mass spectrometry were significantly different within and outside of the Hermes range ($p<0.05$; Figure 3; Table 3). These 10 compounds were analyzed further using structure elucidation to identify the class of compound. Plant compound classes were identified as: tocopherol, peptide, hexose, alkaloid, terpene, flavonoid, and iridoid. Based on analysis from structure elucidation three

different types of alkaloids were identified, labeled alkaloid 1, alkaloid 2, and alkaloid 3, as well as two different types of hexoses, labeled glucose 1 and glucose 2. Interestingly, all of the compounds are not from the same class. Five of the ten compounds including: tocopherol, peptide, glucose 2, alkaloid 1, and alkaloid 3 were found in higher quantities inside of the hermes habitat range, while the remaining five compounds including: glucose 1, flavonoid, terpene, alkaloid 2, and iridoid were found in higher quantities outside of the hermes habitat range. For example, tocopherol was two times higher inside of the hermes habitat range, whereas terpene was two times higher outside of the hermes habitat range. Interestingly, the two hexose compounds were found in contrasting quantities where glucose 1 was two times higher outside of the hermes habitat range, but glucose 2 was nearly two and a half times higher inside the hermes habitat range. Similarly, the three alkaloid compounds were found in differing quantities with alkaloid 2 being the only type found in higher quantities outside of the hermes range (Figure 3). Without further investigation of the specific compound it cannot be fully understood what the implications are for the host plant and butterfly, but the basic role and function of these compounds can be discussed.

Climate Variables Within and Outside Hermes Habitat Range

To test for the effects of climatic conditions on the Hermes copper butterfly host plant, temperature and precipitation data were collected from weather stations adjacent to the sampling sites and averages were calculated using an inverse-distance weighting function. Climate variables: average monthly temperature, maximum average monthly temperature, minimum average monthly temperature, and total precipitation were also graphed to show the difference within and outside of the Hermes copper habitat range

(Figure 4). Average monthly temperature, maximum and minimum average monthly temperature were all higher inside of the habitat range, although the variation in maximum average monthly temperature within and outside of the range was minimal. Precipitation was the sole climate variable found to be higher outside of the habitat range. These climate variables will be further utilized to assess impacts on plant secondary compounds within the host plant.

Canonical Correspondence Analysis

Canonical correspondence analysis was used to determine the effect of environmental variables on the ten plant compound classes that were identified. The data were separated into three separate categories: climate, soil, and foliage; and plotted against the ten plant compounds.

For the climate variables, CCA1 was primarily a function of average temperature and precipitation. CCA2 was primarily a function of average maximum temperature as that environmental variable is almost parallel with the axis (Figure 5). The proportion of variation explained in the data by the climate variables was 75.3% and 14.6% for the CCA1 and CCA2 axes, respectively, with a cumulative explained proportion of 89.9% (Table 4). The right side of the plot, where alkaloid 2, flavonoid, and glu 1 were found, was a wetter and cooler environment. The top left side of the plot, where glu 2, peptide, and tocopherol were found, represented a drier and warmer environment. Plant class compounds on the left side of the plot tended to be associated with a drier and warmer environment. Plant compounds found in higher quantities inside of the Hermes habitat range were restricted to the left side of the plot, while compounds found in higher quantities outside of the hermes range were restricted primarily to the right side of the plot (Figure 3; Figure 5).

For the soil variables, the proportion of variation explained was 59.5% and 16.6% for the CCA1 and CCA2 axes, respectively, with a cumulative explained proportion of 76.1% (Table 4). Nitrogen deposition and pH were shown to be negatively related with a majority of the plant class compounds in the lower left hand quadrant (Figure 6). Compounds, such as tocopherol, peptide, and glucose 2 seemed to be driven in part by a decline in total N and total C and an increase in total inorganic N (TIN). Glucose 2, iridoid, and flavonoid were associated with an increase in pH and TIN.

For the foliage chemistry analyses, the proportion of variation explained was 53.3% and 21.2% for the CCA1 and CCA2 axes, respectively, with a cumulative explained proportion of 74.5% (Table 4). Leaf N, leaf C, and lignin were negatively related to leaf C:N (Figure 7). Compounds such as iridoids, terpenes, and alkaloid 2 were positively related to an increase in leaf N and leaf C, as well as lignin. Glucose 2 and peptide were highly influenced by the holocellulose content in leaf tissue and negatively related to solubles and lignin. Alkaloid 1, alkaloid 3, and tocopherol were positively related with solubles and an increase in the leaf C:N ratio.

DISCUSSION

Plant Compound Classes

The results of this study supported the hypothesis that plant secondary compounds varied significantly within and outside of the Hermes copper butterfly range (Figure 3). Although only about 20% (10/49) of the secondary compounds that were encountered in *R. crocea* differed within and outside of the Hermes copper range, other studies also found that plant tissue chemistry is affected by environmental variations, such as chronic nitrogen deposition, temperature, and precipitation (Kula et al. 2014; Bale et al. 2002;

Wilson and Maclean 2011). Chemical composition is a primary factor affecting foliar quality (Schultz 1988; Ehrlich and Murphy 1988), which strongly impacts insect performance (Slansky and Scriber 1985; Scriber and Ayres 1988). Host plant quality, which is defined by the nutritional and defensive components of the plant that affect larval development, is crucial for oviposition site selection (Awmack and Leather 2002).

As noted above, only 10 of the identified plant secondary compounds were found to be significantly different within and outside of the Hermes copper habitat range (Figure 3). Of those 10 compounds, 3 were alkaloids, and while alkaloid 1 and alkaloid 3 were found in higher quantities within the Hermes copper butterfly range, alkaloid 2 was higher outside of the butterfly range (Figure 3). The most familiar class of plant toxins are the alkaloids, which are nitrogen containing organic compounds (Robinson 1974). Most alkaloids, of which there are at least 6500 of known structure, are thought to be toxic or act as a repellant to insects and mammals if ingested over an extended period of time (Harborne 1988). However, this may be an over-generalization as there are thousands of alkaloids and a lack of evidentiary support (Robinson 1974) of their role as repellants. There are documented cases in which alkaloids provided benefits in the form of defense mechanisms or breeding advantages for its insect predator. This is true of pyrrolizidine alkaloids, which are needed to manufacture aphrodisiacal substances, which the male butterfly stores in its wing hair pencils and uses in its courtship display to attract the female (Harborne 1988). It is now clear, for example, that the pyrrolizidine alkaloids are dual purpose: they are both protective and serve as essential pheromone precursors. Furthermore, many lepidopteran species including *Danaus plexippus* and *D. chrysippus* have been found to store these alkaloids in their tissues during much of their adult lives (Edgar et al. 1979; Harborne

1988). Specialist herbivores, in particular, have evolved adaptations to deal with secondary plant compounds in their host plants, which allow them to utilize these compounds for their own purposes (Aplin and Rothschild 1972; Naumann et al. 2002; Wittstock et al. 2004). In some cases, they can also use alkaloids as a cue when identifying their host plant (Macel 2010). Therefore, while speculative, the two alkaloids found in higher quantities within the Hermes copper habitat could very well be advantageous to the survival of the larvae and adult butterfly, while alkaloid 2 could have deleterious effects that adversely affect the survival and fecundity of the Hermes copper.

In this study, a single terpene was found to be in higher concentrations outside of the Hermes copper habitat (Figure 3). Terpenes or terpenoids constitute a diverse set of metabolites (>40,000 structures) that are associated with both mutualistic and antagonistic plant-herbivore interactions (Gershezon and Dudareva 2007; Bedoya-Pérez et al. 2014). Terpenes, along with aromatic compounds, constitute the essential oils of plants, with the highest concentration found in the specialized storage cavities of leaves (Mewalal et al. 2017). Many terpenoids function as plant defense compounds against both biotic and abiotic stresses, however they can also act as signal molecules to attract pollinators (Singh and Sharma 2015). Terpenes have a distinctive odor and taste (Lawler et al. 1999) and can act as toxic defenses against both invertebrate and vertebrate species. Odor can mediate a wide variety of plant-herbivore interactions, such as the attraction of pollinators to odor emitting flowers (Plepys et al. 2002; Cunningham et al. 2004). Additionally, pheromones are involved in almost every aspect of insect life, including feeding, sex, aggregation, oviposition, defense and laying trails. Some plants release volatile compounds, such as terpenes when attacked by herbivores (Bedoya-Pérez 2014), which can result in a few

outcomes. Release of these volatile compounds can signal predators that subsequently prey on the herbivore (Mattiacci et al. 1995; Takabayashi and Dicke 1996). Volatile emissions can signal other herbivorous species to avoid that plant to prevent competition with another individual (Pallini et al. 1997). Finally, the volatiles released by the damaged plant can signal neighboring plants to increase their levels of defensive compounds (Dicke and Baldwin 2010). Although the specific terpene in this study could not be identified, it is reasonable to infer that the terpene was likely deleterious to the butterfly species and served only to benefit the host plant.

A single iridoid compound was found to be in higher quantities outside of the Hermes copper butterfly range (Figure 3). Investigations of iridoids, which are a group of non-nitrogenous terpene-derived compounds (Harborne 1988), have greatly contributed to a better understanding of the ecology and evolution of host plant-insect herbivore interactions (Bowers 1988; Farrel and Mitter 1990) and insect defenses against predators (Bowers 1991; Bowers and Farley 1990; Pasteels et al. 1990). Nayar and Fraenkel (1963) suggested that iridoids were responsible for host plant specificity in certain herbivorous insect species. Iridoids have been found to be effective defensive compounds for certain plants against generalist and nonadapted specialist insect herbivores (Bernays and DeLuca 1981; Kubo et al. 1985; Puttick and Bowers 1988); however this may not be relevant in the case of the Hermes copper, which is a specialist lepidopteran species. Bowers (1980) found that larvae and adults that fed on plants containing iridoids were unpalatable to predators. Many insect species are able to sequester iridoids as a measure of defense against predators (Bowers 1988); however this may not be the primary function of iridoid compounds in spiny redberry as it is found in higher quantities where the Hermes larvae

are not present. Although an important function of plant secondary compounds is the defense against insect herbivores (Fraenkel 1969; Spencer 1988; Nahrstedt 1989), these compounds may have other roles, such as control of competitive interactions among plants (Thompson 1985) or storage of nitrogen (Baldwin 1989). They were also found to inhibit germination and growth of seeds and seedlings (Adam et al. 1979) and may work as anti-fungal (Van der Sluis et al. 1983) and microbial (Kubo et al. 1985) toxins. The amounts of iridoids in plants has been found to vary with plant genotype (Fajer et al. 1991) and environmental conditions. In one study total iridoid content of plants increased through the growing season. In a study by Fajer et al. (1991), it was discovered that plantain plants grown at elevated carbon dioxide levels (700 ppm) had significantly lower iridoid concentrations than those grown under ambient carbon dioxide levels (350 ppm). Additionally, iridoid content is also influenced by the age of the leaf, and new and intermediate aged leaves had significantly higher iridoid concentrations than mature leaves. It is not surprising that an iridoid compound was found in the spiny redberry, however, its actual function for this particular plant species, and its role in plant-herbivore interactions, requires further investigation.

Two hexose compounds were identified in this study with Glu 1 in higher concentrations outside of the habitat and Glu 2 in higher concentrations inside of the habitat (Figure 3). Sugars, such as the hexoses glucose and fructose, are the primary products of photosynthesis and the building blocks of most natural organic matter (Granot et al. 2013). Carbon autotrophy is a prominent feature of plant function and the disaccharide sucrose plays an important role in plant metabolism (Roitsch and González 2004). Carbohydrates in plants are synthesized in source leaves and allocated to sink

tissues for either growth and development or storage (Roitsch and González 2004). Hexose compounds are often allocated throughout the plant for a diverse number of functions based on biotic selection pressures (Luu et al. 2017). Hexose compounds in the form of O-acyl sugars, for example, play important roles in plant defense against insect herbivores (Luu et al. 2017) and pathogens (Kato and Arima 1971). Additionally, plant response to cell-wall stresses is dependent on hexose compounds, which aid in pathogen response genes, lesion formation (Hamann et al. 2008) and lignin deposition (CanoDelgado et al. 2000, 2003; Ellis and Turner 2001; Ellis et al. 2002). Based on these examples, the two hexose compounds found within *R. crocea* could provide any number of roles relating to plant defense or maintenance. With the two compounds sharing the same molecular weight it is possible their function within the plant are very similar, but without further analysis to identify the specific compound it would be difficult to speculate as to the exact function.

A single peptide, which are short chains of amino acids that are the building blocks for proteins, was found to be in higher quantities inside of the Hermes butterfly range in this study (Figure 3). Many plants respond to attacks from herbivores by producing defensive proteins in their leaves and stems (Green and Ryan 1972; Ryan 1990). These proteins are often produced not only near the site of the attack, but also in undamaged leaves far from the wounded area, which indicates the use of peptide signaling as a plant defense mechanism (Matsubayashi and Sakagami 2006). Williamson (1950) documented that herbivore attacks elicit an ethylene burst through peptide signaling. A number of different plant species have been documented to respond to herbivore attacks by synthesizing defensive chemicals to protect themselves from herbivores. Systemins, which are a functionally defined family of peptide signals that regulate defensive genes were the

first peptide signals found in plants (Ryan and Pearce 2003). Peptides play an important role in plant defense and although the specific type of peptide is not known it can be assumed that the peptide found in this study is a key component in the host plant's defense against herbivores.

This study found a flavonoid in significantly higher quantities outside of the butterfly's habitat (Figure 3), which could be a potential factor limiting Hermes from expanding its range. Flavonoids are plant secondary metabolites that protect plants from various biotic and abiotic stresses and exhibit a diverse set of functions (Samanta et al. 2011). Flavonoids are responsible for flower color (Griesbach 2005), protection against microbes (Bohm 1998), and defense against herbivore attacks (Mitchell et al. 1993; Putnam 1988). Plant flavonoids were found to inhibit cytochrome activity in insects, such as the larvae of *Drosophila melanogaster* (Mitchell et al. 1993). Flavonoids are incorporated into a number of plant functions, including defense against herbivores. They can, however, also act as attractants to enhance pollination and seed dispersal (Koes et al. 2005; Grotewold 2006). Identification of the specific subgroup would allow for a better understanding as to how this secondary metabolite may be impacting Hermes butterfly populations.

This study found tocopherol in significantly higher quantities within the range of the butterfly (Figure 3), and although the exact benefits to Hermes are unknown, it can be assumed that it plays a significant role in the survivability of the butterfly. Tocopherols are a group of four lipophilic antioxidants that are found primarily in the leaves and seeds of plants (Munné-Bosch and Alegre 2002). These compounds play a basic role in insect physiology, especially for insects that have very specific diet requirements (Vanderzant et

al. 1957; Fraenkel 1959; Dadd 1963). Zwolinska-Sniatalowa (1976) found that tocopherol acted as a stimulant of fecundity for the Colorado potato beetle. It was surmised that metabolic function of tocopherols is so important it can be assumed that they occur in all living organisms (Zwolinska-Sniatalowa 1976). The biological benefits of tocopherol to organisms, including species of Lepidopteran, have been widely demonstrated (Cerqueira et al. 2007).

Effects of Temperature and Precipitation

Analysis of climate variables in this study exhibit large differences between the Hermes range and outside of the range. This study found significantly lower average monthly temperatures and lower minimum average monthly temperatures outside of the range of the butterfly. Although maximum average monthly temperature didn't have much of an effect, outside of the range was found to be wetter and cooler when compared to inside of the range. These results agree with those reported by Deutschman et al. (2011) and although this study did not assess direct effects of climate variables on the Hermes copper it is clear from this study and previous studies that there is a significant impact on the survivability of the butterfly. The results of this study supported the hypothesis that trends in plant secondary compounds will be affected by variations in climatic conditions. Plant secondary compounds found in higher quantities within the Hermes copper habitat range were found to be associated with warmer and drier conditions, while compounds found in significantly higher quantities outside of the range were found to be associated with cooler and wetter conditions (Figure 3; Figure 5). Sites located outside of the Hermes copper habitat range were on average at a higher elevation and exposed to higher rates of precipitation and lower temperatures (Table 1; Figure 4). Although plant secondary

compounds were not examined in direct relation to temperature and precipitation in this study, other studies have found that temperature (Dong et al. 2011; Zhao et al. 2017) and precipitation (Herrera et al. 2017) affect plant chemistry. As stated previously, Deutschman et al. (2011) observed the effect of temperature on both Hermes larvae and adults with an optimum temperature of 22°C. Eilers et al. (2013) confirmed that female butterflies had a preference for oviposition sites with a warmer than average micro-climate. Higher than average rates of precipitation usually equates to higher densities of Hermes copper populations in San Diego County (Deutschman et al. 2011; Pollard 1988; Roy et al. 2001), even though the Hermes range is restricted to warmer and drier parts of southern California (Figure 1). However, results from this study suggest that concentrations of plant secondary compounds are also impacted by variations in climatic conditions as previous studies have expressed and could be a limiting factor for Hermes distribution.

Canonical correspondence analysis (CCA) was used to determine major trends in the data and identify which environmental variables were most influential to distribution of plant secondary compounds. The manner in which the compounds are grouped in the CCA plot emphasizes the impact of climate conditions on concentrations of these compounds within the butterfly host plant. The two hexose compounds (Glu 1 and Glu 2) were found on opposing sides of the CCA plot (Figure 5) and thus, may perform different functions within the plant. Davies et al. (1989) found that hexose content was 60% lower in potato tubers grown under water stress compared to controls. In regards to temperature, previous studies have found that *Arabidopsis thaliana* leaves developed under lower temperatures accumulate large soluble sugar pools without any associated suppression of photosynthetic capabilities (Strand et al. 1997).

Tocopherol was found to be in significantly higher concentrations in habitats that are warmer and drier (Figure 5). Increased concentrations of tocopherol have been found to contribute to the prevention of oxidative damage to plants exposed to drought conditions, temperature stresses (Munné-Bosch et al. 1999; Munné-Bosch 2005; Sadiq et al. 2017), and phytophagous insects (Cela et al. 2018). Keles and Öncel (2002) found that natural environmental stresses can induce oxidative damage, but the combination of stresses (i.e. temperature, water, and salt) can affect how a plant reacts.

Alkaloid 1 and alkaloid 3 were found to be temperature driven while alkaloid 2 was more effected by precipitation (Figure 5). It has been well observed that alkaloids are more potent in plants that occur in hotter and drier climates (Briske and Camp 1982; Gershenzon 1984). Interestingly, alkaloid 2 differs from the other alkaloid compounds and increases with higher precipitation, however, only marginally. Further identification of alkaloid 2 is necessary to understand why concentrations increase with higher precipitation.

Terpenes react to abiotic stresses in such a variety of ways that it is necessary to focus on the specific compound to understand the pattern of response (Gershenzon 1984). In this study, the terpene was found in the warmer and drier spectrum of the CCA plot (Figure 5). Flück (1955) found that higher monoterpenoid concentrations were associated with lower water stress due to the dominating influence of temperature. Unfortunately, further analysis is difficult since terpenes display such a wide variety of behaviors when acted upon by abiotic stresses.

In this study, temperature and precipitation did not seem to affect the iridoid compound in any meaningful way as it sits in the center of the CCA plot (Figure 5). This

indicates that no climatic variable was particular influential in altering or affecting the iridoid content in the host plant. However, in previous studies drought stress was found to increase concentrations of iridoids (Wang et al. 2010). Martz et al. (2009) found that low freezing temperatures were associated with an increase in the content of iridoids. It is important to note that the same plant secondary compound may react in a different manner to abiotic stresses based on the plant species (Gershenzon 1984).

The peptide in this study was found to be positively influenced by temperature and negatively influenced by precipitation (Figure 5). Under water stress, the synthesis of polypeptides was induced in a variety of plant species (Bray 1988; Guerrero and Mullet 1988; Bensen et al. 1988; Mason et al. 1988; Vartanian et al. 1987), although the functional role of the stress response was not established. Similar to terpenes mentioned previously, peptide is a broad class of plant secondary compounds and is not easily analyzed unless further identification is completed. Osborn et al. (1995) analyzed the effect of temperature on peptides and found the results to be mixed, which solidifies the point that a known compound and its plant counterpart need to be studied together under the desired conditions for an accurate understanding.

The flavonoid identified in this study was found to be more heavily influenced by precipitation (Figure 5). A decline in temperature has been reported to cause qualitative changes in flavonoid content (Jaakola and Hohtola 2010). Reported studies have shown that plants will adapt to low or high temperatures by altering flavonoid synthesis (Albert et al. 2009; Schmidt et al. 2010), however, in this study temperature was not found to be a main factor influencing flavonoid content. These results were consistent with previous findings that the total flavonoid content of a plant is enhanced under higher levels of

precipitation compared to lower levels (Zhang et al. 2012; Yang et al. 2010). However, similar to the previous metabolites discussed, flavonoids display a variety of behaviors when exposed to water stress. Water stress is known to increase the amount of secondary metabolites, including flavonoids, in a variety of plants (Zobayed et al. 2007; He and Zhong 2003).

The effect of variations in these plant secondary compounds on the Hermes copper butterfly are currently unknown. However, trends in these compounds can be examined in conjunction with climatic conditions to better understand how these compounds may be influencing the range of the butterfly. Abiotic factors, such as temperature and precipitation are known to influence annual populations of butterflies (Pollard 1988; Roy et al. 2001). Preston et al. (2008) found that increased rainfall at higher elevations acted as a buffer to protect habitat and butterfly populations from temperatures that exceeded an optimal range. Interestingly, the compounds that were found in higher quantities within the range of the butterfly had a positive relationship with temperature and a negative relationship with precipitation (Figure 5). Drought and increased fire frequency are driving Hermes populations into higher altitudes (Marschalek and Deutschman 2019). The Hermes copper host plant may be reacting to environmental stressors by altering its chemical composition, but it may not be enough to sustain a butterfly population in conditions of extreme drought and increased fire regime.

Effects of N Deposition on Plant Tissue Chemistry

The results of this study did not support the hypothesis that spatial variations in plant secondary compounds are affected by N availability. The assimilation of N and C is a process closely related to the growth and function of a plant. Plant growth and

development is frequently limited by N (Chapin 1980; Ågren 1985; Vitousek et al. 1997). Plant responses to increased N availability include increased foliar N concentrations, photosynthetic rates, and reproduction (Padgett and Allen 1999; Magill et al. 2000). Additionally, increased N has been documented to affect plant allelochemistry (Wilkens et al. 1996; Osier and Lindroth 2004), however, the manner in which the changes occur depends on the plant species and the types of compounds (Jamieson and Bowers 2012). Deficiencies of N has been well documented to reduce plant growth and alter allocations of nutrients to various plant structures (Asher and Loneragan 1967; Bastow-Wilson 1988). Changes in N availability may indirectly affect herbivores by modifying phenology and quality of the host plant (Throop and Lerdau 2004). All compounds found in higher quantities within the Hermes copper habitat range were driven more by an increase in N deposition, with many of those compounds also being influenced by total inorganic N (TIN) (Figure 6). Plant secondary compounds were not separated as evenly when assessing leaf variables, such as C and N (Figure 7).

In this study, the two hexose metabolites were found on opposing sides of the soil and foliage CCA plots (Figure 6; Figure 7). Glu 1 was influenced more significantly by a decline in N deposition and an increasing pH and soil C:N ratio, while Glu 2 was inversely related to total N and total C (Figure 6). Glu 1 had a positive relationship with leaf N and leaf C, while Glu 2 increased as the holocellulose content of the leaf increased (Figure 7). Huber et al. (1989) found that leaf growth of N limited plants was reduced, partly due to a decline in sucrose utilization within the plant. Sucrose formation had a positive relationship with an increased N supply, due to a heightened capacity for photosynthesis in N sufficient plants. Another study, however, found that hexose metabolites were

significantly higher in plants grown in low levels of N with substantial hexose accumulation in the roots of tobacco seedlings (Paul and Stitt 1993). Interestingly, internally and externally supplied sucrose did not induce the same isoenzymes of sucrose synthases when applied to the roots of plants (Koch et al. 1992). This result could have meaningful applications for this study where the two hexose metabolites were often found to be inversely related.

Tocopherol was driven in part by both N deposition and TIN (Figure 6), and leaf C and N (Figure 7). Tocopherol content has been shown to increase with decreasing N concentrations, however, the growth rate of the plant declined as well (Durmaz 2007). Previous studies have found that differences in tocopherol content were dependent on factors such as, N concentration, N source (Durmaz 2007), light, temperature (Huo et al. 1997; Bandarra et al. 2003), and growth phase of the plant (Donato et al. 2003). Szalai et al. (2010) found that tocopherol concentrations increase with increasing N fertilization, however, the ratio of ammonium to nitrate is a determining factor in how much the tocopherol content changes in the plant.

As stated previously in the paper, three alkaloids were identified with alkaloid 1 and alkaloid 3 being more strongly correlated with increasing N deposition, while alkaloid 2 is driven by total N and total C (Figure 6). Alkaloid 1 and 3 are driven by the leaf C:N ratio and alkaloid 2 is driven by leaf N and leaf C (Figure 7). Alkaloids have been well documented to increase with increased N assimilation (Johnson et al. 1987). Alkaloid concentration and composition can be altered by N availability, defoliation, developmental state of the plant (Johnson et al. 1987), temperature, and precipitation (Briske and Camp 1982; Gershenzon 1984).

The terpene metabolite identified was found to be primarily driven by total N and total C (Figure 6) and leaf N and leaf C (Figure 7). In an environment characterized by increasing levels of N deposition, plant species, such as *P. sylvestris*, have been documented to respond to this increase by allocating resources to the production of terpenes (Ormeño et al. 2008; Lu et al. 2015). In another study, the effect of nitrogen on terpenes varied with the authors suggesting that not all C or N based chemicals react to environmental variables in the same manner (Muzika 1993). These results highlight the unpredictability and variability of these compounds when exposed to a combination of environmental variables that act to alter the allocation of resources within the plant.

The peptide compound was driven by N deposition and TIN (Figure 6), while also being influenced by holocellulose content of the leaf (Figure 7). Peptides frequently act as organic N sources for plants and are assimilated by a number of plant species for use in plant growth and development (Person et al. 2003). In N deficient environments, signaling peptides that are responsive to N have been known to affect the expansion of root systems in favor of other plant processes (Araya et al. 2014).

In this study, the iridoid compound was driven by pH and soil C:N ratio (Figure 6) and lignin content in the leaf (Figure 7). Darrow and Bowers (1999) found that nutrient limitation resulted in an increase in iridoid glycosides, and while the availability of nutrients had no of iridoid glycosides.

The flavonoid metabolite was driven by TIN (Figure 6) and holocellulose content in the leaf (Figure 7). An increase of flavonoids has been documented under conditions of cold treatment (Winkel-Shirley 2002; Hannah et al. 2006) and N depletion (Lillo et al. 2008;

Peng et al. 2008). Olsen et al. (2009) found that addition of N was sufficient to halt flavonoid synthesis in *Arabidopsis* plant species.

CONCLUSION

The results of this study suggest that plant secondary compounds within the Hermes copper host plant are sensitive to variations in climate conditions. Although N availability in this study showed no significant effects on plant secondary compounds, additional research is suggested in order to better understand effects of N on the host plant and the Hermes copper larvae. This study demonstrated the ways in which plant secondary compounds are influenced by abiotic factors. Future research is necessary to identify the specific secondary compounds for further analysis and discussion.

The Hermes copper has restricted geographic ranges, narrow habitat tolerances, and small population sizes that make it highly vulnerable to extinction (Rabinowitz 1981). Insects, specifically lepidoptera, show rapid responses to climate change due to short life cycles and wide variation in population sizes (Bale et al. 2002), thus they are ideal candidates when assessing impacts of climate change. Butterfly species are especially influenced by environmental changes at differing spatial scales, with increasing atmospheric N loads causing remarkable changes in community assemblages, such as a decline in specialist species that are unable to adapt to a changing environment (Habel et al. 2016). In situ conservation of extant populations remains an important method and is crucial for the success of translocations, including relocations to extirpated habitats (Thomas et al. 2009; Wilson and Maclean 2011). For several protected butterfly species, it has been suggested that dispersal capacity of 10-50km would prevent recolonizations, which emphasizes the importance of managing habitat networks or entire landscapes

where the protected species resides (Wilson and Maclean 2011). Understanding the habitat requirements for the Hermes copper butterfly is crucial in order to develop conservation strategies to mitigate potential threats to the remaining populations in San Diego County.

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FIGURES AND TABLES

Table 1. Location and selected characteristics for all study sites. N deposition estimates are from a high-resolution (4 km) model (Tonnesen et al. 2007).

Site	Latitude	Longitude	Elevation (m)	Est. N Deposition (kg N $ha^{-1}yr^{-1}$)
Within butterfly habitat				
Elfin Forest (EF)	33.074	-117.157	138	11.73
Black Mountain (BM)	32.977	-117.123	281	12.38
Meadowbrook (MB)	32.964	-117.069	187	12.13
Mission Trails (MT)	32.833	-117.038	144	10.77
McGinty Peak (MP)	32.758	-116.851	352	9.87
mg20763	33.063	-117.083	122	11.91
cbo86186	33.025	-117.171	49	12.13
SD195367	32.938	-117.213	49	12.83
cbo29765	32.938	-117.134	72	12.23
oe3104	32.951	-117.017	210	10.43
SD211218	32.869	-116.968	152	10.94
SD208086	32.711	-117.079	29	9.79
cbo37640	32.925	-117.162	109	11.50
SD182404	33.044	-117.153	49	11.73
in:9513993	32.832	-117.104	95	12.15
Outside butterfly habitat				
UCR102732	33.781	-117.056	610	10.84
in:8546144	33.807	-117.354	595	10.45
UCR241774	33.725	-117.392	378	8.37
UCR100298	33.800	-117.061	610	11.20
UCR260565	33.641	-117.226	537	9.47
UCR249823	33.598	-117.142	402	11.19
cbo43336	33.308	-117.232	129	13.36
cbo43271	33.315	-117.234	80	13.36
cbo53916	33.366	-117.153	224	12.34
SD201912	33.168	-117.094	232	13.33
cbo73769	33.171	-117.275	96	12.93
UCR270175	33.466	-117.042	488	10.08
UCR1131	33.386	-116.790	846	6.32
SD163649	33.259	-117.141	N/A	12.48
cbo76139	33.093	-117.298	28	10.44

Table 2. Location and elevation of all weather stations used for climate data. Data are from the Western Regional Climate Center (https://wrcc.dri.edu/) accessed on 30 May, 2019.

Site	Latitude	Longitude	Elevation (ft.)
Clark	33° 52' 36"	117° 18' 32"	1720
El Cariso	33° 38' 50"	117° 24' 40"	3038
Sage	33° 36' 20"	116° 56' 21"	2560
Santa Rosa Plateau	33° 31' 43"	117° 13' 50"	1980
Pala	33° 21' 40"	117° 06' 21"	455
Valley Center	33° 14' 14"	117° 00' 31"	1483
Wire Mountain	33° 14' 08"	117° 22' 05"	245
San Pasqual	33° 05' 29"	117° 00' 44"	255
Miramar East	32° 52' 32"	117° 03' 34"	900
Camp Elliot	32° 51' 33"	117° 06' 20"	539
Mission Valley	32° 46' 48"	117° 12' 00"	300
San Miguel	32° 41' 06"	116° 58' 25"	425
Alpine	32° 50' 01"	116° 44' 21"	2041
Barrett	32° 40' 03"	116° 41' 58"	2395

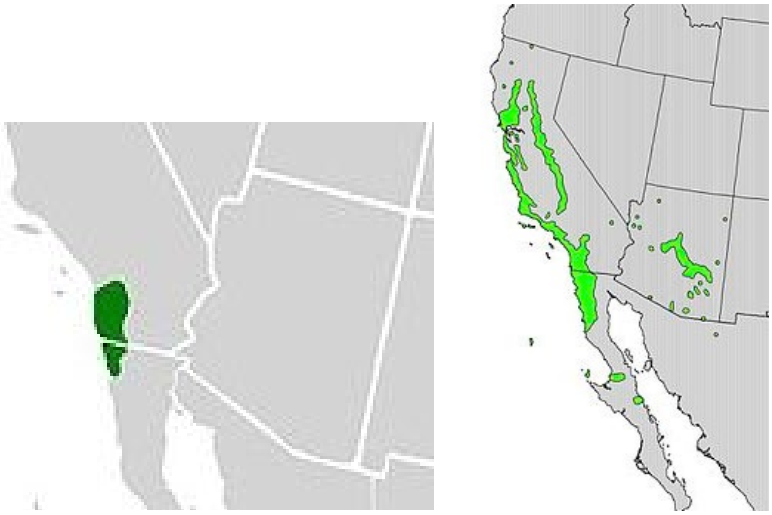

Figure 1. Example of the range of the Hermes copper (*Lycaena hermes*) (left-panel) and the range of the Spiny redberry (*Rhamnus crocea*) (right-panel). Left panel is from Edwards (1970) and the right panel is from Little (1976).

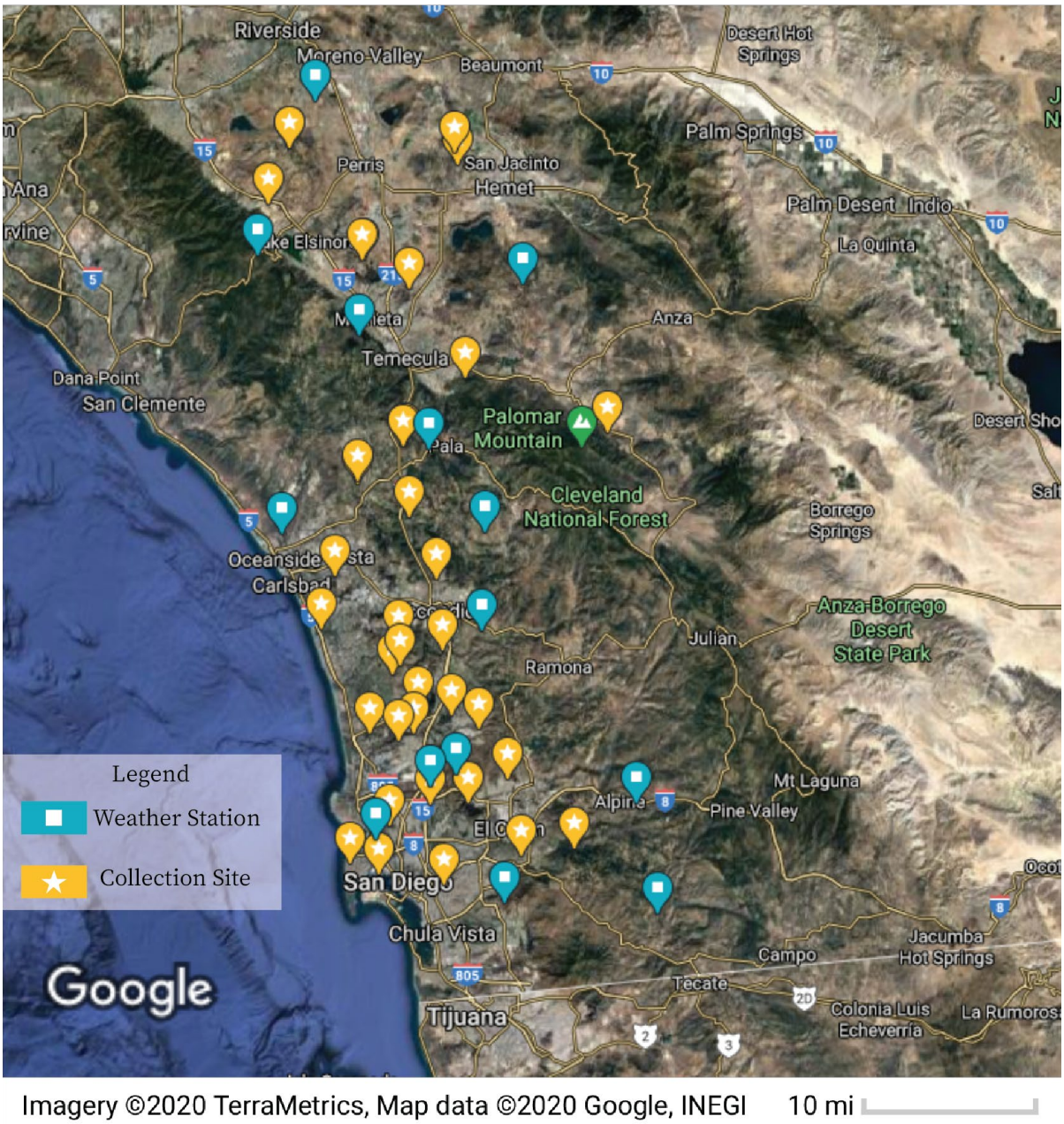

Figure 2. Distribution of sites and weather stations throughout San Diego County and Riverside County.

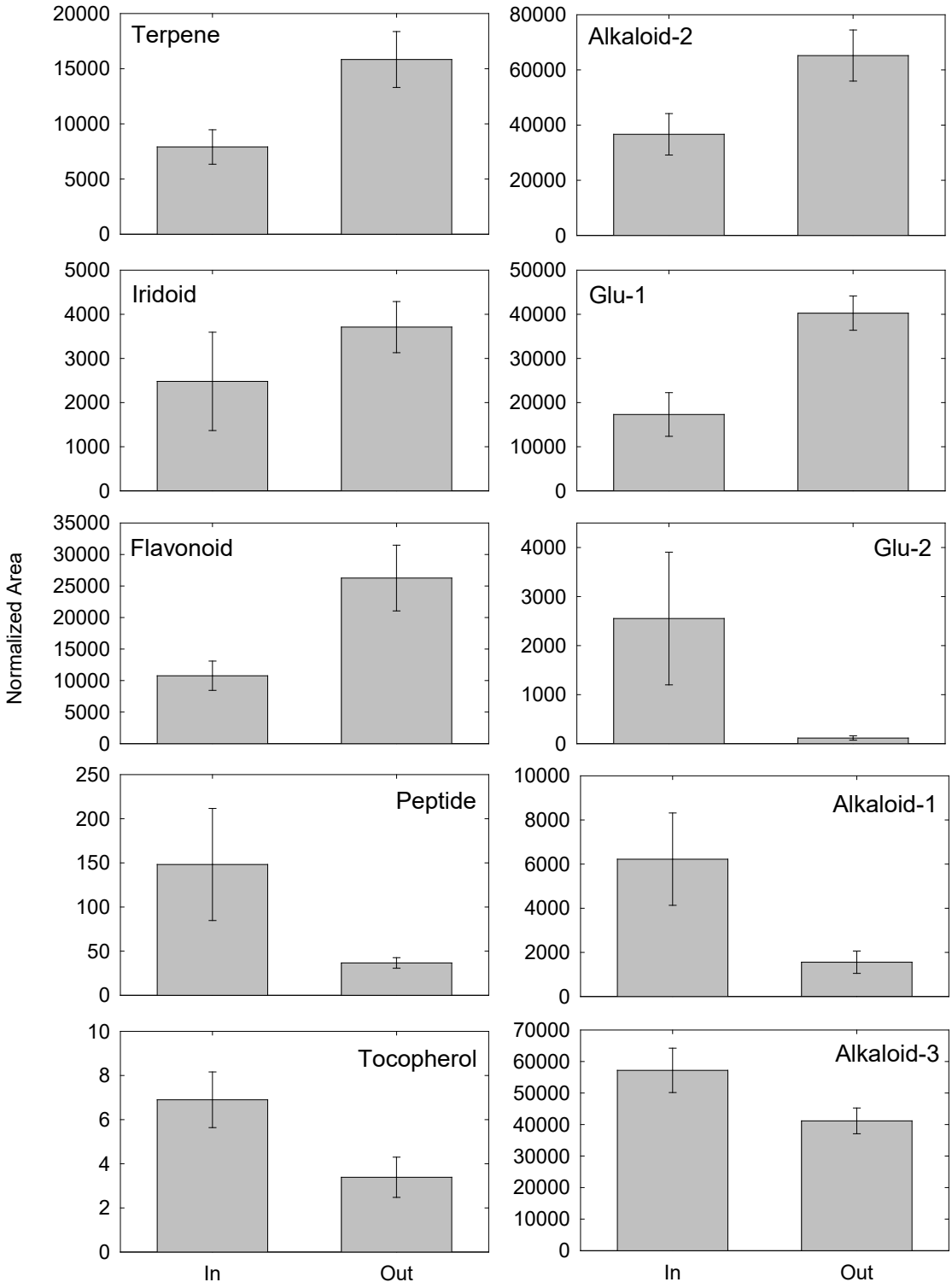

Figure 3. Mean (±se; n=15) normalized area of the 10 compounds that were found to be significantly different inside and outside of the range of the Hermes copper butterfly.

Table 3. Results from a t-test or Mann-Whitney U-test for comparison of statistically significant compounds inside vs. outside Hermes habitat, including the t- or Mann-Whitney U- values, p-values, and whether the data passed the normality assumption for the t-test. Degrees of freedom for all tests were 28.

Compound/Peak	t-value	p-value	u-value	p-value	Pass normality?
Terpene (11)	2.663	0.0127	56	0.0186	Yes
Alkaloid 2 (12)	2.392	0.0237	63	0.0408	Yes
Alkaloid 3 (16)	1.972	0.0586	59	0.0264	No
Iridoid (19a)	0.9783	0.3363	55	0.0164	No
Hexose (4)	3.658	0.001	32	0.0005	No
Peptide (6b)	1.751	0.0909	54	0.0145	No
Hexose (7e)	1.801	0.0825	53	0.0128	No
Flavonoid (7f)	2.713	0.0113	63	0.0408	Yes
Alkaloid 1 (9c)	2.172	0.0385	63	0.0408	No
Tocopherol	-2.261	0.0159			

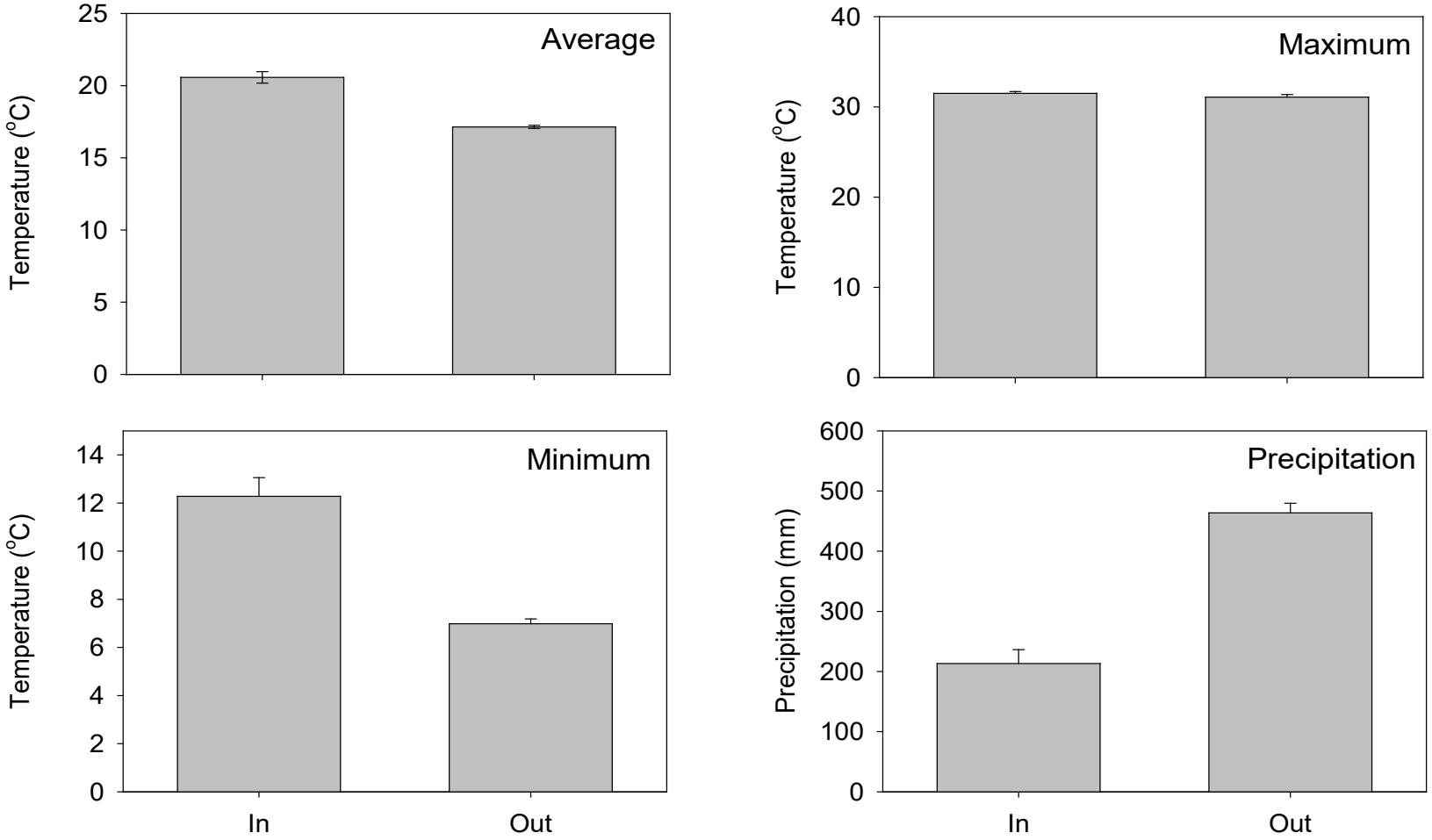

Figure 4. Mean (±se; n = 15) average monthly temperature (Average), maximum average monthly temperature (Maximum), minimum average monthly temperature (Minimum), and total precipitation (Precipitation) estimated for sites within and outside of the Hermes habitat range. Data were calculated for each site using an inverse-distance weighting interpolation from data obtained from the Western Regional Climate Center (see Table 2).

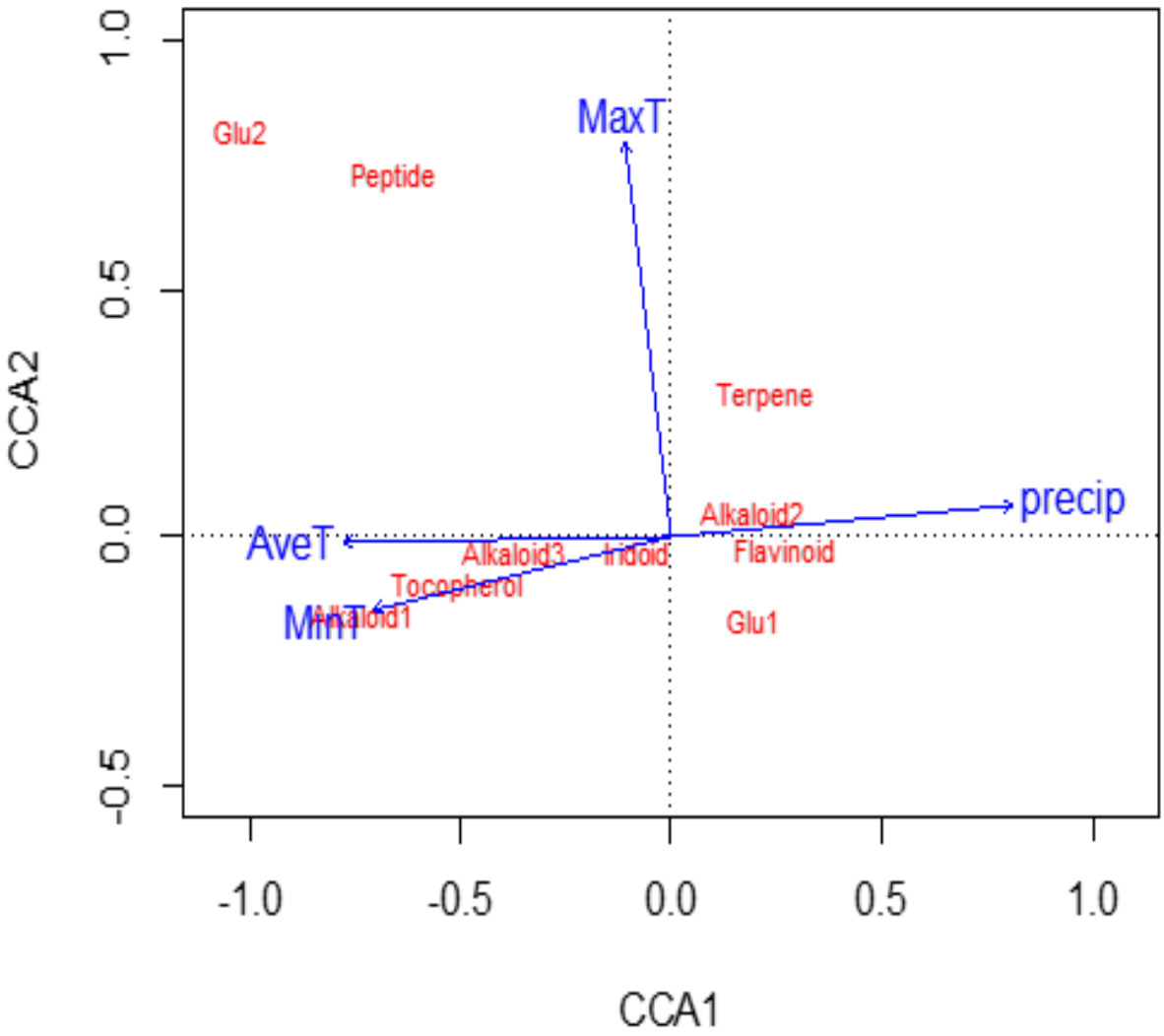

Figure 5. Results of a canonical correspondence analysis for effects of climate variables on identified plant compound classes from samples collected across southern California range of spiny redberry.

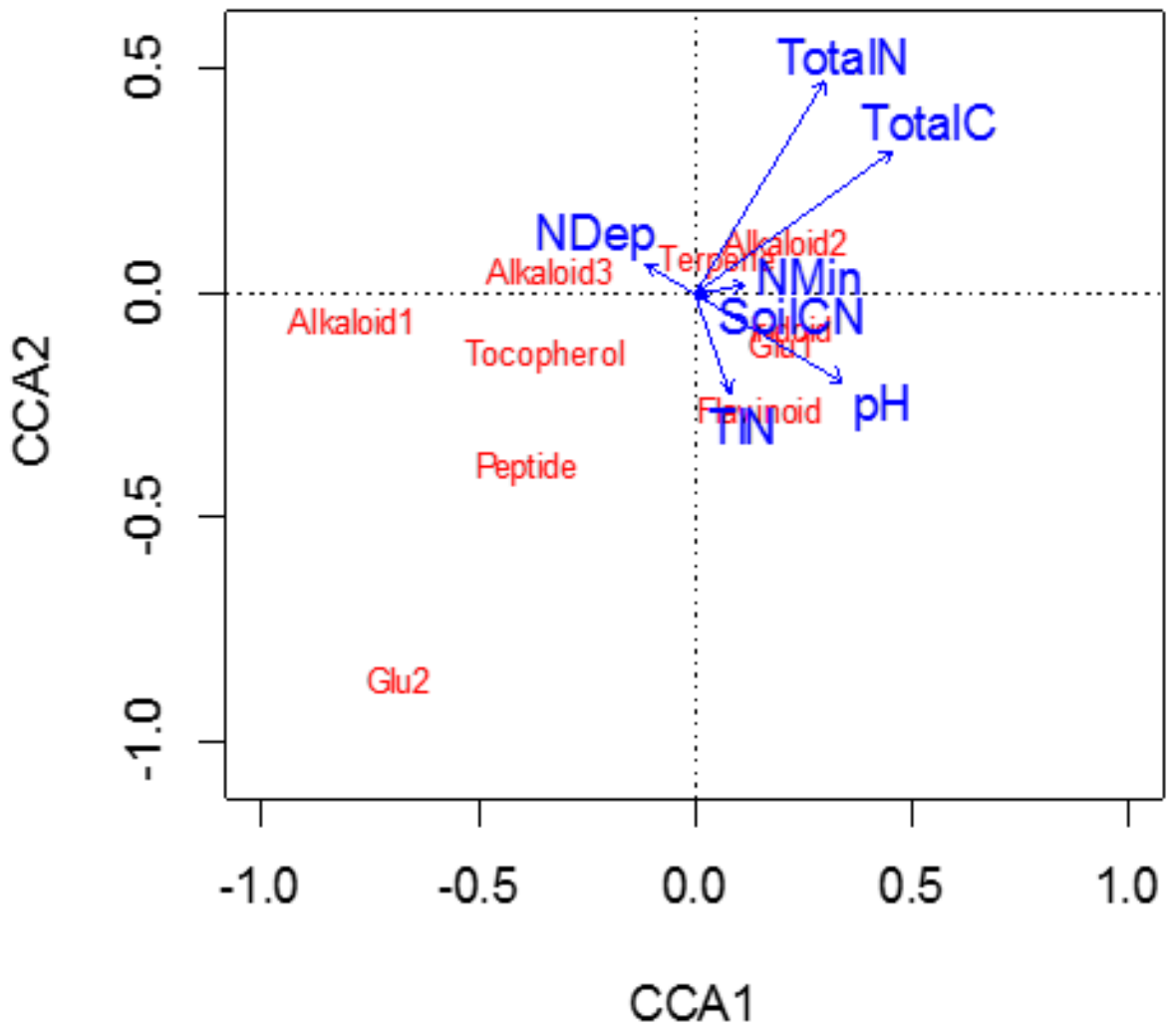

Figure 6. Results of a canonical correspondence analysis for effects of soil variables on identified plant compound classes from samples collected across southern California range of spiny redberry.

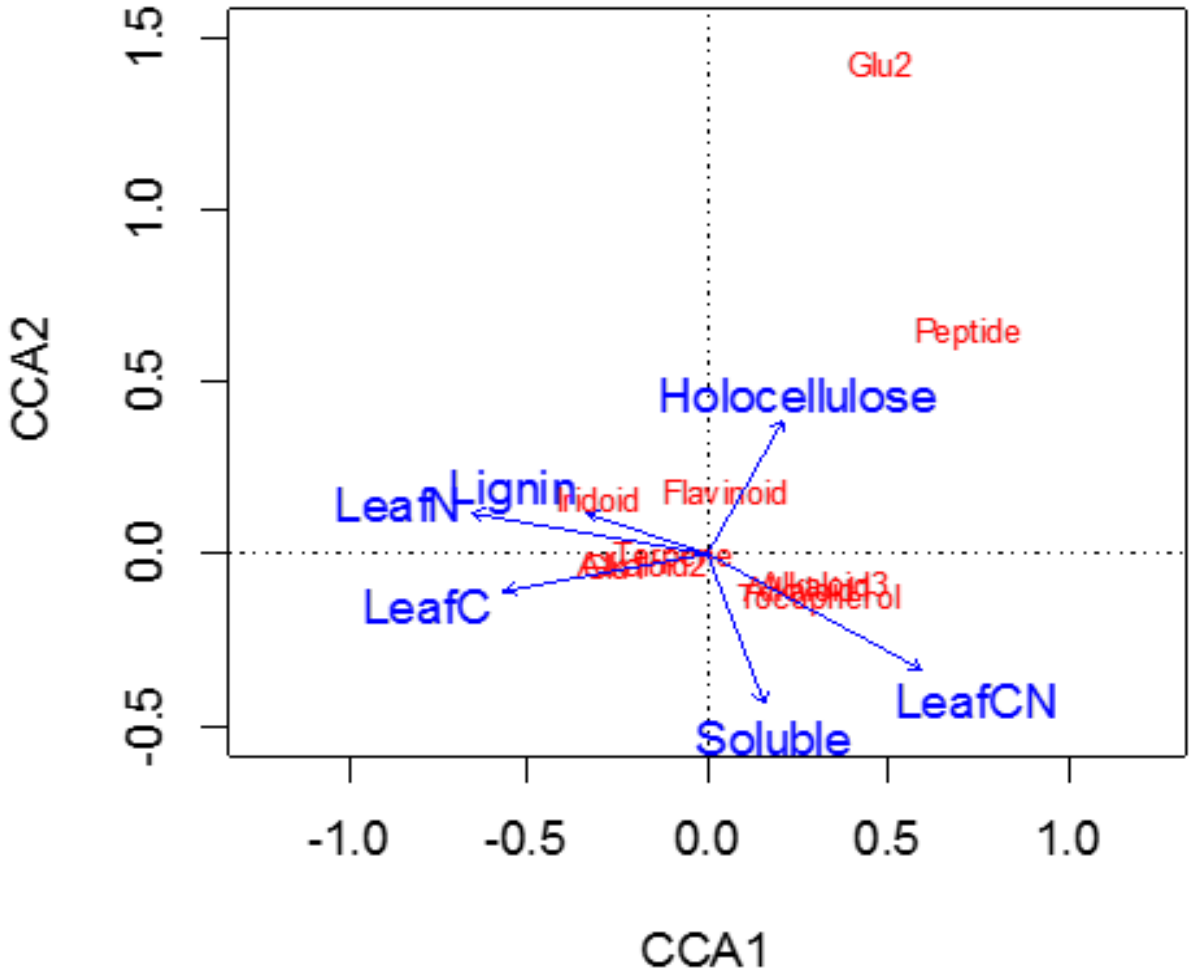

Figure 7. Results of a canonical correspondence analysis for effects of foliage variables on identified plant compound classes from samples collected across southern California range of spiny redberry.

Table 4. Accumulated constrained eigenvalues for the three CCA plots.

	Climate		Soil		Foliage	
Statistic	**CCA1**	**CCA2**	**CCA1**	**CCA2**	**CCA1**	**CCA2**
Eigenvalue	0.093	0.018	0.075	0.021	0.058	0.023
Proportion Explained	0.753	0.146	0.595	0.166	0.533	0.212
Cumulative Proportion	0.899		0.761		0.745	

Table 5. Location and selected characteristics for all study sites. Soil data are mean for the upper 0-10 cm soil layer sampled in May 2018. These data include pH, TIN, total N, total C, soil C/N, and N mineralization. TIN = total inorganic nitrogen (NO_3 + NH_4); Total N and C are the total soil N and C concentrations; soil C/N in the ratio of total soil C and N; N Min. = microbial N conversion of organic N to inorganic N; Leaf N and C are the leaf N and C concentrations; Leaf C/N is the leaf C to N ratio, soluble, lignin, and holocellulose refer to the concentrations of these constituents in *R. crocea* leaves. N deposition estimates are from a high-resolution (4 km) model (Tonneson et al. 2007).

Site	N Deposition (kg N/ha)	pH	TIN (g N/m²)	Total N (gN/m²)	Total C (gN/m²)	Soil C/N	N Min. (gN/m²d)	Leaf N (%)	Leaf C (%)	Leaf C/N (%)	Soluble (%)	Lignin (%)	Holocellulose (%)
UCR100298	11.2	7.05	5.76	276.6	2700.3	9.76	-0.01	2.05	50.9	24.8	60	28	12
UCR241774	8.37	7.03	8.99	274.3	3119.7	11.4	-0.46	2.42	61.7	25.5	52	26.4	21.6
UCR102732	10.8	6.75	4.94	260.8	2260.0	8.67	0.28	1.84	47.8	25.9	52	29.7	18.3
UCR260565	9.47	6.45	8.97	326.4	4232.3	13.0	-0.24	2.09	50.4	24.2	54	25.3	20.7
UCR249823	11.2	6.62	3.50	666.2	2481.8	3.73	0.11	3.17	58.2	18.4	50	26.2	23.8
UCR270175	10.1	7.20	1.27	212.4	1305.8	6.15	2.16	1.23	43.5	35.4	50	27.5	22.5
Cbo53916	12.3	7.45	0.91	374.9	1959.4	5.23	1.32	1.80	43.1	23.9	54	32.2	13.8
In:8546144	10.4	6.56	10.9	219.5	2355.9	10.7	-1.10	1.98	53.9	27.2	48	33.8	18.2
Cbo43271	13.4	7.26	2.00	469.4	2361.8	5.03	0.62	1.90	44.0	23.1	44	40	16
Cbo43336	13.4	6.62	3.00	485.3	3068.9	6.32	-0.05	2.81	49.7	17.7	44	28	28
UCR1131	6.32	7.88	2.86	258.2	3762.3	14.6	0.54	1.89	57.0	30.2	56	19.8	24.2
SD163649	12.5	6.92	1.41	185.7	1686.3	9.08	0.66	1.52	49.6	32.7	60	24.8	15.2
Cbo73769	12.9	7.19	0.75	249.4	2518.9	10.1	1.36	1.15	42.2	36.6	56	25.1	18.9
SD201912	13.3	7.08	1.32	298.8	3349.9	11.2	0.69	1.46	47.9	32.7	60	28	12
Cbo76139	10.4	7.31	2.26	192.3	1366.8	7.11	1.08	1.42	47.2	33.3	50	37.5	12.5
Elfin Forest (EF)	11.7	6.39	0.52	229.4	979.62	4.27	2.20	1.29	49.3	38.3	42	34.8	23
Mg20763	11.9	7.46	4.53	217.6	1750.9	8.04	-0.11	1.94	47.6	24.6	46	29.7	24.3
SD182404	11.7	6.19	1.30	438.1	1552.4	3.54	0.75	1.21	43.9	36.2	68	20.2	11.8
Cbo86186	12.1	7.75	1.84	216.3	2078.3	9.61	0.62	1.63	44.7	27.5	54	32.2	13.8
Black Mountain (BM)	12.4	6.38	3.82	97.10	1924.3	19.8	0.17	1.14	41.1	36.5	47	35.8	17.2
Sd195367	12.8	6.44	5.38	151.1	1288.3	8.53	0.13	1.82	50.6	27.9	58	18.9	23.1
Meadowbrook (MB)	12.1	6.51	0.74	308.4	1568.7	5.09	1.58	1.15	43.9	38.8	48	28.9	23
Cbo29765	12.3	6.00	1.02	203.0	1926.1	9.49	1.01	1.35	41.8	31.0	60	22.9	17.1
Oe3104	10.4	7.81	0.95	237.0	2028.9	8.56	0.67	1.11	46.6	42.1	50	26.2	23.8
Cbo37640	11.5	5.64	3.79	227.3	3571.9	15.7	0.11	1.85	46.0	24.9	50	28.6	21.4
In:9513993	12.2	6.80	1.78	306.0	2988.9	9.77	2.57	1.66	54.4	32.9	52	31.2	16.8
SD211218	10.9	5.76	11.6	201.6	1680.5	8.34	-1.23	1.49	44.5	29.8	54	27.6	18.4
Mission Trails (MT)	10.7	6.49	2.96	147.6	2271.3	15.4	0.58	1.45	52.7	36.8	49	38.6	12.1
SD208086	9.79	6.46	4.88	231.3	2748.6	11.9	-0.17	1.24	46.7	37.6	60	22	18
McGinty Peak (MP)	9.87	6.87	1.29	399.9	2323.1	5.81	1.19	1.28	51.2	40.3	54	28.2	18.2