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**ORIGINAL PAPER**

# Artificial rainfall increases herbivory on an externally defended forb

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**Abstract** Plants have a variety of herbivore resistance traits, including a diverse array of chemicals, either inside plant tissues or on plant surfaces. External chemical defenses are common and widespread, though understudied as a class. One potential selective force on these defenses is their potential for abiotic dislodgement given their exposed position. I tested whether abiotic removal (artificial rainfall) leads to increased herbivory in the annual chenopod *Atriplex rosea*. This plant, like other chenopods, has specialized secretory trichomes, which secrete water-soluble herbivore resistance compounds onto the plant's surfaces. Consistent with this hypothesis, I found significantly greater chewing herbivory in plants which received artificial rainfall compared to no-rainfall controls and a below-leaf water control. This simple experiment demonstrates that abiotic factors can directly change the efficacy of a resistance trait.

**Keywords** External chemical defenses · Herbivore resistance · Salt bladders · *Atriplex* · *Diabrotica*

## Introduction

The efficacy of plant resistance traits depends on a number of both biotic and abiotic factors. Interactions with herbivores and non-herbivores may determine the efficacy of this

relationship in several ways. Certain traits that are highly effective against one herbivore are rendered useless by behavior or morphology of another; for instance, caterpillars, beetles, and orthopterans clip leaf veins or canal systems to prevent the secretion of physically and chemically deterrent latex or resin (Dussourd and Denno 1991). In plants with indirect resistance traits, the identity of mutualistic associates (e.g., an ant species inhabiting an *Acacia* tree) determines the extent to which they control herbivory (Palmer and Brody 2007). The examples of biotic context dependency in plant defense are near-limitless; no resistance trait can be effective against all herbivores.

The abiotic environment may also effect the efficacy of a given resistance trait. This effect is often filtered through the physiology of the plant. For instance, an annual mustard, *Streptanthus polygaloides*, takes up nickel from its native serpentine soils. When grown in soils with less nickel, the plant becomes more palatable to caterpillars and grasshoppers (Martens and Boyd 1994). *Chenopodium album* plants grown in shade treatments (to mimic light competition) were more susceptible to herbivory (Kurashige and Agrawal 2005). However, while there may be clear trends in herbivory across an abiotic gradient or habitat types, the physiological mechanism is not always clear and the effect may be a direct consequence of the abiotic environment. For instance, caterpillar feeding on *Mimulus* [*Diplacus*] *aurantiacus* was primarily driven by sun exposure, not by plant response to the sun exposure (Lincoln and Mooney 1984).

Direct effects of abiotic factors may be especially pronounced when the resistance trait is external to the plant (i.e., outside of the plant's cuticle). Gonzalez-Coloma et al. (1988) demonstrated that elevated levels of ozone break down an important defensive compound found in the surface leaf resins of creosotebush and that this degradation

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may increase herbivore survivorship on the plant. LoPresti (2016) hypothesized that degradation or removal by abiotic factors may be an important ecological difference between secreted resistance compounds and those sequestered in plant tissues.

Plants with external chemical defenses (ECD) are most common in arid or seasonally arid areas, including Mediterranean and desert ecosystems (Dell and McComb 1979; LoPresti 2016), a finding broadly consistent with this hypothesis. If, as hypothesized, defenses are removed or degraded by water, a plant would need to constantly replenish them in a less-arid environment. While certain ECD are secreted constantly (including certain glandular trichome exudates), most occur over a short time or a specific ontogenic stage (LoPresti 2016). This latter category includes many resins secreted at budbreak by glandular trichomes or colleters (Dell and McComb 1979), as well as the secretion of salts and other water-soluble chemicals by salt bladder burst in many chenopods (LoPresti 2013).

Here, I test the effect of simulated rainfall removal on chewing herbivory on a nonnative forb, *Atriplex rosea* (Chenopodiaceae). *A. rosea* is an old-world xeric ruderal (Shomer-Ilan et al. 1981), also established in the western US. It occurs widely through California's central valley and northern interior coast range, germinating in March or April and growing and flowering as most annuals in these locations are senescing (Baldwin et al. 2012; pers. obs.). As it puts on most of its biomass during the Mediterranean summer, it rarely experiences rainfall during its growing season.

At my field site in the California coast range it is fed upon by both internally and externally feeding herbivores, most commonly the adults of a leaf beetle, *Diabrotica undecimpunctata*, which chew its leaves (Fig. 1). Caterpillars of a small butterfly (Lycaenidae: *Brephidium exilis*) chew reproductive tissues and a chrysomelid leaf miner



**Fig. 1** An adult *Diabrotia undecimpunctata* (Coleoptera: Chrysomelidae) on *Atriplex rosea* (Chenopodiaceae) showing characteristic feeding damage. Lake County, California, photo: EFL

(*Monoxia angularis*) feeds internally on leaves (Eiseman 2014). Like other *Atriplex* and *Chenopodium* species, it has salt bladders on its surface, which may be physiologically important in ionic balance (Osmond et al. 1980), but also function as a resistance trait in many species deterring insect herbivores (LoPresti 2013). The plant shunts dissolved compounds into these bladders, until they burst, whereupon the water quickly evaporates, leaving a residue of various compounds (often salts) (Osmond et al. 1980). Using a moist sponge, LoPresti (2013) gently wiped off the salt bladder residue from *A. rosea* in the field and found increased chewing herbivory on manipulated leaves compared to control neighbors. While this manipulation demonstrated a resistance function of the bladders in the field, whether this sort of removal could occur naturally due to rainfall or other abiotic factors was still unknown.

## Materials and methods

To determine the effect of rainfall on the efficacy of a plant external defense, I performed a manipulative experiment on the summer annual chenopod *Atriplex rosea*. I marked 75 plants in a seasonally wet meadow (on the shores of an abandoned mine tailing pond) on 5 June 2013 at the UC Davis McLaughlin Reserve, Lake County, CA (38.874763°N, 122.444043°W). The plot was far from the lake shore and covered <0.1 ha of well-spaced plants. Especially large or small plants were not selected, nor were plants within <10 cm from others' canopies. Moving around the plot, I measured basal area of each plant and alternated assigning plants to three treatments: a simulated rainfall (the plant drizzled with 3 $cm^3$ of water per each $cm^2$ of basal area: RM), a water addition without rainfall (the same amount of water applied below all leaves: WA), and an unmanipulated control which received no water (C). The former two treatments were also shaken gently to mimic any physical disturbance a weather event involving rain might provide. I reapplied these treatments once (14 June 2013) and collected all plants on 17 and 18 August. The only precipitation during the experiment fell on 25 June, and was <5 mm.

At this time, I assessed whether the 50 largest basal leaves of each plant had sustained chewing herbivore damage; these leaves were likely present at the time of manipulation (the plants had grown roughly an order of magnitude in volume since manipulation). I chose the response variable of chewing herbivory for several reasons: (1) it can be assessed on dry leaves, which many were by the time of collection, (2) sucking herbivory may appear superficially similar to leaf senescence or disease (and sucking herbivores were not common on *A. rosea*), and (3) the bladders are likely only effective against chewing

herbivory, as sucking herbivores or miners may avoid them (furthermore, neither were observed in the experimental plants, pers. obs.). I collected all aboveground biomass of each plant, dried them in a 60 degree drying oven for >72 h, and weighed all to the nearest 0.1 g. All statistics were done in R version 3.1.3. Leaf herbivory was analyzed with binomial regression and biomass with a one-way ANOVA.

## Results and discussion

As hypothesized, simulated rainfall increased the proportion of leaves suffering chewing herbivory over that in each of the two control groups (Fig. 2, upper panel: Tukey's comparison of means: RM-C, $p = 0.03$; RM-WA, $p = 0.01$). Notably, this effect was not due to watering, as the rainfall manipulation group received significantly more herbivore damage than the water addition group. Below-leaf water addition did not significantly alter the palatability compared to control plants (C-WA, $p = 0.93$). The differences between the rainfall manipulation group and the other two was striking in the field; plants in the rainfall group had noticeably less accumulation of the whitish crust of burst bladders that accumulates on leaves and stems.

Because it lessened herbivory, this trait is properly referred to as a resistance trait (Karban and Myers 1989). No fitness differences were found; biomass did not differ between treatments (Fig. 2, lower panel: all treatment comparisons of means, $p > 0.80$). Small amounts of chewing herbivory are unlikely to have significant fitness costs in this case; *D. undecimpunctata* feeds on usually mature leaves and not reproductive tissues or meristem (pers. obs.). (During the summer of 2013, I found both *Brephidium exilis* and *Monoxia angularis* in the area, but not in the patch of *A. rosea* upon which I was experimenting. All the chewing damage noted was consistent with *D. undecimpunctata* as shown in Fig. 1).

While rain is a rare phenomenon in California summers and it would be easy to dismiss this result as ecologically unrealistic, rare storms do happen in the summer and may be ecologically important (Raguse et al. 1977). Additionally, *A. rosea* is widespread in the western US, including areas which get a summer rainy season. More broadly, external chemical defenses are very common, and biogeographically and phylogenetically widespread and their interactions with the abiotic environment have been rarely investigated (LoPresti 2016). Gonzalez-Coloma et al. (1988) showed observationally that ozone degrades leaf resins of creosote, which are an important compound in resistance to herbivores (Gonzalez-Coloma et al. 1990). Similarly, allelochemicals secreted into the soil from plant roots are liable to degradation (Gimsing et al. 2009) and
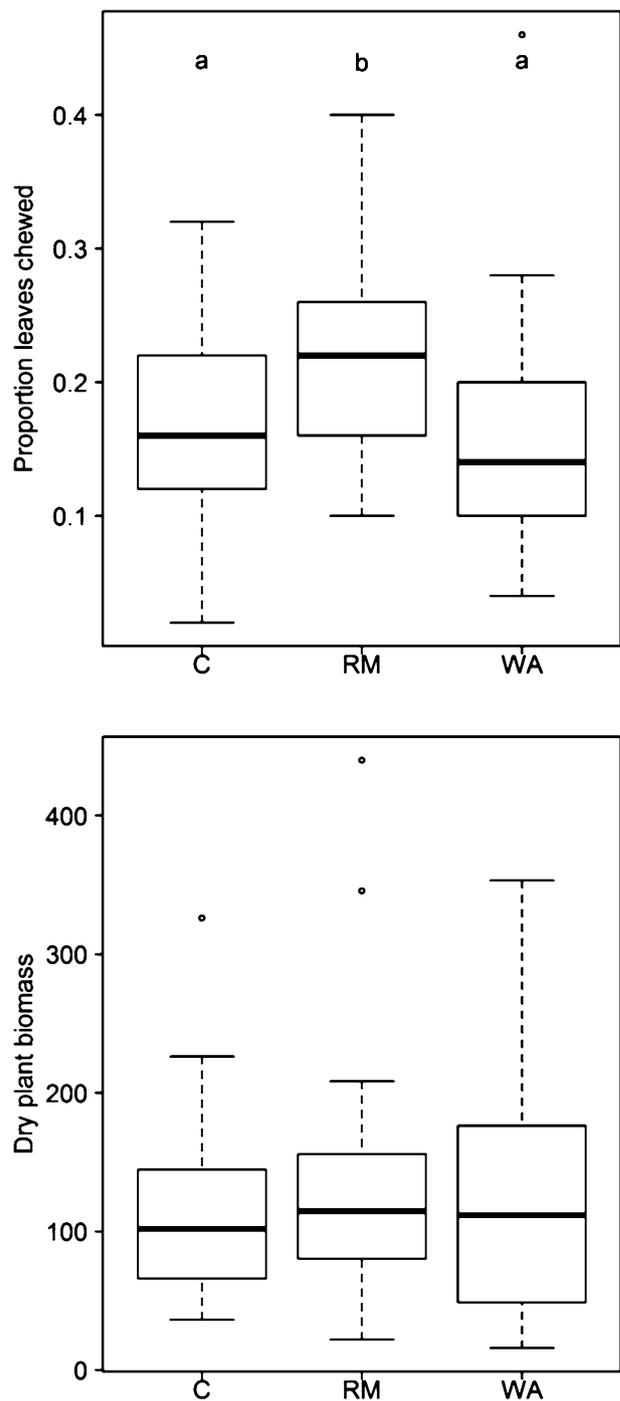


**Fig. 2** *Upper* proportion of the 50 leaves sampled per plant chewed in each of the three treatments. *Letters* indicate significant differences. *Lower* dry biomass (in grams) of each plant in each of the three treatments. Box and whisker plot shows median (*heavy line*), and 25/75% quartiles (*box*), with whiskers to minimum and maximum (*outliers plotted outside*) for each treatment

surely their effectiveness is reduced by physical removal by water or soil movement. However, this study represents the first attempt to experimentally determine the effects of

an abiotic factor directly on the efficacy of a plant resistance trait in the field.

This interaction demands further attention on other externally defended plants, a compendium of which is provided in LoPresti (2016). This distinction—external versus internal chemical defenses—should not be thought of as a dichotomy, instead a continuum. No plant will excrete every molecule of defensive compound, indeed, some of the best examples of external defenses come from various *Solanum*, *Nicotania*, and *Datura* (Solanaceae) species, which are heavily defended internally as well (e.g., Van Dam and Hare 1998). The proportion of defenses excreted versus sequestered may be under local selection; I hypothesize that populations of excreting plants in rainfall-prone areas will sequester a greater proportion of defenses in their tissues than populations in arid areas.

In what looks like a stark contrast to this hypothesis, in *Datura* studied by Elle and Hare (2000), rainfall correlated positively with proportion of the population with ECDs; however, *Datura* grows largely during the California summers (Elle et al. 1999), when rainfall does not wash off the exudates. In this case, water scarcity probably makes it harder for plants to allocate resources to glandular exudates, an assertion supported experimentally (Elle et al. 1999). Furthermore, local adaptation and coevolution may depend on the suites of herbivores in a local area (Thompson 2005); common and widespread species such as *Atriplex rosea* or *Chenopodium album* may be useful systems to examine the interplay between the environment, defense strategy, and local herbivores. This interplay is likely particularly pronounced in external defenses and may be an important mechanism for fluctuating herbivore and plant interactions across space and time.

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