

Climate change and anti-herbivory resistance communication in *Artemisia tridentata*

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ABSTRACT

It has been suggested that anthropogenic climate change may greatly influence plant communities, particularly at high elevations. We asked whether climate change and/or elevation gradient influence volatile intra-plant communication for purposes of anti-herbivory resistance and whether air contact is required for such communication in Artemisia tridentata. Plants at high and low elevation sites received one of three treatments: leaf clipping, leaf clipping and covering with plastic bag and no clipping with plastic bag. Distance from a focal stem to the nearest leaf damage and mean number of damaged leaves were measured before and after treatments. Previous leaf damage was shown to influence distribution of future herbivory damage, and free air flow influenced the distribution of subsequent herbivory damage. However, we did not find evidence for greater elevational influence on amount or distribution of herbivory damage.

INTRODUCTION

In the past century, global-average surface temperatures have risen by approximately 0.6°C – the largest increase of any century in the last 1000 years (IPCC 2001). The 1990s was the warmest decade and 1998 the warmest year on record since the beginning of instrumental records (Houghton et al. 2001). The International Panel on Climate Change predicted in its third report that global-average surface temperatures will increase by a further 1.4-5.8°C by 2100 and atmospheric carbon dioxide (CO₂) concentrations will rise 540-970 p.p.m. over the same period (IPCC 2001).

Among the numerous predictions for the effects of global warming is an expected increase in herbivore pressure on plants (Coley 1999, Wilf et al. 2001, Bale et al. 2002, Roy et al. 2004). With temperature as the predominant abiotic factor influencing herbivorous insects, global warming is expected to have a considerable effect on insect communities (Bale et al. 2001, Roy et al. 2004). Increased temperature and photoperiod interact to determine the phenology of insects, directly affecting development, survival, range and abundance. For example, warmer temperatures result in an increase in an

insect's thermal budget for growth and reproduction (Bale et al. 2001). Developing a more thorough understanding of how insect herbivores and plant populations interact may shed light on how ecosystems may be indirectly shaped by a changing climate.

Sagebrush (*Artemisia tridentata* Nutt. ssp. *vaseyana*) is a perennial shrub that is widespread throughout much of the western U.S. and reaches its upper altitudinal limit at approximately 9500' (Langenheim 1962). Sagebrush possesses exceptional morphological and physiological traits such as deep roots, capacity for hydraulic lift, overwintering foliage and allelopathic capabilities which make it a highly effective competitor. Additionally, sagebrush has been shown to have the capacity for intra- and interplant communication for the purpose of damage-induced resistance to herbivory (Preston et al. 2002, Karban, et al. 2006). This ability to communicate between conspecifics, and even with plants of other species, has been attributed primarily to the chemical signaling molecule methyl jasmonate, a methyl ester of the fatty acid derivative jasmonic acid (Preston et al. 2002, Karban et al. 2006). Release of this compound from damaged leaves appears to be transmitted via air flow to other plants, or to leaves on the same individual (Karbon et al. 2006). It has been suggested that this communication is likely to increase inclusive fitness, as the conspecifics closest to an emitting plant are more likely to be genetically related (Grafen 1984, Karban et al. 2004). This may be particularly true for sagebrush, since it is known to frequently reproduce vegetatively.

Evidence suggests that under projected climate change models, sagebrush range may expand in areas where it is currently patchily distributed (Harte & Shaw 1995, Loik & Harte 1996, Shaw et al. 2000). This expectation is based on evidence of this species' abilities as an effective competitor in environments that are particularly resource limited,

especially with respect to water (Perfors et al. 2003). However, no studies to date have asked whether communication for anti-herbivore defense in sagebrush is influenced by climate change. This phenomenon will likely further alter availability of resources across ecosystems (Harte et al. 1995, Harte & Shaw 1995, Saleska et al. 1999, De Valpine & Harte 2001, Perfors et al. 2003, Harte et al. 2006) as well as the range and abundance of herbivorous insect populations (Bale et al. 2001).

Faced with increased stress from expanding into particularly resource-limited areas, such as montane and subalpine meadows, and greater herbivory pressure, sagebrush may benefit from increased anti-herbivory defense via volatile communication. Few laboratory studies have analyzed the costs and benefits to plants of anti-herbivore defense in terms of a framework of trade-offs between resource allocation for growth and reproduction versus defense (Sagers & Coley 1995, Agrawal 2000, Siemens et al. 2002). Several studies have looked at the intra- and interspecies communication abilities of plants with respect to herbivory-induced resistance and the potential implications for plant fitness and ecology (Mahall & Callaway 1991, Karban & Maron 2002, Preston et al. 2002, Karban et al. 2004, Karban et al. 2006). Additionally, while numerous laboratory studies have shown that volatiles are important for *interplant* anti-herbivory defense communication (Karbon & Baldwin 1997, Preston et al. 2002), only one field study has investigated the role of volatiles in *intraplant* communication (Karbon et al. 2006).

Our principle question was whether lower elevation sagebrush will have increased anti-herbivory defense communication compared to those at high elevation. The low elevation site served as a proxy for expected climate change due to its warmer

temperatures, longer growing season and greater resource limitation. We also asked whether air contact is required for anti-herbivory defense communication in low and high elevation sagebrush individuals.

Hypotheses:

H₁: Previous damage to leaves influences future herbivore damage in sagebrush.

We predicted that sagebrush individuals with experimentally-damaged leaves would have significantly fewer herbivore-damaged leaves per stem than undamaged individuals. We also expected that the distance between herbivore damage sites would be greater in individuals that were previously damaged.

H₂: Herbivore damage to low elevation sagebrush is more strongly influenced by previous damage than high elevation sagebrush.

We predicted that experimentally-damaged sagebrush at low elevation would have fewer herbivore-damaged leaves and the distance between herbivore damage sites would be greater on individuals at low elevation than at high elevation.

H₃: Free air flow between damaged and undamaged leaves is required to influence future herbivory damage.

We predicted that experimentally-damaged individuals with free air flow between damaged and undamaged leaves would have fewer herbivore-damaged leaves compared to damaged individuals with blocked air flow. We also predicted that the distance between leaves with damage would be greater in individuals with free air flow compared to those without.

METHODS

Study sites.

We chose two field sites on an elevation gradient as a proxy for climate variation. The low elevation site was located on U.S. Forest Service property at approximately 2770m on the southeast slope of Crested Butte Mountain, Gunnison Co., CO, USA (38°52'32.92"N, 106°54'33.65"W). The vegetation here is predominantly sagebrush, bunch grass (*Festuca thurberi*) and forbs, and the area is not generally grazed. The high elevation site was located at the Rocky Mountain Biological Laboratory at Gothic, Colorado (38°57'37.61"N, 106°58'59.16"W) at approximately 3000m on a southwest facing slope dominated primarily by sagebrush.

Does elevation influence intraplant communication?

We chose 100 sagebrush plants at both sites (total of 200 plants). Plants were systematically assigned to one of four groups to evenly distribute treatments across the sites' microhabitats. The first treatment group was selected for artificial foliage damage, which consisted of clipping the distal half of all leaves on one stem. Plants assigned to the first control group were not clipped. The second treatment group was selected for clipping of leaves on a focal stem as in the first group, with damaged stems covered with an opaque plastic bag secured with a plastic tie. The control group was not clipped, but one stem was covered with a bag as in the treatment group. Each treatment and control group consisted of 25 plants per site.

Before treatments were applied, we counted the number of damaged leaves on the focal stem and the distance to the three nearest stems with damaged leaves. The type of

leaf damage (holes, mines, leaf cutting) and type of insects present on stems were also noted. These measurements were repeated approximately 10-14 days after treatments were applied. Data were analyzed via analysis of variance tests using NCSS statistical software.

RESULTS

Results from the ANOVA tests showed a significant site effect on both the mean distance to herbivory damage ($F=110.39$, $P<0.005$, $df=1,198$) from the focal stem and the mean number of herbivore-damaged leaves ($F=273.41$, $P<0.005$, $df=1,198$). Mean distance to herbivore-damaged leaves at the high elevation site was 2.0 cm (± 0.5596 SE), and 10.3 cm (± 0.5596 SE) at the low elevation site. The mean number of herbivore-damaged leaves at the high elevation site was 5.9 (± 0.1878 SE) and 1.5 (± 0.1878 SE) at the low elevation site. A treatment effect was found for clipped and unbagged control plants at the low elevation site with regard to the mean distance (12.2 cm) from focal stem to herbivore-caused damage, a 10.9% greater distance than that of the unclipped and bagged controls (11.0 cm) and 38.9% greater than unclipped and unbagged controls (8.8 cm). A slight treatment effect was also found for clipped and unbagged plants at the high elevation site with regard to the mean number of herbivore-damaged leaves (5) per plant compared to controls (6).

DISCUSSION

Our first hypothesis was supported by the data. Treated individuals at low elevation had a greater distance to subsequent herbivore damage compared to controls. Treatment did not have a significant effect on the number of herbivore-damaged leaves, however. Our second hypothesis was inconclusively supported. The fact that there was

less herbivory damage at the low elevation site does suggest the possibility of some climate effect. However, the scope of this study did not allow an examination of other possible causative factors. For example, it was noted that the *types* of herbivory differed between the two sites, with more observations of a higher frequency of ant-aphid mutualisms at the lower site compared with the high elevation site. Enhanced plant defense may be a beneficial result of these ant-aphid mutualisms, as suggested by some field studies (Floate & Whitham 1993).

Additionally, the heterogeneity of the plant communities at each site may serve to influence insect herbivores. The high elevation site was dominated primarily by *A. tridentata*, while the low elevation site had a variety of grasses and forbs. The variety of plant species and their staggered phenologies may provide a more suitable habitat for insect herbivores, with more resources such as forage of higher nutritional value, greater numbers of regugia, mates and nesting sites.

In terms of a costs-benefits analysis, it is possible that the costs of anti-herbivory defense communication outweigh the benefits for higher elevation individuals, and that possibly conditions allow lower elevation individuals to allocate more resources to defense. Alternatively, perhaps due to the shorter growing season and limited resources at higher elevation, sagebrush individuals there allocate more resources to growth and reproduction than anti-herbivory defense. As suggested by numerous studies on the costs and benefits of plant defense, herbivores may act on plants in the process of natural selection, thus helping to shape plant defense evolution (Sagers & Coley 1995).

Our third hypothesis that free air flow between damaged and undamaged leaves is required to influence future herbivory damage was also somewhat supported by results

from the low elevation site. Individuals in the clipped and unbagged group exhibited a greater distance between the treated stem and the nearest stems with herbivory damage, suggesting that air contact between adjacent parts of the plant is beneficial, if not necessary to transmit anti-herbivory signals via plant volatiles. While there was a slight treatment effect on the number of damaged leaves at high elevation, it was not significant enough to suggest the equivalent effect.

As noted above, there are numerous possible factors beyond the scope of this study that could explain these results. One factor that may help to explain lack of a stronger treatment effect may be due to the timing of our study. Previous studies testing the importance of volatile chemicals in anti-herbivory defense involved treatments being applied in the spring or very early summer (Karban et al. 2006), whereas the treatments in this study were not applied until mid-summer. The phenological stage of the plant may influence the necessity of volatile signaling such that once plants have put out reproductive shoots, the need for defense isn't as great. Alternatively, with resources such as water being scarcer later in the growing season, shifting of resource allocation may diminish volatiles.

CONCLUSION

Results from this experiment suggest that there may be possible influences of elevation as a proxy for climate change on plant defense mechanisms in *A. tridentata*. However, our results were not comprehensive enough to rule out other factors influencing herbivory patterns within plant populations and communities. While some treatment effect was shown at both sites, more study would be needed to look at possible

seasonal influence on herbivory and plant defenses as well as the impacts of greater interspecific diversity within the insect herbivore and plant communities in question.

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