4-26-2020

Direct and Indirect Effects of Additional Sodium on Grasshopper Growth and Development

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Abstract:

In order to investigate why sodium, combined with macronutrients, led to a spike in grasshopper abundance in a Texas prairie field, laboratory and field studies were conducted in Ohio and Texas to understand if sodium has an effect on grasshopper growth and development. These effects could happen directly, by altering grasshopper physiology, or indirectly by altering the plant communities that grasshoppers eat. To examine direct effects, grasshoppers were captured, reared, and fed diets with varying amounts of sodium. Indirect effects were examined by collecting the most dominant plants within plots treated with different micro and macronutrients, and feeding them to grasshoppers. The growth and development of each individual was tracked and treatment groups were compared. Direct effects could not be tested, but we did find an indirect significant difference in the change in weight of one species that fed off plants grown in a plot treated with nitrogen, phosphorus, and sodium. We speculate this result could be due to the high nutrient content of the plants and look into other factors that could have affected the results. The potential direct effects of sodium are also discussed along with the economic and environmental implications they could have.

Disclaimer:

This presentation is solely for academic purpose and does not have any commercial value.

Acknowledgements:

I would like to thank Dr. Chelse Prather and the Prather Lab.

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Introduction

Micronutrients are minerals that organisms need in small amounts, as opposed to macronutrients like nitrogen and phosphorus, which organisms require in large amounts (Jones 2008). Sodium is a very important micronutrient to animals, which depend on it for their physiological functioning (Prather et al., 2018). Some processes sodium is responsible for is managing the sodium pump, cell signaling, maintaining hydrologic homeostasis, and neural and brain development (Chown & Nicolson 2004; Snell-Rood et al., 2014). In terms of insects, sodium has been found to influence the structure of termites and to increase the abundance of prairie insect communities (Kaspari et al., 2014, Kaspari et al., 2017). More recently, and the basis of this study, Prather et al. (2018) discovered that sodium, in combination with macronutrients, are limiting nutrients to grasshopper abundance. Despite the recent findings of sodium's effects on insects, this area of study has largely been ignored in favor of other macronutrient effects like nitrogen and phosphorus.

Nutrient limitation of herbivores can happen directly or indirectly (Daufresne & Loreau, 2001). Directly, it can happen by changing the organism's growth and physiology (Collier et al., 2005; McDowell & Wilcock, 2008). Phosphorus, for example, can directly limit herbivore somatic cell growth, and when grasshopper species *C. curtipennis* was fed diets with enriched phosphorus, their growth rate increased by 30% (Rode et al., 2017). Also herbivorous insects struggle with a stoichiometric imbalance in regards to the amount of nitrogen in their bodies versus the amount found in foliage (Rode et al., 2017). When insects fed on nitrogen-enriched plants, they yielded higher growth, survival, and reproductive rates likely due to the increase of protein synthesis that nitrogen assists with (Rode et al., 2017; Lemoine & Shantz, 2016; Kainulaninen et al., 1996; Saxena, 1991; Townsend, 2001). Indirectly, nutrient limitation happens by altering plant communities that herbivores eat (Fukui, 2018). Nitrogen is the most limiting nutrient for plant growth (Lawlor et al., 2001; Zhao et al., 2005). When soils were enriched, the plant community saw an increase in net primary production, biomass, and biodiversity (LesBauer & Treseder, 2008; Humbert et al., 2015), and furthermore, a study by Harry Olde Venterink found that phosphorus is "likely" a limiting factor in species richness and productivity (2011). These factors all, in turn, affect insects of an ecosystem because most insects rely on plants for food, and more plant species richness also supports more insect species richness (Haddad et al., 2001; Prather et al., 2018; Siemann 1998).

Some studies have shown that besides nitrogen and phosphorus, sodium might be important for herbivore growth and development (Kaspari et al., 2008; Kaspari et al., 2017; Prather et al., 2018; Joern et al., 2012). Studies by Kaspari et. al (2008, 2017) revealed that sodium deposits in prairie communities increased terrestrial invertebrate abundance both below and above ground, and that ant communities are more active in coastal areas because of the higher frequency of sodium deposits via rainfall. Furthermore, Joern et al. (2012) found that when plant biomass, diversity, and macronutrient concentrations are varied, there is little change in grasshopper communities, but when nutrients are added to these components, significant change occurrs in the grasshopper communities. Prather et al. (2018) further solidifies the theory

of sodium being important to herbivores when the results showed that grasshopper population rose when exposed to higher levels of sodium with macronutrients.

Although this finding illuminates the importance of sodium limitation, we still do not know whether the effects of sodium are mediated through direct or indirect mechanisms. I will use a series of laboratory studies in Ohio and in Texas to determine this. This experiment is important because grasshoppers play a significant role in ecosystems and the economy (Belovsky & Slade, 2000; Belovsky & Slade, 2018; Prather et al., 2018; Branson et al., 2006). Although grasshoppers can help ecosystems by assisting plant growth through nutrient cycling and soil fertilization (Belovsky & Slade, 2000), in some species, an overabundance can result in large crop plantations becoming diminished, therefore causing economic damage (Branson et al. 2006). Therefore, it is important to stabilize their populations. I hypothesize that the direct effects of sodium will cause significant changes to grasshopper growth and development, responsible for the spike in abundance found by Prather et al., while the indirect effects of sodium will not yield any significant changes. I believe this mainly because of the overwhelming literature emphasizing how crucial sodium is for animal functioning. The spike in abundance could very well be due to the physiological changes regarding behavior. I do not think grasshoppers will be affected by any indirect means because an overabundance of sodium does not help plant production or growth, and can potentially hurt it because added sodium can alter plant ion ratios which can result in sodium toxicity (Blumwald et al. 1999).

Methods

Direct

We tested for the direct effects of additional sodium by collecting 60 total 2nd-3rd-instar grasshoppers. The grasshoppers were kept individually in mason jars capped with a mesh lid and divided into six groups of ten, with each group being fed artificial diets with varying amounts of sodium. Group one had no additional sodium and every group beyond had 10% more sodium than the last. Artificial diets were composed of sodium, Horse Charge, casein, starch, protein powder, egg powder, sucrose, a vitamin mixture, and methyl-4-hydroxybenzoate. These ingredients were put in a blender with a solution of boiled water and agar gel. Each artificial diet was then poured into four petri dishes, labeled with their respective amount of sodium, and kept in a refrigerator. Water was also provided for moisture by adding a soaked cotton ball in a soufflé cup in each cage, which was re-wetted or replaced every two days. Before the grasshoppers were put into their cages, their femur lengths, from the most anterior portion to the most distal portion were measured, and their wet weight was measured. These were their initial measurements, and they would be measured again in the same ways every week, with their measurements recorded in a notebook, until the end of the experiment. To account for development, the dates of molts were recorded for each individual. Individuals were fed every two days by having their food smeared on the mesh lid of the mason jar. When deaths occurred, the dead individual's cage was thoroughly cleaned out. All data was recorded in a Microsoft Excel data sheet with the treatment, number of molts, date of molts, and date of death.

Indirect

We tested how plant composition affected grasshopper growth and development by collecting plants representative of micronutrient plot plant compositions. Plants chosen accounted for 50% of the plant composition in each treatment. All plant species were collected at least three meters from the micronutrient plots, but not from the plots themselves. There were four micronutrient plots with the treatments of sodium, nitrogen and phosphorus, sodium and nitrogen and phosphorus, and the control. One leaf from each species was taken from each respective plant, and each leaf was checked to ensure it was high quality and free from apparent damage. Plants were then taken back to the lab where they were clipped at the torn end and placed immediately in water for rehydration. As a vehicle for feeding, each treatment was assigned a vial filled with water with the treatment's leaves' petioles fully emerged in the water. Each vial was placed in a pint-sized mason jar with one grasshopper in it. The leaves were spread evenly along the rim of the vial to ensure the grasshoppers equal access and parafilm was wrapped around the rim to prevent grasshoppers from drowning. The leaves in each "bouquet" were replaced every two days by new leaves, which were also acquired every two days. There was also one soaked cotton ball in a soufflé cup in each cage. These were re-wetted or replaced every two days as well.

Table 1.1

Control	Na	NP	NPNa
Rhychospora caduca	*Rhychospora caduca*	*Helianthus angustifolia*	*Eryngium yuccafolia*
Morella cerifera	*Longbeak sedge*	*Rudbeckia grandiflora*	*Helianthus angustafolia*
Eryngium yuccifolium	*Panicum sp.*	*Rubus argutus*	*Ambrosia psilostachya*
Longbeak sedge	*Paspalum plicatulum*	*Tripsacum dactyloides*	*Rubus argutus*
Schizachrium scoparium	*Helianthus angustifolius*	*Ambriosa psilostachya*	*Paspalum plicatulum*
Setaria parviflora	*Eryngium yuccafolium*	*Lovegrass*	*Centella erecta*
Panicum sp.	*Schizachrium scoparium*	*Eryngium yuccafolia*	*Boltonia*
Lovegrass sp.	*Fimbry*	*Boltonia*	*Panicum sp.*

Table 1.1: Each treatment plot gets one leaf of each of the eight most dominant species in the plot.

The grasshopper species used were 4th instar *Melanoplus femurrubrum* and *Paroxya atlantica.* 40 individuals of each species were used at a time (20 male, 20 female), with a total of 20 individuals per treatment- five of each sex of each species. Grasshoppers were collected Monday through Thursday every week until there were enough replicates of each species. Grasshoppers without intact hind legs were not collected, as this would affect wet weight considerably. Before the grasshoppers were put into their cages, their femur lengths, from the most anterior portion to the most distal portion were measured, and their wet weight was measured. These were their initial measurements, and they would be measured again in the same ways every week until the end of the experiment,

with their final dry weight recorded as well. To account for their molts, the dates of their molts were recorded. If an individual died within the first three days of the experiment, the experiment was restarted with a new individual. The dates of death were recorded, their cage was checked for parasitoid larvae, and the dead individual was kept for two days to check for any parasitoid emergence. All data was recorded in a Microsoft Excel data sheet with the species, sex, treatment, instar, and date of capture of each grasshopper.

Table 1.2

Plant Type	**Species Name**	**Characteristics**
Forb	*Ambriosa psilostachya*	Hairy, 1-3% silica
Forb	*Boltonia sp*	Medium C:N ratio
Forb	*Centella erecta*	Fleshy, soft leaves
Forb	*Eryngium yuccifolium*	Spiny, thick leaves
Forb	*Helianthus angustifolius*	Medium foliage texture, narrow leaves
Forb	*Morella cerifera*	High C:N ratio, coarse foliage, waxy leaves
Forb	*Redbeckia grandiflora*	Rough and hairy leaves
Forb	*Rubus argutus*	Rough and prickly leaves
Grass/Sedge	*Fimbry*	Rough, hairy leaves
Grass/Sedge	*Longbeak sedge*	Fine texture foliage, loose leafy tufts
Grass/Sedge	*Lovegrass sp.*	Fine foliage texture, medium C:N ratio
Grass/Sedge	*Panicum sp.*	Medium N content, low P content
Grass/Sedge	*Paspalum plicatulum*	Fine foliage texture
Grass/Sedge	*Rhynchospora caduca*	High N content, low P content
Grass/Sedge	*Schizachrium scoparium*	Medium texture foliage and C:N ratio, smooth leaves
Grass/Sedge	*Setaria parviflora*	Very bristly leaves
Grass/Sedge	*Tripsacum dactyloides*	Coarse foliage, low C:N ratio,

Table 1.2: All forbs and grasses & sedges, respectively, used in the experiment with their defining characteristics in terms of edibility.

To determine whether there were significant differences in femur growth and change in weight between treatments, A histogram, QQ plot, Shapiro test, Bartlett test, and Kruskal-Wills/ANOVA were all used (via R ver. 1.69), and box plots were made to visualize these results.

The change in femur length and weight for each treatment per species was found by taking the average of each individual's change in length and weight in the respective treatment and species.

Results

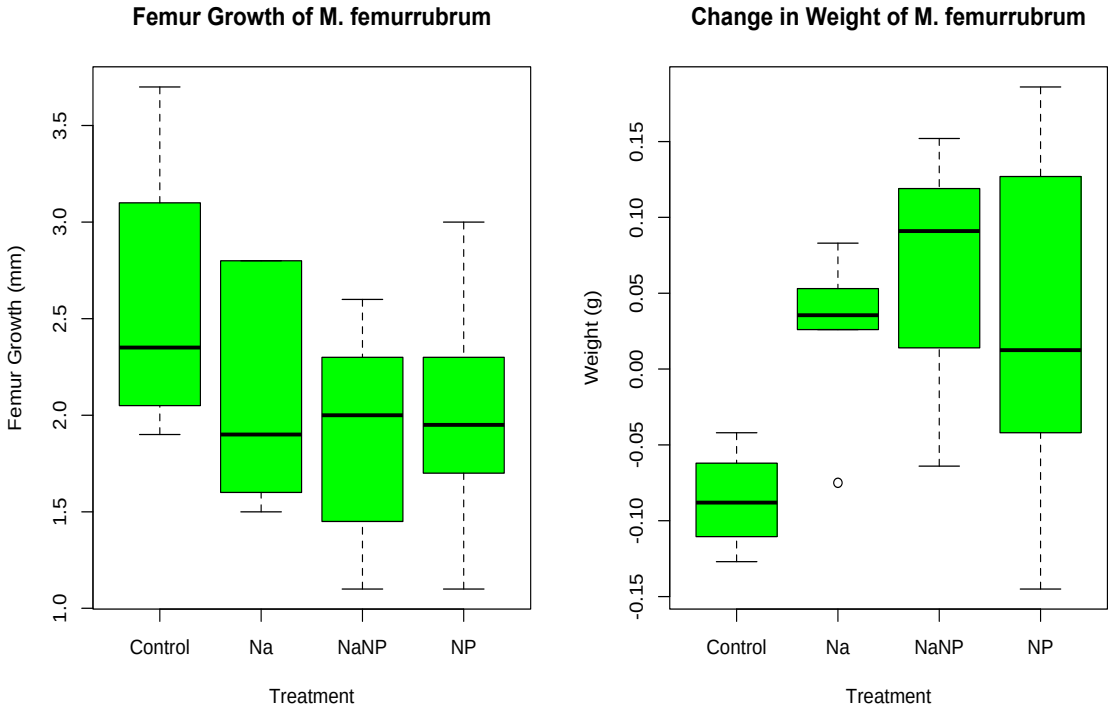
Direct

The proposed experiment for testing the direct effects of additional sodium on grasshopper growth and development unfortunately was unable to be complete due to a

bacteria repeatedly wiping through the grasshopper jars. Four separate sets of 60 individuals were collected and reared, and on all four occasions, the individuals died overnight. Anti-bacterial soap was used for sterilization of the lab room, but this was not effective in keeping the individuals alive.

Indirect

Figure 1.1: The average change in femur length (mm) and weight (g) of *M. femurrubrum* after the two weeks of the experiment.



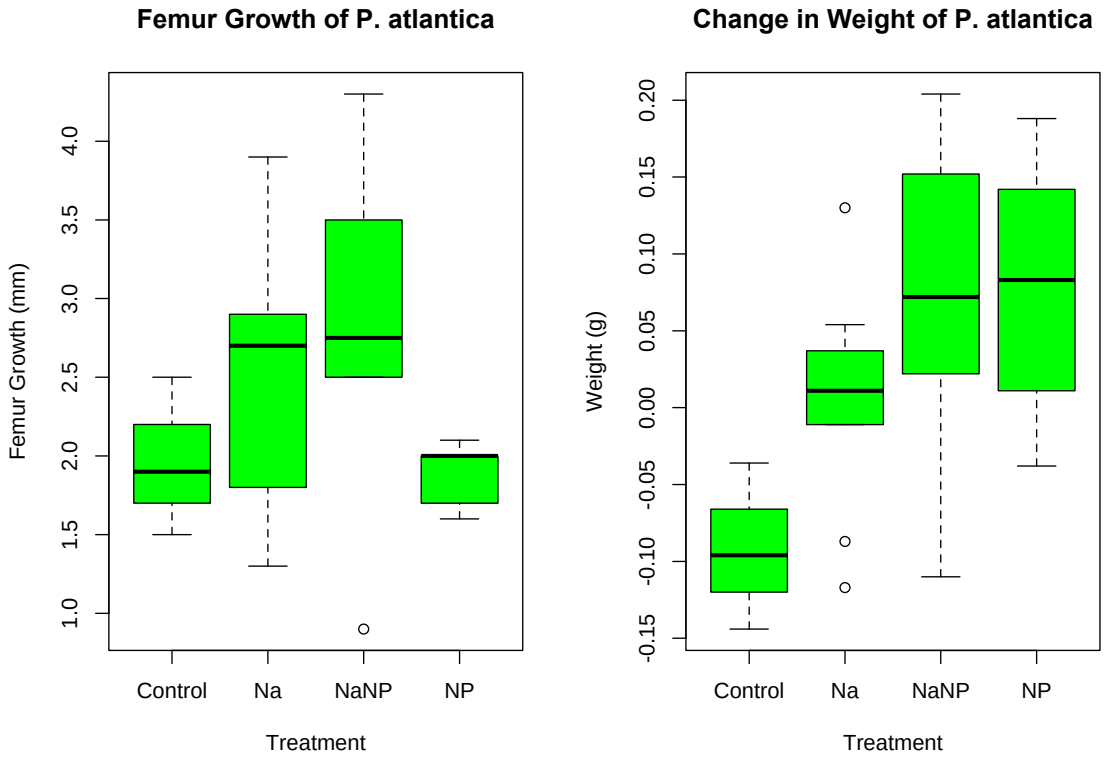
M. femurrubrum femur length: (Control= 2.56 ± .789mm, Na= 2.08 ± .581mm, NP= 1.99 ± .608mm, NaNP= 1.90 ± .532mm).
Weight: (Control= -.086 ± .035g, Na= .0263 ± .0536g, NP= .0301 ± .106g, NaNP= .067 ± .074g).

For *M. femurrubrum*, there were no significant differences in femur growth between any treatments (p-value= .497). The NP and control treatment groups had the widest spreads, and the Na and control treatments had the interquartile ranges. For change in weight, the control group resulted with an average weight of significantly less than the NaNP treatment, and the controls actually lost weight (p-value NaNP-Control= .024).

P. atlantica femur length: (Control= 1.97± .503mm, Na= 2.59 ± .868mm, NP= 1.88 ± .217mm, NaNP= 2.78 ± 1.14mm).
Weight: (Control= -.092 ± .0541g, Na= .00556 ± .0733g, NP= .0772 ± .0924g, NaNP= .0687 ± .0109g).

For *P. atlantica*, there were no significant differences in femur length (p-value= .206) or weight (p-value= .0518) between any treatments. The widest spreads and interquartile ranges for femur growth were for the Na and NaNP treatments, and for change in weight, they were the NaNP and NP treatments.

Figure 1.2: The average change in femur length (mm) and weight (g) of *P. atlantica* after the two weeks of the experiment.



Parasitoids killed total of six individuals, three of which were *M. femurrubrum* in the Na treatment.

Discussion

This experiment found a significant difference in the change of weight of *M. femurrubrum* between the control and NaNP treatments, with the individuals feeding off plants grown in the NaNP plot ending up heavier. This result means that the plants grown in each plot did in fact yield significant differences on the growth and development of this species. For *P. atlantica*, no significant differences were found. I will mainly speculate as to why these results occurred, and what further implications these results have ecologically, like if the effects of sodium would be higher inland or in coastal areas, and the use of sodium in agricultural settings.

It is likely that the individuals from the NaNP plot ended up heavier because of the total nutrient content of the plants grown in this plot. According to data from Prather

(unpublished, 2012), *A. psilostachya* and *R. artugus* contained the highest total nitrogen percentage (2.05% and 2.12%) out of all plants used and were present only in this plot and the NP plot, which also yielded high average weights. As previously mentioned, nitrogen is the most limiting nutrient for insects, and when grasshopper species *C. curtipennis* fed on nitrogen-enriched plants, their growth rate increased likely due to higher productivity of protein synthesis that nitrogen assists with (Rode et al., 2017; Townsend, 2001). Phosphorus is also a limiting nutrient to growth rate (Rode et al., 2017). *R. artugus* and *A. psilostachya* similarly had the two highest percentages of phosphorus (13.33% and 12.93%), also possibly contributing to the individuals' in the NaNP and NP plots greater change in weight. Meanwhile, the control plot is mainly composed of grasses and sedges instead of forbs, which are relatively much harder to eat and digest because of how thick they are (Evans et al., 2007), likely explaining the negligible increase in weight for *P. atlantica* and decrease in weight for *M. femurrubrum.*

A possible reason why more differences in weight were not seen could be that there was plenty of overlap with plants that grew in each plot. Several plants grew in more than one treatment plot. Out of the 24 types of plants in the four plots, only seven grew in just one plot (*M. cerifera, S. scoparium,* and *S. parviflora* in the control, *Fimbry sp.* in the Na, *R. grandiflora* and *T. dactyloides* in the NP, and *C. erecta* in the NPNa plot), and the grasshoppers were not forced to eat from every species in their plot. This means that for all we know, the grasshoppers could have been eating the same speices. It is also possible that the grasshoppers that ate the plants in the NaNP treatment ended up relatively heavier because the forb *Centella erecta* grew exclusively in this plot. *C. erecta* has soft, fleshy leaves (USDA, NCRS. 2020) making it more edible than almost all of the other plants in the plots, including the nutrient rich *R. artugus* and *R. granndiflora* found in the NP plot.

M. femurrubrum showed significant differences in weight, but *P. atlantica* did not. This could be because although both of these species are polyphagous, meaning they can feed on different types of plants, grasshoppers are often polyphagous to different extents (Mulkern, 1967). *M. femurrubrum* is "highly" polyphagous (Bernays & Chapman, 1994), while *P. atlantica* is not (Squitier & Capinera, 2002). The more flexible diet of *M. femurrubrum* could have caused the individuals to consume more on average, then causing the significant difference in weight.

We could not test for direct effects of sodium, but they could certainly be taking place through physiological means. Most notably, the NP and NaNP plots grew plants with much higher sodium content, like *Boltonia sp.* (4,097 ppm), *H. angustifolius* (8,515 ppm), *C. erecta* (9,374 ppm), and *R. grandiflora* (11,503 ppm). Higher amounts of sodium could have affected grasshoppers because *R grandiflora* and *C. erecta* had the highest total contents of sodium, and four of the five plants with the highest contents were exclusively in these two plots. Sodium could be influencing brain and neural development and better managing the sodium pump, cell signaling, and maintaining hydrologic homeostasis (Prather et al., 2018; Chown & Nicolson 2004; Snell-Rood et al., 2014). Kaspari et al. (2017) found that excess sodium led to an increase in the abundance of prairie insect communities and an increase in ant population and activity, leading me to believe the same results could occur with grasshoppers.

If sodium ends up assisting grasshopper growth and development via direct effects, provoking a spike in population, our use of salty irrigation water (Ghassemi et al.,

1995) could potentially be encouraging grasshopper outbreaks when they are unwanted, which will in turn negatively affect the economy (Branson et al., 2006). On the other hand, this knowledge could yield positive outcomes. In struggling plant communities, soils can be enriched with sodium to assist plant growth and recycle nutrients (Belovsky & Slade, 2000). Sodium might therefore be a key component to land management. Also, an overabundance of grasshoppers causes some farmers to spray pesticides to save their crop yields (Lomer et al., 1999). This is harmful to the environment because it can cause biomagnification, the reason for the DDT crisis (Evans et al., 1991; Henry et al., 2003; Stansfield et al., 1989). Sodium could also be used as an attractant, which could help farmers use less pesticide by attracting the pests to one side of the field, then spraying just that side instead of the entire field. If we can control grasshopper populations and even other insect populations in agricultural fields by monitoring the amount of sodium that they are exposed to, land management practices could be much more efficient.

I would expect the effects of sodium in bolstering grasshopper population to be more significant in coastal areas because there is more rainfall, and rain deposits sodium (Kaspari et al. 2008; 2017). Kaspari et al (2008) concluded that more sodium deposits from rainfall led to more ant abundance, which leads me to believe the same will follow for grasshoppers.

For future directions, it would be ideal to conduct this experiment on lab reared grasshoppers instead of field caught ones. Having the individuals reared in the lab would make them much more adept to surviving the experiment because they would not have to go through the drastic change of moving from field to lab. It would also be ideal to have a room dedicated to this project. This past summer, when we ran the direct effect experiments, we were sharing a lab room, and this could have been the reason why the grasshoppers kept dying; they could have been contaminated despite our efforts to sterilize the room (Smith et. al, 2013). Lastly, this experiment could also be tested on other insects to help us establish a broader conclusion of sodium's effects on insects. Furthermore, this could be tested on different taxonomic groups and herbivores.

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