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Effect of Increased Carbon Dioxide Levels on Urushiol Production in Toxicodendron radicans and Toxicodendron toxicarium

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By

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Abstract

It is hypothesized that global warming is at least partially due to increased greenhouse gases, one of which is carbon dioxide. An increase in atmospheric carbon dioxide has been shown to stimulate higher rates of plant productivity, including the production of plant toxins like urushiol. Urushiol, a secondary plant compound produced in members of the Anacardiaceae family, causes dermatitis in sensitive individuals. An increase in urushiol production could increase the reactivity of certain individuals to plants like poison oak and poison ivy. To determine how elevated levels of atmospheric carbon dioxide would effect urushiol production, 16 samples of poison ivy and poison oak were grown in environmental chambers. One chamber was maintained at ambient carbon dioxide levels (0.037%) and the second was held at an increased level of 0.057%. Urushiol was extracted and analyzed through thin layer chromatography and ultraviolet spectroscopy to determine the amount present in each plant after sixteen weeks of exposure in the two growth chambers. The overall urushiol production and the average weight of the leaves decreased as time elapsed in both chambers. Poison ivy urushiol absorbance showed a decrease over all 16 weeks of exposure. Poison oak showed a drop in urushiol absorbance through 8 weeks then increased back towards initial levels, indicating that poison oak is a hardier species when dealing with the stress of being transplanted and placed in a new environment. Decreases in both overall leaf size and urushiol production may be due to the lack of grow lights in the chambers and plant stress. In addition, the presence of chlorophyll pigments in early extractions influenced the ultraviolet absorption and may have compromised our results.

Introduction

Currently, there is a great debate among scientists and politicians on the future effects of global warming. It is theorized that the cause of global warming, at least partially, is due to the emission of greenhouse gases, including carbon dioxide (CO_2). The full effect of increased carbon dioxide on plants is unknown. Carbon dioxide is the main carbon source for plant photosynthesis and an increase in atmospheric carbon dioxide, in the presence of bright light, will increase the photosynthetic rate. It has been suggested that plants, including weeds, will respond favorably to higher carbon dioxide levels (Ziska 2005), producing their products at higher amounts. This includes the production of their toxins, increasing the toxicity of the plant.

An increase in the toxicity of the Anacardiaceae family, especially poison ivy (Mohan *et al.* 2006) and poison oak will have a direct impact on many humans. Poison ivy (Figure 1), or *Toxicodendron radicans*, is a common weed that can be found in disturbed forests and suburban areas with woody borders (Caspers 1957). Though it can easily be confused with other woody vines, *T. radicans* is mainly characterized by alternating leaves with the leaflets occurring in triplets (Caspers 1957). The toxin, known as urushiol, is found throughout the entirety of the plant, even when dead (Brunner 1982). Poison oak (Figure 2), or *Toxicodendron toxicarium,* has leaflets occurring in triplet that are oblong and toothed or lobed giving them a shape similar to that of an oak leaf (Gillis 1971). The plants occur at least 1m apart from each other and grow as a shrub, rather than a vine (Gillis 1971). When either of these plant species comes in contact with bare skin, a rash generally erupts. This rash, known as contact dermatitis (Caspers 1957), is an allergic response to primary or secondary contact with the urushiol.

Urushiol (Figure 3) is a pentadecylcatechol hydrocarbon (American Chemical Society 2006) that attaches to membranes it contacts (Byers *et al.* 1979). The variable R group is a variable hydrocarbon chain, giving the compound eight different congeners (ElSohly *et al.*

1982). The molecules of urushiol bind to the membrane of skin cells, inducing a response by the immune system (Caspers 1957). The exact nature of the reaction between the urushiol and cellular membranes is unclear (Byers *et al.* 1979). However, it has been determined that exposure of skin cells to urushiol elicits the development of antigens, which are then passed to the entirety of the cell's progeny and into the immune system through memory cells (Caspers 1957). Any future exposures to urushiol induce an allergic immune response from the antigens. This allergic response is displayed by a rash and itching in the exposed area.

Hypersensitivity to urushiol is common throughout the population of the United States. It is estimated that anywhere between 10 and 50 million people develop contact dermatitis caused by exposure to the poison ivy family each year (American Chemical Society 2006). With an increase in the toxicity of these plants, especially *T. radicans*, the number of cases could greatly increase, as more individuals in the world's population become sensitive to the toxic urushiol. However, it is unknown whether the severity of the resulting contact dermatitis would increase as the amount of toxin produced increases. Urushiol has been isolated and purified through an ethanol extraction process and is capable of being analyzed though thin layer chromotography and UV absorption (ElSohly *et al.* 1982). These methods have been applied to analyze several other toxin level changes in plants, when exposed to elevated carbon dioxide levels. These studies have shown an increase in the toxin production. Studies on *T. radicans* have shown an increase in urushiol levels when exposed to increases in ambient carbon dioxide levels (Mohan *et al.* 2006). While these plants were left in their natural habitat, the effect of increased carbon dioxide concentrated in environmental growth chambers (EGC) is unknown. The focus of this experiment was to compare urushiol production in poison ivy and poison oak leaves exposed to ambient and elevated levels of carbon dioxide. The poison oak and poison ivy plants exposed to increased levels of carbon dioxide were expected to show higher amounts of urushiol production. Poison oak specimens were expected to show higher overall levels of urushiol when compared to poison ivy.

Methods

Plant Collection and Maintenance

A total of thirty-two plants were follected from a single site in Talbot County, GA, sixteen each of *T. radicans* and *T. toxicarium.* All plants were of the same approximate size and had the same number of leaves.. Transplantation occurred on site, using soil surrounding the roots, and plants were staked if needed. Sixteen plants, eight of each species, were maintained in the environmental growth chambers (EGC) for sixteen weeks. They were watered as needed, usually every three days. An initial urushiol extraction was performed to ascertain the amount produced by each species. Urushiol was extracted from both species grown under each condition every four weeks.

Chamber Atmosphere

The EGC used were Percival Controlled Environment Chambers, model PR-127. This type of chamber has a total volume of 11.42 m^3. Each chamber has 48 Philips fluorescent FT2 T12 cool white, high output, 160 watt bulbs that run at 1500 lumens. Each chamber also has twenty-four 25 watt cool white incandescent bulbs. The environment within the chamber was controlled by an Intellus microprocessor programmable controller. The constant environment maintained in the chambers was on a 12 hour light cycle, with the temperature held at 21^{o}C. Initially, all the lights were on in the cambers but it became apparent after approximately ten hours that having all the lights on would be too stressful for the plants. As poison oak and poison ivy are understory plants, they are exposed to lower levels of light than other species higher up in the canopy or in open fields (Mauseth 1998). Over exposure to light could shock the plants too greatly and kill them (Mauseth 1998). Therefore, only the incandescent bulbs were used as a light source. The carbon dioxide levels were the only variant of the chambers' environment. The ambient simulation chamber was maintained at a level of 0.037% carbon dioxide. The global warming simulation chamber was maintained at a level of 0.057% caron dioxide . Carbon dioxide was manually pumped into the global warming simulation chamber from an outside canister through a hose attached to the 1.5 inch, male threaded inlet valve. The amount of carbon dioxide being pumped in was measured by a saturation meter. Carbon dioxide levels remained constant, maintained by the air tight seal on the chamber. Levels were adjusted at the beginning and end of each week to compensate for the opening and closing of the chamber doors.

Extraction

The largest leaf available was removed from each specimen and the wet gram weight taken. Urushiol extraction followed established procedures (Corbett and Billets 1975). The leaves were placed in beakers completely submerged in 100% ethanol and monitored in a 200 ml beaker for four days at -20.0°C. This ethanol solution contained the first extract (E_1).

Under a fume hood, the ethanol in the E_1 extract was allowed to evaporate off. This resulted in a residue, E_2. The E_2 residue was dissolved then further purified using a mixture of 20 ml of toluene and 15 ml of 5% $NaCl/H_20$ in a 250 ml separatory funnel. The new extract (E_3), contained in the organic (top) layer, was siphoned off. The E_3 extract was allowed to evaporate under a fume hood. Approximately 8 ml of dichloromethane (DCM) was used to dissolve the remaining residue resulting in the final extract, E_4, which was analyzed.

Analysis

The E_4 extracts were initially analyzed using thin-layer chromatography (TLC) on silica plates using a 100% methanol solvent (ElSohly *et al.* 1982). Visualization of the bands separated by TLC was accomplished with an ultraviolet light (Ma and ElSohly 1980). This analysis was used to determine if any extractions contained urushiol (ElSohly *et al.* 1982). The extracts showing the presence of urushiol through TLC were then analyzed using a Genesys 10 uv spectrophometer. The absorbance was set at 275 nm, the wavelength determind by ElSohly *et al.* (1982) to be the specific value absorbed by urushiol. The samples were placed in matching quartz cuvets for analysis. One cuvet was filled with DCM and used as a control. The absoption value given by the Genesys 10 is essentially unit less but labeled with the unit C. The concentration of the urushiol extract was determined by taking the absorbance value gained from the UV analysis and dividing it by the initial weight of leaves from the sample plant. This would approximate the absorbance per gram of urushiol produced per plant.

Results

Trends of leaf weight, R_f values, and average absorbance per gram of each plant category were graphed over the four months. The change in the total wet leaf weight in grams over the sixteen week growing period are presented in Figure 3. The leaf weights were averaged together as there were no differences in the leaf weights of the two species examined. There was a rapid drop in the leaf weight for the first eight weeks. During weeks nine through sixteen, the leaf gram weight continued to decline, but at a much slower rate. The average initial leaf weights were approximately 1.1 g, but by week eight, the average leaf weight was approximately 0.28 g and leveling off by week sixteen to 0.26 g.

To determine if urushiol was present in the final extract the R_f values were calculated. Average R_f values for the combined species of each extraction are presented in Figure 4. Over the five extractions, there was a slight increase in the average R_f values. This was unexpected and may indicate a change in the composition of the urushiol present, specifically the ratio of different congeners.

UV absorbance of urushiol per gram of leaf material for each of the four plant categories: Poison Oak and Poison Ivy exposed to elevated carbon dioxide levels and Poison Oak and Poison Ivy exposed to ambient carbon dioxide levels are presented in Figure 5. Poison oak exposed to elevated carbon dioxide levels initially had a urushiol absorption per gram of leaf material at approximately 0.781 C/g and steadily fell to the eight week mark. At eight weeks, the average absorbance was 0.073 C/g. The absorbance trend increased during the last eight weeks, having average absorbencies of 0.108 C/g and 0.298 C/g at twelve and sixteen weeks, respectively.

Poison ivy exposed to elevated carbon dioxide levels had a similar trend. It began with an average urushiol absorption per gram of leaf weight of 0.488 C/g and declined over the next four weeks to an urushiol absorption per gram of leaf weight of 0.089 C/g. This category then saw a slight recovery of urushiol levels, moving up to an urushiol absorption per gram of leaf weight of 0.131 C/g at the eight week mark. Another decline was seen from the eight week to the twelve week extraction. The twelve week urushiol absorption per gram of leaf weight was 0.027 C/g. The urushiol absorption per gram of leaf weight rose again slightly to an average of 0.045 C/g. Overall, there was a drop in urushiol absorbance from the beginning of the experiment to the end.

Poison oak exposed to ambient carbon dioxide levels also saw an overall decrease in average urushiol absorbance per gram of leaf weight. However, there was a severe drop in the absorption then a slight recovery. Initially, the average urushiol absorption per gram of leaf weight was 0.251 C/g then rose slightly to 0.481 C/g at the four week mark. This rise is due to unexplained values obtained from the initial extraction. The decreasing trend began at the eight week mark with an average urushiol absorption per gram of leaf weight of 0.115 C/g. Between weeks eight and twelve, the urushiol absorbance rose slightly to 0.121 C/g. The trend continued to rise at the sixteen week mark, having a final average urushiol absorption per gram of leaf weight of 0.287 C/g.

The final category, poison ivy exposed to ambient carbon dioxide levels, also saw an overall decrease in urushiol absorption per gram of leaf weight. Initially, the ambient poison ivy had an average urushiol absorption per gram of leaf weight of 0.321 C/g and saw a slight decrease in extracts from week four, with an average urushiol absorption per gram of leaf weight of 0.129 C/g. There was a slight increase between weeks four and eight, moving to 0.137 C/g. After week eight, the urushiol absorption levels per gram of leaf weight decreased steadily. Week twelve had an average uruhsiol absorption per gram of leaf weight of 0.025 C/g, and week sixteen had an average urushiol absorption per gram of leaf weight of 0.022 c/g.

Poison oak in elevated levels of carbon dioxide were found to have approximately twice the amount of urushiol than poison ivy in both elevated and ambient carbon dioxide levels in the initial extraction. Poison oak in both ambient and elevated levels of carbon dioxide were found to have approximately ten times the amount of urushiol than poison ivy in both elevated and ambient carbon dioxide levels at the sixteen week interval. The initial absorptions were not considered for the poison oak in ambient carbon dioxide due to the unexplained values of the initial extraction. This was calculated by taking the average urushiol absorbance per gram for the poison oak in ambient and elevated carbon dioxide samples and dividing it by the average for the poison ivy in ambient and elevated carbon dioxide samples.

Discussion

Over the sixteen week growing period, the plant specimens had an overall decrease in both the average leaf gram weight and the average urushiol absorption per gram of leaf tissue. Both of the averages were at their lowest values at eight weeks of exposure in the growth chambers. The drop in the average leaf weight indicated the plants were under stress, due to transplantation shock and conditions in the growth chamber. The plants failed to thrive during the sixteen weeks, eventually dying by the end of the experiment because they were suffering from too much environmental stress to recover. Changes in the composition of the oil extracted, indicated by slight increases in the average R_f values over the growing period, could also indicate the plants were stressed. These changes in the R_f values could indicate compositional changes in the E_4 extract of the urushiol congeners present.

All four experimental groups showed an overall decrease in absorption, indicating a decrease in the amount of urushiol per leaf. Poison ivy samples continued to show decreasing absorbance after week eight, though at a much slower rate, indicating a decrease in urushiol present per leaf. In contrast, the poison oak samples began to recover after eight weeks resulting in an average usushiol absorbance per gram of leaf tissue approximately ten times higher than those found in poison ivy. These values contradicted those previously observed by Mohan *et al.* (2006). It was expected that poison ivy and poison oak exposed to higher levels of carbon dioxide would show an increase in urushiol production when compared to samples exposed to normal carbon dioxide levels. Mohan *et al.* (2006) saw an increase in urushiol production. This contrast is due to the plants dying back from transplantation and the unnatural environment in the growth chambers. The recovery in poison oak indicate that the species may be more hardy than the poison ivy. The increase in urushiol absorbance correlated with a recovery observed for the poison oak specimens.

Mauseth (1998) may help to explain why there was no rise in urushiol production in specimens exposed to the elevated carbon dioxide level. Plants must have a balance in the amount of light they recieve to grow optimally in the presence of excess in carbon dioxide. When plants are lacking that balance, they will respond unfavorably. Specifically, the plant specimens used were exposed to excess carbon dioxide and light levels too low to process the excess gas. This situation elicited a condition in the plants where they respired faster than they could photosynthesize (Mauseth 1998). Because they could not keep respiration and photosynthesis in balance, the plants had to consume their stored nutrients (Mauseth 1998). Being maintained in this environment over an extended period of time, the plants depleted their nutrient stores and eventually died.

When examining the final extractions to determine which species had more urushiol present, poison oak was determined to contain more urushiol based on the average UV absorption. Poison oak samples contained approximately twice as much urushiol at onset of the experiment and ten times more urushiol than the poison ivy samples at week sixteen. The difference in the urushiol amounts was expected, but the sixteen week value was found to be much higher than those reported in a previous study by ElSohly *et al.* (1982). This study found that poison oak contained approximately 3.5 times more urushiol than poison ivy. This is thought to be due to the recovery of the poison oak plants. The composition of the oil extracted was 35% and 10 % urushiol for each respective species for ElSohly *et al.* (1982).

Conclusion

This study was to determine if an increase in atmospheric carbon dioxide levels would cause an increase in urushiol production in both poison ivy and poison oak. Both species showed a drop in the amount of urushiol produced over a sixteen week period of growth in a controlled environment. This contradicted what was found by Mohan *et al.* (2006). Poison oak samples began to show a recovery in weeks twelve and sixteen, indicating that this may be the hardier of the two transplanted species. If the growing time were expanded, and the plants allowed to recover, an increase in urushiol production in response to the increased carbon dioxide level might be detected.

The second purpose of this study was to determine if poison oak contained more urushiol than poison ivy. Initial urusihol levels were approximately twice as much in the poison oak samples than the poison ivy samples. Examining the urushiol levels of the categories at sixteen weeks, poison oak was shown to contain ten times more urushiol than poison ivy. This supported what was expected and but is much higher than what was found previously by ElSohly *et al.* (1982) in the sixteen week extract. This is another indicator that the poison oak plants may have been recovering at the end of the experiment.

Changes in carbon dioxide levels, and global warming in general, have not been assessed. The effects on plant life are not understood. Further research is needed in this area. A repetition of this experiment would be the next step, creating an environment that the plants are able to survive in. This would include the presence of an ultraviolet light source in the chamber and placing the plants into a more nutrient rich substrate.

Appendicies

Figure 1. Columbus State University Herbarium sample of poison ivy (*T. radicans*).

Figure 2. Columbus State University Herbarium sample of poison oak (*T. toxicarium*).

Figure 3. The chemical structure of urusihol.

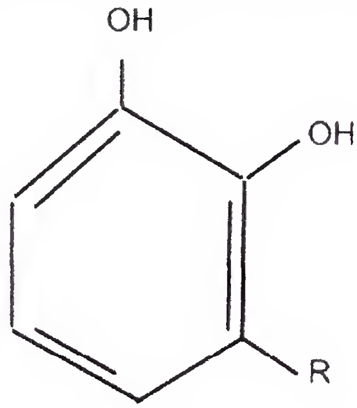


Table 1. The wet leaf gram weights (g) for each sample used for each category for the five extractions completed. (PO= Poison Oak, PI= Poison Ivy, CO_2= 0.057% CO_2, A= 0.037% CO_2)

	Round	Initial	Four Weeks	Eight Weeks	Twelve Weeks	Sixteen Weeks
Sample Type	Sample Number	Sample Weights (g)				
PO CO_2	1	1.06	0.21	0.22	0.4	0.25
	2	0.87	0.39	0.25	0.34	0.08
	3	0.93	0.55	0.18	0.08	0.3
	4	no sample	0.46	0.37	0.48	0.3
	5	0.39	0.4	0.19	0.57	0.31
PI CO_2	11	2.5	0.44	0.31	0.2	0.27
	12	1.33	0.4	0.27	0.42	0.31
	13	0.79	0.53	0.45	0.27	0.23
	14	0.99	0.3	0.42	0.18	0.25
	15	0.6	0.66	0.28	0.38	0.23
PO A	16	1.67	0.38	0.39	0.09	0.11
	17	0.94	0.25	0.19	0.08	0.32
	18	1.11	0.03	0.33	0.21	0.12
	19	1	0.08	0.2	0.1	0.05
	20	1.13	0.24	0.21	0.09	0.35
PI A	21	1.07	2.22	0.12	0.36	0.4
	22	1.17	0.2	0.24	0.21	0.37
	23	1.18	0.2	0.36	0.31	0.39
	24	1.62	0.49	0.36	0.43	0.41
	25	0.75	0.68	0.17	0.55	0.33
	Average Weight	1.11	0.455	0.275	0.287	0.269

Figure 4. Change in the average leaf weight (g) from all plant specimens over the growing period.

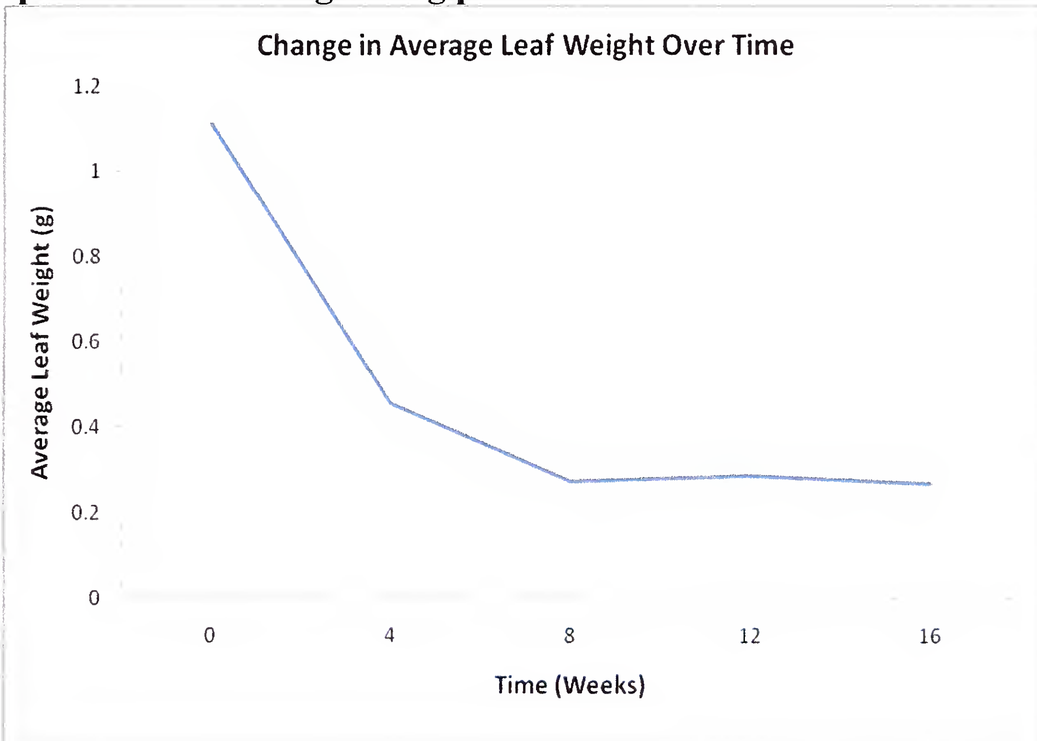


Table 2. The Rf values for each sample used for each category for the five extractions completed.

	Round	Initial	Four Weeks	Eight Weeks	Twelve Weeks	Sixteen Weeks
Sample Type	**Sample Number**	**R_f Values**				
PO CO2	**1**	0.545	0.538	0.580	0.619	0.640
	2	0.545	0.543	0.573	0.633	0.640
	3	0.558	0.538	0.580	0.626	0.646
	4	no sample	0.549	0.587	0.612	0.646
	5	0.552	0.543	0.587	0.604	0.659
PI CO2	**11**	0.558	0.538	0.594	0.612	0.652
	12	0.570	0.549	0.587	0.604	0.665
	13	0.539	0.532	0.594	0.597	0.671
	14	0.545	0.526	0.587	0.604	0.671
	15	0.552	0.532	0.601	0.597	0.677
PO A	**16**	0.561	0.538	0.594	0.604	0.665
	17	0.564	0.520	0.594	0.604	0.683
	18	0.570	0.526	0.601	0.604	0.665
	19	0.558	0.520	0.601	0.612	0.671
	20	0.545	0.520	0.608	0.612	0.683
PI A	**21**	0.545	0.526	0.608	0.604	0.683
	22	0.539	0.532	0.601	0.597	0.677
	23	0.533	0.526	0.608	0.619	0.695
	24	0.533	0.520	0.601	0.576	0.689
	25	0.527	0.514	0.552	0.583	0.695
	Average R_f Value	0.549	0.532	0.592	0.606	0.669

Figure 5. Trend of changes in the average R_f values for all plant specimens over time determined using thin layer chromatography (TLC).

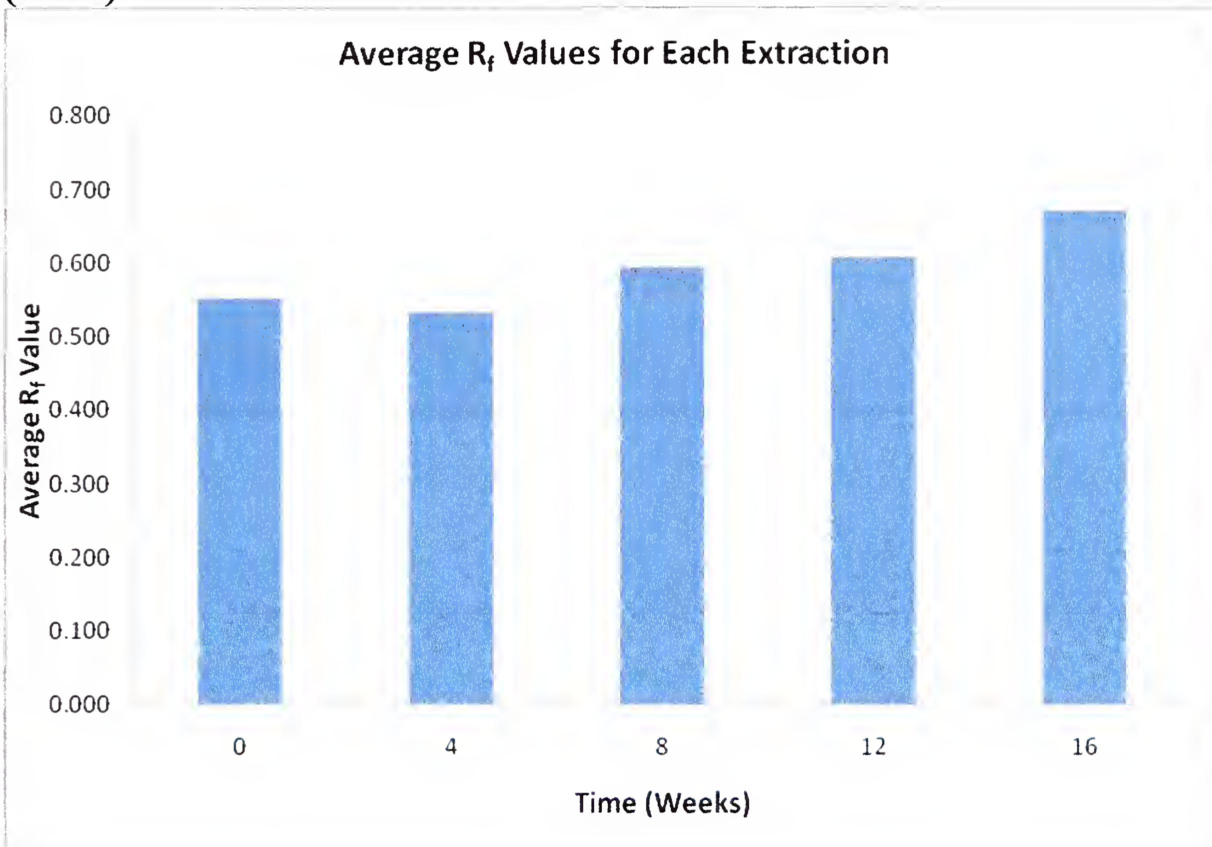
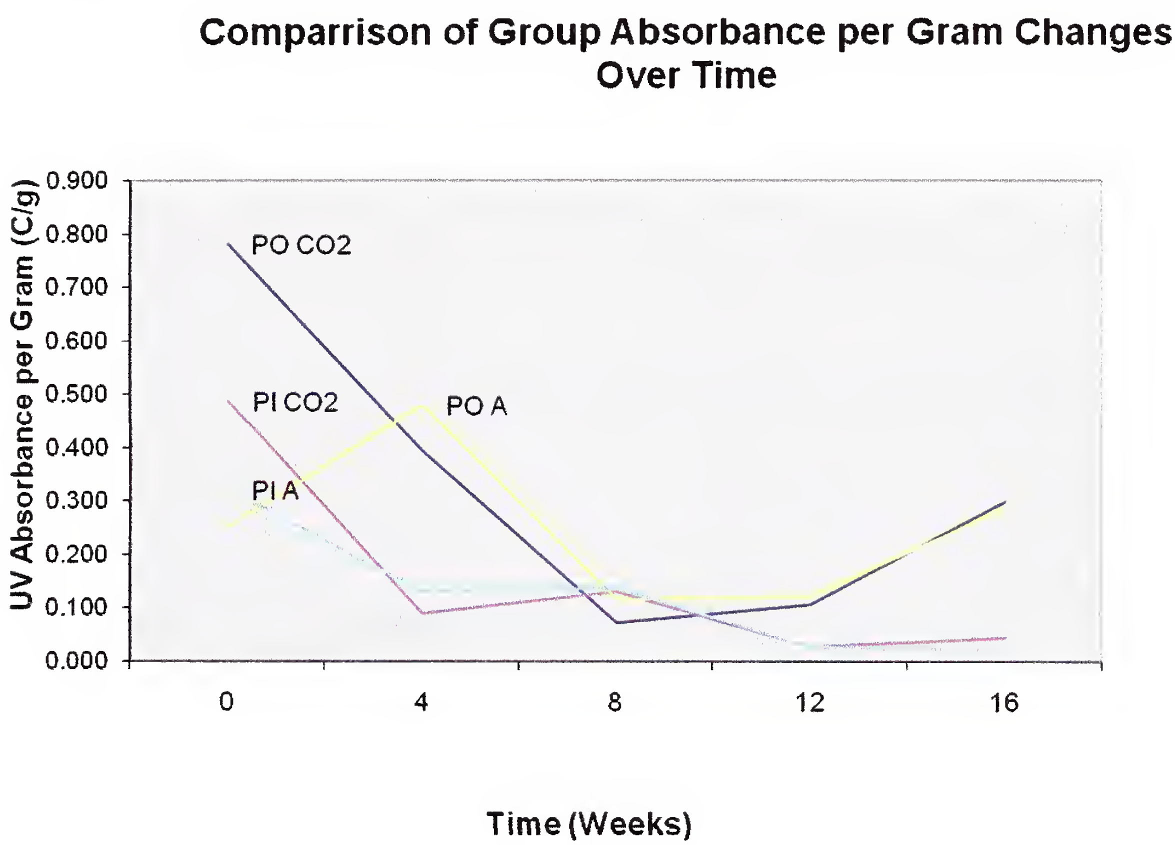


Table 3. The average UV absorbance per gram of urushiol for each sample used for each category for the five extractions completed.

			Initial	Four Weeks	Eight Weeks	Twelve Weeks	Sixteen Weeks
Sample Type			Absorbance per Gram (C/g)				
PO CO2	1		0.677	0.348	0.077	0.200	0.108
	2		0.798	0.131	0.068	0.213	0.725
	3		0.862	0.375	0.100	0.047	0.293
	4		no data available	0.504	0.054	0.050	0.327
	5		0.787	0.610	0.068	0.032	0.042
		Average	0.781	0.393	0.074	0.108	0.299
PI CO2	11		0.341	0.105	0.248	0.000	0.059
	12		0.408	0.053	0.093	0.052	0.023
	13		0.547	0.117	0.209	0.035	0.039
	14		0.541	0.120	0.036	0.032	0.080
	15		0.602	0.053	0.068	0.017	0.022
		Average	0.488	0.089	0.131	0.027	0.045
PO A	16		0.287	1.474	0.138	0.018	0.727
	17		0.309	0.448	0.111	0.034	0.263
	18		0.465	0.333	0.052	0.092	0.175
	19		0.185	0.025	0.170	0.000	0.200
	20		0.008	0.125	0.105	0.460	0.069
		Average	0.251	0.481	0.115	0.121	0.287
PI A	21		0.198	0.056	0.142	0.020	0.008
	22		0.176	0.030	0.158	0.041	0.038
	23		0.280	0.000	0.103	0.015	0.023
	24		0.132	0.455	0.167	0.032	0.017
	25		0.820	0.106	0.118	0.021	0.024
		Average	0.321	0.129	0.137	0.026	0.022

Figure 6. UV absorbance (275 nm) per gram (C/g) for all four plant categories. (PO CO_2= Poison oak in elevated carbon dioxide, PI CO_2= Poison ivy in elevated carbon dioxide, PO A= Poison oak in ambient carbon dioxide, PI A= Poison ivy in ambient carbon dioxide)



References

American Chemical Society (2006). Poison ivy could get worse. *Chemical & Engineering News*, **84**, 9.

Brunner, Lillian S. and Suddarth, Doris S. (1982). *The Lippincott Manual of Nursing Practice.* J.B. Lippincott Company, Philadelphia.

Byers, Vera S., Castagnoli, Neal, Jr., and Epstein, William L.(1979). In Vitro Studies of Poison Oak Immunity. *Journal of Clinical Investigation*, **64**, 1449-1456.

Caspers, A.P. (1957). Poison ivy (*Rus toxicodendron). Canadian Medical Association Journal*, **76,** 852-860.

Corbett, Michael D. and Billets, Stephen (1975). Characterization of poison oak urushiol. *Journal of Pharmaceutical Sciences*, **64**, 1715-1718.

Craig, John C., Waller, Coy W., Billets, Stephen, and Elsohly, Mahmoud A. (1978). New GLC analysis of urushiol congeners in different plant parts of poison ivy, *Toxicodendron adicans. Journal of Pharmaceutical Sciences*, **67,** 483-485.

Elsohly, Mahmoud A., Adawadkar, Prakash D., Ma, Cheng-Yu, and Turner, Carlton E. (1982) Separation and characterization of poison ivy and poison oak urushiol components. *Journal of Natural Products*, **45**, 532-538.

Gillis, W.T. (1971). The systematics and ecology of poison ivy and poison oaks. *Rhodora*, **73**, 72-159, 161-237, 370-443, 465-540.

Ma, Cheng-Yu and Elsohly, Mahmoud A. (1980). High-performance liquid chromatography separation of urushiol congeners in poison ivy and poison oak. *Journal of Chromatography*, **200**, 163-169.

Mauseth, James D. (1998) *Botany: An Introduction to Plant Biology, Second Edition.* James and Bartlett, USA.

Mohan, Jacqueline E., Ziska, Lewis H., Schlesinger, William H., Thomas, Richard B., Sicher, Richard C., George, Kate, and Clark, James S. (2006). Biomass and toxicity responses of poison ivy (*Toxicodendron radicans*) to elevated atmospheric CO2. *Proceedings of the National Academy of Sciences*, **103**, 9086-9089.

Ziska, Lewis H. (2005). Climate change impacts on weeds. *Climate Change and Agriculture: Promoting Practical and Profitable Responses*, **3**, 2-5.