



Keeping the Chicago Region Free of Poison Hemlock: An Interdisciplinary Approach to Studying the Invasion Front of a Known Invasive Plant

Final report to Chicago Wilderness and Illinois Conservation Foundation

December 31, 2009

PRINCIPAL INVESTIGATORS:

Kelly Granberg
University of Illinois at Chicago
Civil and Materials Engineering
ERF 3077 M/C 246
842 W. Taylor St.
Chicago, IL 60607
Phone: (312) 413-7638
Fax: (312) 996-2426
Email: kgranb3@uic.edu

Paul Gulezian
University of Illinois at Chicago
Ecology and Evolution
SES 3474 M/C 066
845 W. Taylor St.
Chicago, IL 60607
Phone: (312) 996-8673
Fax: (312) 413-2435
Email: pgulez2@uic.edu

Jennifer Ison*
University of Illinois at Chicago
Ecology and Evolution
SEL 1031 M/C 067
840 W. Taylor St.
Chicago, IL 60607
Phone: (312) 996-9462
Fax: (312) 413-2435
Email: ison@uic.edu
*fiscal agent and contact person

Summary Paragraph

Invasive species create a multitude of ecological problems. They can drastically alter resource use in ecosystems (Elton 1958; Vitousek et al. 1996); introduce new diseases and pathogens that can negatively affect native species (Mack et al. 2000); and generally change species interactions and ecosystem processes in ways that result in lower levels of biodiversity in the ecosystems in which they invade (Sakai et al. 2001; Batten et al. 2006). When humans disturb the environment (building houses and roads, damming rivers, plowing for agriculture, etc.), often times invasive species exploit these disturbances by colonizing new areas and dramatically increasing in abundance (Hierro 2006, Kneitel and Perrault 2006). Here we show that *Conium maculatum* (poison hemlock), a plant recently detected only along roadsides in Cook County, is not particularly adapted to these disturbed habitats, and is likely to spread to other habitats in the region. We combine analyses that characterize the soil where *Conium* is growing; assess the genetic structure of individuals within and among the nine known populations; and test for tolerance to heavy metals and local adaptation with greenhouse establishment experiments. In short, we find no reason to believe this species is likely to remain confined to disturbed roadside habitats with contaminated soil. We recommend that land managers in the region remain vigilant for the presence of *Conium* at their sites, since *Conium* seems likely to become an ecologically problematic invasive plant in the Chicago region.

Introduction

Many invasive plants have life history traits (fast growth rates, tolerance to pollution, generalist resource use) that can be useful in disturbed habitats (Thuiller et al. 2006). This research project investigates one relatively new, potentially invasive species to the Chicago region that has only been found in one particular kind of disturbed habitat thus far.

Conium maculatum (poison hemlock) is a non-native plant in the carrot family (Umbelliferae) that is often considered an invasive species throughout the United States (Whitson et al. 1992). It is biennial and self-incompatible (Baskin and Baskin 1990). Its seeds do not disperse easily by gravity over long distances, so the plant is often carried by water, in the hair of mammals, and by human activity to colonize new areas (Stephens 1980). The species is native to Europe, North Africa, and Asia, and has been documented in every state and Canadian province except for Mississippi, Florida, Alaska, Hawaii, Manitoba, Yukon, and the Northwest Territories (USDA PLANTS database: <http://plants.usda.gov/>). *Conium* is commonly found in most of Illinois except for the northeastern counties (Cook, McHenry, Lake, etc).

Conium was first observed in Cook County along the Bishop Ford Freeway (I-94) in 2006 (Gulezian and Nyberg 2008). The spread of *Conium* to Cook County appears to be a recent phenomenon, as there are no herbarium records of the species in Cook County (<http://www.vplants.org>). We confirmed its relatively recent arrival in the region through a series of surveys in 2007 and 2008, in which the roadsides of all major interstate highways in southern Cook County and any natural areas within 1 km of the roadway were searched for the plant. We found nine populations, all of which were along roadsides or associated with land owned by the Metropolitan Water Reclamation District (MWRD).

The primary questions posed by this research are:

1. Is soil contaminated where *Conium* is growing?
2. What is the genetic structure, if any, of the plants in Cook County?
3. Is there local adaptation (“home field advantage”) for seeds from particular populations growing in specific soils as demonstrated in the greenhouse?

4. Is *Conium* more tolerant to heavy metals than two other comparable native species (*Desmodium canadense* and *Echinacea purpurea*)?

5. Will *Conium* be likely to spread to natural areas or is it more likely to be confined to a particular disturbed habitat (roadsides)?

Ultimately, this research aims to assess the likelihood that *Conium* will become a widespread, ecologically problematic species in the greater Chicago region.

Results

Soil Characterization

Select contaminants and parameters measured for the 9 field soils and peat control used in the greenhouse experiment are given in Table 1. Arsenic (As), cadmium (Cd), and lead (Pb), are heavy metals that were found at elevated levels relative to contamination thresholds in the EPA Ecological Soil Screening Levels for plants (EPA 2005) from an initial sampling effort. pH and organic matter are shown because they can play major roles in metal sorption and binding in soils, with lower pH likely to increase metal mobility, bioavailability, and toxicity in soil and lower organic matter also tending to boost metal availability in soil. Bulk density, given as an indicator of soil compaction, was measured under field conditions where pore space was often wet or saturated so that clay soils (typically lowest in dry bulk density) have the highest wet bulk densities.

Genetic Structure Analysis

A total of 217 individuals were scored for the patterns produced with 5 ISSR primers, which yielded 114 unique loci (unique bands in the gel). Table 2 summarizes the results of the genetic analysis. We found very high genetic diversity in all eight of our populations. There were no two individuals in any of the populations with the exact same genotype. This is

consistent with *Conium* having a self-incompatible mating system, and seed-based colonization of new habitat. There are also high levels of heterozygosity in all eight populations (table 2). The populations with the highest levels of metal contamination, BFN1, Harlem, and 551 (table 1), do not have low heterozygosity levels (table 2), a finding which would be consistent with local adaptation to high levels of contamination. There is little to no genetic structure between the populations. Hickory estimated θ_{II} , which is a measure of differentiation between populations similar to F_{st} , as only 0.013 indicating that populations are acting more like one large population rather than eight smaller populations (table 2). There are a few private bands (alleles) in the populations 130th/Doty and Stickney (table 2). Except for CalDryBed, all populations had locally common bands, indicating that CalDryBed does have lower genetic diversity (mean heterozygosity = 0.139 or 0.431), especially of rare bands. This lower genetic diversity does not appear to be connected to high levels of contamination nor any other measured soil parameter.

Greenhouse Establishment Experiments

We detected differences in *Conium* growth among the 9 field soils. Mean dry weights (g) of *Conium* above ground biomass (AGB) grown on different field soils ranged from a high for Harlem (0.416) to a low for Calumet (0.051). These means were calculated from the pooled AGB measurements for all 9 seed sources, with 3 replicates per source per soil type (N = 27). Even without advanced statistical analysis, there is evidence for real differences in seedling growth relative to field soil type. There appear to be three clusters of AGB means relative to soil type: high growth (Harlem (0.416 g) and 551 (0.366)); medium growth (BFN1 (0.208), BFN2 (0.188), and 552 (0.177)); and low growth (CalDryBed (0.099), Stickney (0.095), 130th/Doty (0.072), and Calumet (0.051)). The four populations with soil in the low growth cluster were the

four populations with soil that had the highest wet bulk density measurements (table 1). All of these soils were very clay-like, and this parameter probably had a greater negative effect on *Conium* growth in the greenhouse than any other factor, including metal contamination.

Figure 1 summarizes the local adaptation experiment of the greenhouse establishment research. Overall, there does not appear to be evidence in support of a “home field advantage” (local adaptation) for seeds from a given population growing better on their own local soil. The AGB means of growth on home and away soils are virtually indistinguishable for almost every population, with the possible exception of BFN2. In short, there is no compelling evidence for local adaptation (as defined by a home field advantage for seeds growing on their own local soil) from the greenhouse experiment data.

The metal tolerance component of the greenhouse experiment is illustrated by Figure 2. Overall, there is evidence that *Conium* is more tolerant to soil spiked with known concentrations of heavy metals than both *Desmodium* and *Echinacea*. This is most clearly illustrated by the data from the As treatments. In this treatment, the AGB means for all three species appear to be different from each other, with *Conium* growing the best, *Echinacea* growing least well, and *Desmodium* in between. This pattern is repeated in the Cd treatment, although the differences between species are less extreme. *Conium* and *Echinacea* are probably the only two species that are statistically different in this treatment. The Combo treatment (As, Cd, and Pb) shows a similar pattern to Cd, but the differences between *Conium* and *Echinacea* are more pronounced. However, overall, all three species grew less well in the Combo treatment as compared to Cd and Pb alone. The overall growth in the Combo treatment was more similar to the smaller AGB means, irrespective of species, in the As treatment.

It is important to note that the AGB means in the Peat control are probably not statistically different from one another. Additionally, it is important to appreciate that the AGB means are probably not statistically different between the Peat control and the Cd treatment. However, the AGB means for the As and Combo treatments are likely statistically different (and lower) from the Peat control.

All three species grew as well or better in the Pb treatment as compared to the Peat control. This result was initially surprising, but a likely explanation for the apparent promotion of growth is probably due to the fact that we used lead chloride (PbCl_2) to spike the soils of the Pb treatment. Lead chloride, semi-soluble in water, was selected because it was the most soluble lead salt except for lead nitrate that would have also made nitrogen available to promote plant growth in the relatively nitrogen-poor medium (Zimdahl and Skogerboe 1977). Although greenhouse watering was expected to dissociate the spiked lead chloride, its limited solubility (.99g/100ml) combined with other soil interactions may not have made the lead available to the plants.

Discussion

We did not find evidence that the known *Conium* populations in Cook County are locally adapted to the highly disturbed roadsides areas in which we detected the species. The soils of these areas are contaminated, particularly with heavy metals such as As, Cd, and Pb, and *Conium* appears to be more tolerant of heavy metal contamination than two comparable native species, *Desmodium* and *Echinacea* (figure 2). This may potentially explain its presence in areas contaminated with these metals. However, this metal tolerance does not seem to be connected to patterns of genetic diversity in any of the populations, a result which would be consistent with local adaptation to the soils. Nor do we find evidence from the greenhouse establishment

experiments that *Conium* grows better when seeds are grown on the local field soils compared with the soils of the other 8 populations (figure 2). This lack of correlation between growth and specific local soils, in addition to its tolerance of contaminated soils, suggests that its generalist characteristics make it more likely to colonize many disturbed places along roadways. *Conium* may then use these roadside populations to colonize diverse habitats in the region (Thuiller et al. 2006, von der Lippe and Kowarik 2007).

Moreover, *Conium* has relatively high levels of genetic diversity (table 2), and we do not detect any clear genetic structure among populations, or connections between specific populations and any of our measured field soil parameters. The fact that there are distinct differences among field soils with respect to *Conium* growth in the greenhouse (growth ranged widely from a high of 0.444 g to a low of 0.051 g) also suggests that *Conium* has colonized roadside areas with diverse soil characteristics (table 1) that greatly influence its growth. This is further evidence that the species does not have very specific habitat requirements, which may make it more likely to spread to natural areas (Christen and Matlack 2006).

In short, these results indicate that: 1) *Conium* is likely in an early phase of its invasion to the region; 2) it is not locally adapted to the disturbed roadside habitats where we detected the populations; and 3) it is likely to spread to other habitats in the region--including natural areas of conservation value. Given this conclusion, we would recommend that natural area land managers remain vigilant for the appearance of this species at their sites, and devote resources to remove it after it is detected. *Conium* has the potential to become an ecologically problematic species in the Chicago region.

Methods Summary

This research was organized into three major sections: a soil characterization analysis that quantified various chemical and physical parameters of the soil in which each *Conium* population was growing; a genetic structure analysis that quantified the levels of genetic relatedness within and among individual *Conium* plants among the 9 known populations; and greenhouse establishment experiments that tested for local adaptation (as defined as seeds growing better in local soil compared to other soils) and tolerance to heavy metals (as defined as growth of *Conium* relative to *Desmodium* and *Echinacea* in the presence of known levels of As, Cd, and Pb).

Soil Characterization

Common potential pollutants (metals and hydrocarbons) and physical and chemical soil parameters were measured to characterize soil at the 9 populations where *Conium* was growing. To quantify contamination, a total of 28 soil cores were collected in an initial sampling effort and concentrations of 20 metals and 20 PAHs (polycyclic aromatic hydrocarbons) were measured. Subsequent grab samples (1 at each population) and peat used in the greenhouse study were analyzed for As, Cd, and Pb which were observed at elevated levels. All soil samples were also quantified for parameters such as pH, salinity, bulk density, percent solids, organic matter, organic carbon, nitrogen, and black carbon.

Genetic Structure Analysis

The goal of the genetic structure analysis was to assess the level of relatedness among individuals within and between the nine *Conium* field populations. Details on specific procedures are available upon request. The general procedure was as follows. We collected at least 30 individual leaf samples from 7 of the 9 populations. Two nearby populations (551 and

552) did not have 30 plants, so all of the plants from these populations were sampled, and the samples were combined for the genetic analysis. Tissue samples were homogenized and DNA was extracted using a Qiagen DNeasy Plant Mini Kit. We quantified the amount of DNA in each extraction using a NanoDrop 2000. The extracted DNA was amplified with PCR and five different ISSR primers (811, 816, 818, 825, 830). ISSRs are highly variable dominant markers that use microsatellite regions in the genome as primers, and the presence or absence of bands on an agarous gel indicates differences between individuals/populations.

The PCR products were run on high resolution agarous gels to produce banding patterns of DNA fragments based on the specific genomic sequences of each *Conium* individual. This is a standard way to assess genetic structure within and among populations in plants (Wolfe et al. 1998, Esselman et al. 1999). Photos were taken of each gel at several resolutions and printed to hard copy. Gel photos were scored collaboratively by two simultaneous observers with color-coded gel pens. The patterns of colored bands were entered into a computer database and analyzed with two genetic structure programs; GenAlEx 6.2 (Peakall and Smouse 2006) and Hickory 1.1 (Holsinger et al. 2002). GenAlEx utilizes standard statistical methods and assumes the populations are in Hardy-Weinberg equilibrium. Hickory uses Bayesian statistics and does not assume Hardy-Weinberg equilibrium.

Greenhouse Establishment Experiments

The purpose of this part of the project was to set up a controlled experiment to test: 1) whether seeds from a given population grow better in their home soils, and 2) whether *Conium* grows better in soils spiked with As, Cd, and Pb than *Desmodium* and *Echinacea*. The greenhouse experiment was structured as follows. Seeds and soils were collected from the nine known *Conium* field populations. A complete factorial design was implemented, with each

combination of seed source and soil source replicated three times (i.e. the “home field advantage” test). Peat moss was spiked with Pb at 1000 ppm, As at 100 ppm, Cd at 100 ppm (the ‘high’ levels measured from *Conium* field soils within an order of magnitude), and a combination treatment with the three metals at the same respective concentrations. A peat moss control (unspiked) was also used.

Seeds from all nine *Conium* populations and from catalog-bought *Desmodium* and *Echinacea* sources were placed in each spiked treatment in triplicate (i.e. the metal tolerance experiment). Each combination of seed and soil (N=408) was randomly placed in one of fifteen greenhouse trays. Each tray was rotated daily to control for the potential influence of position on the greenhouse bench. The seedlings were monitored daily for germination and growth rates over 45 days. Seedlings were counted as established at the appearance of the first true leaf. Heights were measured and seedlings were placed into two height classes: 0-5 cm and 5-10 cm. At the end of the growth phase, above ground biomass was harvested, dried in an oven, and weighed. These data provide a metric for overall plant performance and are the primary results of the establishment experiment.

Literature Cited

- Baskin, J. M. and C. C. Baskin. 1990. Seed germination ecology of poison hemlock, *Conium maculatum*. The Canadian Journal of Botany **68**:2018-2024.
- Batten, K. M., M. Scow, K. Davies, and S. Harrison. 2006. Two invasive plants alter soil microbial community composition in serpentine grasslands. Biological Invasions. **8**: 217-230.
- Christen, D. and G. Matlack. 2006. The role of roadsides in plant invasions: a demographic approach. Conservation Biology **20**:385-391.
- Elton, C. S. 1958. The Ecology of Invasions by Animals and Plants. 2nd ed. University of Chicago Press: Chicago. 181 p.
- Esselman, E. J., L. JianQiang, D. Crawford, J. Windus, and A. Wolfe. 1999. Clonal diversity in the rare *Calamagrostis porteri* ssp. *insperata* (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and intersimple sequence repeat (ISSR) markers. Molecular Ecology **8**:443-451b.

- Gulezian, P. Z. and D. Nyberg. 2008. Invasion in progress: dispersal limitation, human vehicle vectors, and the invasion of a problematic exotic species: *Conium maculatum*. A report to the Illinois Native Plant Society. Erigenia 2008.
- Hierro, J. L. et al. 2006. Disturbance facilitates invasion: The effects are stronger abroad than at home. American Naturalist **168**: 144-156.
- Holsinger, K. E., P. O. Lewis, and D. K. Dey. 2002. A Bayesian approach to inferring population structure from dominant markers. Molecular Ecology **11**:1157-1164.
- Kneitel, J.M. and D. Perrault. 2006. Disturbance-induced changes in community composition increase species invasion success. Community Ecology **7**: 245-252.
- Mack, R. N., D. Simberloff, W. N. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications **10**: 689-710.
- Peakall, R., Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6**: 288-295. Available via <http://www.anu.edu.au/BoZo/GenALEX>.
- Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, and S. G. Weller. 2001. The population biology of invasive species. Annual Review of Ecology and Systematics. **32**:305-332.
- Stephens, H. A. 1980. Poisonous plants of the central U.S. The Regents Press of Kansas, Lawrence, Kansas.
- Thuiller, W. et al. 2006. Interactions between environment, species traits, and human uses describe patterns of plant invasions. Ecology **87**: 1755-1769.
- Vitousek, P. M., C. M. D'Antonio, L. L. Loope, and R. Westbrooks. 1996. Biological invasions as global environmental change. American Scientist **84**:468-478.
- von der Lippe, M. and I. Kowarik. 2007. Long-distance dispersal of plants by vehicles as a driver of plant invasions. Conservation Biology **21**:986-996.
- Whitson, T. D., L. Burrill, S. Dewey, D. Cudney, B. Nelson, R. Lee, and R. Parker. 1992. Weeds of the West. The Western Society of Weed Science: 22-23.
- Wolfe, A. D., Q.-Y. Xiang, and S. R. Kephart. 1998. Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable intersimple sequence repeat (ISSR) bands. Molecular Ecology **7**:1107-1125.
- Zimdahl, R. L. and R. K. Skogerboe. 1977. Behavior of lead in soil. Environmental Science and Technology **11**: 1202-1207.

Tables and Figures

Table 1: Characterization of the field soil for the 9 *Conium* populations found in Cook County and the peat control used in the greenhouse establishment experiments

	(mg/kg dry soil)			pH	(% dry wt.)	(g/ml wet wt.)
	Arsenic	Cadmium	Lead		Organic matter	Bulk density
551	10	3.6	200	7.484	7.78	0.98
552	9.1	1.2	110	7.783	6.35	1.08
Stickney	8.7	4.1	71	7.403	4.23	1.73
Harlem	14	4	250	7.499	9.81	0.93
CalDryBed	9.3	3.7	64	7.756	4.01	1.45
Calumet	7.8	1.5	60	7.75	6.26	1.58
BFN2	6	1.9	160	7.613	5.36	0.82
BFN1	13	7.6	380	7.759	7.23	1.01
130th/Doty	8.8	1.2	130	8.001	3.42	1.44
Peat	< 1.1	< .53	3.5	5.904	< 1.1	0.21

Table 2: Summary of genetic structure

Population	551&2	Stickney	Harlem	CalDryBed	Calumet	BFN1	BFN2	130/Doty
No. Bands	94	104	97	83	94	100	93	102
No. Private Bands	0	2	0	0	0	0	0	1
No. LComm Bands (<=25%)	4	2	1	0	3	1	2	4
Mean He	0.232	0.227	0.202	0.143	0.215	0.228	0.220	0.189
SE of Mean He	0.018	0.018	0.017	0.017	0.019	0.018	0.018	0.017
Genetic structure of the populations from GenAlEx program. No. Bands is the number of polymorphic bands for each population. No. Private Bands is the number of bands only found in each given population. No. Lcomm Bands is the number of bands that occur are found in 25% or fewer populations but occur in frequency of >5%. Mean He is the mean heterozygosity of each population, and SE of Mean He is the standard error of the mean.								
Population	551&2	Stickney	Harlem	CalDryBed	Calumet	BFN1	BFN2	130/Doty
Mean He	0.431293	0.433888	0.431213	0.430707	0.434749	0.43528	0.435182	0.43282
SD of Mean He	0.012928	0.014064	0.01435	0.013379	0.013291	0.014439	0.013071	0.014193
	Total	SD						
Theta-II	0.01312	0.006577						
Genetic structure of the populations from the Hickory program. Mean He is the mean heterozygosity calculated with Bayesian statistics. The DS of Mean He is the standard deviation of the mean heterozygosity. Theta-II is a measure of total population differentiation (similar is Fst). SD is the standard deviation of theta-II.								

Figure 1: *Conium* growth expressed as mean weights of above ground biomass for seeds grown on home (local) and away (soil of all other *Conium* populations). Error bars are +/- 1 standard error. N = 3 for home soils , N = 24 for away soils.

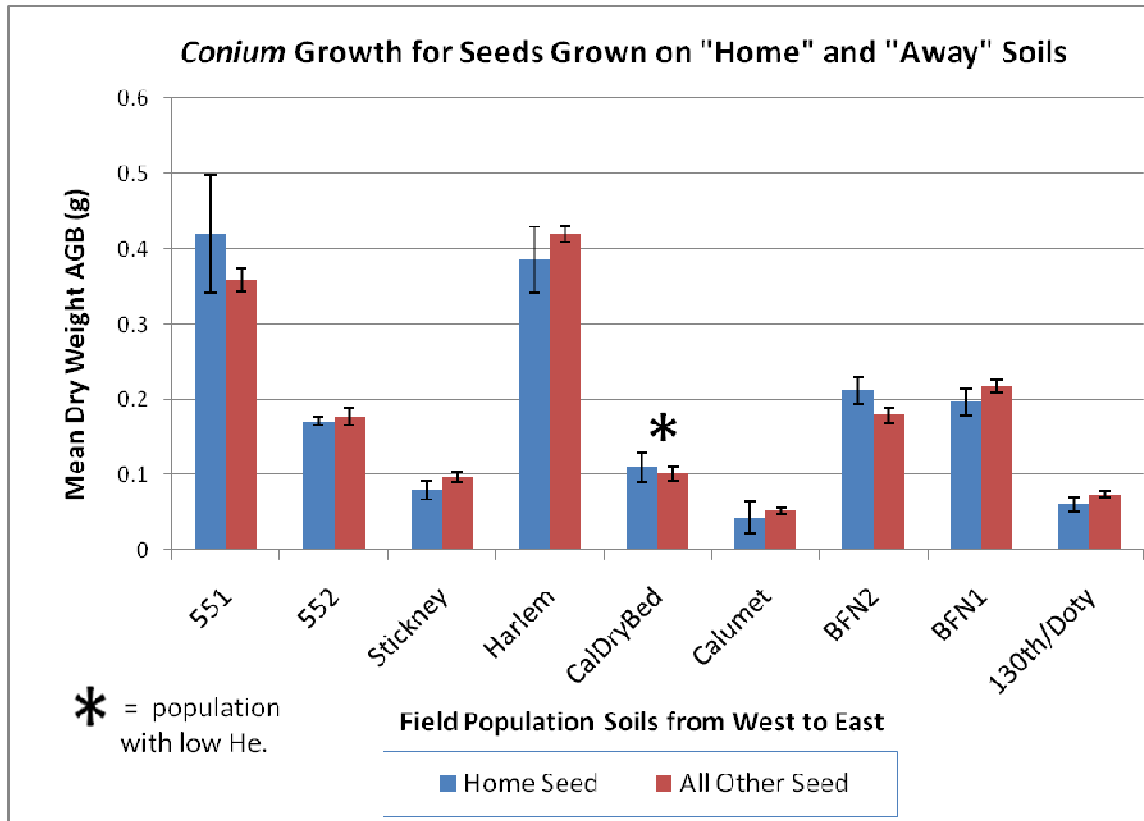


Figure 2: Metal tolerance (As, Cd, Pb, Combo) among three species from the greenhouse experiment. Error bars are +/- 1 standard error. N = 27 for *Conium*, N = 3 for *Desmodium* and *Echinacea*.

