# Establishment of an Invasive Plant Species (Conium maculatum) in Contaminated Roadside Soil in Cook County, Illinois

Author(s): Paul Z. Gulezian, Jennifer L. Ison, and Kelly J. Granberg
Source: The American Midland Naturalist, 168(2):375-395. 2012.
Published By: University of Notre Dame
URL: http://www.bioone.org/doi/full/10.1674/0003-0031-168.2.375

Am. Midl. Nat. (2012) 168:375–395

# Establishment of an Invasive Plant Species (Conium maculatum) in Contaminated Roadside Soil in Cook County, Illinois

PAUL Z. GULEZIAN[1]
*Department of Biological Sciences, University of Illinois at Chicago, 845 West Taylor Street M/C 066, Chicago 60607*

JENNIFER L. ISON[2]
*Department of Biological Sciences, University of Illinois at Chicago, 845 West Taylor Street M/C 066, Chicago, Illinois, 60607 and Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe 60022*

AND

KELLY J. GRANBERG
*Department of Civil and Materials Engineering, University of Illinois at Chicago, 842 West Taylor Street M/C 246, Chicago 60607*

ABSTRACT.—Interactions between environmental variables in anthropogenically disturbed environments and physiological traits of invasive species may help explain reasons for invasive species' establishment in new areas. Here we analyze how soil contamination along roadsides may influence the establishment of *Conium maculatum* (poison hemlock) in Cook County, IL, USA. We combine analyses that: (1) characterize the soil and measure concentrations of heavy metals and polycyclic aromatic hydrocarbons (PAHs) where *Conium* is growing; (2) assess the genetic diversity and structure of individuals among nine known populations; and (3) test for tolerance to heavy metals and evidence for local soil growth advantage with greenhouse establishment experiments. We found elevated levels of metals and PAHs in the soil where *Conium* was growing. Specifically, arsenic (As), cadmium (Cd), and lead (Pb) were found at elevated levels relative to U.S. EPA ecological contamination thresholds. In a greenhouse study we found that *Conium* is more tolerant of soils containing heavy metals (As, Cd, Pb) than two native species. For the genetic analysis a total of 217 individuals (approximately 20–30 per population) were scored with 5 ISSR primers, yielding 114 variable loci. We found high levels of genetic diversity in all populations but little genetic structure or differentiation among populations. Although *Conium* shows a general tolerance to contamination, we found few significant associations between genetic diversity metrics and a suite of measured environmental and spatial parameters. Soil contamination is not driving the peculiar spatial distribution of *Conium* in Cook County, but these findings indicate that *Conium* is likely establishing in the Chicago region partially due to its ability to tolerate high levels of metal contamination.

## INTRODUCTION

Invasive species can 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 and human health (Mack *et al.*, 2000; Juliano and Lounibos, 2005), and change species interactions and ecosystem processes in

---

[1] Corresponding author: e-mail: paul.gulezian@gmail.com

[2] Present address: Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S 3B2 Canada

ways that result in lower levels of biodiversity in the ecosystems they invade (Sakai *et al.*, 2001; Batten *et al.*, 2006). When humans disturb the environment (through construction, damming rivers, plowing for agriculture, etc.), invasive species often exploit these disturbances by colonizing new areas and dramatically increasing in abundance (Hierro, 2006; Kneitel and Perrault, 2006). Interactions between environmental variables in anthropogenically disturbed environments and physiological traits of invasive species may explain invasive species' range expansions and colonization of new areas. This may be particularly true in urban areas since urban ecosystems and landscapes are highly altered by human activity (Sanderson *et al.*, 2002). Here we investigate how soil contamination along roadsides may influence the establishment of *Conium maculatum* (poison hemlock) a plant considered invasive elsewhere and recently detected only along roadsides in Cook County, IL, USA.

Studies of soil contamination and plant invasion have explored relationships between concentrations of contaminants such as heavy metals in both soils and in plant tissues of different species growing near mine sites (Bech *et al.*, 1997; Liu *et al.*, 2005), considered how plants may adapt to contaminated substrates to form monospecific patches that may be associated with biological invasions (Henriques and Fernandes, 1991; Urbansky *et al.*, 2000), and examined how contaminants move through urban soils and/or how plants uptake these compounds for potential use as bioindicators of pollution (Mihaljevič *et al.*, 2010; Keane *et al.*, 2001). Genetic studies have reconstructed invasion histories from an analysis of the genetic structure of multiple invasive populations (Dray Jr. *et al.*, 2009; Henry *et al.*, 2009; Prentis *et al.*, 2009), and evaluated gene flow and/or population differentiation and genetic diversity of multiple invasive populations to predict the potential for future expansion and to inform ecological management (Mandák *et al.*, 2009; Chun *et al.*, 2010).

However, there are few published studies that examine how the establishment or spread of an invasive plant may be associated with soil contamination. Two studies have investigated the interaction of physical soil parameters and either genotype diversity or phenotypic plasticity of an invasive plant species (Morrison and Molofsky, 1998; Pan *et al.*, 2006), but neither addressed anthropogenic soil contamination with naturally establishing populations in the field. Despite a growing interest in genome-environment interactions in ecology, there is a dearth of information on such interactions for invasive species upon establishment in a new region. An assessment of levels of genetic variation at contaminated sites and patterns of genetic structure among these sites coupled with a quantification of specific soil contaminants may provide important insights into the processes associated with biological invasions.

This study was prompted by initial field investigations related to the presence of *Conium maculatum* (hereafter, *Conium*) in Cook County. *Conium*, widespread in the United States and Canada (USDA PLANTS database: http://plants.usda.gov/), was first observed in Cook County along the Bishop Ford Freeway (I-94) in 2006 (Gulezian and Nyberg, 2008). The establishment of *Conium* in Cook County appeared to be a recent phenomenon, as there are no herbarium records of the species in Cook County (http://www.vplants.org). Subsequent surveys in 2007 and 2008 were conducted 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. In total we found nine populations, all along roadsides and/or near land owned by the Metropolitan Water Reclamation District of Greater Chicago (MWRD-GC). The MWRD-GC lands contained wastewater treatment plants (WWTPs), including the largest in the world at Stickney, and biosolids processing and drying facilities where biosolids are spread on large open concrete beds. Biosolids are likely to contain metals and persistent organic pollutants that are removed from the wastewater stream. The *Conium* populations' spatial connection to these facilities suggested soil contamination may be an important factor in *Conium*'s presence in Cook County.

Urban soils are often contaminated by a suite of diverse compounds, but we focused on two major classes of contaminants, heavy metals and polycyclic aromatic hydrocarbons (PAHs), given the populations' locations along interstate highways and biosolids drying beds. Vehicle and road wear, combustion byproducts, and pollutant-enriched biosolid material are likely to deposit in nearby soil and thus enrich its heavy metal and PAH content (Van Bohemen and Janssen van de Laak, 2003; Oliver *et al.*, 2005; Harrison *et al.*, 2006). Chemical analysis demonstrated that soil where *Conium* was growing approached or exceeded contamination thresholds according to EPA ecological soil screening criteria (U.S. EPA, 2005a). Given this result, we examined *Conium*'s tolerance to specific soil contaminants and investigated whether genetic diversity within populations or genetic structure among populations was associated with measured contaminant concentrations.

The primary questions posed by this research are:

1. What is the overall genetic diversity of *Conium* populations in Cook County and what is the level of genetic differentiation among populations?
2. Using collections of seeds and soils, is there any evidence for local soil growth advantage for seeds growing in home soil versus other sites' soil in the greenhouse?
3. Are *Conium* seedlings more tolerant of heavy metals than two native species, *Desmodium canadense* (showy tick trefoil) and *Echinacea purpurea* (purple coneflower; hereafter *Desmodium* and *Echinacea*)?
4. Is there any discernible correlation between concentrations of soil contaminants and/or spatial variables and the genetic diversity (within) and genetic structure (among) the field populations?

Addressing these questions to gain insight into environment/genome interactions is important in understanding the spread of invasive plans in anthropogenically disturbed landscapes.

## METHODS

This research was divided into four principal research methodologies. The first section consisted of two seasons of field surveys to detect *Conium* populations along roadways and in natural areas in southern Cook County. The second was a soil characterization analysis that quantified various contaminants and physical/chemical parameters of the soil in which each *Conium* population grew. The third was a genetic analysis that quantified the levels and patterns of genetic variation in *Conium* plants among the nine populations. The fourth section was the greenhouse establishment experiments that tested for (1) local advantage (defined as seeds germinating and seedlings growing better in their original soil compared to soil from other populations), and (2) tolerance to heavy metals (defined as the growth of individuals from nine *Conium* populations relative to *Desmodium* and *Echinacea* seedlings in the presence of known levels of arsenic (As), cadmium (Cd), and lead (Pb).

*Study species.*—Many invasive plants have life history traits (fast growth rates, tolerance to pollution, generalist resource use) that can be advantageous in disturbed habitats (Thuiller *et al.*, 2006). *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 and a single plant produces thousands of seeds (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

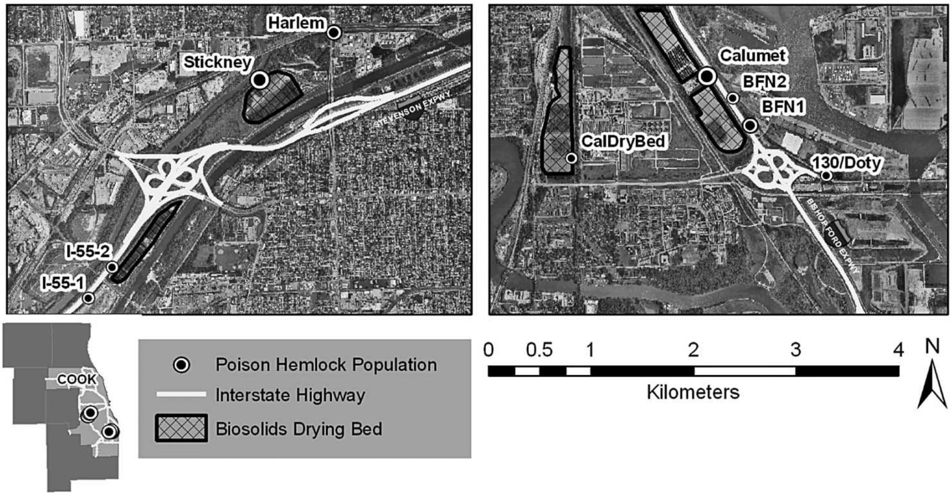

FIG. 1.—Map of *Conium* field populations, MWRD-GC biosolids drying beds, and associated roadways. Upper left is the West Suburbs 'hotspot,' upper right is the Calumet 'hotspot.' Populations are symbolized with respect to size of *Conium* population area, as listed in Table 1. Map projection: Illinois SPCS East NAD83 feet. Data sources: USGS Orthoimagery (2008) and IL Dept of Transportation Highway Data (2009)

activity to colonize new areas (Stephens, 1980). The species is native to Europe, North Africa, and Asia, and has been documented in every American state and Canadian province except for Mississippi, Florida, Alaska, Hawaii, Manitoba, Yukon, and the Northwest Territories. *Conium* is commonly found in most of Illinois except for the northeastern counties (Cook, McHenry, Lake; USDA PLANTS database: http://plants.usda.gov/).

*Field surveys.*—The first population of *Conium* in Cook County (Calumet) was documented in 2006. Detailed systematic surveys were conducted during May and Jun. of 2007 and 2008 and nine populations were detected. Since *Conium* is a biennial, two consecutive years of surveys allowed for better detection of populations since only second year plants are conspicuous. The nine *Conium* populations were located in two distinct 'hotspots' in southern Cook County (Fig. 1). Four populations (Stickney, Harlem, I-55-1, I-55-2) were located near interstate I-55 and the Stickney WWTP near McCook, IL and five populations (Calumet, CalDryBed, BFN-1, BFN-2, 130th/Doty) were located near interstate I-90/94 close to 130th St. and the Calumet WWTP in southeastern Chicago. Given the widespread nature of the species in other places across the continent, it seemed unlikely that it would be regularly associated with a particular habitat in Cook County. This peculiar, localized distribution inspired our investigation.

We conducted surveys by car (with one driver and a second observer), by bicycle, and on foot during *Conium*'s flowering time in May and Jun. Second year plants are 2 m or more in height and produce many large umbels of bright, conspicuous white flowers, permitting easy detection even from the window of a car traveling on an interstate highway. We recorded frequency of occurrence, the location of each population, and estimated densities by walking around the perimeter of each *Conium* patch with a GPS device (Garmin Colorado 400t) that calculated distances and areas. We surveyed all of the roadsides of the five major interstate highways (2-90, 2-94, 90/94, 57, and 55) in southern Cook County, accounting for

272 mi of roadway. In order to survey areas with different types of land use near the roadways, 12 mi were surveyed on foot, and 35 mi were surveyed by bicycle, respectively, on paths within and between natural areas for all of the natural areas adjacent to (a boundary within 1 km of) the surveyed interstate roadways. The surveyed natural areas included: Beaubien Woods (I-94), Bartell Grasslands (I-57), Chicago Portage (I-55), Bemis Woods South (I-294), Thatcher Woods (I-290). No *Conium* populations were detected in any natural area.

*Soil sample collection.*—We sampled soil at each of the nine *Conium* populations in Jun. of 2008 by extracting cores with a stainless steel probe inserted manually at grade. One, two, and three samples were extracted from each population based on its respective size (small; up to 2000 $m^2$, medium; 2000 to 6000 $m^2$, and large; over 6000 $m^2$) for a total of fifteen cores within all populations. Soil cores measured 2 cm in diameter by 30 cm in length, except for one core in each medium and large population that was segmented and only the top 20 cm composited and analyzed (note that no significant differences were found between segments and full cores). In addition to within-population soil cores, soil cores offset 500 m from the boundary of each population were taken to permit comparison of roadside soil where *Conium* was both present and absent, for a total of 24 soil cores collected for analysis in the sampling effort. The probe was rinsed with de-ionized water and wiped clean between each sample. Cores were sealed in labeled glass jars and refrigerated for storage within 8 h of sampling. Samples were prepared for analysis by homogenization and removal of rocks, organic matter, and debris appearing larger than 1 cm in any two directions with a clean stainless steel spoon and spatula. In Jun. 2009 we also collected nearly 10 liters of soil from each population by shovel and bucket at core locations to be used in the greenhouse establishment experiments.

*Soil contamination analysis.*—Soil samples were analyzed for 20 metals and 20 PAHs by National Environmental Laboratory Accreditation Program (NELAP) certified analytical laboratory StatAnalysis, Inc., Chicago, IL. Metals were extracted from subsamples by the acid digestion procedure given in U.S. EPA method 3050B (U.S. EPA, 1996a) and analyzed by inductively coupled plasma-mass spectrometry per EPA method 6020A (U.S. EPA, 2007): except for mercury, which was analyzed by cold vapor atomic absorption given in method 7471A (U.S. EPA, 1994). PAHs were extracted from soil subsamples according to EPA ultrasonic extraction method 3550B (U.S. EPA, 1996b), followed by analysis via gas chromatography/mass spectrometry in selective ion mode per method 8270C (U.S. EPA, 1996c).

*Physical and chemical soil characterization.*—Soil samples were quantified for physical and chemical parameters including: wet bulk density, percent solids (the complement of percent moisture), organic matter, organic carbon, nitrogen, black carbon, pH, and salinity. Bulk density was determined by gravimetrically weighing the soil and measuring its volume in a 50 ml graduated conical tube after shaking and three taps on a hard surface to settle (if clayey soil, after mixing and compaction with pestle to air space similar to original cores). Solids were determined from gravimetric weighing of sub-samples pre- and post-oven drying (105 C, 48 h), while organic matter was determined by gravimetric weighing of dried sub-samples pre and post muffle furnace combustion (375 C, 24 h) as described in Buckley *et al.* (2004). Organic carbon, nitrogen, and black carbon measurements were taken from dried or combusted subsamples by elemental analysis (Buckley *et al.*, 2004). Salinity and pH were measured in a 1:1 soil to de-ionized water suspension by conductivity cell and combination electrode respectively, following vortex and manual shaking for 1 min at 10 min intervals over an hour and settling over an hour (Janzen, 1993; Hendershot *et al.*, 1993).

*Field sampling of plant tissue.*—During the 2008 flowering season we collected leaf samples from all the identified Cook County *Conium* populations. *Conium* plants do not grow clonally and individual flowering plants in the field are easily distinguished from each other. No two tissue samples within a population had the same genotype, confirming our identification of individuals. For five of the populations (130th/Doty, BFN-1, Calumet, Harlem, and Stickney) we assessed the spatial range of the population and estimated total flowering plants. We then systematically walked the population (most were elongated rectangles in shape, given the roadside locations) and sampled every third to tenth plant we came to, depending on population size, so that we would collect around 30 leaf samples upon reaching the end of the population. Two populations (BFN-2 and CalDryBed) had between 20–24 flowering plants so we collected samples from all flowering plants. Two distinct nearby (380 m) populations (I-55-1 and I-55-2) had very few flowering plants, so all of the plants from these populations were sampled, and the samples were combined for the genetic analysis (21 total samples). All tissue samples (217 total samples) were dried immediately in silica gel and stored at room temperature.

*Genetic laboratory analysis.*—Dried tissue samples were homogenized to a fine powder using Talboys High Throughput Homogenizer (Henry Troemner LLC, Thorofare, NJ) and DNA was extracted using a Qiagen DNeasy Plant Mini Kit (Qiagen, Germantown, MD). We quantified the amount of DNA in each extraction using a NanoDrop ND-1000 (version 3.3). Thirty potential inter simple sequence repeat (ISSR) primers were screened for amplification, variability, and consistency of results in *Conium.* ISSRs are highly variable dominant markers that use microsatellite regions in the genome as primers, and the presence or absence of bands on an agarose gel indicates differences in primer binding sites between individuals. We chose five inter-simple sequence repeats (ISSR) primers (Table 2) for our study, which yielded 114 variable bands. A 25 μl volume polymerase chain reaction (PCR) was carried out for each *Conium* sample for each ISSR primer. We used the following 25 μL polymerase chain reaction mixture: 10 ng template DNA, 1.0 μm ISSR primer, 12.5 μL PCR MasterMix 2× (Promega:50 U/mL Taq DNA polymerase in a proprietary reaction buffer, pH 8.5, 400 μM of each dNTP, and 3 mM $MgCl_2$), 12.5 μg/μL of bovine serum albumin, and an additional 0.675 mM $MgCl_2$. The thermocycling conditions consisted of: a 5 min denaturing step at 94 C, then 35 cycles of the following 45 sec denaturing step (94 C), 45 sec of annealing step (50 C), 2 min elongation step (72 C), then a final 5 min elongation step at 72 C. If a PCR reaction failed for any given *Conium* sample up to two additional reactions were conducted. Five samples required re-extraction of DNA.

PCR products were electrophoresed on high resolution 1.25% agarous gels (Sigma-Aldrich) containing ethidium bromide to visualize DNA. Every sixth well of the agarose gel had a 100–1000 base pair ladder. Gels were run for 200 min at 100 volts. Gels were then placed in GeneFlash imaging box equipped with an ultraviolet light and a camera (syngene bio imaging synoptics Ltd version 0.34). To ensure that we captured every band regardless of brightness we took four pictures per gel at 4, 8, 12, and 16 sec exposures. Gel images were printed and scored by eye independently by two researchers. Individual bands were scored for presence or absence. To ensure that banding pattern differences were unrelated to the PCR reactions and agarose gel we mixed up individuals from populations in each PCR reaction and on agarose gels.

*Genetic data analysis.*—Observed and expected heterozygosities were calculated using GenAlEx6.2 (Peakall and Smouse, 2005). Population structure was evaluated using the Bayesian approach implemented in Hickory 1.1 (Holsinger and Lewis, 2003) and AFLPDAT (Ehrich, 2006). GenAlEx utilizes standard statistical methods and assumes the populations are in Hardy-Weinberg equilibrium. We used GenAlEx to get an estimate of population heterozygosities and Nei's unbiased pairwise genetic distance (Nei, 1978). We also used GenAlEx to perform an analysis of molecular variance (AMOVA) to determine how molecular variation was apportioned among and within populations, and a Mantel test to investigate the relationship between genetic distance and geographic distance for our molecular data. However, since our populations have recently established in the region, the populations may violate Hardy-Weinberg equilibrium. Therefore we also used a Bayesian statistic program, Hickory 1.1, which does not assume Hardy-Weinberg equilibrium, to get an overall estimate of population structure. We used AFLPDAT, which is a group of functions for R to get proportion polymorphic bands and intra-population genetic diversity for each population. We ran AFLPDAT in R 2.10.1 (R Development Core Team, 2009).

*Greenhouse establishment experiments.*—The goals of this part of the study were to set up controlled experiments 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*. Seeds and soils were collected from the nine known *Conium* field populations. *Conium* seeds were collected from each field population from a minimum of 10 individuals evenly distributed throughout each population, and all seeds from a given population were pooled together en masse. *Desmodium* and *Echinacea* were selected as comparable species because they have similar seed sizes to *Conium*, are often used in roadside plantings of native species by the Illinois Department of Transportation, and commonly occur in many of the natural ecosystems of the Chicago region.

A complete factorial design was implemented for the *Conium*-only local advantage test, with each combination of field population seed source and field population soil source replicated three times. Thirty seeds from a given population were placed on the top of soil from each population in a small greenhouse cup (volume = 388 mL per cup) for each replicate and watered once a day. Each combination of seed and soil (30 seeds per population per soil replicate) from both the *Conium*-only local advantage and the three-species metal tolerance experiments (n = 408) was randomly placed in one of fifteen greenhouse trays. Each tray was randomly rotated daily to control for the potential influence of position on the greenhouse bench.

For the metal tolerance experiment, a complete factorial design was used as well. Peat moss was spiked with Pb, Cd, and As to 1000 mg/kg, 100 mg/kg, and 100 mg/kg (dry weight basis) respectively, elevated concentrations within an order of magnitude of those measured in *Conium* field soil cores. Spiking salts lead(II) chloride, cadmium chloride, and sodium arsenate (dibasic heptahydrate) were chosen to maximize metal bioavailability in terms of water solubility and to minimize nutrient enrichment by presence of nitrogen or phosphorous (Janzen, 1993). In addition to three individual metal treatments, a combination treatment of all three metals and a control treatment (without metal pollutants) were prepared for a total of five treatments.

Soil spiking was done by fully dissolving a known mass of each metal salt in beakers of deionized water and mixing thoroughly with a known mass of peat moss for each soil treatment: Pb, Cd, As, and Combo (Pb, Cd, and As). A Peat control was mixed with pure deionized water. Saturated soil treatments were allowed to dry and equilibrate for 48 h under a fume hood with regular mixing before being potted for seeding. Seeds from all nine *Conium* populations and from catalog-bought *Desmodium* and *Echinacea* sources (Prairie Moon Nursery, Winona, MN) were placed in each spiked treatment in triplicate to conduct the metal tolerance experiment. Prairie Moon Nursery is a nursery specializing in native plants, and the *Desmodium* and *Echinacea* seeds we used were not seeds from cultivars but rather from native plants that were collected from prairie remnant populations in the field. We did analysis of seedling growth at the population level and at the species level (with *Conium* populations pooled). Each seed population had 15 replicates among five treatments: Pb, As, Cd, Combo, and control (peat moss without addition of metals).

The seedlings were monitored daily for germination and growth rates over 45 d. 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 (45 d), above ground biomass (AGB) was harvested, dried in an oven for 48 h at 90 C, and weighed. These data provide a metric for overall plant performance and are the primary results of the establishment experiment. We used final counts of germinated and ungerminated seeds and numbers of individuals in different size classes to characterize the relative growth among the 11 populations as well.

*Quantitative and statistical analysis.*—Statistical analyses were done with SYSTAT software package (v. 12, Systat Software, Inc., Chicago, IL USA). The AGB measurements for each population of seeds in each spiked treatment (As, Cd, Pb, Combo) were standardized to the mean AGB of a given population of seeds in the Peat control to account for differences among species in germination times and growth rates.

To investigate the relationships between metrics of genetic diversity and soil and/or spatial variables measured in the field for each population, we used rank correlations. Significant negative correlations between genetic diversity and soil contaminant concentrations would be evidence for locally adapted *Conium* genotypes. Our correlations were at the population scale, with n = 8 since the I-55-1 and I-55-2 populations were combined for all genetic analyses. Correlation of ranks was calculated using the Spearman correlation coefficient, or rho ($\rho$), a nonparametric statistic, as recommended by Zar (1996). For all variables the lowest value was given the rank of 1 (if there was a single instance of that value) and large values had higher ranks. Positive correlations imply that low values of one variable are associated with low values of the other variable. We used three genetic metrics (mean heterozygosity ($H_e$), proportion of variable loci, and intra-population genetic diversity) and compared those variables to 20 soil or spatial variables measured for each field population. A t-distribution is usually used to determine the significance of the rank correlations, but is a poor approximation of the sample distribution of $\rho$ when $n < 10$. In our analysis with $n = 8$ populations (df = 6), therefore, significant results at the 0.05 level are obtained when the absolute value of $\rho$ is at least 0.62 and 0.72, for directional and non-directional tests, respectively (Zar, 1996).

## RESULTS

*Field populations and soil characterization.*—The relative locations of the nine *Conium* field populations, the MWRD-GC biosolids drying beds, and the associated roadways are shown in Figure 1. The nine *Conium* populations were located in two distinct 'hotspots' in southern Cook County. Four populations (Stickney, Harlem, I-55-1, I-55-2) were located near interstate I-55 close to McCook, IL and the Stickney WWTP, and five populations (Calumet, CalDryBed, BFN-1, BFN-2, 130th/Doty) were located near interstate I-90/94 close to 130th St. and the Calumet WWTP in southeastern Chicago. Table 1 summarizes several of the key soil and spatial measurements for each population. The total area covered by each *Conium* population varied considerably from 50 to 60,000 $m^2$. The distances to the nearest population ranged from 100 to 1450 m. The Calumet and Western Suburbs hotspots shown in Figure 1 are about 20 km apart.

In general, soil parameter and contamination data seldom differentiated significantly between the populations, hotspots, or population interiors and exteriors. Arsenic, barium, cadmium, chromium, copper, iron, lead, manganese, mercury, nickel, potassium,

TABLE 1.—Select field soil and spatial parameters for the nine *Conium* populations found in Cook County. Pop. Area is the total area covered by all the *Conium* individuals in a population (defined as a contiguous area of at least 50% *Conium* cover) and Distance to Nearest Pop. is the distance measured to the nearest known *Conium* population. For the soil parameters, single values are reported for small populations (55-1, 55-2, CalDryBed, BFN-2, and 130th/Doty). Mean values for large and medium populations are accompanied by the standard deviation (Stickney, Calumet, n = 3) and the range (Harlem, BFN1, n = 2), respectively

| | Soil parameters | | | | | | Spatial parameters | |
|---|---|---|---|---|---|---|---|---|
| | (mg/kg dry soil) | | | | (% dry wt.) | (g/ml wet wt.) | ($m^2$) | (m) |
| | As | Cd | Pb | pH | Organic matter | Bulk density | Pop. area | Distance to nearest Pop. |
| I-55-1 | 6.70 | 1.30 | 78.0 | 7.86 | 3.559 | 1.263 | 100 | 380 |
| I-55-2 | 9.50 | 1.50 | 92.0 | 8.11 | 3.847 | 1.163 | 50 | 380 |
| Stickney | 11.43 ± 3.87 | 6.50 ± 1.25 | 107.0 ± 53.05 | 7.47 ± 0.19 | 5.641 ± 1.79 | 1.071 ± 0.14 | 6000 | 660 |
| Harlem | 6.65 ± 0.25 | 1.80 ± 0.60 | 152.5 ± 22.5 | 7.60 ± 0.13 | 6.474 ± 1.22 | 1.034 ± 0.03 | 3250 | 660 |
| CalDryBed | 18.0 | 16.0 | 240.0 | 6.63 | 15.079 | 0.801 | 200 | 1450 |
| Calumet | 18.50 ± 8.26 | 5.52 ± 2.51 | 356.7 ± 253.84 | 7.89 ± 0.21 | 3.733 ± 1.19 | 1.160 ± 0.17 | 60,000 | 100 |
| BFN-1 | 11.68 ± 7.33 | 24.53 ± 23.48 | 334.8 ± 255.25 | 7.70 ± 0.05 | 3.890 ± 1.29 | 1.109 ± 0.03 | 4000 | 100 |
| BFN-2 | 13.0 | 2.60 | 280.0 | 7.78 | 8.307 | 1.049 | 150 | 100 |
| 130th/Doty | 9.60 | 1.30 | 140.0 | 7.39 | 2.057 | 1.447 | 2000 | 900 |

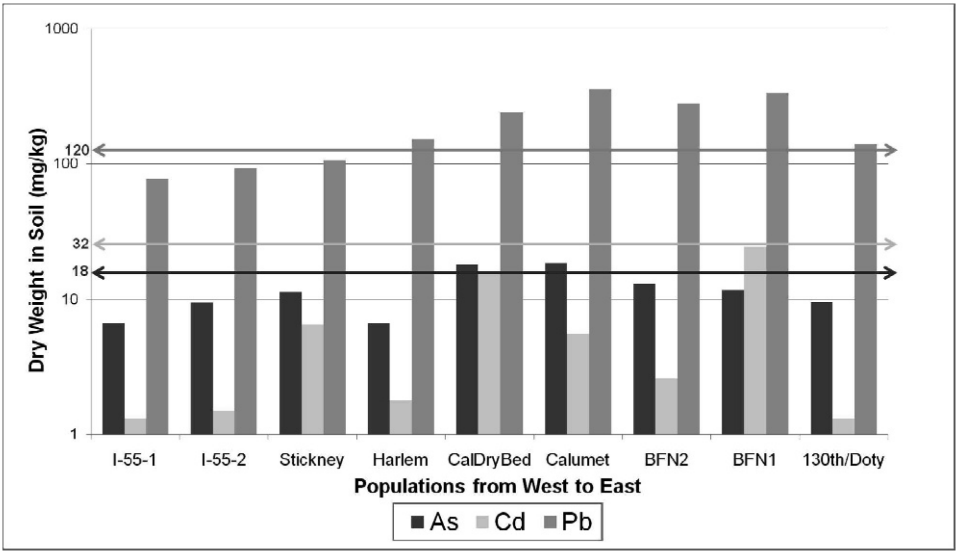

FIG. 2.—Soil concentrations of As, Cd, and Pb at each *Conium* population. Solid lines are contamination thresholds from the U.S. EPA Ecological Soil Screening Levels for plants (U.S. EPA, 2005a). Note log scale

vanadium, and zinc among metals; and anthracene, phenanthrene, pyrene, fluoranthene, chrysene, benz(a)anthracene, benzo(b)fluoranthene, benzo(a)pyrene, benzo(k)fluoranthene, indeno(1,2,3-cd)pyrene, benzo(g,h,i)perylene, and dibenz(a,h)anthracene among PAHs were frequently detected, while other metals and lightweight PAHs were less so. Table 1 shows single or mean soil population concentrations (recall the number of samples were relative to *Conium* population area) of As, Cd, and Pb ranging from 6.7 to 18.5, 1.3 to 24.5, and 78.0 to 356.7 mg/kg dry weight, respectively. Figure 2 depicts the soil concentrations of As, Cd, and Pb with solid lines indicating soil screening levels of concern for plants (U.S. EPA, 2005a), illustrating that at least one population nearly reaches or exceeds contamination thresholds for each of the metals. While the average concentration of total PAHs (11.1 mg/kg, not shown) and levels of individual PAHs may exceed human health risk standards, the risk to plant life is not as clear as for the metals (U.S. EPA, 2005b). The relatively high As, Cd, and Pb concentrations shown and their known interactions with plants (Keane *et al.*, 2001; Uveges *et al.*, 2002; Liu *et al.*, 2005) motivated our interest in a controlled test of metal tolerance for *Conium* in the greenhouse. All soil parameters, PAH, and metal data were used in correlation analyses with genetics metrics.

*Genetic analysis.*—The primer nucleotide sequences, the number of individuals successfully amplified, and the total number of polymorphic bands found in all the individuals analyzed for each of the five ISSR markers are reported in Table 2. Table 3 describes the genetic diversity found in nine *Conium* populations ($n = 217$) using 114 loci from five ISSR markers. The observed number of bands ranged from 83 to 104, with very few bands being unique to a single population. Heterozygosities ranged from 0.143 to 0.232 and the proportion of variable loci (PVl) ranged from 0.523 to 0.798.

The analysis of molecular variance (AMOVA) demonstrated significant levels of variation ($P < 0.01$) among populations, with 16% of the total variation among populations while

TABLE 2.—ISSR marker information. All markers had an annealing temperature of 55 C

| Primer | Sequence | Individuals scored | Total polymorphic bands |
|---|---|---|---|
| 811 | $[GA]_8C$ | 172 | 25 |
| 816 | $[CA]_8T$ | 209 | 22 |
| 818 | $[CA]_8G$ | 209 | 33 |
| 825 | $[AC]_8T$ | 210 | 18 |
| 830 | $[TG]_8G$ | 183 | 16 |

84% was due to differences between individuals within populations. A neighbor-joining phenogram (Fig. 3) based on the pairwise values of Nei's unbiased genetic distance indicates that genetic distances among populations are small ($<0.04$) compared to previous studies of non-invasive plants in their home range (Ma *et al.*, 2008), but similar to studies of invasive plants like miconia (*Miconia calvescens*; Le Roux *et al.*, 2008). The phenogram shows that CalDryBed, the most physically isolated population (Table 1), is clearly the most genetically distinct population, but there is no overall geographic pattern to the distribution of the populations in the figure. In several cases, genetic distances clearly do not reflect physical proximity (Fig. 1). An example is the close clustering of the Harlem and 130th/Doty populations, two populations in separate invasion 'hotspots,' located over 30 km apart. Moreover, the results of the Mantel test ($r = 0.062$, $P = 0.01$) revealed no pattern of genetic differentiation by geographic distance.

*Rank correlations between genetic metrics and soil/spatial field parameters.*—Rank correlations at the population scale ($n = 8$) between metrics of genetic diversity and variables measured in the field for soil and spatial attributes of each population are given in Table 4. There are few significant correlations, and overall, it appears that the populations' genetic diversity does not correlate with most of the measured soil and spatial attributes. However, there are several significant associations of note. Mean heterozygosity and distance to nearest population are negatively correlated, as are intra-population genetic diversity and distance

TABLE 3.—Summary of the genetic structure for the nine *Conium* populations ($n = 217$) using 114 loci from 5 ISSR markers. I-55-1 and I-55-2 populations were grouped into one I-55 population for all genetic analysis due to small numbers of individuals. Genetic analysis 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 a given population. Mean He is the mean heterozygosity of each population, and SE of Mean He is the standard error of the mean. Proportion of Variable Loci is the mean proportion of loci (for 5 ISSR markers) in a population that are polymorphic. Intra-Population Genetic Diversity is the mean proportion of loci (for 5 ISSR markers) that are different between two individuals in each population (average number of pairwise differences between individuals within the same population)

| Population | No. of individuals | No. of bands | No. of private bands | Mean $H_e$ | SE of mean $H_e$ | Proportion of variable loci | Intra-Population Genetic Diversity |
|---|---|---|---|---|---|---|---|
| I-55 | 20 | 94 | 0 | 0.232 | 0.018 | 0.722 | 0.251 |
| Stickney | 32 | 104 | 2 | 0.227 | 0.018 | 0.798 | 0.228 |
| Harlem | 30 | 97 | 0 | 0.202 | 0.017 | 0.730 | 0.196 |
| CalDryBed | 20 | 83 | 0 | 0.143 | 0.017 | 0.523 | 0.148 |
| Calumet | 24 | 94 | 0 | 0.215 | 0.019 | 0.690 | 0.213 |
| BFN-1 | 23 | 100 | 0 | 0.228 | 0.018 | 0.791 | 0.223 |
| BFN-2 | 34 | 93 | 0 | 0.220 | 0.018 | 0.706 | 0.231 |
| 130th/Doty | 31 | 102 | 1 | 0.189 | 0.017 | 0.758 | 0.183 |

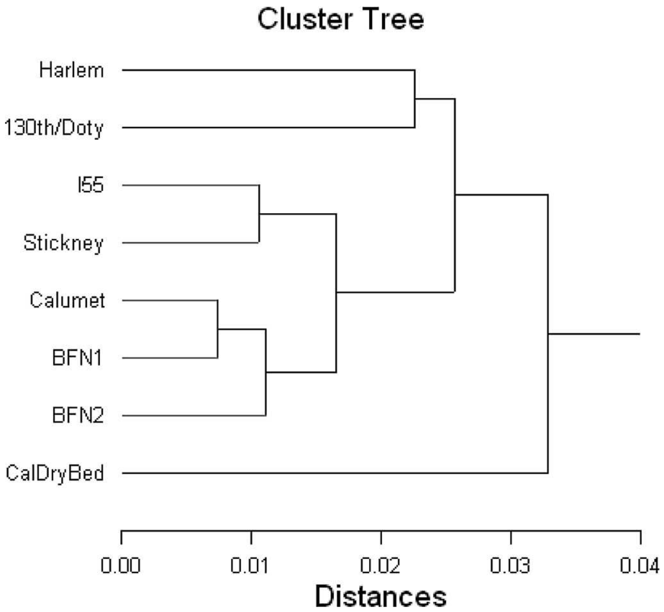

FIG. 3.—Phenogram of genetic differentiation between *Conium* populations measured by Nei's unbiased pairwise genetic distance

to nearest population. Mean heterozygosity and intra-population genetic diversity are both positively correlated to pH. Although concentrations of barium and potassium were the only metal measurements to correlate significantly (negatively) with genetic metrics, there is a general trend of negative correlations between many of the metal concentrations and metrics like mean heterozygosity, proportion of variable loci, and intra-population genetic diversity. Of the 24 correlations shown in Table 4 between metal concentrations (using the eight metals with the highest mean concentrations across all samples) and genetic diversity metrics, 23 are negative correlations, so while we found few significant correlations, the trend is a consistent negative association between genetic diversity and levels of soil contamination.

*Greenhouse establishment local advantage experiment.*—Figure 4 illustrates *Conium* growth expressed as mean weights of above ground biomass for seeds grown on local population soil and on soil from all other *Conium* populations. There is no clear local advantage for any of the seed and soil pairs.

*Greenhouse metal tolerance experiment.*—Figure 5 shows the metal tolerance (As, Cd, Pb, Combo) among three species from the greenhouse experiment. We report mean AGB which has been divided by the mean weight from the peat control for each population of seeds. *Conium* grows significantly better in the As treatment than *Echinacea* (ANOVA $P = 0.002$, $F = 7.82$, $df = 2$, pairwise comparisons significant at the 0.05 level). *Conium* also grows significantly better in Combo (As, Cd, Pb) treatment than both *Desmodium* and *Echinacea* (ANOVA $P < 0.0001$, $F = 13.19$, $df = 2$, pairwise comparisons significant at the 0.05 level).

## DISCUSSION

Given that *Conium* is widespread in much of North America (USDA) and is a known colonizer of wetland margins and riparian areas (Stephens, 1980; Whitson *et al.*, 1992), the

TABLE 4.—Correlations at the population scale (n = 8) between metrics of genetic diversity and variables measured in the field for soil and spatial attributes of each population. With n = 8 (df = 6), significant results at the 0.05 level are given when the absolute value of ρ is at least 0.62 (marked with $^X$) and 0.72 (marked with *), for directional and non-directional tests, respectively

| Variable | Mean $H_e$ | Proportion of variable loci | Intra-Population Genetic Diversity |
|---|---|---|---|
| As | −0.21 | −0.54 | −0.17 |
| Ba | −0.45 | $-0.64^X$ | −0.40 |
| Cd | 0.095 | 0 | −0.095 |
| Cu | −0.38 | −0.57 | −0.33 |
| Pb | −0.19 | −0.38 | −0.24 |
| Hg | −0.095 | −0.29 | −0.24 |
| K | $-0.71^X$ | −0.52 | −0.76* |
| Zn | −0.26 | −0.36 | −0.36 |
| Total Metals | −0.48 | 0.33 | −0.43 |
| Total PAHs | −0.048 | 0 | −0.19 |
| pH | $0.71^X$ | −0.14 | 0.77* |
| Bulk Density | 0.36 | 0.31 | 0.26 |
| Liquid % Salinity | −0.36 | −0.12 | −0.21 |
| % Solids | 0.48 | 0.43 | 0.24 |
| % Nitrogen | −0.30 | −0.44 | −0.084 |
| % Organic Carbon | −0.071 | −0.50 | 0.26 |
| % Black Carbon | 0.38 | −0.048 | $0.67^X$ |
| % Organic Matter | −0.31 | −0.36 | −0.17 |
| Population Area | 0.007 | 0.33 | −0.21 |
| Dist. to Nearest Pop. | $-0.65^X$ | −0.012 | $-0.65^X$ |

historical absence of *Conium* in Cook County, IL suggests that *Conium* would not be expected to be exclusively associated with contaminated sites along the roadways of a major urban area. The interesting question, therefore, is why *Conium* is associated with such sites in Cook County. *Conium* clearly shows at least a general tolerance to anthropogenic soil contamination in both the field (Fig. 2) and the greenhouse (Fig. 5), but it is unclear that this tolerance to contaminated soils is driving its establishment in the Chicago region. Although we found high levels of genetic diversity in our populations, which is consistent with the expectation for successful, non-clonal, invasive plants in adverse environments (Lee, 2002), we did not find significant positive correlations between genetic diversity and concentrations of soil contaminants (Table 4). Such a result would be expected for successfully colonizing populations under adverse conditions (high soil contaminants in our study). In fact, though not significant, the vast majority of the correlations between genetic diversity and soil contaminant concentrations reported in Table 4 were negative, suggesting that high diversity was more likely associated with low levels of contamination. Moreover, we found little population genetic differentiation and structure among all known populations (Fig. 3). We also found no significant evidence for a particular *Conium* population associated with high metal or PAH contamination (Table 4).

Considered alone, the genetic analysis serves as an interesting assessment of the genetics of an invasive species that has recently established populations in a new region. Ward *et al.* (2008) summarized the ecological genetics of invasive plants and found that invasive plant populations in the invaded range can have both high and low levels of genetic diversity. When genetic structure is measured, the results tend to reflect the reproductive biology of

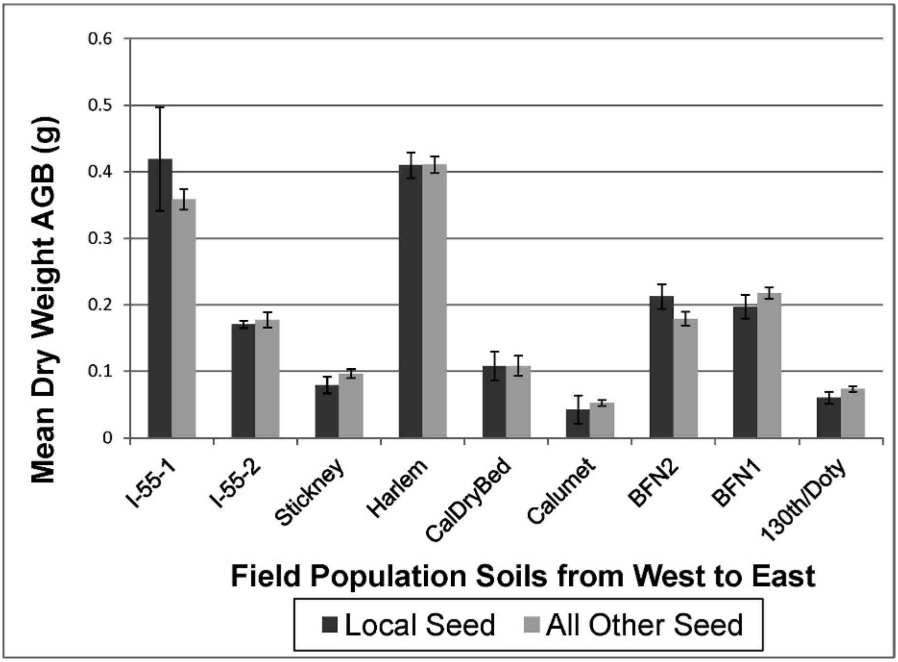

FIG. 4.—*Conium* growth expressed as mean weights of above ground biomass for seeds grown on home (local) and away (soil of all other *Conium* populations). Error bars are ±1 standard error. n = 3 for home soils, n = 24 for away soils

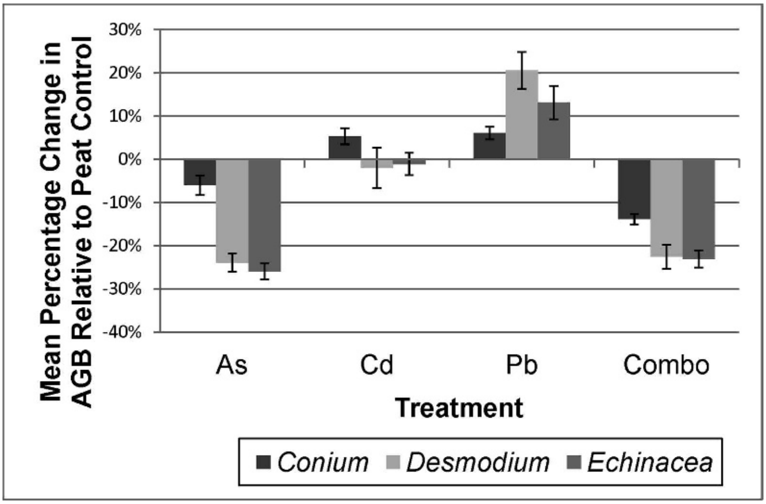

FIG. 5.—Metal tolerance (As, Cd, Pb, Combo) among three species from the greenhouse experiment. Depicted is the percentage change in above ground biomass (AGB) for a given metal treatment relative to the growth in the peat control without added metals. Error bars are ±1 standard error. n = 27 for *Conium*, n = 3 for *Desmodium* and *Echinacea*

the species in question. Cross-breeding species like *Conium* tend to have high genetic diversity within populations and low genetic differentiation among populations. This is indeed what we found, which is not surprising. For example, all of the values for the genetic diversity metrics in our populations are generally high when compared to values from previous studies on other invasive species including: buffelgrass (*Pennisetum ciliare* L. Link; PVI ranged from 0.222 to 0.519; Gutierrez-Ozuna *et al.*, 2009); alligator weed (*Alternanthera philoxeroides*; $H_e$ = 0.0056, PVI was 0.0648; Geng *et al.*, 2007); fountain grass (*Pennisetum setaceum*; no genetic variability detected with 18 ISSR primers; Poulin *et al.*, 2005); and cat's claw creeper (*Macfadyena unguis-cati* L. Gentry; Prentis *et al.*, 2009) and bladder campion (*Silene latifolia*; Taylor and Keller, 2007). This is true even for the *Conium* populations with relatively low numbers of individuals. Table 3 shows that all populations had proportions of variable loci of at least 0.50, with several populations reporting values close to 0.80. This is a large amount of variability for relatively small, relatively isolated, and relatively young (from a colonization standpoint) populations. It seems very likely that *Conium* is not clonal even though it forms dense monospecific stands. Although we sampled plants in a stratified manner (*i.e.*, did not sample two plants next to each other), we still found that no two individuals had exactly the same genotype, even in populations where we censused all plants. Consequently, all plants seem to be the result of sexual reproduction by seed, consistent with evidence that *Conium* is self-incompatible (Baskin and Baskin, 1990).

Also notable is the virtual lack of any genetic differentiation among *Conium* populations. Figure 3 shows a cluster tree of the structure of the eight populations, which is remarkable for the relatively short lengths of the branches between nodes given the numerical scale. CalDryBed is the most genetically unique population. Even relatively small populations like CalDryBed and I-55 (comprised of I-55-1 and I-55-2 for genetic analysis due to small population sizes) that do not show much differentiation from larger populations do not seem to suffer much loss of genetic diversity. Although small populations may not show reduced diversity, there may be evidence that more isolated populations do. Table 4 shows significant negative correlations between distance to nearest population and both mean heterozygosity and intra-population gene diversity. This apparent reduction in diversity with increasing spatial isolation is more consistent with expectations for colonizing populations, but the general lack of genetic differentiation and the relatively high genetic diversity are more unusual and an especially remarkable attribute for the invasive *Conium* populations in Cook County.

Although we did not find significant correlations between levels of genetic diversity and soil contaminant concentrations, it is likely that *Conium*'s general tolerance to contaminants like As, Cd, and Pb (as compared to other species; Fig. 5) may allow it to colonize and reproduce in roadside areas that harbor these pollutants. The results of our spiked metal treatments in the greenhouse seedling establishment experiment are consistent with the idea that *Conium*'s tolerance to soil contamination allows it to do well enough in disturbed soils and roadside locations that may serve as 'footholds' in a new region. Whether *Conium* will remain exclusively associated with these roadside habitats or whether it will expand to other kinds of habitats and micro-environments is unclear. What is clear, however, is that our results showing populations with consistently high genetic diversity compared to previous studies of invasive plants (Poulin *et al.*, 2005; Geng *et al.*, 2007; Gutierrez-Ozuna *et al.*, 2009) indicate that *Conium* is only able to successfully colonize areas along roadsides in Cook County that receive a large amount of seed from a diverse source, or multiple introductions of seed from several genetically distinct sources. This established high diversity (Table 3) and *Conium*'s general tolerance to particular heavy metals (Fig. 5) only increases the species' adaptability to new environmental conditions. Therefore we find no evidence that indicates *Conium* will remain limited to the particular roadside habitats where we detected it.

The principal goal of this study was to combine ananalysis of the genetic variation of populations of a recently established invasive specieswith an assessment of a specific kind of anthropogenic environmental contamination. The only other similar study to investigate how environmental contamination (copper, tributyltin) may be promoting biological invasion concerns marine sessile invertebrates in New South Wales, Australia, but that study did not incorporate a genetic assessment of the invading populations (Dafforn *et al.*, 2009). Although we had good reason to believe that tolerance to soil contamination, and particularly contamination by heavy metals (Table 1, Figs. 1, 2), may be aiding *Conium*'s establishment in Cook County, we did not find specific evidence to conclude that a tolerance to soil contamination is actively driving the invasion process for this species in this region.

However, an alternative hypothesis that we did not explicitly test is provided by research on fungal endophytes that impart habitat-specific stress tolerances to their plant hosts which can increase plant survival in high stress environments, such as contaminated soils. Kim *et al.* (2008) found that *Phytolacca americana* (pokeweed) plants from sites with contaminated soils in South Korea contained a single dominant fungal endophyte that was missing from pokeweed plants that grew in uncontaminated sites. Moreover, the endophyte was measured in highest abundance in leaves where the highest concentrations of metals were found. It is possible that the *Conium* plants that grow in the contaminated roadside habitats we studied also harbor endophytes that promote their survival in such high-stress habitats. Other studies have demonstrated that fungal symbionts have the ability to grant metal tolerance to plants (Monnet *et al.*, 2001; Wilhite *et al.*, 2001; Al-Karaki *et al.*, 2001), so this is a possibility worth investigating for the *Conium* populations in Cook County.

What is striking is the overall lack of relationship between the genetic diversity metrics and the levels of soil contamination, even in light of the peculiar spatial association with sites with demonstrated metal contamination. As indicated in Table 4, few measures of genetic diversity were correlated (positively or negatively) with levels of soil contamination. In fact, we only found two metals (barium and potassium) that were significantly negatively associated with any genetic diversity metric (mean heterozygosity, proportion of variable loci, or intra-population genetic diversity). Neither metal was found in very high concentrations in the field, nor is either regarded as particularly toxic, as compared to metals like arsenic, cadmium, lead, and mercury, although potassium is an essential plant nutrient. The general lack of significant relationships between soil and genetics variables may indicate that *Conium* is just particularly opportunistic, with some capacity to tolerate disturbed soils that harbor relatively high levels of contaminants. So while our soil and genetics characterizations generated a good baseline data set, they did not clarify specific reasons for why *Conium* may be occurring in these particular locations in the region. Our dataset may benefit from an analysis using roadside dispersal models (Floerl and Inglis, 2005; Drake and Lodge, 2006; Pysek *et al.*, 2008) if the distribution and genetics of *Conium* populations along roadsides in regions near the Chicago area was also known.

The greenhouse experiments did not provide any significant evidence of local advantage (Fig. 4), further supporting the idea that the *Conium* populations in Cook County are not specifically promoted by the soils in which they are growing. In fact, only two of the nine seed populations (I-55-1, BFN-1) grew better in their own local soil than they grew in all of the other eight field soils. Even CalDryBed, the population with the lowest mean heterozygosity (Table 3) and the basal root on the cluster tree (Fig. 3), did not show any difference in AGB growth between local and other soils. Hypothetically, if one expected to observe local adaptation to soil that was reflected in the genetic diversity of the individuals of a population, a reduction in genetic variation would be consistent with natural selection for a particular ecotype having higher fitness (seed set, etc) in a particular soil (Hufford and Mazer, 2003; Lopez *et al.*, 2009). Although we did not do range-wide sampling that would allow us to test for a contamination-resistant *Conium* ecotype, we did not even find evidence for the mere promotion of seedling growth by local soil for any of our populations. Thus, it would seem unlikely that we would detect a contamination-resistant *Conium* ecotype even if we had done the requisite range-wide sampling and analysis.

However, we did find evidence for *Conium*'s tolerance to soils spiked with known concentrations of As, Cd, and Pb, as compared to *Desmodium* and *Echinacea. Conium* seedlings were more tolerant of the As and combo (As, Cd, and Pb) treatments ($P < 0.05$, Fig. 5). These are important results because they demonstrate that *Conium* has a general tolerance to some of the contaminants that we found at elevated levels in the roadside soils were *Conium* has colonized the region. This general tolerance to metal contamination may serve to allow most *Conium* individuals to grow well enough in highly disturbed, contaminated areas to form self-sustaining populations that export seed to colonize new areas in the region. Since this tolerance does not seem to be the exclusive attribute of a particular *Conium* population with low diversity, this may also allow *Conium* to maintain its observed relatively high genetic diversity, an attribute of an invasive species that is often viewed as an advantage when colonizing new areas with lots of habitat heterogeneity and environmental variability (Lee, 2002).

This study sought to generate relevant information about *Conium*'s recent establishment in the Chicago region's urban landscapes through a characterization of the soil and the genetics of the known *Conium* populations. Our specific ideas about soil contamination promoting the invasion of this species were motivated by our field surveys showing *Conium* populations to be associated with contaminated soil characteristic of roadway and biosolid influence. We did find evidence that *Conium*'s general tolerance to metals like As, Cd, and Pb may allow it to colonize and thrive in highly disturbed areas with contaminated soil along roadways, but we did not find convincing evidence that genetic diversity and levels of soil contamination were positively correlated. We cannot claim, therefore, that metal contamination is driving the distribution of *Conium* in Cook County in any clear way. The peculiar spatial association with MWRD land and roadsides remains poorly understood. Even so, the results of our greenhouse seedling experiments may help clarify reasons for *Conium*'s initial success in colonizing the region. Other studies have shown that invasive plant species like *Solidago canadensis* (Canada goldenrod) can out-compete native species in lead-contaminated soil in greenhouse competition experiments (Zhang *et al.*, 2008). In our study, *Conium*'s apparent relative advantage over *Desmodium* and *Echinacea* in soils contaminated with As and with a combination of As, Cd, and Pb may partially explain its success in colonizing roadside soils with relatively high levels of metal contamination.

Although we were not able to clearly explain the processes that generated the curious distribution patterns of *Conium* populations in Cook County, this interdisciplinary approach to explaining invasive plant establishment is likely to be successful if enough reasonably relevant possibilities (such as fungal endosymbionts) are investigated. Weencourage others to modify our techniques and refine our central approach to suit their research questions while preserving the combination of a genetic analysis with assessments of environmental contamination or other kinds of anthropogenic alterations. Invasive species are clearly connected to human activities and the disruption of 'natural' ecosystem processes (Vitousek *et al.*, 1996; Mack *et al.*, 2000; Hierro *et al.*, 2006), and specific investigations into genome and environment interactions are likely to help explain reasons for many invasive species' success in colonizing and succeeding in new areas.

*Acknowledgments.*—We thank Chicago Wilderness and the NSF IGERT LEAP (Landscape Ecological and Anthropogenic Processes, http://www.uic.edu/depts/bios/leap/) program at UIC for research funds and stipend support (grants CW-2008-04275 and NSF-0549245). The Illinois Native Plant Society (INPS) provided support for the initial field survey work. MWRD-GC provided access to their land and permission to collect plant and soil samples. H. Findlay assisted with greenhouse data collection and scoring ISSR bands. J. Hassan and D. Reitz assisted with genetic laboratory work. We thank M. V. Ashley, D. Nyberg, K. Rockne, and D. Zaya for valuable guidance throughout this project and for helpful feedback on earlier drafts of the manuscript.

## LITERATURE CITED

AL-KARAKI, G. N., R. HAMMAD, AND M. RUSAN. 2001. Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. *Mycorrhiza*, **11**:43–47.

BASKIN, J. M. AND C. C. BASKIN. 1990. Seed germination ecology of poison hemlock, *Conium maculatum. Can. J. Bot.*, **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. *Biol. Invas.*, **8**:217–230.

BECH, J., C. POSCHENRIEDER, M. LLUGANY, J. BARCELO, P. TUME, F. J. TOBIAS, J. L. BARRANZUELA, AND E. R. VASQUEZ. 1997. Arsenic and heavy metal contamination of soil and vegetation around a copper mine in Northern Peru. *Sci. Total Environ.*, **203**:83–91.

BUCKLEY, D. R., K. J. ROCKNE, A. LI, AND W. J. MILLS. 2004. Soot Deposition in the Great Lakes: Implications for semi-volatile hydrophobic organic pollutant deposition. *Environ. Sci. Technol.*, **38**:1732–1739.

CHRISTEN, D. AND G. MATLACK. 2006. The role of roadsides in plant invasions: a demographic approach. *Conserv. Biol.*, **20**:385–391.

CHUN, Y. J., B. FUMANAL, B. LAITUNG, AND F. BRETAGNOLLE. 2010. Gene flow and population admixture as the primary post-invasion processes in common ragweed (*Ambrosia artemisiifolia*) populations in France. *New Phytol.*, **185**:1100–1107.

DAFFORN, K. A., T. M. GLASBY, AND E. L. JOHNSTON. 2009. Links between estuarine condition and spatial distributions of marine invaders. *Divers Distrib.*, **15**:807–821.

DRAKE, J. M. AND D. M. LODGE. 2006. Alle effects, propagule pressure and the probability of establishment: risk analysis for biological invasions. *Biol. Invas.*, **8**:365–375.

DRAY, JR. F. A., R. E. HALE, P. T. MADEIRA, B. C. BENNETT, AND T. D. CENTER. 2009. Concordance between life history traits, invasion history, and allozyme diversity of the Everglades invader *Melaleuca quinquenervia. Aquat. Bot.*, **90**:296–302.

EHRICH, D. 2006. AFLPDAT: a collection of r functions for convenient handling of AFLP data. *Mol. Ecol. Notes*, **6**:603–604.

ELTON, C. S. 1958. The ecology of invasions by animals and plants. University of Chicago Press, Chicago.

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. *Mol. Ecol.*, **8**:443–451.

FLOERL, O. AND G. J. INGLIS. 2005. Starting the invasion pathway: the interaction between source populations and human transport vectors. *Biol. Invas.*, **7**:589–606.

GENG, Y. P., X. Y. PAN, C. Y. XU, W. J. ZHANG, B. LI, J. K. CHEN, B. R. LU, AND Z. P. SONG. 2007. Phenotypic plasticity rather than locally adapted ecotypes allows the invasive alligator weed to colonize a wide range of habitats. *Biol. Invas.*, **9**:245–256.

GULEZIAN, P. Z. AND D. W. 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.*

GUTIERREZ-OZUNA, R., L. E. EGUIARTE, AND F. MOLINA-FREANER. 2009. Genotypic diversity among pasture and roadside populations of the invasive buffelgrass (*Pennisetum ciliare* L. Link) in northwestern Mexico. *J. Arid Environ.*, **73**:26–32.

HARRISON, E. Z., S. R. OAKES, M. HYSELL, AND A. HAY. 2006. Organic chemicals in sewage sludges. *Sci. Total Environ.*, **367**:481–497.

HENDERSHOT, W. H., H. LALANDE, AND M. DUQUETTE. 1993. Soil reaction and exchangeable acidity. *In:* M. R. Carter (ed.). Soil sampling and methods of analysis. Lewis Publishers, Boca Raton, Florida.

HENRIQUES, F. S., AND J. C. FERNANDES. 1991. Metal uptake and distribution in rush (*Juncus conglomeratus* L.) plants growing in pyrites mine tailings at Lousal, Portugal. *Sci. Total Environ.*, **102**:253–260.

HENRY, P., G. LE LAY, J. GOUDET, A. GUISAN, S. JAHODOVÁ, AND G. BESNARD. 2009. Reduced genetic diversity, increased isolation and multiple introductions of invasive giant hogweed in the western Swiss Alps. *Mol. Ecol.*, **18**:2819–2831.

HIERRO, J. L., D. VILLARREAL, Ö. EREN, J. M. GRAHAM, AND R. M. CALLAWAY. 2006. Disturbance facilitates invasion: the effects are stronger abroad than at home. *Am. Nat.*, **168**:144–156.

HOLSINGER, K. E., P. O. LEWIS, AND D. K. DEY. 2002. A Bayesian approach to inferring population structure from dominant markers. *Mol. Ecol.*, **11**:1157–1164.

——— AND ———. 2003. HICKORY: a package for analysis of population genetic data. V. 8. University of Connecticut, Storrs.

HUFFORD, K. M. AND S. J. MAZER. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends Ecol. Evol.*, **19**:147–155.

JANZEN, H. H. 1993. Soluble salts. *In:* M. R. Carter (ed.). Soil sampling and methods of analysis. Lewis Publishers, Boca Raton, Florida.

JULIANO, S. A. AND L. P. LOUNIBOS. 2005. Ecology of invasive mosquitoes: effects on resident species and human health. *Ecol. Lett.*, **8**:588–574.

KEANE, B., M. H. COLLIER, J. R. SHANN, AND S. H. ROGSTAD. 2001. Metal content of dandelion (*Taraxacum officinale*) leaves in relation to soil contamination and airborne particulate matter. *Sci. Total Environ.*, **281**:63–78.

KIM, Y. O., R. J. RODRIGUEZ, E. J. LEE, AND R. S. REDMAN. 2008. *Phytolacca americana* from contaminated and noncontaminated soils of South Korea: effects of elevated temperature, $CO_2$ and simulated acid rain on plant growth response. *J. Chem. Ecol.*, **34**:1501–1509.

KNEITEL, J. M. AND D. PERRAULT. 2006. Disturbance-induced changes in community composition increase species invasion success. *Comm. Ecol.*, **7**:245–252.

LEE, C. E. 2002. Evolutionary genetics of invasive species. *Trends Ecol. Evol.*, **17**:386–391.

LE ROUX, J. J., A. M. WIECZOREK, AND J. Y. MEYER. 2008. Genetic diversity and structure of the invasive tree *Miconia calvescens* in Pacific islands. *Divers Distrib.*, **14**:935–948.

LIU, H., A. PROBST, AND B. LIAO. 2005. Metal contamination of soils and crops affected by the Chenzhou lead/zinc mine spill (Hunan, China). *Sci. Total Environ.*, **339**:153–166.

LOPEZ, S., F. ROUSSET, F. H. SHAW, R. G. SHAW, AND O. RONCE. 2009. Joint effects of inbreeding and local adaptation on the evolution of genetic load after fragmentation. *Conserv. Biol.*, **23**:1618–1627.

MA, X., X. Q. ZHANG, Y. H. ZHOU, S. Q. BAI, AND W. LIU. 2008. Assessing genetic diversity of *Elymus sibiricus* (Poaceae: Triticeae) populations from Qinghai-Tibet Plateau by ISSR markers. *Biochem. Syst. Ecol.*, **36**:514–522.

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. *Ecol. Appl.*, **10**:689–710.

MAL, T. AND J. LOVETT-DOUST. 2005. Phenotypic plasticity in vegetative and reproductive traits in an invasive weed, *Lythrum salicaria* (Lythraceae), in response to soil moisture. *Am. J. Bot.*, **92**:819–825.

MANDÁK, B., P. ZÁKRAVSKÝ, D. KOŘÍNKOVÁ, P. DOSTÁL, AND I. PLAČKOVÁ. 2009. Low population differentiation and high genetic diversity in the invasive species *Carduus acanthoides* L. (Asteraceae) within its native range in the Czech Republic. *Biol. J. Linn. Soc.*, **98**:596–607.

MIHALJEVIČ, M., V. ETTLER, O. ŠEBEK, P. DRAHOTA, L. STRNAD, R. PROCHÁZKA, J. ZEMAN, AND O. SRACEK. 2010. Alteration of arsenopyrite in soils under different vegetation covers. *Sci. Total Environ.*, **408**:1286–1294.

MONNET, F., N. VAILLANT, A. HITMI, A. COUDRET, AND H. SALLANON. 2001. Endophytic *Neotyphodium lolii* induced tolerance to Zn stress in *Lolium perenne. Physiol. Plantarum*, **113**:557–563.

MORRISON, S. L. AND J. MOLOFSKY. 1998. Effects of genotypes, soil moisture, and competition on the growth of an invasive grass, *Phalaris arundinacea* (reed canary grass). *Can. J. Bot.*, **76**:1939–1946.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**:538–590.

OLIVER, I. W., M. J. MCLAUGHLIN, AND G. MERRINGTON. 2005. Temporal trends of total and potentially available element concentrations in sewage biosolids: a comparison of biosolid surveys conducted 18 years apart. *Sci. Total Environ.*, **337**:139–145.

PAN, X., Y. GENG, W. ZHANG, B. LI, AND J. CHEN. 2006. The influence of abiotic stress and phenotypic plasticity on the distribution of invasive *Alternanthera philoxeroides* along a riparian zone. *Acta Oecol.*, **3**:333–341.

PEAKALL, R. AND P. E. SMOUSE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes*, **6**:288–295. Available via http://www.anu.edu.au/BoZo/GenAlEx.

POULIN, J., S. G. WELLER, AND A. K. SAKAI. 2005. Genetic diversity does not affect the invasiveness of fountain grass (*Pennisetum setaceum*) in Arizona, California and Hawaii. *Divers Distrib.*, **11**:241–247.

PRENTIS, P. J., D. P. SIGG, S. RAGHU, K. DHILEEPAN, A. PAVASOVIC, AND A. J. LOWE. 2009. Understanding invasion history: genetic structure and diversity of two globally invasive plants and implications for their management. *Divers Distrib.*, **15**:822–830.

PYSEK, P., V. JAROSIK, J. MULLEROVA, J. PERGL, AND J. WILD. 2008. Comparing the rate of invasion by *Heracleum mantegazzianum* at continental, regional, and local scales. *Divers Distrib.*, **14**:355–363.

R DEVELOPMENT CORE TEAM. 2009. R: a language and environment for statistical computing. R Foundation for Statistical Computer, Vienna. http://www.r-project.org.

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. *Ann. Rev. Ecol. Syst.*, **32**:305–332.

SALTONSTALL, K. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *P. Natl. Acad. Sci. USA*, **99**:2445–2449.

SANDERSON, E. W., J. JAITEH, M. A. LEVY, K. H. REDFORD, A. V. WANNEBO, AND G. WOOLMER. 2002. The human footprint and the last of the wild. *Bioscience*, **52**:891–904.

STEPHENS, H. A. 1980. Poisonous plants of the central U.S. The Regents Press of Kansas, Lawrence, Kansas.

SWINK, F. AND G. WILHELM. 1994. Plants of the Chicago region. Indiana Academy of Science, Indianapolis.

TAYLOR, D. R. AND S. R. KELLER. 2007. Historical range expansion determines the phylogenetic diversity introduced during contemporary species invasion. *Evolution*, **61**:334–345.

THUILLER, W., D. M. RICHARDSON, M. ROUGET, Ş. PROCHES, AND J. R. WILSON. 2006. Interactions between environment, species traits, and human uses describe patterns of plant invasions. *Ecology*, **87**:1755–1769.

URBANSKY, E. T., M. L. MAGNUSON, C. A. KELTY, AND S. K. BROWN. 2000. Perchlorate uptake by salt cedar (*Tamarix ramosissima*) in the Las Vegas Wash riparian ecosystem. *Sci. Total Environ.*, **256**:227–232.

U.S. EPA. 1994. (United States Environmental Protection Agency). Method 7471A, Revision 1 - Mercury in solid or semisolid waste (manual cold-vapor technique). *In:* SW-846 manual (Test methods for evaluating solid waste, physical/chemical methods) 3rd ed. http://www.epa.gov/epaoswer/hazwaste/testmethods/sw846/index.htm.

———. 1996a. Method 3050B, Revision 3 - Acid digestion of sediments, sludges, and soil. *In:* SW-846 manual (Test methods for evaluating solid waste, physical/chemical methods) 3rd ed. http://www.epa.gov/epaoswer/hazwaste/testmethods/sw846/index.htm.

———. 1996b. Method 3550B, Revision 3 - Ultrasonic extraction. *In:* SW-846 manual (Test methods for evaluating solid waste, physical/chemical methods) 3rd ed. http://www.epa.gov/epaoswer/hazwaste/testmethods/sw846/index.htm.

———. 1996c. Method 8270C, Revision 3 – Semivolatile organic compounds by gas chromatography/mass spectrometry (GC/MS). *In:* SW-846 manual (Test methods for evaluating solid waste, physical/chemical methods) 3rd ed. http://www.epa.gov/epaoswer/hazwaste/testmethods/sw846/index.htm.

———. 2005a. Ecological Soil Screening Levels for As, Cd, Pb. Interim Final. OSWER Directive 9285.7-62 (As), -65 (Cd), -70 (Pb). USEPA. Office of Solid Waste and Emergency Response. Washington, DC. Mar. http://www.epa.gov/ecotox/ecossl/.

———. 2005b. Ecological Soil Screening Levels for Polycyclic Aromatic Hydrocarbons. Interim Final. OSWER Directive 9285.7-78. USEPA. Office of Solid Waste and Emergency Response. Washington, DC. Mar. http://www.epa.gov/ecotox/ecossl/.

———. 2007. Method 6020A, Revision 4- Inductively coupled plasma-mass spectrometry. *In:* SW-846 manual (Test methods for evaluating solid waste, physical/chemical methods) 3rd ed. http://www.epa.gov/epaoswer/hazwaste/testmethods/sw846/index.htm.

UVEGES, J. L., A. L. CORBETT, AND T. K. MAL. 2002. Effects of lead contamination on the growth of *Lythrum salicaria* (purple loosestrife). *Environ. Pollut.*, **120**:319–323.

VAN BOHEMEN, H. D. AND W. H. JANSSEN VAN DE LAAK. 2003. The influence of road infrastructure and traffic on soil, water, and air quality. *Environ. Manage.*, **31**:50–68.

VITOUSEK, P. M., C. M. D'ANTONIO, L. L. LOOPE, AND R. WESTBROOKS. 1996. Biological invasions as global environmental change. *Am. Sci.*, **84**:468–478.

VON DER LIPPE, M. AND I. KOWARIK. 2007. Long-distance dispersal of plants by vehicles as a driver of plant invasions. *Conserv. Biol.*, **21**:986–996.

WARD, S. M., J. F. GASKIN, AND L. M. WILSON. 2008. Ecological genetics of plant invasion: What do we know? *Invas. Plant Sci. Manage.*, **1**:98–109.

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, University of Wyoming, Laramie.

WILHITE, S. E., R. D. LUMSDEN, AND D. C. STRANEY. 2001. Peptide synthetase gene in *Trichoderman virens. Appl. Environ. Microbiol,* **11**:5055–5062.

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. *Mol. Ecol.*, **7**:1107–1125.

ZAR, J. H. 1996. Biostatistical analysis. Prentice Hall, Upper Saddle River, New Jersey.

ZHANG, Q., R. YANG, J. TANG, AND X. CHEN. 2008. Competitive interaction between the invasive *Solidago canadensis* and native *Kummerowia striata* in lead contaminated soil. *Bot. Stud.*, **49**:385–391.

SUBMITTED 21 OCTOBER 2011 ACCEPTED 10 FEBRUARY 2012