INVESTIGATIONS IN WEED BIOLOGY: STUDIES AT THE PLANT, POPULATION, AND COMMUNITY LEVELS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for

the Degree Doctor of Philosophy in the

Graduate School of The Ohio State University

By

Lynn Marie Sosnoskie, M.S.

* * * *

The Ohio State University
2005

Dissertation Committee:

Dr. John Cardina, Adviser

Dr. Douglas Doohan

Dr. David Francis

Dr. P. Charles Goebel

Approved by

_____

Adviser

Graduate Program in Horticulture and Crop Science

ABSTRACT

Studies were conducted at the plant, population, and community level to address questions concerning seed germination in *Alliaria petiolata* (Bieb) Cavara and Grande, weed community composition and structure in response to tillage, rotation and herbicide, and variation in *Abutilon theophrasti* Medicus. The purpose of the research detailed in this dissertation was to improve knowledge of weed biology and ecology with the hopes of developing more effective management strategies that are compatible with sustainable agriculture and environmentally responsible natural area maintenance, and answering questions about various population processes including: allele frequency, mating system, genetic drift, and natural selection.

Alliaria petiolata (garlic mustard) seeds are dormant at maturity, requiring approximately 90 to 105 days cold-moist stratification at 4 to 5 °C for germination to occur. Greenhouse and laboratory studies are hindered by this prerequisite; therefore, the goal was to develop a fast and easy method to break dormancy. Hand-harvested *A. petiolata* seeds stored at room temperature were subjected to chemical and mechanical scarification treatments, placed in plastic Petri dishes on filter paper saturated with either gibberellic acid (GA_3, 10^{-3} M) or distilled water only, and incubated at 20/10 °C (12:12 hrs) and 15/6 °C (12:12 hrs). Control seeds failed to germinate under all conditions. Seed germination percentages for mechanically scarified, 3% H_2O_2, and H_2SO_4 treated

seeds improved when GA_3 was applied exogenously instead of water. Intact *A. petiolata* seed coats appear to be permeable to water, but not GA_3, suggesting that undamaged coats may serve as a chemical and/or physical barrier to the diffusion of large exogenously applied biomolecules. To overcome dormancy and stimulate germination, without relying on cold-moist stratification, *A. petiolata* seed coats must be chemically or mechanically scarified to remove permeability barriers and treated with exogenous gibberellins to stimulate germination.

The composition of the soil weed-seedbank community, with respect to species density and diversity, was characterized 35 years after the implementation of a long-term study involving cropping sequence (continuous corn, corn-soybean, corn-oat-hay) and tillage system (conventional-, minimum- and no-tillage). We identified 35, 42, and 37 weed species in 1997, 1998, and 1999, respectively. Analyses of variance indicated that crop sequence influenced species richness (S), evenness (J) and the Shannon-Weiner index (H'), for all three years ($P \leq 0.01$). Values of S, J, and H' recorded for all combinations of the three-crop sequence were typically greater than the values of S, J, and H' reported for either the corn monoculture, or the two-crop rotation. Species richness was affected by tillage all three years. As the intensity of soil disturbance decreased, values for S increased ($P \leq 0.10$). Mean total germinable weed seed density was greater in the no-tillage treatments than in the minimum- and conventional-tillage treatments, averaged across rotations and years. Differences in community composition

among treatments, for each year, were examined using a multi response permutation procedure (MRPP). Results suggest that the weed seed community in a corn-oat-hay rotational system differs in structure and composition from communities associated with continuous corn and corn-soybean systems ($P \leq 0.003$). No-tillage systems were different in composition as compared to conventional-tillage and minimum-tillage treatments ($P \leq 0.01$). Understanding how a greater range in the type, timing, and arrangement of cultural practices shift weed species composition is important in designing alternative crop management systems.

There is concern that the widespread use of genetically-modified glyphosate-(N-phosphonomethyl glycine)-tolerant crops (GTCs) will alter agricultural weed community dynamics with respect to glyphosate-tolerance and emergence phenology if accompanied by increased applications of a single herbicide mode-of-action across space and time. The composition of weed and weed seedbank communities in systems planted to glyphosate-tolerant crops has not been adequately described. Of particular importance is the need for baseline studies from which to measure future trends. The composition of the soil weed-seedbank community, with respect to species density and diversity, was characterized four, five, and six years (2002 to 2004) after the adoption of glyphosate-tolerant corn and soybeans in a continuing long-term tillage and rotation study. Results indicate that the density and diversity of species in the seedbank and field were influenced by tillage and rotation, and were similar to trends observed in the preceding

study. In particular: mean total germinable weed and weed seed density was greater in the no-tillage treatments than the minimum- and conventional-tillage treatment; species richness and weed and weed seed density were higher in more diverse rotations. Species associated with individual treatments were not different from species recorded in the same plots prior to the exclusive use of GTCs and glyphosate, suggesting that significant changes in weed community composition and structure have not occurred.

A. theophrasti (velvetleaf) is a noxious weed in modern row-crop agriculture. This study characterized the morphological and phenological variation present in 80 velvetleaf accessions from Asia, Japan, India, Europe, Eastern Africa and North America to determine whether "crop" and "weedy" biotypes exist and are easily differentiated. Principal components and univariate analyses indicate that accessions producing yellow-colored seed capsules are significantly taller, flower later and were longer-lived than their brown-colored counterparts were. This finding supports simultaneous assertions that the yellow-colored-capsule varieties were domesticated for use as a fiber crop. Thirty-four conserved ortholog set (COS) markers were developed *in silico* to evaluate genetic variation in *A. theophrasti*. Eight primer pairs failed to amplify *A. theophrasti* genomic DNA, 15 yielded products with multiple bands, and 11 produced single bands. One primer pair gave a polymorphic product that was detected consistently. Putative single nucleotide polymorphisms (SNPs) were associated with unique Type II restriction enzyme cleavage sites. Continuing work in our lab will be aimed at verifying the

candidate SNPs, developing and screening additional primers, and characterizing the genetic diversity among *A. theophrasti* populations in order to make inferences about allele frequency, mating system, genetic drift, and natural selection.

Dedicated to the memories of
Bernard and Ida Sosnoskie,
and Mary Lewis Lark

ACKNOWLEDGMENTS

I wish to thank:

My advisor, John Cardina, for his guidance, support, and friendship during my graduate program.

My student advisory committee members: Doug Doohan, Dave Francis and Charles Goebel, for their insight, instruction, and patience.

Bert Bishop and Larry Madden for their assistance with the statistical analyses.

Cathy Herms for being my sounding board, spellchecker, psychiatrist, and friend.

The weed ecology lab and former summer students: Tim, Joel, Paul, Brian, Karen, Rodrigo, Mark, Kerry, Sarah, Amanda, Jason, Sajal, Ben, Matt, Jessica, and Sarah, for plating seeds, counting weeds and processing soil cores.

The greenhouse and field crews and OARDC library staff.

My friends and families, especially all of the Sosnoskies, Varanos, and LaForests.

Charles and Patricia Sosnoskie, my parents, who always believed that I'd finish school….someday.

My fiancé, Joe LaForest. You will always be the best thing about Ohio State.

VITA

December 8, 1972	Born – Shamokin, Pennsylvania
1994	B.S. Biology, Lebanon Valley College
2000	M.S. Plant Science, University of Delaware
1994	Horticulture Intern, Hershey Nursery
1994-1995	Arboriculture Intern, Longwood Gardens
1995	Horticulture Intern, Mt. Cuba Center for the Study of Piedmont Flora
1995-1996	Quality Control Supervisor, Conard Pyle Nurseries
1996-1997	Horticulturist, Tyler Arboretum
1997-1999	Graduate Research Assistant University of Delaware
1999 – present	Graduate Research Assistant The Ohio State University
2000 – 2001	Vice-Chair - Graduate Student Committee American Phytopathological Society
2001 – 2002	Chair - Graduate Student Committee American Phytopathological Society
2002 – 2003	Member – Council of Graduate Students The Ohio State University
2002 – 2003	Member – University Senate The Ohio State University

2003 – 2004 .....Chair – Public Relations Committee
Council of Graduate Students
The Ohio State University

2003 – 2004 .....Member – Search Committee
Executive Vice-President for Research
The Ohio State University

PUBLICATIONS

Sosnoskie, L. M., J. Cardina, S. Sthapit, D. Francis, and A.R. Miller. 2004. Morphological and phenological variation in world collections of velvetleaf (*A. theophrasti*). HortScience 39:753.

Sosnoskie, L. M., J. Cardina, C. P. Herms. 2004. Weed seedbank community composition in a 35-year-old tillage and rotation study. HortScience 39:845.

Cardina, J., L. M. Sosnoskie, and C.P. Herms. 2003. Seedbank changes following the adoption of glyphosate-tolerant crops. Aspects of Applied Biology, Seedbanks: Determination, Dynamics and Management. 69:77-82.

FIELDS OF STUDY

Major Field: Horticulture and Crop Science
Minor Field: Weed Ecology

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CHAPTER 1

INTRODUCTION

Weedy and invasive plants are a serious economic and environmental problem in natural areas and agroecosystems. The relationship among weeds, crops, native flora and fauna, and the environment are complex and continually changing. An increased understanding of weedy and invasive plant biology and ecology is required to develop more effective management strategies that are compatible with sustainable agriculture and environmentally responsible natural area maintenance (Wyse 1992, Abernathy and Bridges 1994, Norris 1997, Hall *et al.* 2000). The Weed Science Society of America (WSSA) developed an inventory of research needs comparable to those established by the United States Department of Agriculture (USDA) sponsored Coalition for Research on Plant Systems '99 (CROPS) workshop (Hall *et al.* 2000). Priorities based on this inventory include: basic research to increase the body of knowledge regarding the biology and ecology of invasive alien weed species, investigations into species shifts in the aboveground and weed seedbank communities in response to agricultural disturbance, and using molecular biology and genomics techniques to address questions relating to genetic variation and phenotypic plasticity as they relate to weedy characteristics (Hall *et al.* 2000). The research presented in this dissertation contributes to the subject areas of seed dormancy in an invasive species (*Alliaria petiolata* (Cavara)

Bieb. and Grande), community ecology and weed species shifts in response to tillage, rotation and genetically-modified crops, and variation in an economically damaging agronomic weed (*Abutilon theophrasti* Medicus).

Specifically, the studies address the following questions:

Chapter 2 – Can we break dormancy and induce germination in *A. petiolata* seeds using chemical and mechanical scarification techniques in conjunction with gibberellic acid? The ultimate goal of this study was to develop a quick and easy germination protocol to facilitate laboratory and greenhouse research with *A. petiolata*, the seeds of which have a strong physiological dormancy.

Chapter 3 – Do diversity, density, and composition of weed species vary with respect to tillage and rotation? The goal of this research was to describe community composition and structure of the weed seedbank in the Triplett-VanDoren No-Tillage Experimental Plots, which have been managed with varied cultural and chemical strategies since 1963.

Chapter 4 – Do diversity, density, and composition of weed species vary with respect to tillage and rotation three to six years after adoption of glyphosate-tolerant crops? The goal of this study was to establish a baseline for monitoring future potential shifts in weed and weed seedbank communities in response to exclusive use of glyphosate in a long-term tillage and rotation system.

Chapter 5 – Do populations of *A. theophrasti* from around the world vary morphologically, phenologically, and genetically? The goal of this study was to describe the total phenetic variation present in 77 *A. theophrasti* accessions, to determine if crop and weedy biotypes differ phenotypically, and to develop genetic tools for use in population genetic analysis.

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CHAPTER 2

CHEMICAL AND PHYSICAL TREATENTS FOR BREAKING DORMANCY AND INDUCING GERMINATIONTION IN *ALLIARIA PETIOLATA* (BIEB) CAVARA AND GRANDE SEEDS UNDER LABORATORY CONDITIONS

Lynn M. Sosnoskie and John Cardina

INTRODUCTION

Biological invasions account for greater losses in biodiversity worldwide than do climate change, increased atmospheric CO_2, and declining ozone (Randall 1996). *Alliaria petiolata* (Bieb) Cavara and Grande (Garlic Mustard, Brassicaceae), a naturalized European biennial, is widely recognized as a serious threat to the structure and function of forested ecosystems in North America (http://www.invasivespecies.gov/profiles/ main.shtml, http://nature.org/wherewework/northamerica/stats/ohio/science/art6279.html, http://www.dnr.state.oh.us/wildlife/resources/mgtplans/invasives.htm). It is believed that *A. petiolata* was introduced, deliberately, to North America by European settlers for culinary and medicinal purposes (Cavers *et al.* 1979, Nuzzo 1993). Currently, the species has been identified in 34 U.S. states and four Canadian provinces ranging from New England and Ontario south to Georgia, Tennessee, and Arkansas; the

area of infestation extends from the eastern seaboard across the great plains to Oregon and British Colombia (Nuzzo 1993, http://tncweeds.ucdavis.edu/alert/alrtalli.html).

Anderson *et al.* (1996) reported that the density and diversity of native herbs declined in sites where *A. petiolata* was the most prevalent species, implying that *A. petiolata* is a superior competitor in wooded habitats. Meekins and McCarthy (1999) demonstrated that *A. petiolata* reduced the aboveground biomass accumulation of *Quercus prinus* seedlings, suggesting that *A. petiolata* may be capable of interfering with oak forest regeneration. Root exudates from *A. petiolata* can also inhibit the germination of *Gigaspora rosea* spores, potentially preventing indigenous forest species from forming mycorrhizal associations (Roberts 1997, cited in Dhillion and Anderson 1999). White-tailed deer (*Odocoileus virginianus*) preferentially consume native species over *A. petiolata*, creating "holes," which *A. petiolata* can colonize (http://tncweeds.ucdavis.edu/esadocs/documents/allipet.html).

Garlic mustard seeds are well-developed, but physiologically dormant, at maturity requiring a period of cold/moist stratification before germination occurs (Klykken 1937, cited in Cavers *et al.* 1979, Murley 1951, cited in Cavers *et al.* 1979, Lhotská 1975, Baskin and Baskin 1992). In order to perform laboratory- or greenhouse-based studies, researchers have either: (1) stratified *A. petiolata* seeds on moist filter paper, at 4 to 5 °C, for a minimum of 90 to 105 days (Meekins and McCarthy 1999, Prati and Bossdorf 2004) or (2) harvested naturally germinated *A. petiolata* seedlings (Dhillion and Anderson 1994).

Hydrogen peroxide (H_2O_2), sulfuric acid (H_2SO_4), and mechanical scarification have been reported to alleviate both physical and some forms of physiological

dormancy in a variety of plant species (McDonald and Khan 1977, Hsiao and Quick 1984, Chen and Lin 1994, Kindinger 1994, Naredo *et al.* 1998, Akinola *et al.* 2000, Boscagli and Sette 2001, El-Siddig *et al.* 2001, Katzman *et al.* 2001, Ogawa and Iwabuchi 2001, Baskin and Baskin 2004). The goal of this study was to determine if H_2O_2, H_2SO_4, and mechanical scarification could be used in combination with gibberellin A_3 (GA_3), as a substitute for cold/moist stratification, to alleviate dormancy and induce rapid germination in *A. petiolata* seeds.

MATERIALS AND METHODS

Seed source:

Alliaria petiolata seeds used in this investigation were harvested in July, 2000 from the Wooster Memorial Park (82° 1.91' W and 40° 48.89' N), Wooster, OH. Seeds were collected in paper bags, air-dried for one week on greenhouse benches, and stored at room temperature (23 °C) in brown paper envelopes until required.

Seed mass and seed size:

Ten replicates of 50 seeds each were weighed to determine dry seed mass, transferred to 9-cm (diameter) plastic Petri dishes containing 25% rag, steel blue blotter paper (Anchor Paper Co., St Paul, MN) saturated with 5-ml deionized water, and incubated at 22 °C for 24-hr. After 24-hr, each replicate was blotted dry to remove excess moisture and re-weighed to establish post-imbibition mass. Additionally, 50 seeds were scanned at 600 dpi, using a Hewlett Packard C8500A flatbed scanner to

estimate pre-imbibition seed size. Length, width, and area were calculated, for each seed, using the SeedClassifier software developed at The Ohio State University for the characterization and classification of *Ambrosia trifida* L. seeds (Sako *et al.* 2001). The 50-seed sample was incubated at 22 °C for 24-hr in deionized water, and then re-scanned to assess changes in seed size with respect to water uptake. Seed mass, length, width, and area measurements were natural-log transformed to improve normality. Differences between dry and imbibed seeds with respect to mass, size, and area, were evaluated with paired t-tests (Minitab, Release 13.1).

Effect of H_2O_2, H_2SO_4, mechanical scarification and GA_3 on seed germination:

Five-gram samples of *Alliaria petiolata* seeds were subjected to each of the following treatments: (1) immersion in 50-ml 3% (v/v) H_2O_2 for 24-hr with constant shaking, (2) immersion in 50-ml 3% (v/v) H_2O_2 for 48-hr with constant shaking, (3) immersion in 200-ml concentrated (95-97%) H_2SO_4 for 1-min, (4) immersion in 200-ml concentrated H_2SO_4 for 5-min, (5) scarified for 1-sec in a mechanical, sandpaper-lined tumbler, (6) scarified for 3-sec in a mechanical, sandpaper-lined tumbler, and (7) no chemical or mechanical scarification (untreated control). Following each treatment, seeds were rinsed for 10-min with running deionized water and blotted dry.

Treated and control seeds were immediately placed in 9-cm plastic Petri dishes on blue blotter paper saturated with either 5-ml 10^{-3} M GA_3 solution (pH 3.7; Alfa Aesar, Ward Hill, MA) or 5-ml deionized water (pH 7.5). Six replicates of 35 seeds per dish were observed for each treatment-by-germination solution combination under two alternating temperature regimens (12-hr/12hr) of 20/10 °C and 15/6 °C for 35-days.

Fluorescent light was applied during the warm periods. Dishes were arranged in a completely randomized design within the germinators (Hoffman Manufacturing, Albany, OR). The temperature cycles used in the experiment were chosen based on the work of Baskin and Baskin (1992) who reported that 20/10 °C and 15/6 °C were the most favorable thermoperiods for germination of cold-stratified *A. petiolata* seeds. Gibberellic acid and water solutions were refreshed as needed.

Germinated seeds were counted and removed weekly. Seeds were considered germinated when either a minimum of 2-mm of radicle, or else the cotyledons, protruded beyond the seed coat. Results were reported as cumulative percent germination and arcsine transformed prior to analysis to improve normality and homogeneity of variance. The effect of germination solution on cumulative percent germination for treatment within temperature, was evaluated using t-tests when the data could be normalized, and non-parametric sign tests when one member of a paired comparison had a mean and standard deviation equal to zero (Minitab, release 13.1). The combined effect of treatment and germination solution on cumulative percent germination was evaluated using one-way analysis of variance (ANOVA) (SAS, version 9.1). Means were separated by least significant differences.

Scanning electron microscopy to determine the effects of chemical and mechanical scarification on seed coat structure:

Air-dried control and treated seeds were fixed to aluminum specimen mounts using carbon coated adhesive pads, coated with platinum, and observed and photographed using a Hitachi S3500N variable pressure scanning electron microscope.

Tetrazolium testing:

Seedlot viability was determined using standard tetrazolium testing protocols. Three replicates of 25 intact seeds and three replicates of 25 seeds nicked with a razor blade were placed on blue blotter papers in 9-cm plastic Petri dishes saturated with 5-ml 1% (w/v) 2,3,5-triphenyl tetrazolium chloride (Fisher Scientific, Fair Lawn, NJ). The dishes were incubated in the dark at 23 °C for 24-hrs. At the end of the incubation period, seeds were sliced longitudinally, through the embryo, to detect the formazan product. Seed halves that were colorless were incubated for an additional 6-hr and re-evaluated.

RESULTS AND DISCUSSION

Seed mass and seed size:

Mean seed mass, length, width and area increased after 24-hr imbibition in H_2O, by 25-, 37-, 16- and 34%, respectively ($P \leq 0.01$) (Table 2.1). These results are consistent with Kokron (unpublished, cited in Cavers *et al.* 1979) who demonstrated that freshly dormant *A. petiolata* seeds imbibe water, nearly doubling in mass. Both studies are in disagreement with Klykken (1937, cited in Cavers *et al.* 1979) who reported that the seed coat of *A. petiolata* is relatively impervious to water.

Effects of H_2O_2, H_2SO_4, mechanical scarification, and GA_3 seed germination:

Non-scarified (control) seeds failed to germinate within the 35-day observation period, regardless of temperature and solution, suggesting that the release of dormancy

and the initiation of germination cannot be achieved with exogenous applications of water or GA_3, alone (Table 2.2). Generally, chemically and physically scarified *A. petiolata* seeds did not germinate unless treated with GA_3 (Table 2.2). Only seeds treated for 24- or 48-hr with 3% H_2O_2 germinated in deionized water (7-9%) (Table 2.2). This suggests that H_2O_2 reduced the germination requirement for GA_3, possibly through the direct physiological stimulation of the embryo. It has been suggested that H_2O_2 induces seed germination by (1) activating the oxidative pentose phospate pathway, (2) oxidizing germination inhibitors, and/or (3) modifications to the seed coat and associated membranes (Hsiao and Quick 1984, Ogawa and Iwabuchi 2001). Mean cumulative germination percentages were higher for H_2SO_4 (66-77 %) and physically (30-82 %) scarified seeds than for H_2O_2 (30-43 %) treated seeds, in GA_3 for both thermoperiods (Table 2.2). Immersion in H_2SO_4 for 5 min and mechanical scarification for 3 sec were the best treatments overall for breaking dormancy and initiating germination at both temperature cycles ($P \leq 0.05$) (Table 2.2). Differences between thermoperiods were not evaluated.

Effects of H_2O_2, H_2SO_4, and mechanical scarification on seed coat structure:

Scanning electron micrographs revealed that the H_2O_2, H_2SO_4, and mechanical scarification treatments damaged the *A. petiolata* seed coat by creating fissures and abrasions or by corroding and removing the outermost layer of the seed coat (Figures 2.1, 2.2, 2.3, and 2.4). Treatment with H_2O_2 usually resulted in the formation of an embryo-exposing crack that extended along the longest axis of the seed (Figure 2.2). Chen and Lin (1994) and Duval and NeSmith (2000) reported similar observations for

H_2O_2-treated *Cinnamomum camphora* L. and *Citrullus lanatus* (Thunb.) Matsum and Nakai 'Genesis' seeds, respectively. In addition, H_2O_2 treated seeds were bleached slightly, suggesting that pigmented molecules, of unknown chemistry and biological functionality, had been leached from the seed coat or been chemically modified. Sulfuric acid dissolved the outermost layers of the seed coat, whereas mechanical scarification either ripped or the removed the seed coat, often exposing the embryo, at the longitudinal poles (Figures 2.3 and 2.4). For the most part, bacteria and fungi parasitized non-germinated chemically and physically scarified seeds, suggesting that resistance mechanisms were jeopardized during the course of treatment. Many intact (control) seed coats were also covered with fungal mycelium, although it is not known to what extent the interior of the seeds had been penetrated.

Tetrazolium testing:

Formazan is an insoluble, red-colored pigment generated when 2,3,5-triphenyl tetrazolium chloride reacts with hydrogen ions produced by dehydrogenase activity in living cells. Only scarified seeds exhibited formazan production after the 24-hr incubation period; formazan was not detected in seeds with intact coats. Embryos excised from intact seeds were able to produce formazan when returned to a 1% tetrazolium chloride solution for 6-hr. *Alliaria petiolata* seed viability, as determined by tetrazolium staining, ranged from 70 to 100%, with a mean viability of 83%.

Conclusions:

According to Baskin and Baskin (1992), *A. petiolata* seeds possess a form of physiological dormancy that is overcome naturally by a period of cold (~4 °C) moist stratification for a minimum of 90 to 105 days. Cold/moist stratification induces the degradation of abscisic acid and promotes the mobilization of embryo-derived gibberellins. Gibberellic acids stimulate cell wall hydrolases to convert starch into sugar, which fuels cellular respiration and embryo growth (McDonald and Khan 1975, Baskin and Baskin 2004).

Alliaria petiolata seeds failed to germinate unless GA_3 was used in concert with treatments that physically damaged the seed coat. Imbibition data indicates that water is able to enter the seed coat unimpeded. This suggests that intact coats may serve as a chemical and/or physical barrier to the diffusion of exogenous gibberellins. Tetrazolium tests support this hypothesis. Intact *A. petiolata* seeds failed to produce formazan when imbibed in a 1% 2,3,5-triphenyl tetrazolium chloride solution. Both GA_3 and 2,3,5-triphenyl tetrazolium chloride are large (346.38 MW and 334.81 MW, respectively), acidic molecules and are expected to have comparable diffusion properties. Scanning electron micrographs reveal that H_2O_2, H_2SO_4, and mechanical scarification alter the integrity of the *A. petiolata* seed coat; these modifications may facilitate the movement of large biomolecules into the seed interior. Similar results were reported by McDonald and Khan (1977) who showed that Indian ricegrass (*Oryzopsis hymenoides* (Roem. & Shult.) Ricker) seed coats inhibited the diffusion of labeled GA_3 and 2,3,5-triphenyl tetrazolium chloride into the embryo. Results from that study showed that exogenous

applications of GA_3 were insufficient to stimulate germination unless seed coats were breached chemically or mechanically.

The results presented here detail only the most successful treatments for breaking dormancy and inducing germination in *A. petiolata*. Unsuccessful treatments included: immersion in KNO_3; bleaching with sodium hypochlorite, 2-, 4-, 12- hour immersions in 1%, 3%, and 30% H_2O_2, 5-sec mechanical scarification; 10-min treatments in concentrated sulfuric acid; and incubation at constant 4 °C and 23 °C.

Laboratory and greenhouse experiments with *A. petiolata* are more complicated to plan and execute because of the strong physiological dormancy of the seeds. A minimum of 90 days cold-stratification is required before experiments can be initiated on whole plants. To overcome dormancy and stimulate germination, without relying on cold-moist stratification, *A. petiolata* seeds must be (1) chemically or mechanically scarified and (2) treated with exogenous gibberellins. Data from this study indicate that 3% H_2O_2, concentrated H_2SO_4, and mechanical scarification, in concert with GA_3, can be used to alleviate seed dormancy and induce germination in *A. petiolata* seeds within 35 days.

Trait	Pre-imbibition	Post-imbibition	*P*-value	Percent (%) change
50-seed Mass (g)	0.175 (± 0.002)	0.219 (± 0.003)	≤ 0.05	25
Length (mm)	3.11 (± 0.04)	3.56 (± 0.04)	≤ 0.05	37
Width (mm)	1.21 (± 0.05)	1.41 (± 0.06)	≤ 0.05	16
Area (mm^2)	2.85 (± 0.05)	3.82 (± 0.06)	≤ 0.05	34

Table 2.1: Mean mass, length, width, and area (± 1SE), and *P*-values for tests of significance, of *A. petiolata* seeds before and after a 24-hr imbibition period in deionized water. Seeds were imbibed as described in the Materials and Methods. Values for mass are the means of ten replicates of 50 seeds each. Values for length, width, and area are means of 50 seeds.

	Temperature			
	20/10 °C		15/6 °C	
	Germination solution		Germination solution	
Treatment	GA_3	H_2O	GA_3	H_2O
Untreated	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
24-hr 3% H_2O_2	41.42 c (± 2.83)	7.14 (± 2.42)	31.01 b (± 4.20)	9.05 (± 1.36)
48-hr 3% H_2O_2	36.19 c (± 4.76)	8.57 (±2.21)	30.00 b (± 2.83)	9.05 (± 1.72)
1-min H_2SO_4	66.00 b (± 5.54)	0 (± 0)	66.67 a (± 4.34)	0 (± 0)
5-min H_2SO_4	72.00 ab (± 4.38)	0 (± 0)	77.01 a (± 5.53)	0 (± 0)
1-sec scarification	65.71 b (± 1.95)	0 (± 0)	30.48 b (± 7.83)	0 (± 0)
3-sec scarification	82.38 a (± 1.36)	0 (± 0)	71.43 a (± 2.66)	0 (± 0)

Table 2.2: Mean cumulative percent germination (±1 SE) for 18 treatment-by-germination solution combinations at 20/10 °C and 15/6 °C. Values are the means of six replicates of 35 seeds/dish for each treatment-solution-temperature combination. Mean germination percentages differed significantly with respect to solution within treatments and temperatures as determined by t-tests and non-parametric sign tests. Means followed by the same letter are not different at the $\alpha = 0.05$ level. Water and control treatments were not included in one-way ANOVA.

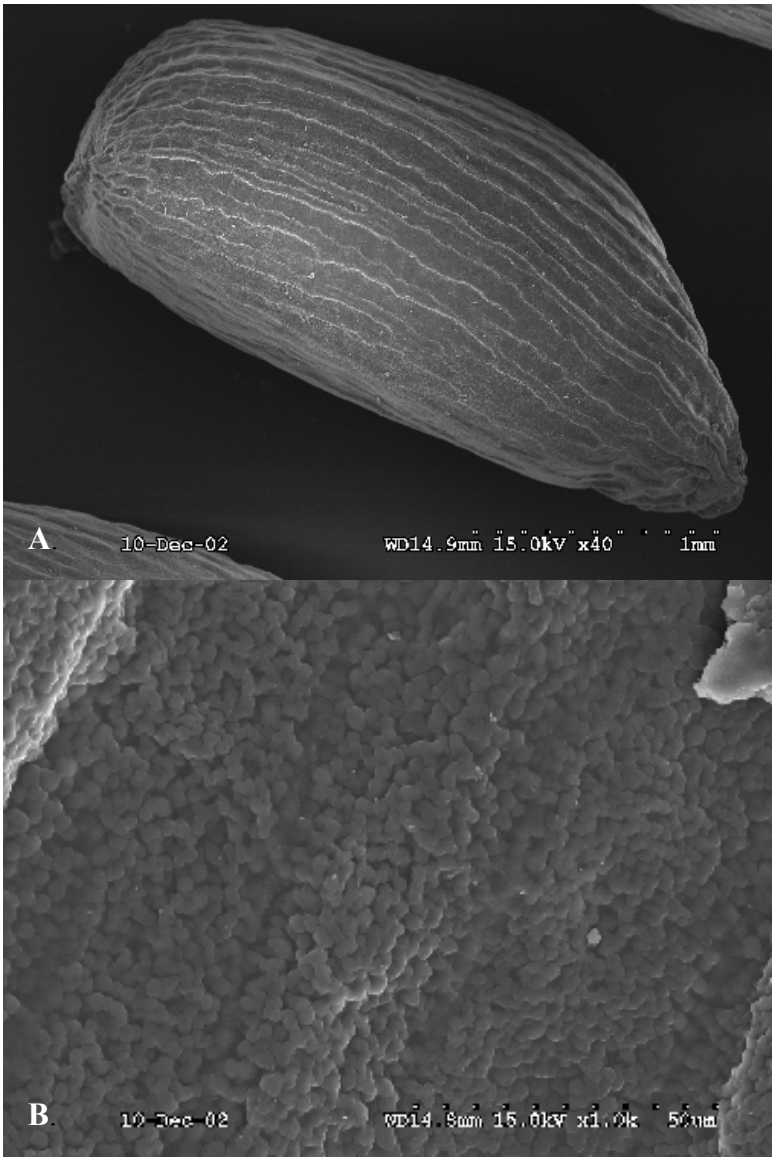

Figure 2.1: Scanning electron micrograph of an untreated (control) *A. petiolata* seed at 40x (A) and 1000x (B) magnification.

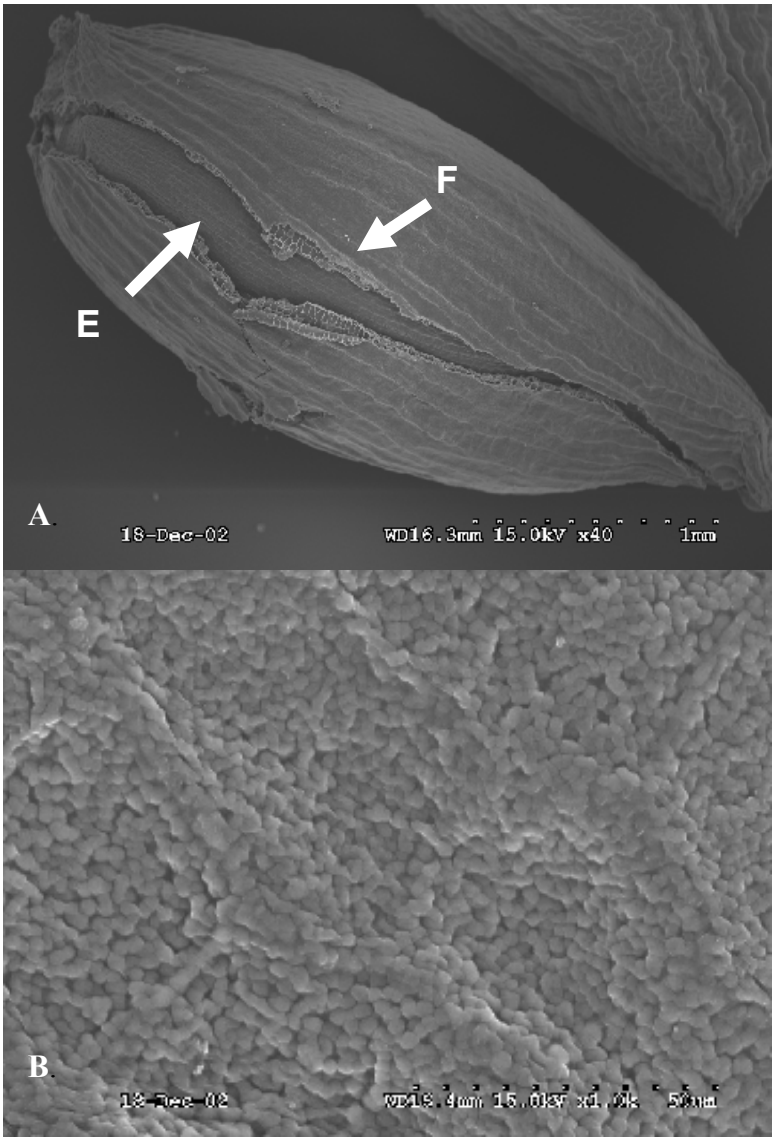

Figure 2.2: Scanning electron micrograph of an *A. petiolata* seed treated with 3% H_2O_2 for 24-hr at 40x (A) and 1000x (B) magnification. Note the H_2O_2-induced fissure (F) that is exposing the embryo (E). Also, note that the surface of the seed coat appears to be physically unchanged by the treatment as compared to the control.

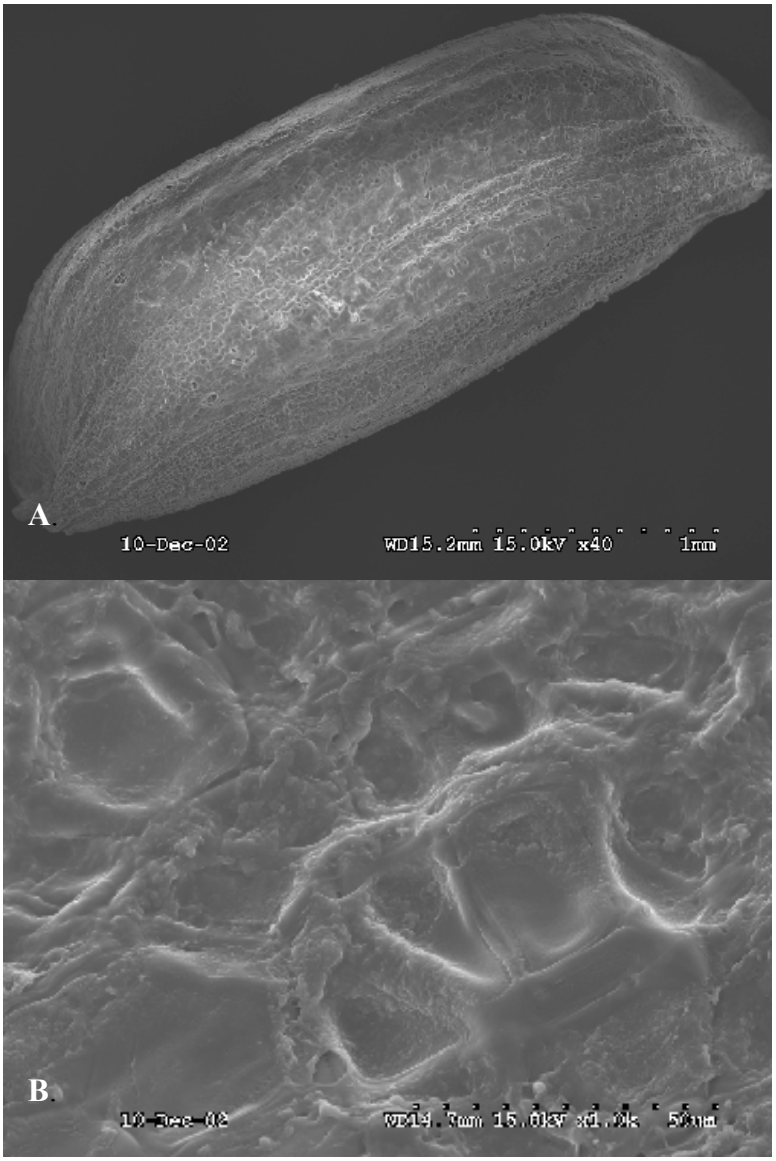

Figure 2.3: Scanning electron micrograph of an *A. petiolata* seed treated with concentrated H_2SO_4 for 3-min at 40x (A) and 1000x (B) magnification. Note that the exterior of the seed coat appears to have been removed by the corrosive activity of H_2SO_4, revealing sub-surface cellular layers.

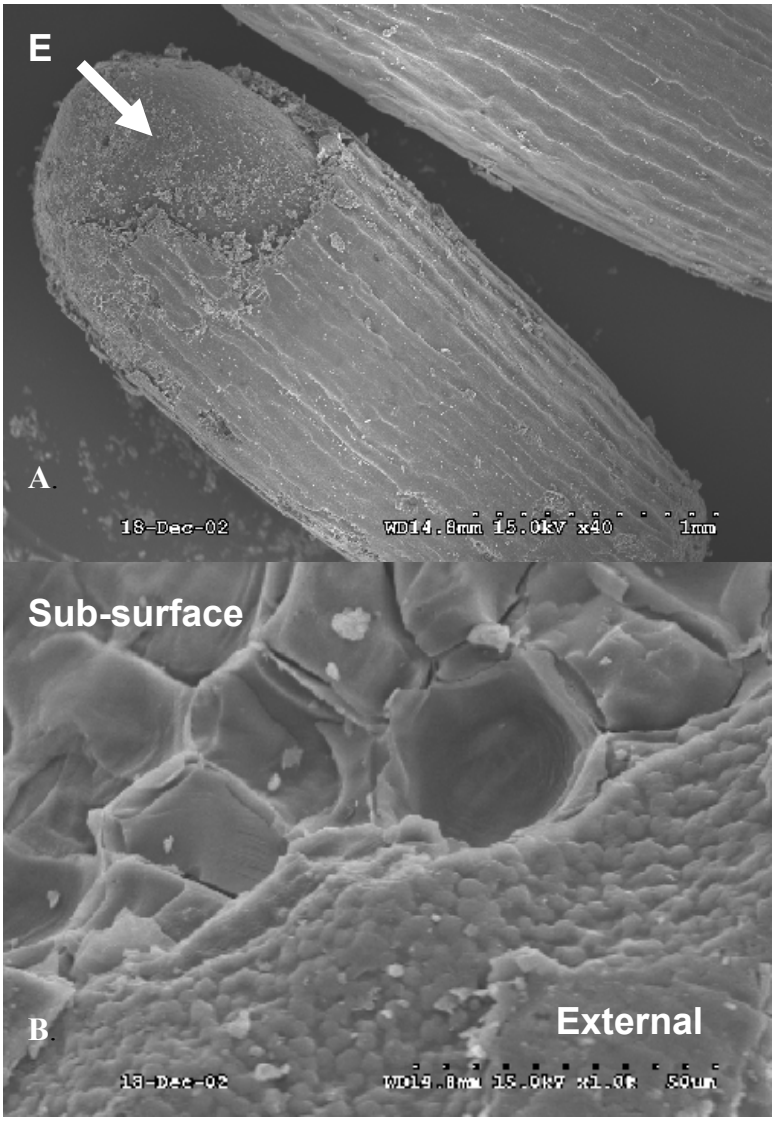

Figure 2.4: Scanning electron micrograph of an *A. petiolata* seed mechanically scarified for 3-min at 40x (A) and 1000x (B) magnification. Note that mechanical scarification abraded the seed coat at the longitudinal poles, exposing the subsurface cellular layers and the embryo (E).

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CHAPTER 3

WEED SEEDBANK COMMUNITY COMPOSITION IN A 35-YEAR-OLD TILLAGE AND ROTATION EXPERIMENT

Lynn M. Sosnoskie, Catherine P. Herms, and John Cardina

INTRODUCTION

For over 50 years, weeds of row-crop agriculture in the United States have been managed using synthetic herbicides in combination with mechanical cultivation. Yet weeds remain a significant problem for farmers. Many of the plant species and biotypes characterized as being 'weedy' in modern crop production were not considered troublesome, and may not even have been present, one century ago (Selby 1897, Runnels and Schaffner 1931).

Weed control efforts have been followed by the rapid selection of individual species or biotypes that require additional control measures. The adoption of reduced-tillage systems resulted in increases in the appearance of herbaceous and woody perennials (Cardina *et al.* 1991, Swanton *et al.* 1993, Zanin *et al.* 1997), to which farmers responded by increasing the number of herbicide applications (Johnson 1994, Aspelin and Grube 1999). Mounting public awareness of the health and environmental hazards associated with intense herbicide use spurred the development of highly

specific, low-rate, low-toxicity herbicides, to which many weeds rapidly developed resistance (Zoschke 1994, Powles and Shaner 2001).

A better understanding of how weed populations and communities change with agricultural practices should allow us to develop improved models for prediction in weed species shifts. Decision-making tools based on these models will, in turn, enable farmers to make more ecologically- and economically-sound weed management decisions. These decisions would consider the impact of present practices on future weed communities, and would aim to delay evolutionary changes in weeds, inhibit the establishment and spread of economically damaging species, and prevent the substitution of one harmful species for another. The adoption of cultural and chemical management practices that yield weed communities that are less damaging to crops and/or are more beneficial to the ecosystem, with respect to nutrient cycling, maintaining floral and faunal diversity, preventing soil erosion, or enhancing populations of favorable microflora, is an important objective of modern weed management (Swift and Anderson 1993, Feldman and Boyle 1998, Spahillari *et al.* 1999, Liebman 2001, Sturz *et al.* 2001, Hyvonen and Salonen 2002, Gerowitt *et al.* 2003). To achieve these goals, information describing how weed communities respond over time to multiple, and interacting, forces will be needed (Zoschke 1994, Booth and Swanton 2002).

Previously published work from the weed ecology lab at The Ohio State University / Ohio State Agricultural Research and Development Center described seed depth distribution and abundance of individual species in an experiment where three tillage systems and three crop sequences have been in place for over 30 years (Cardina *et al.* 2002). Here, we report results on the diversity and composition of the weed

seedbank community in the same long-term experiment. Specifically, we address how total weed seed density and weed species diversity vary with management system, and how tillage system and crop sequence influence the community composition, as a whole, as represented in the germinable seedbank.

MATERIALS AND METHODS

Site description and treatment establishment:

This investigation was conducted in a long-term tillage and rotation experiment (Triplett-VanDoren No-Tillage Experimental Plots) initiated in 1963 in Wooster, Ohio, USA (81º 56' W; 40º 42' N, elev. 310 m). Soil at this site is classified as a fine, mixed, Typic Fragiaqualf (Luvisols) of the Wooster series, which is a deep, gently sloping, moderately to well-drained silt loam soil, with a relatively impermeable fragipan at a depth of 400-750 mm. The soil is comprised of 2.9 % organic matter, 11 % sand, 75 % silt, and 14 % clay. The climate is continental; annual precipitation averages 905 mm. The average minimum (January) and maximum (July) temperatures are -4.8 and 29.2 ºC, respectively.

Individual plots were 5 m wide and 21 m long, and were arranged in a randomized complete block with three replications. The three tillage systems are: no-tillage (NT), minimum-tillage (MT) and conventional-tillage (CT). No-tillage treatments received only the in-row disturbance caused by a coulter-type planter. Minimum-tillage changed from shallow moldboard plowing without subsequent disking (1963-1982), to a single paraplow operation (1983-1984), to a single pass with a chisel

plow (1985-present). Conventional-tillage consists of moldboard plowing to a depth of 20 to 25 cm followed by two 10 cm deep secondary tillage (disking) operations before planting.

The crop sequences are: continuous corn (*Zea mays* L.), corn-soybean (*Glycine max* L.), and corn-oat (*Avena sativa* L.)-hay (alfalfa (*Medicago sativa* L.) + ryegrass (*Lolium perenne* L.)). To avoid confounding with weather conditions, each crop in each sequence appears every year. For plots maintained in the corn-soybean rotation, there are separate plots planted to both corn and soybean in each tillage system. Likewise, there are separate plots planted to corn, oat, and hay, for the three-crop rotation. Because there may be significant within-sequence variation, each crop of each rotation is considered separately in the statistical analyses, i.e. plots presently planted to corn following soybean [designated C(S)] are differentiated from plots currently planted to soybean following corn [S(C)]. Similarly, plots planted to corn following hay and oats [C(OH)], oats following corn and hay [O(HC)], and hay following oats and corn [H(CO)], were analyzed separately. This allows us to identify differences, within rotations, related to crop specific management practices.

In addition to plowing (above), some management variables have been modified over time to conform to accepted agronomic practices. These include crop variety, fertilizer rate, crop density, row spacing, and herbicide type. For example, corn was first planted in rows 102 cm apart, but has been planted in rows 76 cm apart since 1973. Soybean was planted at these same row spacings until 1985, and has since been drilled in rows 18 cm apart. Oat and alfalfa hay crops, planted with conventional grain drills until 1979, have since been planted with no-tillage drills. Every year, corn populations

have been determined and thinned to a common density. Fertilizer was applied following Ohio State University soil test recommendations, and the same rates of N, P, and K have been used in each tillage system. Lime has been broadcast as required to maintain a pH of about 6.0 in the plowed A (Ap) horizon.

Historically, weeds were controlled at the discretion of the farm manager, with the goal of achieving exceptional weed control in all crops. Within a crop and year, the same weed management measures were used in all tillage systems, except that an additional herbicide application was made before planting in the NT system. Initially, amitrole (1*H*-1,2,4-triazol-3-amine) was applied for this purpose, but glyphosate (*N*-[phosphonomethyl]glycine) has been used since 1974, except in 1985 and 1989, when paraquat (1,1'-dimethyl-4,4'-bipyridinium ion) was substituted. Prior to 1985, the MT and CT plots were occasionally cultivated for weed management. No herbicides were applied to the oat or hay crops, except to kill existing vegetation before planting in the NT system. Herbicides and rates of application, in addition to information on cultivars, fertilizer rates, and insecticide rates have been documented (Dick and Van Doren 1985, Dick *et al.* 1986, Dick and Durkalski 1998, Cardina *et al.* 2002).

Sample collection and preparation:

Soil seedbank samples were obtained in the third week of March 1997, 1998, and 1999, before emerging seedlings appeared in the fields. Plots were divided into thirds, and six soil cores, 3.5 cm in diameter and 10 cm deep, were obtained at random from a 3- x 4-m sampling area located in the center of each third (total of 18 cores per plot).

Soil was pooled within sampling areas and was processed by sieving through a 0.64-cm screen to break up large soil clods.

Each composite sample, minus large stones and root fragments, was spread in a 22-cm-square tray and placed on a sand bed. The soil samples were kept moist by daily applications of water to the sand bed. This was necessary to avoid splashing seeds from the trays and to reduce formation of a crust, which can impede seedling emergence. Positions of trays on the benches were re-randomized weekly. The greenhouse was set to day/night temperatures of 18/8 °C to simulate spring field conditions, with no artificial lighting. The direct germination method used here requires significant time and space, but provides a more complete detection of species than do sieving or elutriation methods (Gross and Renner 1989, Cardina and Sparrow 1996).

Data collection:

Emerged weed seedlings were identified, counted, and removed. Seedlings too small to identify were marked with coded sticks or transplanted, and grown until their identity could be assured. Seedlings were counted weekly. After emergence ceased (about 4 months), samples were placed in a 4 °C cold room for 3 weeks to reestablish springtime conditions and break secondary dormancy in the remaining seeds. Soil samples were sieved, and exposed to alternating temperatures (15/4 °C) for one week before being returned to the greenhouse. This process was repeated twice, after which no additional seedlings emerged. In the current study, we did not sieve or elutriate the soil in search of viable, ungerminated seeds, as past research has shown that such

methods do not result in the retrieval of a substantial number of additional seeds (Cardina and Sparrow 1996).

Data analysis:

Total germinable weed seed density for each plot was expressed as the number of seeds/m^2 of field soil to a depth of 10 cm. The diversity measures of species richness (total number of species, S), the Shannon-Weiner index (H'):

$$H' = -\Sigma P_i \, x \, (lnP_i),$$

where P_i = proportion of total sample belonging to i^{th} species, and evenness (J):

$$J = H'/lnS,$$

where H' = the Shannon-Weiner index and S = total number of species, were calculated for each plot from the species density data (PC-ORD version 3.01).

Seed density data were log-transformed prior to any statistical analyses in order to meet the assumptions of ANOVA. Evenness values were transformed prior to analysis using a power transformation:

$$b_y = x^p,$$

where p = 2, to improve normality and homogeneity of variance. Seeds/m^2 and S, J, and H' were compared across the three tillage systems (NT, MT and CT) and six cropping sequences [CC, C(S), S(C), C(OH), O(HC), H(CO)] using the mixed model procedure in SAS (version 9.1). Rotation, tillage, and the rotation-by-tillage interaction were regarded as fixed factors, while block and the block-by-tillage interaction were considered as random. Years were analyzed separately.

A relative importance (RI) index was calculated for each species, for each plot, to obtain a single, synthetic measure:

$$RI = (rD + rF) / 2,$$

that incorporates both relative density (rD), where:

$$rD = \textit{density of weed } x \textit{ / total weed density},$$

and relative frequency (rF), where:

$$rF = \textit{absolute frequency of weed } x \textit{ / total absolute frequency of all weeds},$$

and where absolute frequency is the number of samples with weed *x* / total number of samples. The RI index has been used by other researchers to characterize the composition of weed flora (Derksen *et al.* 1993, Swanton *et al.* 1999). Because the RI index is a proportion, values were arcsine square root-transformed prior to any statistical analyses.

Differences in community composition among treatments, for each year, were examined statistically using the multi response permutation procedure (MRPP). The MRPP is a robust, non-parametric multivariate classification technique that tests the hypothesis that samples representing an a priori defined group cluster in multivariate space based on composition (Zimmerman *et al.* 1984, Biondini *et al.* 1988). Unlike parametric analyses, MRPP does not require variables to meet distributional assumptions such as multivariate normality, linearity, or homogeneity of variances.

We used the Sørensen distance measure (x_i):

$$x_i = 1 - (2w / a+b),$$

where a = species abundance in community *x*, b = species abundance in community *y* and w = shared abundances, and the Mielke weighting factor (C_i):

$$C_i = n_i / \Sigma n_i,$$

where n_i = number of species within a group, to calculate the weighted mean within group index of clumping (δ):

$$\delta = \Sigma C_i x_i,$$

for all comparisons (McCune and Grace 2002). The degree of separation between communities was determined by the T statistic:

$$T = (\text{observed } \delta - \text{expected } \delta) / \text{std. dev. of } \delta.$$

The more distinct the communities, the more negative the value of T (McCune and Grace 2002). The probability of obtaining a value for δ as small or smaller is determined by comparing the observed delta (δ) with a Pearson type III distribution of possible δs, which are derived from sample permutations (McCune and Grace 2002). The MRPP analyses were performed on the transformed RI index values to evaluate the main effects of tillage and rotation on community composition (PC-ORD version 3.01). Alpha levels were adjusted using a Bonferroni correction (α/n, where n = total number of pair-wise comparisons) to account for multiple and simultaneous comparisons needed to evaluate treatment main effects.

Smilar studies have used Canonical Discriminant Analysis (CDA) to distinguish statistically among weed communities developing under diverse agronomic practices (Derksen *et al.* 1993, Bàrberi *et al.* 1998, Swanton *et al.* 1999, Bàrberi and Lo Cascio 2001, Bàrberi and Mazoncini 2001, Shrestha *et al.* 2002, Streit *et al.* 2003). Canonical Discriminant Analysis is a parametric multivariate ordination technique that differentiates two or more distinct groups with respect to a set of predictor variables,

through the generation of discriminating (canonical) functions. Because CDA assumes that the data are multivariate normal, the within group variances are equal, and that linearity exists among all pairs of variables, the technique may not be appropriate for evaluating abundance data (Kenkel *et al.* 2002, McCune and Grace 2002). Because CDA generates treatment means that act similar to position coordinates when graphed in a multi-dimensional space defined by the canonical functions, we chose to use the technique as a tool for data visualization. In the graph, management systems with similar groups of species cluster together, whereas those with dissimilar communities are more widely separated.

Canonical discriminant analyses were performed on the transformed RI values of all species found in greater than 5% of the sampled quadrats, using the CANDISC procedure in SAS (version 9.1) following the approach used by Benoit *et al.* (1992), Derksen *et al.* (1993, 1994), and Bàrberi and Lo Cascio (2001). Because CDA is unable to accommodate data with multiple levels of structure, 18 novel treatment categories were generated by combining the two main experimental factors of tillage and rotation (Table 3.1) (Derksen *et al.* 1994). Treatment means were determined and graphed on axes defined by the first and second canonical functions to visually portray the degree of similarity in species composition among experimental groups.

RESULTS AND DISCUSSION

Seedbank structure:

We identified 35, 42, and 38 weed species in 1997, 1998, and 1999, respectively. Forty-six different species representing 19 families were documented during the three years of the study (Table 3.2). Of the 46, twelve are monocots and 34 are dicots (Table 3.2). Twenty-seven species are classified as annuals, 13 are perennials and six act as annuals/perennials/biennials, depending on local climate (Table 3.2) (Uva *et al.* 1997). Eight species, *Amaranthus retroflexus* L., *Capsella bursa-pastoris* (L.) Medicus, *Chenopodium album* L., *Digitaria sanguinalis* (L.) Scop., *Oxalis stricta* L., *Panicum dichotomiflorum* Michnx., *Setaria faberi* Herrm., and members of the genus *Veronica*, accounted for greater than 75% of the total number of germinated seeds, for all three years (Table 3.2). The total number of germinable seeds counted and identified, across all treatments, for the years 1997, 1998, and 1999 were 3081, 3931 and 2710, respectively.

Seedbank density and diversity:

Analyses of variance showed that the number of weed seeds/m^2 for field soil, to a depth of 10 cm, was influenced by tillage, rotation, and the tillage-by-rotation interaction for all three years (Table 3.3). Mean seed density declined as soil disturbance increased (NT $\geq$ MT > CT) ($P \leq 0.01$) (Table 3.4). Averaged across all three years, plots managed in the NT system contained 5955 seeds/m^2, whereas the MT and CT treatments contained 3080 seeds/m^2 and 1384 seeds/m^2, respectively.

Except for 1998, mean seed density was 1 to 1.5 times greater in the CC rotation than in the C(S) and S(C) plots (Table 3.5). Averaged across tillage and year, the CC sequence contained 4553 seeds/m^2, as compared to 2576 and 3729 seeds/m^2 in the C(S) and S(C) treatments, respectively (Table 3.5) ($P \leq 0.01$). Although the treatments were not statistically distinct, there was a trend towards more germinable seeds/m^2 in the C(OH) treatment, than in the O(HC) and H(CO) rotations. Plots planted to corn following oats and hay [C(OH)], regardless of tillage, averaged 4248 seeds/m^2 over all three years, whereas O(HC) and H(CO) plots averaged 3525 and 2865 seeds/m^2, respectively.

Species richness (S) was influenced by tillage and rotation all three years (Table 3.3) ($P \leq 0.10$). As the intensity of soil disturbance decreased, values for S increased (NT $\geq$ MT > CT) (Table 3.6), with an average across years of seven species per plot occurring where soil was plowed and disked (CT), nine where soil was chisel plowed (MT), and 10 where soil was disturbed only by the coulter units on the planter (NT). The three-crop rotations averaged about 12 species per plot, whereas half that number were found in fields planted to continuous corn or corn-soybean rotations (Table 3.6). Analyses of variance indicated that crop sequence and the tillage-rotation interaction influenced evenness (J) and the Shannon-Weiner index (H'), for all three years (Table 3.3) ($P \leq 0.01$). Generally, values of J and H' for the three-crop rotations were greater than those for the corn monoculture and corn-soybean rotations (Table 3.6).

Because mixed model ANOVA indicated significant interactions between tillage and rotation for seed density for all three years, we graphed the within-year density means for rotation versus tillage to identify the basis for the interactions (data not

shown). The ranking of seed density means for crop sequences within tillage treatments was not consistent over years. For example, in the NT system, seed density was highest in CC in 1997 and 1999, but in 1998, it was highest in the S(C) sequence. The interactions were the result of lower values of J and H' in the NT – CC, MT – CC, and MT – C(S) systems. The biological and ecological significance of these interactions are unknown.

Community composition:

Multi-response permutation procedure analyses indicate that the structure and composition of the seedbank communities assayed in the study were influenced by tillage and rotation (Tables 3.7 and 3.8). Except for NT versus MT in 1998, the germinable weed seed community in NT treatments differed from the communities present in the MT and CT treatments, across all rotations ($P \leq 0.015$) (Table 3.7). *Alpha* (α) values were Bonferroni adjusted (0.05/3) to maintain an overall significance of 0.05. Analyses indicate that the weed seed community in NT was more similar to the weed seed community in the MT than the CT systems (Table 3.7). Except for 1997, weed seed communities in CT and MT plots did not differ significantly from each other ($P \geq 0.015$) (Table 3.7).

Weed seed communities in the C(OH), O(HC) and H(CO) sequences differed from the communities present in the continuous corn and corn-soybean rotations, for all three years ($P \leq 0.003$) (Table 3.8). *Alpha* (α) values were Bonferroni adjusted (0.05/15) to maintain an overall significance of 0.05. The weed seed community observed in the continuous corn sequence did not differ significantly in composition

from the communities recorded for the C(S) or S(C) sequences, excepting the CC versus S(C) comparison in 1997 ($P \geq 0.003$) (Table 3.8). Likewise, C(S) and S(C) treatments did not differ in community composition from each other, for all three years ($P \geq 0.003$). Except for the C(OH) versus O(HC) comparison in 1997, none of the comparisons among the C(OH), O(HC), and H(CO) treatments were significantly different ($P \geq 0.003$).

For all three years, the first canonical function, which represents the maximum degree of variation among the experimental environments, explained between 49 and 61% of the between-treatment versus within-treatment variance. The first two functions combined explained 67 to 71% of the variation observed. Taking into consideration the high level of variation in weed seedbanks, we deemed these percentages to be adequate for using only the first two discriminating functions as axes in the graphs. Previous studies using CDA to analyze the composition of weed seedbanks in agricultural settings have reported that the first two canonical functions explained about 70% of total relative variation (Benoit *et al.* 1992, Barberi and Lo Cascio 2001).

Plots of treatment means for the first and second canonical functions against axes defined by the first two functions indicated that community composition of the weed seedbank differed among the management treatments (Figures 3.1, 3.2, and 3.3). The first canonical axis was more strongly associated with crop sequence than with tillage system for all three years. The three-crop systems clustered at the positive end of the first axis, while most of the one- and two-crop systems aggregated at the negative pole. The obvious separation of the C(OH), O(HC), and H(CO) treatments from the CC, C(S), and S(C) rotations suggests that the three-crop sequence, regardless of tillage system,

consisted of a weed community that was distinguishable from the other sequences. The second canonical axis appeared to be associated weakly with tillage. Typically, NT treatments were positioned at the positive end of the second axis, whereas CT and MT treatments were located at the negative end (Figures 3.1, 3.2, and 3.3).

Results indicate that the density, diversity, and composition of weed communities differ with respect to tillage and rotation. Weed communities change in response to tillage, as mechanical disturbance is the primary means by which weed seeds become incorporated into the soil (Yenish *et al.* 1992). Anderson *et al.* (1998), Feldman *et al.* (1997), and Bàrberi and Lo Cascio (2001) reported an increase in the number of aboveground weeds and germinable weed seeds in the surface soil layer with reduced tillage intensity. Feldman *et al.* (1997) suggested that greater numbers of weed seeds resulted from an accumulation of crop residue that prevented the movement of seeds through the soil profile. Seed survival in response to tillage is also influenced by biological factors such as predation, which mostly occurs near the surface, and disease, which is dependent upon inoculum levels and micro-environmental conditions that can be modified by tillage (Stroo *et al.* 1988, Mohler and Callaway 1992). Additionally, higher weed seed densities in NT plots may have occurred in response to reduced herbicide availability because of adsorption to near-surface organic matter (Dick 1983, Isensee and Sadeghi 1994, Sadeghi and Isensee 1996, Dick *et al.* 1997, Shelton *et al.* 1998, Dick and Gregorich 2004).

Through burial and exhumation, seeds are moved to environments that can either facilitate or inhibit their germination, depending on a species' specific light and temperature requirements (Buhler and Mester 1991, Gallagher and Cardina 1998).

Species whose seeds survive, germinate, and emerge near the soil surface increase in reduced tillage systems, whereas species whose seeds depend on burial to break dormancy, protect them from surface predators, or ensure successful establishment tend to increase in tilled systems (Cardina *et al.* 1991, Ball 1992, Yenish *et al.* 1992, Buhler 1995, Feldman *et al.* 1997). The decrease in the number of species with increased intensity of soil disturbance supports the hypothesis that plant community diversity should be low in habitats that are repeatedly and highly disturbed (Huston 1979, Menge and Sutherland 1987). Menalled *et al.* (2001) and Mas and Verdú (2003) reported similar results for both the surface seedbank and the aboveground weed community, respectively.

Crop sequence also influences the composition of weed communities, largely due to varying environments provided by associated cultural practices (Légerè and Samson 1999, Nalewaja 1999, Menalled *et al.* 2001). Selectivity of chemical herbicides used in a cropping system, for example, has caused significant shifts in weed species dominance (Hauser *et al.* 1974, Menges 1987). There is evidence to suggest that crop rotation, independent of herbicides, is important in altering weed communities (Liebman and Dyck 1993). Different crops allow for weeds adapted to different emergence times, sub-canopy light conditions, microbial communities, and allelopathic chemicals to persist (Leroux *et al.* 1996, Menalled *et al.* 2001). The size and composition of the germinable seedbank community has been linked to shifts in the aboveground community in several studies (Cardina and Sparrow 1996, Mulugeta and Stoltenberg 1997, Menalled *et al.* 2001).

The various crops and crop rotations used in our study create diverse environments that differentially influence species emergence, survival, and growth. Weeds that survive in a given crop produce seeds that contribute to the seedbank, from which seedlings are recruited during the next crop in the sequence. The continuous corn sequence, for example, provides a relatively predictable springtime environment where winter annual weeds have been killed by tillage or herbicide to provide an opening for emergence of summer annuals; those individuals that escape weed management efforts are left to produce seeds in late summer. *Chenopodium album, S. faberi*, *A. retroflexus*, and *P. dichotomifolium*, all summer annuals, were a significant component of the C(S), S(C), and CC sequences (Cardina *et al.* 2002).

In contrast, the three-crop rotations require a disturbance (herbicide or tillage) during early spring in the oat year, mid spring in the corn year, and late summer before planting the hay crop. These disturbances provide for a varying habitat that may be suiitable for infestation by different species adapted to germination and emergence at different times of the year. *Oxalis stricta*, *Veronica* spp*., L. purpureum, C. bursa-pastoris*, *E. annuus* and *S. oleraceus* possessed higher relative importance values in the three-crop rotation as compared to the corn monoculture and corn-soybean sequences. These species are diverse biologically and morphologically: *O. stricta* is a summer germinating perennial, *Veronica* spp. are mat-forming winter annuals, *L. purpureum* is an erect winter annual, C. *bursa-pastoris* is a rosette-forming winter annual that also germinates in spring in Ohio, and *E. annuus* and *S. oleraceus* are summer annuals.

The composition of species at a location is the result of climatic conditions and physical, chemical, and biological disturbances acting at a site (Diaz *et al.* 1999). These

effects are hierarchical; long-term climatic conditions determine the taxonomic pool from which communities develop while local perturbations influence the species that become dominant within an ecosystem (Légère and Samson 1999). Numerous studies have reported that site is the most important factor determining weed community composition (Derksen *et al.* 1993, Andersson and Milberg 1998, Squire *et al.* 2000, Cardina *et al.* 2002). Because selective forces act on traits, the presence or absence of a species from a normally suitable habitat is related to the attributes that it does or does not possess (Duckworth *et al.* 2000). If the attributes selected for by a particular management practice remain constant across environments, it should be feasible to develop models to predict weed species shifts in response to chemical and cultural agricultural practices.

Zanin *et al.* (1997) classified weed communities according to life-form, periodicity type, dispersal type, and seed longevity with the aim of linking disturbance regimes with specific biological attributes. They reported that herbaceous perennials and woody shrubs increased in reduced tillage systems, whereas wind-dispersed species were more prevalent where the soil was regularly disturbed (Zanin *et al.* 1997). Légère and Samson (1999) observed that annual dicots were most abundant where weed management intensity was at a minimum. Kleyer (1999) studied the distribution of plant biological characteristics along a disturbance gradient in an agricultural landscape and noted that annual forbs were the dominant life form when disturbance was most intense, whereas perennials were favored in areas where disturbance was minimal. It remains uncertain which morphological, physiological, and phenological properties are being

selected for in each of our treatment systems. The degree of intraspecific variation among populations growing in the different tillage and rotation systems is also unknown.

Implications for weed management:

After years of varying crop sequence, the resulting seedbank community likely reflects the historical balance between opportunities for successful weed emergence, establishment, and seed production, as well as seed and seedling mortality (Martin and Felton 1993, Dorado *et al.* 1999). Effects of crop rotations and tillage systems on soil characteristics and crop productivity require several years to stabilize (Dick and Daniel 1987), and the same is likely true for weed communities. It has been suggested that increased weed species diversity is beneficial to an agroecosystem if the resident species are providing some benefit such as facilitating nutrient cycling or supporting faunal diversity, *etc*, without affecting yield (Swift and Anderson 1993, Feldman and Boyle 1998, Sturz *et al.* 2001). However, the benefits of increased diversity would be diminished if a concomitant increase in the number of weeds resulted in reductions in the quantity and quality of crop yield.

We conclude that the design of crop, soil, and weed management systems can influence the size and composition of the weed seedbank. Understanding how preceding agricultural practices have influenced the constitution of the present-day seedbank may allow us to predict future problems in weed management, and perhaps may enable us to selectively favor weed communities beneficial to agroecosystems (Zanin *et al.* 1997, Anderson *et al.* 1998, Bàrberi *et al.* 1998, Swanton *et al.* 1999, Bàrberi and Lo Cascio

2001, Liebman 2001, Menalled *et al.* 2001, Tuesca *et al.* 2001, Torresen and Skuterud 2002).

Tillage	Rotation	Abbreviation
Conventional-tillage	Continuous corn	CT-CC
Conventional-tillage	Corn-soybean	CT-C(S)
Conventional-tillage	Soybean-corn	CT-S(C)
Conventional-tillage	Corn-oat-hay	CT-C(OH)
Conventional-tillage	Oat-hay-corn	CT-O(HC)
Conventional-tillage	Hay-corn-oat	CT-H(CO)
Minimum-tillage	Continuous corn	MT-CC
Minimum-tillage	Corn-soybean	MT-C(S)
Minimum-tillage	Soybean-corn	MT-S(C)
Minimum-tillage	Corn-oat-hay	MT-C(OH)
Minimum-tillage	Oat-hay-corn	MT-O(HC)
Minimum-tillage	Hay-corn-oat	MT-H(CO)
No-tillage	Continuous corn	NT-CC
No-tillage	Corn-soybean	NT-C(S)
No-tillage	Soybean-corn	NT-S(C)
No-tillage	Corn-oat-hay	NT-C(OH)
No-tillage	Oat-hay-corn	NT-O(HC)
No-tillage	Hay-corn-oat	NT-H(CO)

Table 3.1: Abbreviations for the 18 novel treatments, generated from a factorial combination of the tillage and rotation variables, used in canonical discriminant analysis (CDA).

Latin name	Bayer code	Monocot/ Dicot	Life History	1997	1998	1999
Abutilon theophrasti Medicus	ABUTH	D	SA	-	0.07	0.11
Acalypha viginica L.	ACCVI	D	SA	-	0.07	0.07
Amaranthus retroflexus L.	AMARE	D	SA	4.77	4.12	4.53
Anagallis arvensis L.	ANGAR	D	W/S	-	0.03	-
Capsella bursa-pastoris (L.) Medicus	CAPBP	D	WA	0.16	3.61	4.80
Cardamine hirsute L.	CARHI	D	W/S	0.06	0.20	0.74
Chenopodium album L.	CHEAL	D	SA	27.00	18.14	36.02
Cirsium arvense (L.) Scop.	CIRAR	D	P	-	0.03	-
Conyza canadensis (L.) Cronq.	ERICA	D	W/S	0.07	0.46	0.07
Cyperus esculentus L.	CYPES	M	P	0.10	0.08	0.07
Digitaria sanguinalis (L.) Scop.	DIGSA	M	SA	3.79	4.12	3.14
Echinochloa crus-galli (L.) Beauv.	ECHCG	M	SA	0.62	0.23	-
Elytrigia repens (L.) Nevski	AGRRE	M	P	0.22	0.08	0.04
Erechtites hieracifolia (L.) Raf. Ex DC.	ERECHI	D	SA	0.03	-	0.04
Erigeron annuua (L.) Pers.	ERIAN	D	W/S	5.32	0.08	0.07
Euphorbia maculate L.	EPHMA	D	SA	0.03	0.05	0.04
Festuca arundinacea Schreb.	FESAR	M	P	0.10	0.03	-
Gleochoma hederacea L.	GLEHE	D	P	-	0.79	-
Lactuca serriola L.	LACSE	D	W/S/B	0.55	0.03	0.11
Lamium amplexicaule L.	LAMAM	D	WA	0.84	0.69	0.26
Lamium pupureum L.	LAMPU	D	WA	-	-	4.13
Lobelia inflata L.	LOBIN	D	S/B	-	0.05	0.15

Continued

Table 3.2: Relative density of weed species (as percentage of total number of seeds) occurring in the sampled springtime seedbank of a 35-year-old tillage and rotation study in Wooster, OH, for the years 1997, 1998 and 1999. Latin binomials and Bayer codes conform to WSSA Composite List of Weeds (1989). A "–" indicates that the species was not present in a given year. Monocot/dicot classifications and life history designations are according to Uva *et al.* (1997).

Table 3.2 (continued)

Malva neglecta Wallar.	MALNE	D	W/S/B	0.03	0.10	0.04
Muhlenbergia schreberi J.F.Gmel.	MUHSC	M	P	0.78	0.69	0.70
Oxalis stricta L.	OXAST	D	SA/P	21.07	15.31	16.35
Panicum capillare L.	PANCA	M	SA	1.42	2.24	1.44
Panicum dichotomiflorum Michnx.	PANDI	M	SA	2.43	14.65	6.50
Plantago major L.	PLAMA	D	P	0.16	1.86	2.44
Poa annua L.	POAAN	M	WA	1.55	0.78	3.03
Polygonum arviculare L.	POLAV	D	SA	.032	-	-
Polygonum pennsylvanicum L.	POLPY	D	SA	2.33	0.71	1.03
Portulaca oleracea L.	POROL	D	SA	0.32	0.28	0.89
Rumex acetosella L.	RUMAA	D	P	0.06	0.03	-
Rumex obtusifolius L.	RUMOB	D	P	0.16	0.05	0.07
Senecio vulgaris L.	SENVU	D	W/S	-	3.64	-
Setaria faberi Herrm.	SETFA	M	SA	14.51	18.55	4.72
Setaria glauca (L.) Beauv.	SETLU	M	SA	4.77	0.53	1.11
Solanum ptycanthum Dun.	SOLPT	D	S/P	0.49	0.48	0.74
Sonchus oleraceus L.	SONOL	D	SA	-	2.01	0.92
Stellaria media (L.) Vill.	STEME	D	W/P	0.99	2.04	0.78
Taraxacum officianale Weber in Wiggers	TAROF	D	P	0.32	0.31	0.12
Thlaspi arvense L.	THLAR	D	W/S	0.23	0.46	0.07
Trifolium pretense L.	TRFPR	D	P	0.12	0.07	0.63
Trifolium repens L.	TRFRE	D	P	-	-	0.26
Juncus spp.		M	P	-	0.18	0.44
Veronica spp.		D	WA	4.58	2.01	2.97

Source	df	Richness	Evenness	Shannon-Weiner Index	Density
1997					
Tillage	2	32.30**	3.87	1.42	46.34**
Rotation	5	26.12***	12.13***	29.00***	4.56**
Tillage x Rotation	10	1.44	1.36	2.57*	2.50*
1998					
Tillage	2	7.79*	3.66	2.10	44.40**
Rotation	5	16.99***	2.92**	15.81***	5.62***
Tillage x Rotation	10	1.60	1.53	1.48	4.30***
1999					
Tillage	2	5.11*	3.32	3.03	23.17**
Rotation	5	16.15***	4.30**	16.19***	2.34*
Tillage x Rotation	10	1.16	2.14*	2.36*	3.51**

Table 3.3: Analysis of variance for the main effects of tillage system and crop sequence on species richness (S), evenness (J), diversity (H'), and density (seed/m^2) of the springtime weed seedbanks for the years 1997, 1998 and 1999 in a 35-year-old study in Wooster, OH. Significance is designated as * = $P < 0.10$, ** = $P < 0.01$, *** = $P < 0.001$.

Tillage	Herbicide (kg ai/ha/5 yr)	Mean seed density (no/m^2)		
		1997	1998	1999
Conventional tillage	16.4	1411 c	1389 c	1350 b
Minimum Tillage	18.3	2726 a	3459 b	3054 a
No tillage	23.3	5768 a	7790 a	4308 a

Table 3.4: Total amount of herbicide applied to each tillage system (1994 to 1998), and mean total germinable weed seeds (± 1 SE) in a 35-year-old study in Wooster, OH, sampled from 1997 to 1999. Means followed by the same letter are not significantly different at the $\alpha = 0.05$

Rotation	Mean seed density (no/m^2)					
	1997		1998		1999	
CC	4469	a	3196	cd	5967	a
C(S)	2321	c	2559	d	2848	ab
S(C)	2469	bc	6920	a	1800	b
C(OH)	4366	ab	5318	ab	3061	ab
O(HC)	3234	abc	4610	bc	2733	ab
H(CO)	2958	abc	2623	d	3016	ab

Table 3.5: Mean seed density (± 1SE) for each rotation treatment) in a 35-year-old study in Wooster, OH, sampled from 1997 to 1999. Continuous corn (CC), corn-soybean-corn [C(S)], soybean-corn-soybean [S(C)], corn-oat-hay [C(OH)], oat-hay-corn [O(HC)], hay-corn-oat [H(CO)]. Means followed by the same letter are not significantly different at the $\alpha = 0.05$

	1997			1998			1999		
	S	J	H'	S	J	H'	S	J	H'
Tillage main effects									
CT	6.11 c	0.70 a	1.26 a	8.05 b	0.77 a	1.57 ab	6.83 b	0.78 a	1.43 ab
MT	8.38 b	0.71 a	1.47 a	10.72 a	0.80 a	1.81 a	8.50 a	0.66 b	1.40 b
NT	11.27 a	0.63 a	1.49 a	10.94 a	0.61 b	1.47 b	10.11 a	0.72 ba	1.64 a
Rotation main effects									
CC	6.00 b	0.49 b	0.84 b	6.44 c	0.67 bc	1.19 b	6.33 b	0.62 b	1.08 b
C(S)	6.00 b	0.50 b	0.92 b	7.33 c	0.71 bc	1.34 b	6.00 b	0.57 b	0.99 b
S(C)	4.77 b	0.78 a	1.09 b	7.22 c	0.62 c	1.12 b	5.55 b	0.76 a	1.26 b
C(OH)	12.22 a	0.73 a	1.79 a	14.66 a	0.82 a	2.18 a	10.11 a	0.80 a	1.77 a
O(HC)	11.44 a	0.75 a	1.80 a	12.00 b	0.78 ab	1.91 a	11.22 a	0.82 a	1.97 a
H(CO)	11.11 a	0.84 a	1.99 a	11.77 b	0.80 ab	1.95 a	11.67 a	0.76 a	1.87 a

Table 3.6: Means of species richness (S), evenness (J) and the Shannon-Weiner index (H') (± 1 SE) for the springtime weed seedbank, for the main effects of three tillage systems and six crop sequences, in a 35-year-old study in Wooster, OH. Samples were taken over three years (1997-1999). Conventional-tillage (CT), minimum-tillage (MT), no-tillage (NT), continuous corn (CC), corn-soybean-corn [C(S)], soybean-corn-soybean [S(C)], corn-oat-hay [C(OH)], oat-hay-corn [O(HC)], hay-corn-oat [H(CO)]. Values of H' can range from 0 to lnS (H'_{max}). Maximum diversity usually occurs when all species are similarly abundant. Values of J can range from 0 to 1.

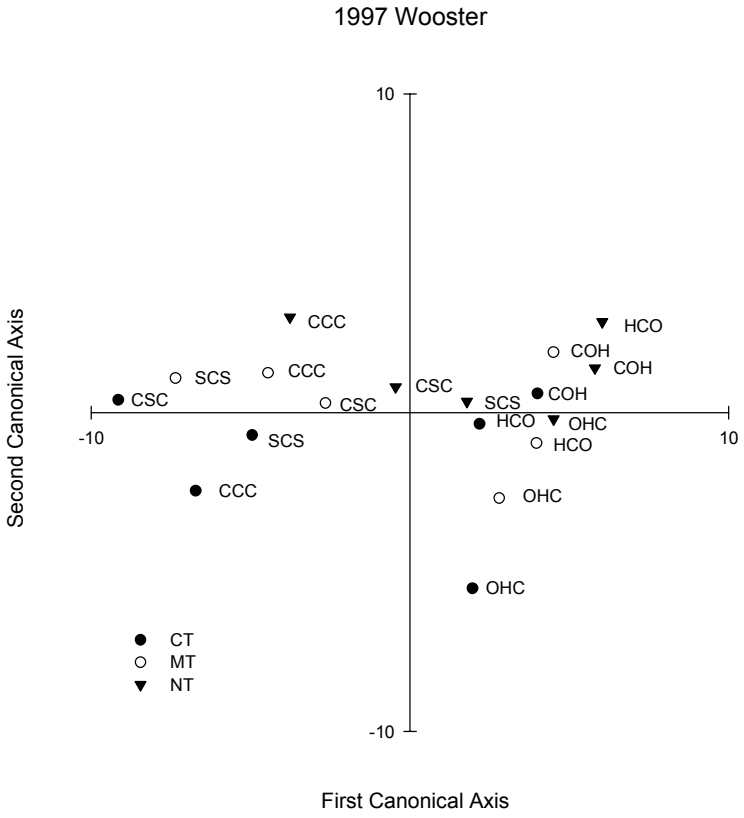

Figure 3.1: CDA ordination plots of treatment means positioned relative to the first and second canonical discriminating functions for the year 1997. Treatment means represent the combination of tillage system and crop sequence. Conventional-tillage (CT), minimum-tillage (MT), no-tillage (NT), continuous corn (CC), corn-soybean-corn [C(S)], soybean-corn-soybean [S(C)], corn-oat-hay [C(OH)], oat-hay-corn [O(HC)], hay-corn-oat [H(CO)].

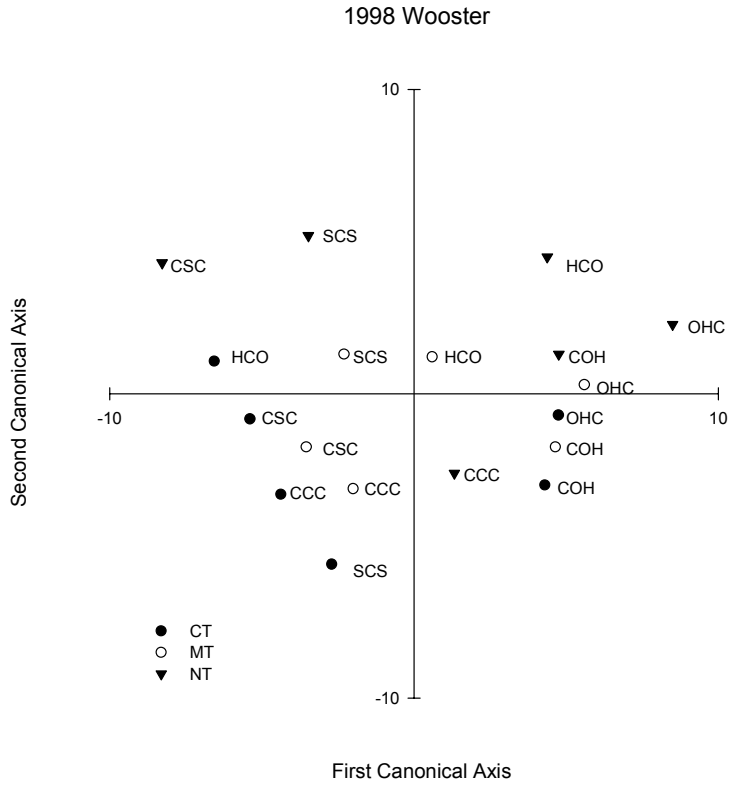

Figure 3.2: CDA ordination plots of treatment means positioned relative to the first and second canonical discriminating functions for the year 1998. Treatment means represent the combination of tillage system and crop sequence. Conventional-tillage (CT), minimum-tillage (MT), no-tillage (NT), continuous corn (CC), corn-soybean-corn [C(S)], soybean-corn-soybean [S(C)], corn-oat-hay [C(OH)], oat-hay-corn [O(HC)], hay-corn-oat [H(CO)].

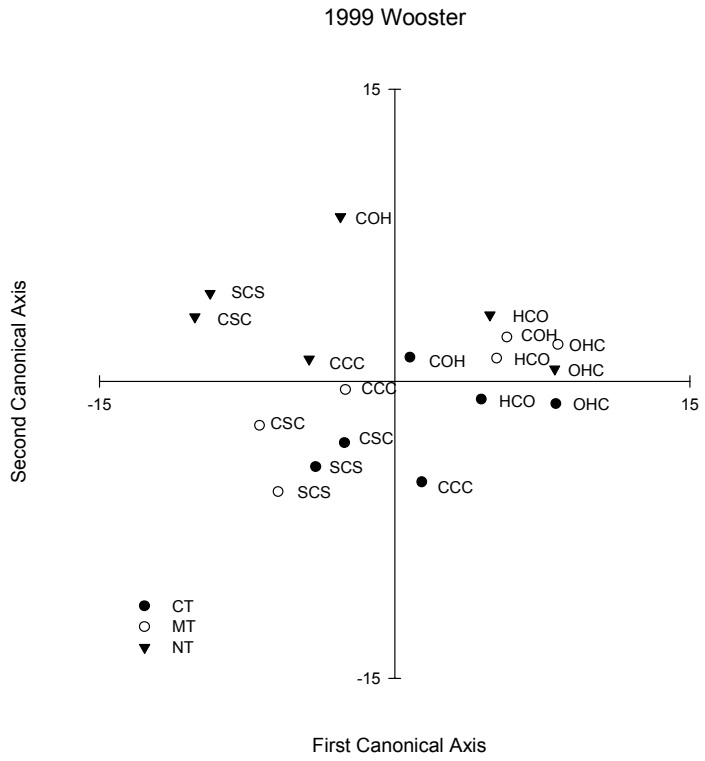

Figure 3.3: CDA ordination plots of treatment means positioned relative to the first and second canonical discriminating functions for the year 1999. Treatment means represent the combination of tillage system and crop sequence. Conventional-tillage (CT), minimum-tillage (MT), no-tillage (NT), continuous corn (CC), corn-soybean-corn [C(S)], soybean-corn-soybean [S(C)], corn-oat-hay [C(OH)], oat-hay-corn [O(HC)], hay-corn-oat [H(CO)].

	1997			1998			1999	
	MT	NT		MT	NT		MT	NT
CT	T = -1.39 *P* = 0.092	T = -6.12 *P* ≤ 0.015	CT	T = -0.86 *P* = 0.363	T = -3.00 *P* ≤ 0.015	CT	T = -0.43 *P* = 0.257	T = -9.01 *P* ≤ 0.015
	MT	T = -3.48 *P* ≤ 0.015		MT	T = -1.15 *P* = 0.121		MT	T = -3.14 *P* ≤ 0.015

Table 3.7: Results of MRPP analyses. Test statistic (T) and probability (*P*) of a smaller or equal delta for main effect of tillage on weed seedbank community composition over three years (1997 to 1999) in a 35-year-old study in Wooster, OH. The probability value of 0.015 reflects a Bonferroni correction for multiple within-year comparisons (α/n where $\alpha = 0.05$, $n = 3$). Conventional-tillage (CT), minimum-tillage (MT), no-tillage (NT).

	C(S)	S(C)	C(OH)	O(HC)	H(CO)
1997					
CC[a]	T = 0.56 *P* = 0.662	T = -4.02 *P* ≤ 0.003	T = -8.86 *P* ≤ 0.003	T = -8.90 *P* ≤ 0.003	T = -8.01 *P* ≤ 0.003
	C(S)	T = -1.12 *P* = 0.385	T = -7.89 *P* ≤ 0.003	T = -7.77 *P* ≤ 0.003	T = -7.05 *P* ≤ 0.003
		S(C)	T = -8.43 *P* ≤ 0.003	T = -7.97 *P* ≤ 0.003	T = -7.68 *P* ≤ 0.003
			C(OH)	T = -4.00 *P* ≤ 0.003	T = -0.81 *P* = 0.197
				O(HC)	T = -1.86 *P* = 0.037
1998					
CC	T = -3.17 *P* = 0.004	T = -2.71 *P* = 0.14	T = -8.50 *P* ≤ 0.003	T = -8.15 *P* ≤ 0.003	T = -7.65 *P* ≤ 0.003
	C(S)	T = -0.23 *P* = 0.346	T = -7.87 *P* ≤ 0.003	T = -7.44 *P* ≤ 0.003	T = -7.26 *P* ≤ 0.003
		S(C)	T = -7.88 *P* ≤ 0.003	T = -6.87 *P* ≤ 0.003	T = -6.49 *P* ≤ 0.003
			C(OH)	T = -0.75 *P* = 0.210	T = -2.09 *P* = 0.288
				O(HC)	T = --2.51 *P* = 0.014
1999					
CC	T = -0.69 *P* = 0.217	T = -0.75 *P* = 0.199	T = -7.11 *P* ≤ 0.003	T = -8.46 *P* ≤ 0.003	T = -7.53 *P* ≤ 0.003
	C(S)	T = -2.08 *P* = 0.037	T = -7.24 *P* ≤ 0.003	T = -8.93 *P* ≤ 0.003	T = -8.34 *P* ≤ 0.003
		S(C)	T = -6.55 *P* ≤ 0.003	T = -8.53 *P* ≤ 0.003	T = -7.84 *P* ≤ 0.003
			C(OH)	T = -1.34 *P* = 0.097	T = -0.60 *P* = 0.243
				O(HC)	T = -1.79 *P* = 0.049

Table 3.8: Results of MRPP analyses. Test statistic (T) and probability (*P*) of a smaller or equal delta for main effect of rotation on weed seedbank community composition over three years (1997 to 1999) in a 35-year-old study in Wooster, OH. The probability value of 0.003 reflects a Bonferroni correction for multiple within-year comparisons (α/n where $\alpha = 0.05$, n = 15). Continuous corn (CC), corn-soybean-corn [C(S)], soybean-corn-soybean [S(C)], corn-oat-hay [C(OH)], oat-hay-corn [O(HC)], hay-corn-oat [H(CO)].

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CHAPTER 4.

COMPOSITION OF THE SPRINGTIME WEED SEEDBANK AND SUMMER FIELD WEED COMMUNITY IN A LONG-TERM TILLAGE AND ROTATION STUDY FOLLOWING ADOPTION OF GLYPHOSATE-TOLERANT CROPS

Lynn M. Sosnoskie, Catherine P. Herms and John Cardina

INTRODUCTION

The abundance and composition of species in arable weed seedbanks reflect past and current management of soils, crops, and weeds (Cavers and Benoit 1989, Feldman *et al.* 1997, Anderson *et al.* 1998, Bàrberi *et al.* 1998, Légerè and Samson 1999, Nalewaja 1999, Bàrberi and Lo Cascio 2001, Menalled *et al.* 2001, Törresen and Skuterud 2002). As agronomic practices change, new niches are created, which become occupied by species adapted to conditions in the newly formed habitat (Lyon *et al.* 2002). The principal species flourishing under intense tillage can differ from those dominating systems with little or no soil disturbance (Feldman *et al.* 1997, Anderson *et al.* 1998, Bàrberi *et al.* 1998, Bàrberi and Lo Cascio 2001, Törresen and Skuterud 2002). Crop rotation can also exert a strong influence on the composition of the weed community, as reflected in the weed seedbank (Légerè and Samson 1999, Nalewaja 1999, Cardina *et al.* 2002). Crop and weed management practices have brought about species shifts that

have further complicated weed control efforts. For example, herbaceous and woody perennial weeds increased in fields where reduced-tillage practices were adopted (Johnson 1994). Herbicide resistance quickly followed the development of acetolactate synthase (ALS) inhibitors and similar herbicides (Powles and Shaner 2001).

Glyphosate (N-phosphonomethyl glycine) is the primary non-selective herbicide for control of annual and perennial weeds in many horticultural and agricultural settings (Woodburn 2000). With the introduction of genetically-modified glyphosate-tolerant crops (GTCs), there is concern that increased applications of a single herbicide mode-of-action across space and time will alter agricultural weed community dynamics in crop fields and their margins (Shaner 2000, Lyon *et al.* 2002, Blackburn and Boutin 2003). In some states, glyphosate-resistant soybeans represent up to 78% of the total hectares planted (National Agricultural Statistics Service 2002). Potential benefits of GTCs, such as flexible timing of control, reduced tillage, and increased yield (Kuiper *et al.* 2000, Riches and Valverde 2002, Freyssinet 2003), would be offset if species shifts resulted in weed communities that are more difficult to manage (Kuiper *et al.* 2000, Lyon *et al.* 2002, Riches and Valverde 2002, Martinez-Ghersa *et al.* 2003). Repetitive use of glyphosate could lead to the evolution and spread of herbicide-resistant/tolerant weeds, selection of weeds with altered morphologies and phenologies, and an increase in the frequency of volunteer crops (Kuiper *et al.* 2000, Shaner 2000, Lyon *et al.* 2002, Riches and Valverde 2002, Martinez-Ghersa *et al.* 2003). Furthermore, the continued use of a non-selective herbicide could result in changes in biodiversity in agroecosystems across all trophic levels (Lyon *et al.* 2002, Blackburn and Boutin 2003). Currently, there is an insufficient amount of literature describing the composition of weed and weed seedbank

communities in systems planted, predominantly, to glyphosate-tolerant crops. Of particular importance is the need for baseline studies from which to measure historical trends (Squire *et al.* 2003).

Previously, we described the effect of tillage and rotation on weed seedbank species diversity and community composition in a continuing long-term study (Cardina *et al.* 2002). Beginning in 1999, corn and soybeans in the Triplett-VanDoren No-Tillage Experimental Plots were planted to GTC varieties, and weeds in those crops were managed with glyphosate, exclusively. Therefore, the purpose of this study was to characterize the density and diversity of species in the springtime weed seedbank and summer weed field communities in a long-term tillage and rotation study that had been converted to glyphosate-tolerant crops four, five, and six years prior. Our goal was to develop a basis of comparison for future studies in GTC systems.

MATERIALS AND METHODS

Site description and treatment establishment:

The study was conducted in a long-term tillage and rotation experiment initiated in 1963 at Wooster, Ohio, USA. Climate, soil, and site characteristics, as well as details of the experimental design were described previously (Dick and Van Doren 1985, Cardina *et al.* 2002).

Fifty-four individual plots (5 m wide and 21 m long) were arranged in a randomized complete block with three replications. Three tillage systems were used: no-tillage (NT), minimum-tillage (MT) and conventional-tillage (CT). No-tillage

treatments were disturbed in-row by the coulter blades on the planter, only. Minimum-tillage changed from shallow moldboard plowing without subsequent disking (1963-1982), to a single paraplow operation (1983-1984), to a single pass with a chisel plow (1985-present). Conventionally tilled plots were moldboard plowed to a depth of 20 to 25 cm followed by two 10 cm deep secondary tillage (disking) operations before planting.

The crop sequences were continuous corn (*Zea mays*) (CC), corn-soybean (*Glycine max*), and corn-oat (*Avena sativa*)-hay {alfalfa (*Medicago sativa*) + ryegrass (*Lolium perenne* L.)}. All crops appear each year; therefore, there are separate plots planted to both corn (C(S)) and soybean (S(C)) in each tillage system, in each block, every year. Likewise, for the three-crop rotation, there are individual plots planted to corn (C(OH)), oat (O(HC)) and hay (H(CO)), every year.

Glyphosate-tolerant corn (Dekalb DKC58-53RR) and soybean (Pioneer 93B36RR) varieties were planted at rates of 87,851 seeds/ha and 77 kg/ha, respectively, for all three years. Corn has been planted in rows 76 cm apart since 1973. Soybeans have been drilled in rows 18 cm apart since 1985. Corn and soybeans were planted April 28, 2002 and April 30, 2004. Planting was delayed until May 23 in 2003 because of wet soil conditions. Oat (77 kg/ha 'Armor'), alfalfa (13 kg/ha 'Croplan Rocket') and ryegrass (7 kg/ha) were planted with no-tillage drills in early- to mid-April, all three years. Fertilizer was applied following Ohio State University soil test recommendations, and the same rates of N, P, and K have been used in each tillage system. Lime was broadcast as required to maintain a pH of about 6.0 in the Ap horizon.

Weeds were controlled at the discretion of the farm manager. All NT plots received a single application of glyphosate (Roundup Ultra, Monsanto Company, St. Louis, MO), plus ammonium sulfate, prior to planting (pre-plant), except in 2004 when this application occurred 20 days after planting (Table 4.1). In oats, Bromoxynil (3,5-dibromo-4-hydroxybenzonitrile, Buctril, Bayer Crop Science, Research Triangle Park, NC) was used approximately 20 days post-emergence to control broadleaf weeds, except in 2004 when no herbicides were applied. One application of glyphosate per year was made post-emergence (POST) in corn and soybean crops (Table 4.1).

Sample and data collection:

Soil seedbank samples were collected in March 2002, 2003, and 2004. Twelve samples, of two soil cores each, were obtained at random from each plot. Each soil core was 3.5 cm in diameter and 5 cm deep. The sampling areas were spaced at regular intervals (approximately 3-4.5 m apart) within each plot, and at least 2 m from the borders. Samples were processed by sieving through a 0.64-cm screen. An entire sample (two cores), minus large stones and root fragments, was spread over a 2.5 cm layer of ProMix BX (Premier Horticulture, Quakertown, PA) in a 15-cm^2 tray, and placed on a mist bench. Soil samples were kept moist by hourly applications of water.

Emerged weed seedlings were identified, counted, and removed weekly. After emergence ceased (about 4 months), samples were placed in a 4 °C cold room for 8 weeks to reestablish springtime conditions and break secondary dormancy in the remaining seeds. Soil samples were stirred and re-sieved, before being returned to the greenhouse.

Weed field counts were completed in June 2002, 2003, and 2004, prior to the first POST applications of glyphosate in corn and soybeans, but after POST applications of bromoxynil in oats. All of the weeds in four (0.25 m^2) sampling areas/plot were identified and counted, but not removed. The sampling areas were spaced at regular intervals (approximately 3-4.5 m apart) within each plot, and at least 2 m from the borders.

Data analysis:

Germinable seed and aboveground weed densities were expressed as the number of seeds and weeds/m^2, respectively. Diversity was described by species richness (total number of species, S), the Shannon-Weiner index (H'):

$$H' = -\Sigma P_i \, x \, (lnP_i),$$

where P_i = proportion of total sample belonging to i^{th} species, and evenness (J):

$$J = H'/lnS,$$

where H' = the Shannon-Weiner index and S = total number of species. These indices were calculated for each plot from the species density data (PC-ORD version 3.01).

Richness and evenness values and the Shannon-Weiner index were transformed prior to analysis using a power transformation:

$$b_y = x^p,$$

where p = 2, to improve normality and homogeneity of variance, when necessary. Seeds/m^2, weeds/m^2, S, J, and H' were compared across the three tillage systems (NT, MT and CT) and six cropping sequences (CC, C(S), S(C), C(OH), O(HC), H(CO)) using

PROC MIXED in SAS (version 9.0). Block (replication) and the block-by-tillage interaction were considered as random factors. Years were analyzed separately.

Indicator species analysis (PC-ORD, version 3.01) was used to describe the association of individual weed species with tillage and rotation. Indicator values (IV) for each species in each treatment were calculated:

$$IV = 100\ (rA \times rF),$$

where rA is the relative abundance of a species in each treatment, and rF is the relative frequency of a species in each treatment. The rA value is calculated:

$$rA = x / \Sigma x,$$

where x is the mean abundance of a species in each treatment. The rF value is calculated:

$$rF = \Sigma b / n,$$

where b = matrix of presence-absence data and n = the number of sample units in a treatment (McCune and Grace 2002). Indicator values can range from zero (no relationship) to 100 (perfect relationship). The greater the IV, the more strongly a species performs as a predictor of an environmental or treatment condition. The statistical significance of IV_{max} for each species was evaluated using a Monte Carlo test with 1000 permutations (McCune and Grace 2002).

RESULTS AND DISCUSSION

Seedbank community structure:

We identified 51 and 50 weed species in the 2002 and 2003 seedbanks, respectively (Figures 4.1 and 4.3). Seedbank data for 2004 are not reported here. The total number of germinable seeds in the weed seedbank summed across all treatments was 4915 and 7057 for the years 2002 and 2003, respectively. The ten most prevalent species each year accounted for greater than 85 % of the total number of germinated seeds (Figures 4.1 and 4.3). *Chenopodium album* L. (CHEAL) and *Oxalis stricta* L. (OXAST), together, accounted for 40 and 38% of the 2002 and 2003 seedbanks, respectively (Figures 4.1 and 4.3). Additionally, *Cardamine hirsuta* L. (CARHI), *Digitaria* spp., *Lobelia inflata* L. (LOBIN), *Plantago* spp., *Poa annua* L. (POANN), *Setaria* spp., and *Veronica peregrina* L. (VERPG) were important components of the total seedbank for either one or both years (Figures 4.1 and 4.3).

Field community structure:

Fifty-one, 50, and 47 species were documented in the 2002, 2003, and 2004 field studies, respectively (Figures 4.2, 4.4 and 4.5). The total number of weeds in the field at the time of sampling summed across all treatments was 11478 in 2002, 13726 in 2003, and 6454 in 2004. The ten most prevalent species each year accounted for 89, 83, and 78 % of the total number of weeds for 2002, 2003, and 2004, respectively (Figures 4.2, 4.4 and 4.5). *Amaranthus retroflexus* L. (AMARE), *C. album*, *Digitaria* spp., *O. stricta*, *Panicum dichotomiflorum* Michx (PANDI), *Plantago* spp., *P. annua*, *Setaria* spp.,

Stellaria media (L.) Vill. (STEME), *Taraxacum officianale* Weber in Wiggers (TAROF), and *Veronica* spp. were important members of at least one to all of the aboveground field communities from 2002 to 2004 (Figures 4.2, 4.4 and 4.5). In 2002 and 2004, *Digitaria* spp. and *T. officinale*, together, accounted for 47 and 33 % of the observed weeds in the field, respectively (Figures 4.2 and 4.5). In 2003, *C. album* and *T. officianale*, together, comprised 44 % of the total weed density.

Tillage effects on density and diversity:

Analyses of variance indicate that the number of weed seeds/m^2 in the 2002 and 2003 seedbanks for field soil to a depth of 5 cm was influenced by tillage (Table 4.2). Mean seed density declined as soil disturbance increased (NT > MT > CT) in both years (Table 4.3). The same trends held when data were averaged across rotation and year. Plots managed in the NT system had the greatest mean seed density (6963 seeds/m^2), followed by plots in MT (4993 seeds/m^2) and CT (2440 seeds/m^2) (Table 4.5). Evenness (J) and diversity, as measured by the Shannon-Weiner index (H'), of the weed seedbank were also affected by tillage in 2002 and 2003 (Table 4.2). Values for J and H' decreased as tillage intensity decreased (CT ≥ MT > NT) (Tables 4.3).

The number of weeds/m^2 in the 2002 and 2004 field counts was influenced by tillage (Table 4.2). Mean weed density in 2002 and 2004 increased as soil disturbances decreased (NT > MT ≥ CT), except in 2004 when mean weed densities were almost two times higher in the MT system than in the NT and CT systems (Table 4.4). Averaged across rotation and year, plots managed to NT had about 50 and 130 more weeds/m^2 than MT and CT treatments, respectively (Table 4.5). Weed species richness in the field

was influenced by tillage in 2004, where S was greatest in the MT system (Table 4.2 and 4.4).

Rotation affects on density and diversity:

Analyses of variance indicate that the number of weed seeds/m^2 was influenced by rotation in 2002 and 2003 (Table 4.2). Mean seed density was greatest in the C(OH) rotation (8788 seeds/m^2) in 2002 and the C(OH) and O(HC) rotations (7888 and 8663 seeds/m^2, respectively) in 2003 (Table 4.3). For both years, mean germinable weed seed densities were greater in the C(OH), O(HC), and H(CO) treatments as compared to the CC, C(S) and S(C) sequences (Table 4.3). When data were averaged across tillage and year, mean weed seed densities for the three-crop rotation ranged from 5303-8338 seeds/m^2, compared to 1936-3567 seeds/m^2 for the one- and two-year rotations (Table 4.5).

Analyses of variance indicate that the number of species in the 2002 and 2003 seedbanks for field soil to a depth of 5 cm was influenced by rotation (Table 4.2). For both years, species richness (S) of the weed seedbank was greater in the C(OH), O(HC), and H(CO) plots, than in the corn monoculture and corn-soybean rotations (Table 4.3). Averaged across tillage and year, 16 to 17 species were found in the three-crop rotation, as compared to 12 to 13 species in the CC, C(S), and S(C) seedbanks (Table 4.5). Evenness (J) in 2002 and H' in 2003 were influenced by rotation and the tillage-rotation interactions (Table 4.2). Values of J were greater in the CC, C(S), and S(C) rotations (0.80-0.87) as compared to C(OH) and H(CO) rotations (0.69-0.71) in 2002, whereas the reverse was generally true for H' in 2003 (Table 4.3). Values of H' were greater in the

O(HC) and H(CO) rotations (2.0) than in the one- and two-crop rotations (1.5-1.7) (Table 4.3). The two-way interactions for J and H' were the result of high values of J and H' for C(S) plots in the NT system (data not shown).

The number of weeds/m^2 was influenced by rotation in 2002, 2003, and 2004, and the tillage-rotation interaction (Table 4.2). Mean weed density was greatest in the C(OH) rotation in 2002 and 2003, and the O(HC) rotation in 2004 (Table 4.4). Averaged across tillage and year, mean weed densities were higherfor the C(OH) rotation (374 weeds/m^2), as compared to the O(HC) (159 weeds/m^2)and H(CO) (145 weeds/m^2) plots (Table 4.5). The tillage-rotation interaction for weed density in 2004 was the result of greater weed numbers in C(S) and C(OH) in NT and MT systems, respectively.

The number of weed species recorded in the 2002, 2003, and 2004 field surveys was influenced by rotation, and the tillage-rotation interaction (Table 4.2). Species richness values were greater in the C(OH) treatments (15.3 and 18.7, respectively) in the 2002 and 2003 field counts, and in the O(HC) rotation (19.4) in 2004 (Table 4.4). Values of J were influenced by rotation in 2003, while values for H' were affected by rotation all three years (Table 4.2). Additionally, H' was affected by the tillage-rotation interaction in 2003 and 2004 (Table 4.2). Values of J were greater in the three-crop rotation (0.71-0.74), than the CC, C(S), and C(S) rotations (0.55-0.66) in 2003 (Table 4.4). Averaged across tillage and year, the value of J was 0.78 for the O(HC) rotation and 0.67-0.71 for the other sequences (Table 4.5). The value for H' was greatest in the C(OH) sequence (2.2) in 2003 and in the O(HC) sequence (2.4) in 2004 (Table 4.4). Averaged across tillage and year, values of H' for the C(OH) and O(HC) rotations were

1.9, compared to values of 1.6-1.7 for the H(CO), one- and two-crop rotations (Table 4.5).

Indicator species analysis:

Indicator species analysis creates indicator values (IV) for each species based on its abundance and frequency, relative to all other species, within a treatment class; the larger the IV the more likely a species is to function as an indicator of a particular management system (McCune and Grace, 2002). The statistical significance of the maximum IV for each species for each treatment class can be assessed using a Monte Carlo test (McCune and Grace, 2002). Species that serve as indicators of reduced and no tillage systems in the seedbank and/or field included *A. retroflexus, C. hirsuta, Cerastium vulgatum* L. (CERVU), *Chenopodium album, Cirsium arvense* (L.) Scop. (CIRAR), *Digitaria* species*, Erigeron annuus* (L.) Pers. (ERIAN)*, Lamium amplixicaule* L. (LAMAM), O. *stricta, Poa annua, Polygonum pensylvanicum* L. (POLPY)*, Senecio vulgaris, Setaria* species*, Sonchus oleraceus* L. (SONOL)*, S. media, T. officinale,* and *V. peregrina* (Tables 4.6 to 4.10). Cardina *et al.* (2002) found that *Digitaria* species*, E. annuus, O. stricta, P. annua, Setaria* species*,* and *S. media* were relatively important species of the NT and MT seedbanks from 1997 to 1999, prior to the use of GTCs and POST applied glyphosate. The CT plots were not characterized by any one species in any year of the study except *C. album* in 2004. Indicator species of continuous corn and corn-soybean plots included *C. album*, *P. annua, Portulaca oleracea* L. (POROL)*, A. retroflexus,* and *Abutilon theophrasti* Medicus (ABUTH) (Tables 4.6 to 4.10). Cardina *et al.* (2002) reported that *C. album* and *A. retroflexus* were important components of the

continuous corn rotation from 1997 to 1999. Species indicative of the three crop rotation were, *Capsella bursa-pastoris* (L.) Medicus (CAPBP)*, C. hirsuta, O. stricta, P. pensylvanicum*, *Setaria* species*, S. oleraceus, T. officinale, Thlaspi arvense* L. (THLAR), and *V. peregrina* among others. Many of the same species were significant members of the corn-oat-hay rotations prior to the inclusion of GTCs.

Implications for weed management:

Results from this study show that tillage and rotation, and the chemical and cultural practices associated with each, influence the density and diversity of weed species that develop in row-crop agriculture. Seeds/m^2, to a depth of 5 cm, declined as depth and intensity of tillage increased. Similar results were reported by Anderson *et al.* (1998), Feldman *et al.* (1997), and Bàrberi and Lo Cascio (2001). Increases in mean weed seed density in the NT seedbanks were not associated with increases in the values of S, J, and H', suggesting that the weed seedbank communities in NT systems were dominated by one or a few species. Tillage influences germinable weed seed density by affecting seed accumulation, dormancy, predation, and pathogenesis. Because our sampling depth did not exceed 5-cm, seeds incorporated to greater depths by moldboard or chisel plowing would not have been surveyed in our study. Through tillage, seeds are repositioned to environments that can either facilitate or inhibit germination (Buhler and Mester 1991, Gallagher and Cardina 1998). Accumulation of residue on the soil surface can protect weed seeds from predation and lead to an increase in weed seed density (Feldman *et al.* 1997). Pathogenesis is dependent upon inoculum levels and

environmental conditions that can be modified by tillage (Stroo *et al.* 1988, Mohler and Callaway 1992).

The application of pre-plant herbicides for control of aboveground weeds to each plot was determined by tillage system. All no-tillage plots, regardless of rotation, received a pre-plant application of glyphosate (1.12 kg ai/ha). In 2004, this application occurred 20 days after planting, and most likely accounted for the reduction in the mean number of species and weeds/m^2 counted in the NT system in that year (Tables 4.4). Weed species that normally avoid pre-plant applications of glyphosate because of delayed emergence would not have evaded the belated treatment (Hilgenfeld *et al.* 2004a,b). The positive association between weed and weed seed densities in the NT plots suggests that the field weed community is maintaining the seedbank community, and vice versa. Supplementary field counts taken in September of 2002 and 2003 (data not shown) also show an increase in the mean number of weeds/m^2 in the NT plots.

In general, plots planted to corn-oats-hay supported weed seed communities that were more dense and more diverse than all other plots, suggesting that cumulative management practices influence seedbank dynamics. Variability in crop rotation allows for the development of weed communities adapted to different emergence times, sub-canopy light conditions, and herbicides (Leroux *et al.* 1996, Légerè and Samson 1999, Nalewaja 1999, Menalled *et al.* 2001). The three rotations used in this study vary with respect to planting and harvest dates; crop morphology; use, amount, and selectivity of chemical herbicides; and timing of weed control operations. For example, in corn and soybean plots winter annuals were controlled with tillage or herbicide in early spring prior to planting, which occurred in late April or May, and summer annuals were

managed with one POST application of glyphosate (1.12 kg ai/ha) approximately 30 days after planting. Hay and oat plots were planted in the late summer and early spring, respectively. Hay crops did not receive any herbicide applications, but were cut two to three times a year. Oats were treated with one POST application of bromoxynil approximately 30 days after planting (0.42 kg ai/ha), except in 2004 (Table 4.1). In 2004, no herbicide applications were made to the oat crops prior to field counts. This missed application of bromoxynil probably accounts in part for the relative increase in the average number of weeds/m^2 observed in the O(HC) system relative to the other rotations in that year. While weed seedbanks reflect the history of crop and weed management practices, aboveground weed communities represent current production strategies.

Bàrberi and Mazzoncini (2001), Hyvönen and Salonen (2002) and Hyvönen *et al.* (2003) reported that species diversity, as measured by species number, and the Shannon-Weiner index, was greatest when physical and chemical inputs were lowest. Our data support these findings in that weed seed species diversity was greatest in the three-crop rotations, which received the least amount of applied herbicides. However, the corn-oat-hay rotations received the most machine traffic because of multiple planting and/or harvest times within a growing season, and in this respect, are the most disturbed rotation system.

Because of differences in sample size and weed seed germination protocols, we cannot make a statistical comparison between these results and the results from previous studies conducted in the Triplett-VanDoren No-Tillage Experimental Plots, although it appears as though the structure and composition of the weed seedbank has changed little

since glyphosate-tolerant corn and soybeans were planted starting in 1999 (Cardina *et al.* 19991, Cardina *et al.* 2002, Sosnoskie *et al.* unpublished). Continuous corn and corn-soybean plots transitioned from an herbicide management program with varied modes-of-action (ALS inhibitors, microtubule inhibitors, shoot inhibitors, pigment inhibitors, photosystem II inhibitors, synthetic auxins) to a system dominated by glyphosate (aromatic amino acid synthesis inhibitor). The lack of a noticeable shift suggests that the residual seedbank may be acting as a buffering agent in the short-term. Various authors have reported occurrences of glyphosate-resistant weed species (VanGessel 2001, Perez and Kogan 2003). As anticipated, we did not detect evidence of resistance during the short time that glyphosate was used almost exclusively. Currently, our understanding of if/how weed and weed seedbank communities shift in response to glyphosate suggests that limited changes in species composition in grower fields are likely to be subtle; we would not expect dramatic changes that would lead to dominace by a group of species that would cause severe problems for crop producion. Results from these investigations will serve as a baseline for future trends in weed community composition following the change to glyphosate tolerant crops

	Corn		Soybean		Oat	
Year	Herbicide	rate	Herbicide	rate	Herbicide	rate
		kg /ha		kg /ha		kg /ha
2000	glyphosate	1.12	glyphosate	1.12	bromoxynil	0.42
2001	glyphosate	1.12	glyphosate	1.12	bromoxynil	0.42
2002	glyphosate	1.12	glyphosate	1.12	bromoxynil	0.42
2003	glyphosate	1.12	glyphosate	1.12	bromoxynil	0.42
					+ 2,4-D	0.14
2004	glyphosate	1.12	glyphosate	1.12		
Total		5.60		5.60		1.82

Table 4.1: Herbicides applied postemergence to corn, soybean, and oat crops from the years 2000 to 2004. Oat plots did not receive any postemergence herbicide applications in 2004. All no-till plots (including hay crops) received pre-plant applications of glyphosate (1.12 kg/ha) to kill existing vegetation before planting every year, except in 2004 when this application occurred 20 days after planting. Glyphosate = *N*-phosphonomethyl glycine; applied with ammonium sulfate as per label instructions. Bromoxynil = 3,5-dibromo-4-hydroxybenzonitrile. 2,4-D = (2,4-dichlorophenoxy)acetic acid.

Figure 4.1: Density of individual weed species (n = 51) (as percentage of total number of weed seed) occurring in the sampled springtime seedbank in a long-term tillage and rotation study in Wooster, OH, for 2002. Latin binomials and Bayer codes conform to WSSA Composite List of Weeds (1989). Species with zero values appeared in the 2002 field count only.

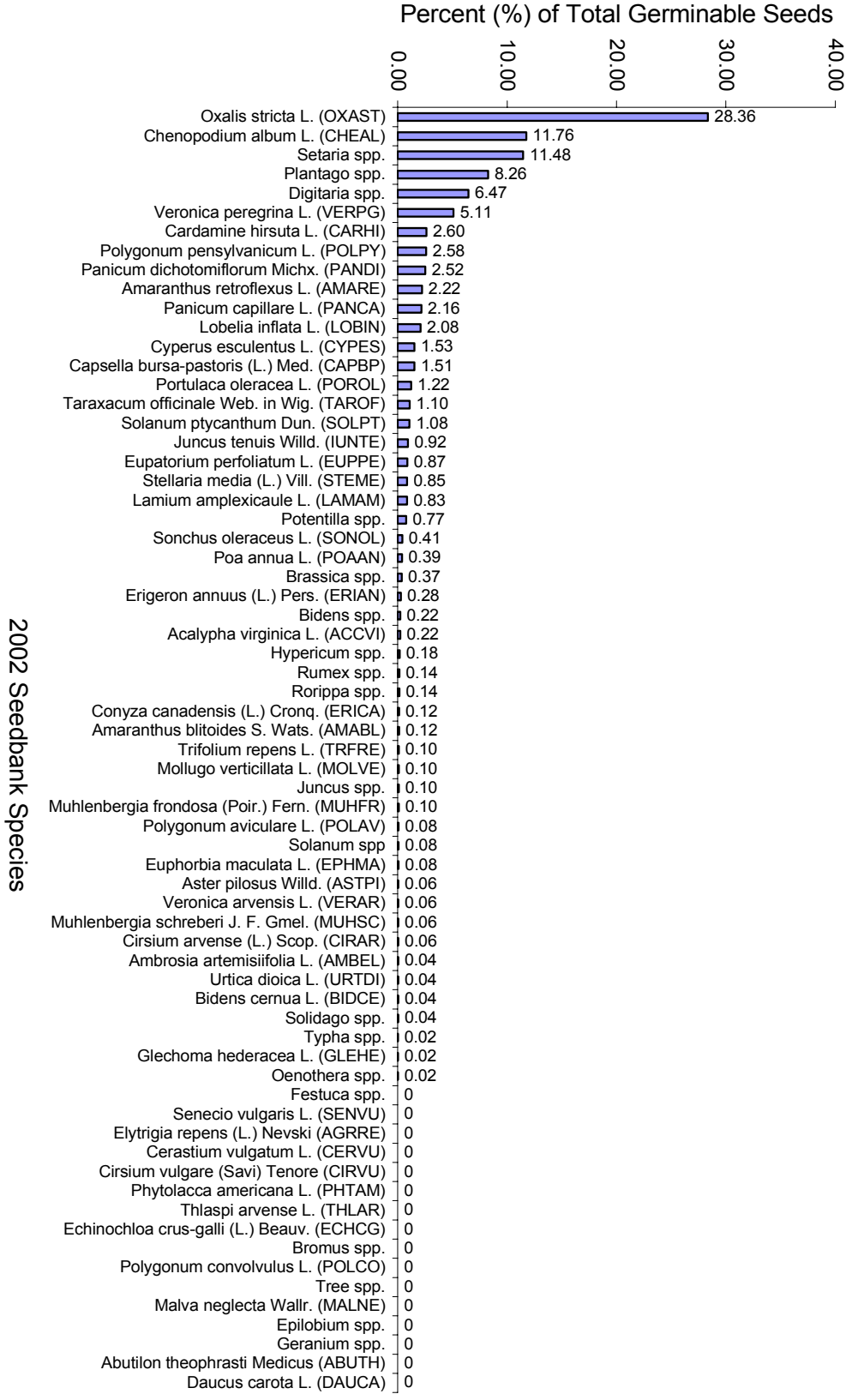
Percent (%) of Total Germinable Seeds
0.00
10.00
20.00
30.00
40.00
2002 Seedbank Species
Oxalis stricta L. (OXAST) 28.36
Chenopodium album L. (CHEAL) 11.76
Setaria spp. 11.48
Plantago spp. 8.26
Digitaria spp. 6.47
Veronica peregrina L. (VERPG) 5.11
Cardamine hirsuta L. (CARHI) 2.60
Polygonum pensylvanicum L. (POLPY) 2.58
Panicum dichotomiflorum Michx. (PANDI) 2.52
Amaranthus retroflexus L. (AMARE) 2.22
Panicum capillare L. (PANCA) 2.16
Lobelia inflata L. (LOBIN) 2.08
Cyperus esculentus L. (CYPES) 1.53
Capsella bursa-pastoris (L.) Med. (CAPBP) 1.51
Portulaca oleracea L. (POROL) 1.22
Taraxacum officinale Web. in Wig. (TAROF) 1.10
Solanum ptycanthum Dun. (SOLPT) 1.08
Juncus tenuis Willd. (IUNTE) 0.92
Eupatorium perfoliatum L. (EUPPE) 0.87
Stellaria media (L.) Vill. (STEME) 0.85
Lamium amplexicaule L. (LAMAM) 0.83
Potentilla spp. 0.77
Sonchus oleraceus L. (SONOL) 0.41
Poa annua L. (POAAN) 0.39
Brassica spp. 0.37
Erigeron annuus (L.) Pers. (ERIAN) 0.28
Bidens spp. 0.22
Acalypha virginica L. (ACCVI) 0.22
Hypericum spp. 0.18
Rumex spp. 0.14
Rorippa spp. 0.14
Conyza canadensis (L.) Cronq. (ERICA) 0.12
Amaranthus blitoides S. Wats. (AMABL) 0.12
Trifolium repens L. (TRFRE) 0.10
Mollugo verticillata L. (MOLVE) 0.10
Juncus spp. 0.10
Muhlenbergia frondosa (Poir.) Fern. (MUHFR) 0.10
Polygonum aviculare L. (POLAV) 0.08
Solanum spp 0.08
Euphorbia maculata L. (EPHMA) 0.08
Aster pilosus Willd. (ASTPI) 0.06
Veronica arvensis L. (VERAR) 0.06
Muhlenbergia schreberi J. F. Gmel. (MUHSC) 0.06
Cirsium arvense (L.) Scop. (CIRAR) 0.06
Ambrosia artemisiifolia L. (AMBEL) 0.04
Urtica dioica L. (URTDI) 0.04
Bidens cernua L. (BIDCE) 0.04
Solidago spp. 0.04
Typha spp. 0.02
Glechoma hederacea L. (GLEHE) 0.02
Oenothera spp. 0.02
Festuca spp. 0
Senecio vulgaris L. (SENVU) 0
Elytrigia repens (L.) Nevski (AGRRE) 0
Cerastium vulgatum L. (CERVU) 0
Cirsium vulgare (Savi) Tenore (CIRVU) 0
Phytolacca americana L. (PHTAM) 0
Thlaspi arvense L. (THLAR) 0
Echinochloa crus-galli (L.) Beauv. (ECHCG) 0
Bromus spp. 0
Polygonum convolvulus L. (POLCO) 0
Tree spp. 0
Malva neglecta Wallr. (MALNE) 0
Epilobium spp. 0
Geranium spp. 0
Abutilon theophrasti Medicus (ABUTH) 0
Daucus carota L. (DAUCA) 0

Figure 4.2: Density of individual weed species (n = 51) (as percentage of total number of weeds) occurring in the summer field count in a 35-year-old tillage and rotation study in Wooster, OH, for 2002. Latin binomials and Bayer codes conform to WSSA Composite List of Weeds (1989). Species are presented in order from highest to lowest as they appeared in the 2002 seedbank. Species with zero values appeared in the 2002 sampled seedbank only.

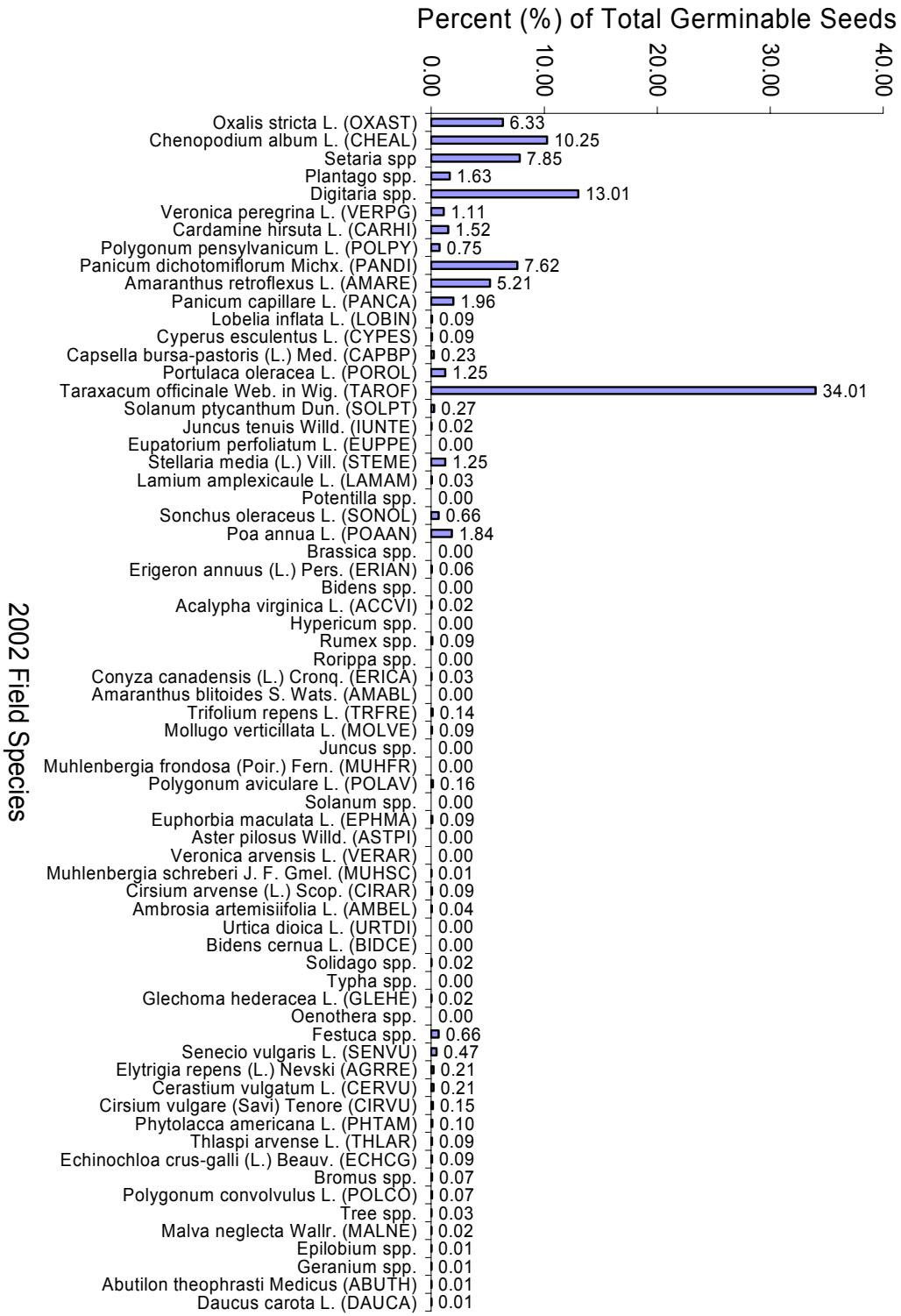
Percent (%) of Total Germinable Seeds
0.00
10.00
20.00
30.00
40.00
2002 Field Species
Oxalis stricta L. (OXAST) 6.33
Chenopodium album L. (CHEAL) 10.25
Setaria spp 7.85
Plantago spp. 1.63
Digitaria spp. 13.01
Veronica peregrina L. (VERPG) 1.11
Cardamine hirsuta L. (CARHI) 1.52
Polygonum pensylvanicum L. (POLPY) 0.75
Panicum dichotomiflorum Michx. (PANDI) 7.62
Amaranthus retroflexus L. (AMARE) 5.21
Panicum capillare L. (PANCA) 1.96
Lobelia inflata L. (LOBIN) 0.09
Cyperus esculentus L. (CYPES) 0.09
Capsella bursa-pastoris (L.) Med. (CAPBP) 0.23
Portulaca oleracea L. (POROL) 1.25
Taraxacum officinale Web. in Wig. (TAROF) 34.01
Solanum ptycanthum Dun. (SOLPT) 0.27
Juncus tenuis Willd. (IUNTE) 0.02
Eupatorium perfoliatum L. (EUPPE) 0.00
Stellaria media (L.) Vill. (STEME) 1.25
Lamium amplexicaule L. (LAMAM) 0.03
Potentilla spp. 0.00
Sonchus oleraceus L. (SONOL) 0.66
Poa annua L. (POAAN) 1.84
Brassica spp. 0.00
Erigeron annuus (L.) Pers. (ERIAN) 0.06
Bidens spp. 0.00
Acalypha virginica L. (ACCVI) 0.02
Hypericum spp. 0.00
Rumex spp. 0.09
Rorippa spp. 0.00
Conyza canadensis (L.) Cronq. (ERICA) 0.03
Amaranthus blitoides S. Wats. (AMABL) 0.00
Trifolium repens L. (TRFRE) 0.14
Mollugo verticillata L. (MOLVE) 0.09
Juncus spp. 0.00
Muhlenbergia frondosa (Poir.) Fern. (MUHFR) 0.00
Polygonum aviculare L. (POLAV) 0.16
Solanum spp. 0.00
Euphorbia maculata L. (EPHMA) 0.09
Aster pilosus Willd. (ASTPI) 0.00
Veronica arvensis L. (VERAR) 0.00
Muhlenbergia schreberi J. F. Gmel. (MUHSC) 0.01
Cirsium arvense (L.) Scop. (CIRAR) 0.09
Ambrosia artemisiifolia L. (AMBEL) 0.04
Urtica dioica L. (URTDI) 0.00
Bidens cernua L. (BIDCE) 0.00
Solidago spp. 0.02
Typha spp. 0.00
Glechoma hederacea L. (GLEHE) 0.02
Oenothera spp. 0.00
Festuca spp. 0.66
Senecio vulgaris L. (SENVU) 0.47
Elytrigia repens (L.) Nevski (AGRRE) 0.21
Cerastium vulgatum L. (CERVU) 0.21
Cirsium vulgare (Savi) Tenore (CIRVU) 0.15
Phytolacca americana L. (PHTAM) 0.10
Thlaspi arvense L. (THLAR) 0.09
Echinochloa crus-galli (L.) Beauv. (ECHCG) 0.09
Bromus spp. 0.07
Polygonum convolvulus L. (POLCO) 0.07
Tree spp. 0.03
Malva neglecta Wallr. (MALNE) 0.02
Epilobium spp. 0.01
Geranium spp. 0.01
Abutilon theophrasti Medicus (ABUTH) 0.01
Daucus carota L. (DAUCA) 0.01

Figure 4.3: Density of individual weed species (n = 50) (as percentage of total number of weed seeds) occurring in the sampled springtime seedbank in a 35-year-old tillage and rotation study in Wooster, OH, for 2003. Latin binomials and Bayer codes conform to WSSA Composite List of Weeds (1989). Species with zero values appeared in the 2003 field count only.

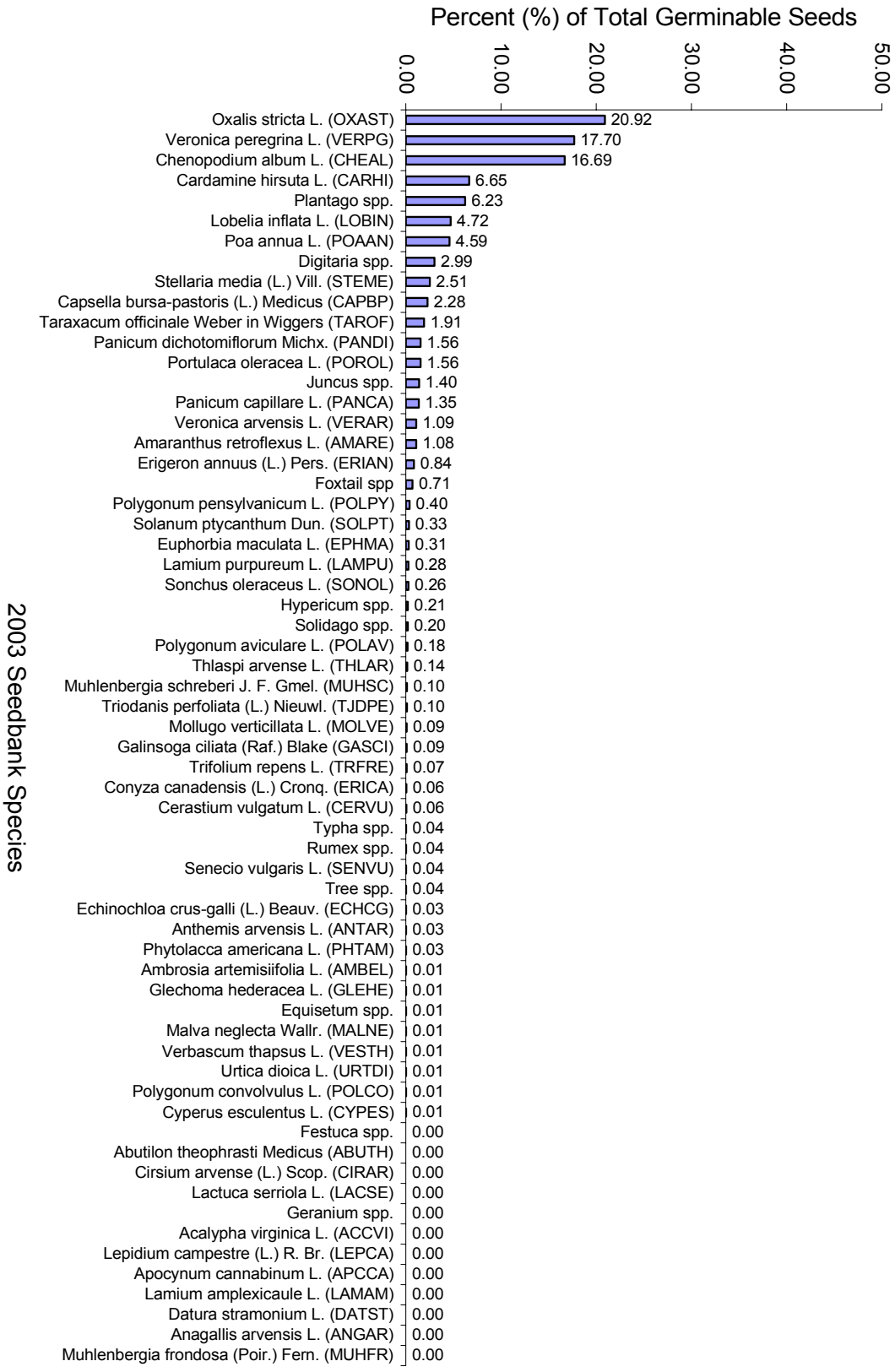
Percent (%) of Total Germinable Seeds
0.00
10.00
20.00
30.00
40.00
50.00
2003 Seedbank Species
Oxalis stricta L. (OXAST) 20.92
Veronica peregrina L. (VERPG) 17.70
Chenopodium album L. (CHEAL) 16.69
Cardamine hirsuta L. (CARHI) 6.65
Plantago spp. 6.23
Lobelia inflata L. (LOBIN) 4.72
Poa annua L. (POAAN) 4.59
Digitaria spp. 2.99
Stellaria media (L.) Vill. (STEME) 2.51
Capsella bursa-pastoris (L.) Medicus (CAPBP) 2.28
Taraxacum officinale Weber in Wiggers (TAROF) 1.91
Panicum dichotomiflorum Michx. (PANDI) 1.56
Portulaca oleracea L. (POROL) 1.56
Juncus spp. 1.40
Panicum capillare L. (PANCA) 1.35
Veronica arvensis L. (VERAR) 1.09
Amaranthus retroflexus L. (AMARE) 1.08
Erigeron annuus (L.) Pers. (ERIAN) 0.84
Foxtail spp 0.71
Polygonum pensylvanicum L. (POLPY) 0.40
Solanum ptycanthum Dun. (SOLPT) 0.33
Euphorbia maculata L. (EPHMA) 0.31
Lamium purpureum L. (LAMPU) 0.28
Sonchus oleraceus L. (SONOL) 0.26
Hypericum spp. 0.21
Solidago spp. 0.20
Polygonum aviculare L. (POLAV) 0.18
Thlaspi arvense L. (THLAR) 0.14
Muhlenbergia schreberi J. F. Gmel. (MUHSC) 0.10
Triodanis perfoliata (L.) Nieuwl. (TJDPE) 0.10
Mollugo verticillata L. (MOLVE) 0.09
Galinsoga ciliata (Raf.) Blake (GASCI) 0.09
Trifolium repens L. (TRFRE) 0.07
Conyza canadensis (L.) Cronq. (ERICA) 0.06
Cerastium vulgatum L. (CERVU) 0.06
Typha spp. 0.04
Rumex spp. 0.04
Senecio vulgaris L. (SENVU) 0.04
Tree spp. 0.04
Echinochloa crus-galli (L.) Beauv. (ECHCG) 0.03
Anthemis arvensis L. (ANTAR) 0.03
Phytolacca americana L. (PHTAM) 0.03
Ambrosia artemisiifolia L. (AMBEL) 0.01
Glechoma hederacea L. (GLEHE) 0.01
Equisetum spp. 0.01
Malva neglecta Wallr. (MALNE) 0.01
Verbascum thapsus L. (VESTH) 0.01
Urtica dioica L. (URTDI) 0.01
Polygonum convolvulus L. (POLCO) 0.01
Cyperus esculentus L. (CYPES) 0.01
Festuca spp. 0.00
Abutilon theophrasti Medicus (ABUTH) 0.00
Cirsium arvense (L.) Scop. (CIRAR) 0.00
Lactuca serriola L. (LACSE) 0.00
Geranium spp. 0.00
Acalypha virginica L. (ACCVI) 0.00
Lepidium campestre (L.) R. Br. (LEPCA) 0.00
Apocynum cannabinum L. (APCCA) 0.00
Lamium amplexicaule L. (LAMAM) 0.00
Datura stramonium L. (DATST) 0.00
Anagallis arvensis L. (ANGAR) 0.00
Muhlenbergia frondosa (Poir.) Fern. (MUHFR) 0.00

Figure 4.4: Density of individual weed species (n = 50) (as percentage of total number of weeds) occurring in the summer field count in a 35-year-old tillage and rotation study in Wooster, OH, for 2003. Latin binomials and Bayer codes conform to WSSA Composite List of Weeds (1989). Species are presented in order from highest to lowest as they appeared in the 2003 seedbank. Species with zero values appeared in the 2003 sampled seedbank only.

Percent (%) of Total Germinable Seeds
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
2003 Field Species
Oxalis stricta L. (OXAST) 7.96
Veronica peregrina L. (VERPG) 6.61
Chenopodium album L. (CHEAL) 30.20
Cardamine hirsuta L. (CARHI) 2.04
Plantago spp. 2.83
Lobelia inflata L. (LOBIN) 1.91
Poa annua L. (POAAN) 6.96
Digitaria spp. 2.65
Stellaria media (L.) Vill. (STEME) 6.27
Capsella bursa-pastoris (L.) Medicus (CAPBP) 2.60
Taraxacum officinale Weber in Wiggers (TAROF) 14.24
Panicum dichotomiflorum Michx. (PANDI) 2.51
Portulaca oleracea L. (POROL) 1.09
Juncus spp. 0.01
Panicum capillare L. (PANCA) 0.91
Veronica arvensis L. (VERAR) 0.00
Amaranthus retroflexus L. (AMARE) 2.34
Erigeron annuus (L.) Pers. (ERIAN) 0.11
Foxtail total 2.05
Polygonum pensylvanicum L. (POLPY) 1.47
Solanum ptycanthum Dun. (SOLPT) 0.26
Euphorbia maculata L. (EPHMA) 0.01
Lamium purpureum L. (LAMPU) 0.32
Sonchus oleraceus L. (SONOL) 0.43
Hypericum spp. 0.01
Solidago spp. 0.00
Polygonum aviculare L. (POLAV) 0.09
Thlaspi arvense L. (THLAR) 0.34
Muhlenbergia schreberi J. F. Gmel. (MUHSC) 0.00
Triodanis perfoliata (L.) Nieuwl. (TJDPE) 0.00
Mollugo verticillata L. (MOLVE) 0.00
Galinsoga ciliata (Raf.) Blake (GASCI) 0.00
Trifolium repens L. (TRFRE) 1.36
Conyza canadensis (L.) Cronq. (ERICA) 0.00
Cerastium vulgatum L. (CERVU) 0.06
Typha spp. 0.00
Rumex spp. 0.07
Senecio vulgaris L. (SENVU) 0.36
Tree spp. 0.09
Echinochloa crus-galli (L.) Beauv. (ECHCG) 0.36
Anthemis arvensis L. (ANTAR) 0.05
Phytolacca americana L. (PHTAM) 0.01
Ambrosia artemisiifolia L. (AMBEL) 0.03
Glechoma hederacea L. (GLEHE) 0.00
Equisetum spp. 0.00
Malva neglecta Wallr. (MALNE) 0.05
Verbascum thapsus L. (VESTH) 0.00
Urtica dioica L. (URTDI) 0.00
Polygonum convolvulus L. (POLCO) 0.21
Cyperus esculentus L. (CYPES) 0.09
Festuca spp. 0.86
Abutilon theophrasti Medicus (ABUTH) 0.05
Cirsium arvense (L.) Scop. (CIRAR) 0.03
Lactuca serriola L. (LACSE) 0.03
Geranium spp. 0.02
Acalypha virginica L. (ACCVI) 0.01
Lepidium campestre (L.) R. Br. (LEPCA) 0.01
Apocynum cannabinum L. (APCCA) 0.01
Lamium amplexicaule L. (LAMAM) 0.01
Datura stramonium L. (DATST) 0.01
Anagallis arvensis L. (ANGAR) 0.01
Muhlenbergia frondosa (Poir.) Fern. (MUHFR) 0.01

Figure 4.5: Density of individual weed species (n = 47) (as percentage of total number of weeds) occurring in the summer field count in a 35-year-old tillage and rotation study in Wooster, OH, for 2004. Latin binomials and Bayer codes conform to WSSA Composite List of Weeds (1989).

Percent (%) of Total Germinable Seeds
0.00
10.00
20.00
30.00
40.00
50.00
2004 Field Species
Digitaria Spp. 19.49
Taraxacum officinale Web. in Wig. (TAROF) 13.70
Panicum dichotomiflorum Michx. (PANDI) 7.92
Chenopodium album L. (CHEAL) 6.83
Veronica Spp. 5.47
Setaria Spp. 5.30
Oxalis stricta L. (OXAST) 5.28
Stellaria media (L.) Vill. (STEME) 4.83
Plantago Spp. 4.80
Poa annua L. (POAAN) 4.63
Amaranthus retroflexus L. (AMARE) 4.38
Cardamine hirsuta L. (CARHI) 3.33
Erigeron annuus (L.) Pers. (ERIAN) 1.67
Polygonum pensylvanicum L. (POLPY) 1.58
Echinochloa crus-galli (L.) Beauv. (ECHCG) 1.53
Panicum capillare L. (PANCA) 0.99
Cyperus esculentus L. (CYPES) 0.98
Lobelia inflata L. (LOBIN) 0.96
Portulaca oleracea L. (POROL) 0.91
Solanum ptycanthum Dun. (SOLPT) 0.77
Capsella bursa-pastoris (L.) Med. (CAPBP) 0.74
Lamium purpureum L. (LAMPU) 0.70
Thlaspi arvense L. (THLAR) 0.36
Sonchus oleraceus L. (SONOL) 0.33
Polygonum aviculare L. (POLAV) 0.26
Polygonum convolvulus L. (POLCO) 0.22
Senecio vulgaris L. (SENVU) 0.20
Trifolium pratense L. (TRFPR) 0.19
Cerastium vulgatum L. (CERVU) 0.17
Tree Spp. 0.17
Muhlenbergia frondosa (Poir.) Fern. (MUHFR) 0.17
Trifolium repens L. (TRFRE) 0.14
Rumex Spp. 0.12
Festuca Spp. 0.12
Phytolacca americana L. (PHTAM) 0.12
Abutilon theophrasti Medicus (ABUTH) 0.12
Euphorbia humistrata Eng. ex Gray (EPHHT) 0.11
Cirsium arvense (L.) Scop. (CIRAR) 0.09
Ambrosia artemisiifolia L. (AMBEL) 0.05
Glechoma hederacea L. (GLEHE) 0.05
Lactuca serriola L. (LACSE) 0.05
Geranium Spp. 0.03
Conyza canadensis (L.) Crong. (ERICA) 0.03
Acalypha virginica L. (ACCVI) 0.03
Arctium minus (Hill) Bernh. (ARFMI) 0.02
Juncus Spp. 0.02
Impatiens pallida 0.02

Source	df	Richness	Evenness	Diversity	Density
2002 Seedbank					
Tillage	2	0.47	25.77*	19.17*	14.40*
Rotation	5	4.64**	8.14**	1.85	28.60**
Tillage x Rotation	10	1.93	2.16*	2.31*	1.91
2002 Field					
Tillage	2	5.44	4.26	1.03	10.37*
Rotation	5	12.61**	2.08	2.91*	10.79**
Tillage x Rotation	10	1.67	0.92	0.83	2.16*
2003 Seedbank					
Tillage	2	5.91	29.60**	10.19*	29.96**
Rotation	5	11.42**	1.04	5.78**	9.05**
Tillage x Rotation	10	1.52	1.55	1.42	1.05
2003 Field					
Tillage	2	3.82	1.26	0.35	1.33
Rotation	5	10.28**	4.43**	8.63**	3.48*
Tillage x Rotation	10	3.44**	1.27	2.61*	1.07
2004 Field					
Tillage	2	12.42*	0.70	1.95	10.60*
Rotation	5	22.48**	0.65	9.15**	10.42**
Tillage x Rotation	10	4.60**	1.99	2.91*	2.27*

Table 4.2: Analysis of variance for the main effects of tillage system and crop sequence on species richness (S), evenness (J), the Shannon-Weiner index (H'), and density (seeds/m^2 or weeds/m^2) for the springtime weed seedbanks and summertime weed communities for 2002, 2003 and 2004 in a 35-year-old study in Wooster, OH. Significance is designated as * = $P < 0.05$, ** = $P < 0.01$.

Tillage Main Effects

	2002 Seedbank				2003 Seedbank			
	S	J	H'	seeds/m^2	S	J	H'	seeds/m^2
CT	14.94 a	0.85 a	2.27 a	2513 c	11.78 a	0.81 a	1.93 a	2367 c
MT	15.83 a	0.81 a	2.22 a	3766 b	13.83 a	0.70 b	1.79 a	6219 b
NT	15.50 a	0.67 b	1.81 b	5541 a	15.28 a	0.57 c	1.55 b	8386 a

Rotation Main Effects

	2002 Seedbank				2003 Seedbank			
	S	J	H'	seeds/m^2	S	J	H'	seeds/m^2
CC	13.89 b	0.8 ab	2.09 a	2391 c	11.11 de	0.65 a	1.49 c	4743 cd
C(S)	14.11 b	0.87 a	2.28 a	1578 c	9.67 e	0.72 a	1.56 c	2294 d
S(C)	13.56 b	0.83 ab	2.14 a	1775 c	12.89 cd	0.67 a	1.67 bc	4983 bc
C(OH)	17.33 a	0.71 c	2.0 a	8788 a	16.11 ab	0.67 a	1.85 ab	7888 a
O(HC)	15.67 ab	0.77 bc	2.09 a	3877 b	17.67 a	0.71 a	2.01 a	8663 a
H(CO)	18.01 a	0.69 c	2.0 a	5233 b	14.33 bc	0.74 a	1.96 a	5373 ab

Table 4.3: Means of species richness (S), evenness (J), the Shannon-Weiner index (H') and density (seeds/m^2) with respect to tillage and rotation, for the springtime weed seedbank communities for 2002 and 2003. Values of H' can range from 0 to lnS (H'_{max}). Maximum diversity usually occurs when all species are similarly abundant. Values of J can range from 0 to 1. Conventional-tillage (CT), minimum-tillage (MT), no-tillage (NT), continuous corn (CCC), corn-soybean-corn (CSC), soybean-corn-soybean (SCS), corn-oat-hay (COH), oat-hay-corn (OHC), hay-corn-oat (HCO). Means followed by the same letter are not significantly different at the $\alpha = 0.05$.

Table 4.4: Means of species richness (S), evenness (J), the Shannon-Weiner index (H') and density (weeds/m^2) with respect to tillage and rotation, for the summer weed communities for 2002, 2003 and 2004. Conventional-tillage (CT), minimum-tillage (MT), no-tillage (NT), continuous corn (CCC), corn-soybean-corn (CSC), soybean-corn-soybean (SCS), corn-oat-hay (COH), oat-hay-corn (OHC), hay-corn-oat (HCO). Means followed by the same letter are not significantly different at the $\alpha = 0.05$.

Tillage Main Effects

	2002 Field				2003 Field				2004 Field			
	S	J	H'	weeds/m^2	S	J	H'	weeds/m^2	S	J	H'	weeds/m^2
CT	9.33 a	0.72 a	1.58 a	98 b	12.94 a	0.67 a	1.69 a	206 a	11.67 b	0.80 a	1.90 a	99 b
MT	9.33 a	0.73 a	1.54 a	186 b	13.00 a	0.70 a	1.75 a	293 a	14.33 a	0.75 a	1.97 a	168 a
NT	11.83 a	0.61 a	1.44 a	364 a	14.18 a	0.63 a	1.66 a	329 a	10.72 b	0.76 a	1.73 a	92 b

Rotation Main Effects

	2002 Field				2003 Field				2004 Field			
	S	J	H'	weeds/m^2	S	J	H'	weeds/m^2	S	J	H'	weeds/m^2
CC	10.33 b	0.67 a	1.49 b	184 b	12.44 b	0.55 b	1.37 d	345 ab	10.11 c	0.78 a	1.79 bc	101 b
C(S)	11.56 b	0.67 a	1.6 ab	172 b	12.56 b	0.61 b	1.52 cd	231 b	10.33 c	0.78 a	1.81 bc	110 b
S(C)	9.67 bc	0.68 a	1.54 ab	158 bc	12.01 b	0.66 b	1.63 bcd	250 ab	7.78 d	0.77 a	1.51 c	45 c
C(OH)	15.33 a	0.68 a	1.84 a	608 a	18.67 a	0.74 a	2.15 a	421 a	11.00 c	0.71 a	1.70 c	93 b
O(HC)	7.00 c	0.79 a	1.46 b	96 cd	11.89 b	0.74 a	1.87 b	183 b	19.44 a	0.81 a	2.39 a	197 a
H(CO)	7.11 c	0.64 a	1.21 b	79 cd	12.21 b	0.71 a	1.73 b	184 b	14.78 b	0.77 a	2.02 b	172 ab

Seedbank

	S	J	H'	seeds/m^2
CT	13.36	0.83	2.10	2440
MT	14.83	0.76	2.01	4993
NT	15.39	0.62	1.68	6964
CC	12.50	0.73	1.79	3567
C(S)	11.89	0.80	1.92	1936
S(C)	13.23	0.75	1.91	3379
C(OH)	16.72	0.69	1.93	8338
O(HC)	16.67	0.74	2.05	6270
H(CO)	16.17	0.72	1.98	5303

Field

	S	J	H'	weeds/m^2
CT	11.31	0.73	1.72	134
MT	12.22	0.73	1.75	216
NT	12.24	0.67	1.61	262
CC	10.96	0.67	1.55	210
C(S)	11.48	0.69	1.64	171
S(C)	9.82	0.70	1.56	151
C(OH)	15.00	0.71	1.90	374
O(HC)	12.78	0.78	1.91	159
H(CO)	11.37	0.71	1.65	145

Table 4.5: Means, over years, of species richness (S), evenness (J), the Shannon-Weiner index (H') and density (seeds/m^2 and weeds/m^2) for the spring seedbank and summer weed population, for the main effects of tillage and rotation. Seedbank values are the means of two years (2002 and 2003); field values are the means of three years (2002-2004). Conventional-tillage (CT), minimum-tillage (MT), no-tillage (NT), continuous corn (CCC), corn-soybean-corn (CSC), soybean-corn-soybean (SCS), corn-oat-hay (COH), oat-hay-corn (OHC), hay-corn-oat (HCO).

Table 4.6: Indicator values of each species in the 2002 weed seedbank for each treatment rounded to the nearest whole percentage. Values with an "*" are maximum values where $P < 0.05$ as determined by a Monte Carlo test with 1000 permutations

	CT	MT	NT	CC	C(S)	S(C)	C(OH)	O(HC)	H(CO)
Acalypha virginica L. (ACCVI)	2	3	12	6	0	1	15	1	1
Amaranthus blitoides S. Wats. (AMABL)	4	0	15	7	0	7	2	2	0
Amaranthus retroflexus L. (AMARE)	28	23	28	12	20	26	7	4	15
Ambrosia artemisiifolia L. (AMBEL)	11	0	0	0	0	22	0	0	0
Aster pilosus Willd. (ASTPI)	2	0	7	0	0	4	0	4	4
Bidens cernua L. (BIDCE)	11	0	0	0	0	6	0	0	6
Bidens spp.	10	5	1	4	0	0	0	10	12
Brassica spp.	8	22	1	15	6	2	0	6	2
Capsella bursa-pastoris (L.) Med. (CAPBP)	21	18	9	0	0	1	25	29 *	23
Cardamine hirsuta L. (CARHI)	1	17	57 *	7	3	2	23	6	20
Chenopodium album L. (CHEAL)	24	17	50	37	7	16	9	4	20
Cirsium arvense (L.) Scop. (CIRAR)	0	4	2	7	0	0	0	0	4
Conyza canadensis (L.) Cronq. (ERICA)	0	2	11	11	0	0	2	0	4
Cyperus esculentus L. (CYPES)	41	19	11	25	13	17	1	6	12
Digitaria spp.	10	40	18	1	8	1	49 *	16	12
Erigeron annuus (L.) Pers. (ERIAN)	8	0	19	0	3	3	5	12	3
Eupatorium perfoliatum L. (EUPPE)	27	16	8	25	16	3	0	8	8
Euphorbia maculata L. (EPHMA)	6	0	6	3	3	0	0	3	3
Glechoma hederacea L. (GLEHE)	0	6	0	0	11	0	0	0	0
Hypericum spp.	2	1	7	0	4	1	7	2	0
Juncus spp.	1	1	10	2	9	0	2	2	0
Juncus tenuis Willd. (IUNTE)	13	25	13	7	5	6	19	4	12
Lamium amplexicaule L. (LAMAM)	1	6	42 *	0	3	8	30	5	1
Lobelia inflata L. (LOBIN)	2	29	16	3	0	1	63 *	3	3
Mollugo verticillata L. (MOLVE)	7	0	2	4	13	0	0	0	0
Muhlenbergia frondosa (Poir.) Fern. (MUHFR)	0	0	11	0	0	0	0	4	7
Muhlenbergia schreberi J. F. Gmel. (MUHSC)	10	1	1	9	0	9	2	0	0
Oenothera spp.	0	6	0	0	0	0	0	0	11
Oxalis stricta L. (OXAST)	13	24	57 *	2	1	2	47 *	19	28
Panicum capillare L. (PANCA)	8	21	23	3	1	8	4	32	12
Panicum dichotomiflorum Michx. (PANDI)	39	20	14	12	6	5	40 *	1	19
Plantago spp.	18	25	12	0	0	0	38 *	20	22
Poa annua L. (POAAN)	3	26	7	0	12	2	18	1	12
Polygonum aviculare L. (POLAV)	4	1	0	0	0	0	22	0	0
Polygonum pensylvanicum L. (POLPY)	10	9	60 *	10	5	15	21	1	21
Portulaca oleracea L. (POROL)	14	15	2	1	47 *	3	0	1	2
Potentilla spp.	18	25	12	10	11	7	1	20	9
Rorippa spp.	19	1	0	2	13	0	0	2	2
Rumex spp.	1	20	1	0	6	0	6	2	6
Setaria spp.	13	30	39	5	2	3	65 *	10	7
Solanum ptycanthum Dun. (SOLPT)	13	26	9	3	8	12	1	29	5
Solanum spp	0	1	13	0	3	0	3	3	3
Solidago spp.	3	0	3	6	0	0	0	0	6
Sonchus oleraceus L. (SONOL)	2	8	17	1	0	0	11	3	28 *
Stellaria media (L.) Vill. (STEME)	2	23	10	0	8	11	8	6	4
Taraxacum officinale Web. in Wig. (TAROF)	11	12	18	5	0	4	52 *	1	3
Trifolium repens L. (TRFRE)	0	0	17	0	0	2	2	7	0
Typha spp.	0	6	0	0	11	0	0	0	0
Urtica dioica L. (URTDI)	0	3	3	6	6	0	0	0	0
Veronica arvensis L. (VERAR)	2	0	4	0	0	0	22	0	0
Veronica peregrina L. (VERPG)	10	30	41	1	3	2	21	31	36 *

Table 4.7: Indicator values of each species in the 2002 field count for each treatment rounded to the nearest whole percentage. Values with an "*" are maximum values where $P < 0.05$ as determined by a Monte Carlo test with 1000 permutations

	CT	MT	NT	CC	C(S)	S(C)	C(OH)	O(HC)	H(CO)
Abutilon theophrasti Medicus (ABUTH)	0	6	0	0	11	0	0	0	0
Acalypha virginica L. (ACCVI)	0	0	11	0	0	0	22	0	0
Amaranthus retroflexus L. (AMARE)	17	25	20	30	32	12	18	0	0
Ambrosia artemisiifolia L. (AMBEL)	1	0	13	13	0	2	2	0	0
Bromus spp.	0	0	11	1	0	0	0	0	10
Capsella bursa-pastoris (L.) Medicus (CAPBP)	6	9	2	1	1	0	20	7	0
Cardamine hirsuta L. (CARHI)	0	1	52 *	0	1	0	34 *	6	1
Cerastium vulgatum L. (CERVU)	0	11	0	7	4	0	0	0	0
Chenopodium album L. (CHEAL)	26	22	23	34 *	28	24	13	0	0
Cirsium arvense (L.) Scop. (CIRAR)	0	0	28 *	1	2	0	1	0	13
Cirsium vulgare (Savi) Tenore (CIRVU)	0	0	26	3	3	0	1	0	8
Conyza canadensis (L.) Cronq. (ERICA)	0	0	17	4	4	4	0	0	0
Cyperus esculentus L. (CYPES)	10	4	0	0	0	1	1	0	27
Daucus carota L. (DAUCA)	0	0	6	11	0	0	0	0	0
Digitaria spp.	13	31	27	2	7	8	75 *	3	0
Echinochloa crus-galli (L.) Beauv. (ECHCG)	0	1	4	0	0	0	22	0	0
Elytrigia repens (L.) Nevski (AGRRE)	12	0	2	0	0	0	1	29 *	0
Epilobium spp.	0	0	6	0	0	11	0	0	0
Erigeron annuus (L.) Pers. (ERIAN)	0	0	28 *	19	10	0	0	0	0
Euphorbia maculata L. (EPHMA)	6	8	2	6	1	24	1	0	0
Festuca spp.	0	14	2	0	0	0	5	9	1
Geranium spp.	0	6	0	0	0	0	0	11	0
Glechoma hederacea L. (GLEHE)	6	0	0	0	0	0	11	0	0
Juncus tenuis Willd. (IUNTE)	0	0	11	6	0	0	6	0	0
Lamium amplexicaule L. (LAMAM)	4	1	0	0	0	0	22	0	0
Lobelia inflata L. (LOBIN)	0	0	17	0	1	0	20	0	0
Malva neglecta Wallr. (MALNE)	0	0	6	0	0	0	11	0	0
Mollugo verticillata L. (MOLVE)	10	1	0	0	8	7	0	0	0
Muhlenbergia schreberi J. F. Gmel. (MUHSC)	0	0	6	0	11	0	0	0	0
Oxalis stricta L. (OXAST)	7	16	32	1	0	0	46 *	38	4
Panicum capillare L. (PANCA)	20	20	4	1	2	4	18	31	1
Panicum dichotomiflorum Michx. (PANDI)	6	10	35	31	3	1	45	0	1
Phytolacca americana L. (PHTAM)	0	7	2	4	0	0	2	0	6
Plantago spp.	13	18	6	0	1	0	51 *	1	14
Poa annua L. (POAAN)	3	31	2	0	64 *	2	0	1	1
Polygonum aviculare L. (POLAV)	0	12	1	0	0	0	32 *	0	1
Polygonum convolvulus L. (POLCO)	0	0	22	1	1	0	17	0	0
Polygonum pensylvanicum L. (POLPY)	1	3	21	0	0	0	70 *	0	1
Portulaca oleracea L. (POROL)	21	20	2	2	55 *	13	5	0	0
Rumex spp.	2	14	0	0	0	0	3	0	32 *
Senecio vulgaris L. (SENVU)	10	2	16	29	8	4	2	0	0
Setaria spp	2	12	49 *	2	2	3	60 *	3	0
Solanum ptycanthum Dun. (SOLPT)	16	8	5	3	14	3	29 *	0	0
Solidago spp.	0	0	6	0	0	11	0	0	0
Sonchus oleraceus L. (SONOL)	0	3	40 *	4	0	1	4	0	44 *
Stellaria media (L.) Vill. (STEME)	3	42 *	4	1	6	22	3	18	0
Taraxacum officinale Weber in Wiggers (TAROF)	9	13	76 *	12	11	17	48 *	2	10
Thlaspi arvense L. (THLAR)	1	10	0	0	1	0	20	0	0
Tree spp.	0	0	11	22	0	0	0	0	0
Trifolium repens L. (TRFRE)	6	5	2	1	1	0	7	0	17
Veronica peregrina L. (VERPG)	2	1	54 *	0	1	2	7	36 *	3

Table 4.8: Indicator values of each species in the 2003 weed seedbank for each treatment rounded to the nearest whole percentage. Values with an "*" are maximum values where $P < 0.05$ as determined by a Monte Carlo test with 1000 permutations

	CT	MT	NT	CC	C(S)	S(C)	C(OH)	O(HC)	H(CO)
Amaranthus retroflexus L. (AMARE)	9	14	43 *	13	4	14	11	9	10
Ambrosia artemisiifolia L. (AMBEL)	0	0	6	0	11	0	0	0	0
Anthemis arvensis L. (ANTAR)	0	0	11	0	0	0	6	0	6
Capsella bursa-pastoris (L.) Medicus (CAPBP)	31	21	6	0	0	1	40 *	13	35
Cardamine hirsuta L. (CARHI)	2	27	52 *	6	2	8	15	39 *	7
Cerastium vulgatum L. (CERVU)	0	0	17	3	0	0	0	17	0
Chenopodium album L. (CHEAL)	13	21	65 *	51 *	13	16	13	3	2
Conyza canadensis (L.) Cronq. (ERICA)	0	6	6	0	0	3	3	11	0
Cyperus esculentus L. (CYPES)	0	0	6	0	0	0	11	0	0
Digitaria spp.	12	41	9	1	2	1	6	11	65 *
Echinochloa crus-galli (L.) Beauv. (ECHCG)	0	0	6	0	0	0	0	11	0
Equisetum spp.	0	0	6	0	0	11	0	0	0
Erigeron annuus (L.) Pers. (ERIAN)	6	0	69 *	17	3	4	5	8	8
Euphorbia maculata L. (EPHMA)	15	11	2	2	5	20	0	2	8
Foxtail spp	1	1	49 *	4	2	4	1	31 *	0
Galinsoga ciliata (Raf.) Blake (GASCI)	4	4	4	2	0	2	7	7	0
Glechoma hederacea L. (GLEHE)	0	6	0	0	0	0	0	11	0
Hypericum spp.	6	9	2	1	1	7	0	3	12
Juncus spp.	26	33	18	20	12	4	13	12	17
Lamium purpureum L. (LAMPU)	0	11	11	0	2	0	3	13	7
Lobelia inflata L. (LOBIN)	4	44	19	2	2	6	3	51 *	12
Malva neglecta Wallr. (MALNE)	0	0	6	0	0	0	0	11	0
Mollugo verticillata L. (MOLVE)	17	0	0	0	6	11	0	0	0
Muhlenbergia schreberi J. F. Gmel. (MUHSC)	0	3	8	0	0	6	6	2	0
Oxalis stricta L. (OXAST)	6	19	60 *	1	1	1	40 *	34	21
Panicum capillare L. (PANCA)	16	24	20	1	3	2	17	7	46 *
Panicum dichotomiflorum Michx. (PANDI)	15	11	30	3	1	1	23	32	16
Phytolacca americana L. (PHTAM)	0	11	0	0	0	6	0	0	6
Plantago spp.	22	23	20	1	0	1	26	45	17
Poa annua L. (POAAN)	4	49 *	0	2	2	81 *	0	0	0
Polygonum aviculare L. (POLAV)	0	21	0	0	0	1	1	3	8
Polygonum convolvulus L. (POLCO)	0	6	0	0	0	0	11	0	0
Polygonum pensylvanicum L. (POLPY)	3	4	8	0	0	0	43 *	4	0
Portulaca oleracea L. (POROL)	14	14	6	2	8	22	4	1	2
Rumex spp.	0	6	0	0	0	0	11	0	0
Senecio vulgaris L. (SENVU)	2	2	2	4	4	4	0	0	0
Solanum ptycanthum Dun. (SOLPT)	0	1	24	7	0	3	0	2	0
Solidago spp.	14	0	6	10	1	3	3	2	1
Sonchus oleraceus L. (SONOL)	0	4	32 *	2	1	2	22	4	1
Stellaria media (L.) Vill. (STEME)	2	57 *	4	10	15	12	9	1	2
Taraxacum officinale Weber in Wiggers (TAROF)	5	53 *	9	13	3	3	12	46 *	0
Thlaspi arvense L. (THLAR)	16	2	1	0	0	0	44 *	1	1
Tree spp.	0	0	17	4	4	0	0	0	4
Trifolium repens L. (TRFRE)	1	10	1	2	0	0	2	9	2
Triodanis perfoliata (L.) Nieuwl. (TJDPE)	1	1	20	0	2	2	0	6	14
Typha spp.	7	0	2	4	4	0	4	0	0
Urtica dioica L. (URTDI)	0	6	0	0	0	11	0	0	0
Verbascum thapsus L. (VESTH)	0	0	6	0	0	0	0	11	0
Veronica arvensis L. (VERAR)	7	14	31	2	2	8	4	26	17
Veronica peregrina L. (VERPG)	6	30	48	3	5	13	28	27	15

Table 4.9: Indicator values of each species in the 2003 field count for each treatment rounded to the nearest whole percentage. Values with an "*" are maximum values where $P < 0.05$ as determined by a Monte Carlo test with 1000 permutations

	CT	MT	NT	CC	C(S)	S(C)	C(OH)	O(HC)	H(CO)
Abutilon theophrasti Medicus (ABUTH)	0	11	1	2	19	0	0	0	0
Acalypha virginica L. (ACCVI)	0	0	6	0	0	0	0	11	0
Amaranthus retroflexus L. (AMARE)	24	28	23	22	17	28	21	0	2
Ambrosia artemisiifolia L. (AMBEL)	6	0	0	0	0	11	0	0	0
Anagallis arvensis L. (ANGAR)	6	0	0	0	0	0	0	0	20
Anthemis arvensis L. (ANTAR)	0	5	1	0	0	0	22	0	0
Apocynum cannabinum L. (APCCA)	0	0	6	0	0	0	0	0	20
Capsella bursa-pastoris (L.) Medicus (CAPBP)	25	10	2	2	0	0	93 *	0	1
Cardamine hirsuta L. (CARHI)	5	10	52 *	2	3	2	11	50 *	4
Cerastium vulgatum L. (CERVU)	1	0	10	0	0	0	0	18	4
Chenopodium album L. (CHEAL)	16	16	54 *	47 *	23	16	12	0	1
Cirsium arvense (L.) Scop. (CIRAR)	4	0	1	0	0	0	22	0	0
Cyperus esculentus L. (CYPES)	8	4	0	0	0	0	44 *	0	0
Datura stramonium L. (DATST)	6	0	0	11	0	0	0	0	0
Digitaria spp.	30	16	21	5	5	2	13	7	34
Echinochloa crus-galli (L.) Beauv. (ECHCG)	0	6	16	1	3	1	31	0	0
Erigeron annuus (L.) Pers. (ERIAN)	0	0	12	10	1	0	0	0	0
Euphorbia maculata L. (EPHMA)	6	0	0	0	0	0	11	0	0
Festuca spp.	0	12	1	0	0	0	44 *	0	0
Foxtail total	4	13	69 *	10	9	7	25	12	12
Geranium spp.	0	6	0	0	0	0	11	0	0
Hypericum spp.	6	0	0	0	0	0	0	11	0
Juncus spp.	0	0	6	0	0	0	11	0	0
Lactuca serriola L. (LACSE)	0	0	6	0	0	0	0	0	20
Lamium amplexicaule L. (LAMAM)	0	0	6	0	11	0	0	0	0
Lamium purpureum L. (LAMPU)	4	3	11	0	0	0	3	5	56 *
Lepidium campestre (L.) R. Br. (LEPCA)	0	0	6	0	0	0	0	11	0
Lobelia inflata L. (LOBIN)	0	15	6	2	0	3	0	25	1
Malva neglecta Wallr. (MALNE)	0	1	15	9	0	0	0	4	5
Muhlenbergia frondosa (Poir.) Fern. (MUHFR)	0	0	6	0	0	0	0	11	0
Oxalis stricta L. (OXAST)	14	24	29	1	0	0	49 *	31	18
Panicum capillare L. (PANCA)	24	30	4	2	15	3	41 *	2	3
Panicum dichotomiflorum Michx. (PANDI)	51	19	14	12	4	4	45 *	23	0
Phytolacca americana L. (PHTAM)	0	0	12	0	0	0	6	6	0
Plantago spp.	4	14	12	0	0	0	70 *	0	6
Poa annua L. (POAAN)	23	62 *	1	5	6	46	5	1	1
Polygonum aviculare L. (POLAV)	0	2	7	0	0	0	14	9	0
Polygonum convolvulus L. (POLCO)	0	0	10	9	0	0	6	0	0
Polygonum pensylvanicum L. (POLPY)	9	12	3	0	0	0	97	0	0
Portulaca oleracea L. (POROL)	19	23	2	6	23	36 *	0	0	0
Rumex spp.	0	13	0	0	0	0	22	0	0
Senecio vulgaris L. (SENVU)	2	3	34 *	8	7	17	2	0	2
Solanum ptycanthum Dun. (SOLPT)	27	5	4	5	12	12	7	0	1
Sonchus oleraceus L. (SONOL)	2	4	24	2	0	3	34 *	0	5
Stellaria media (L.) Vill. (STEME)	12	71 *	4	8	32	17	3	7	6
Taraxacum officinale Weber in Wiggers (TAROF)	30	24	46	16	8	13	20	17	27
Thlaspi arvense L. (THLAR)	7	9	0	0	0	0	65 *	0	0
Tree spp.	6	5	10	0	8	6	4	11	0
Trifolium repens L. (TRFRE)	12	10	14	1	1	0	2	58 *	4
Veronica peregrina L. (VERPG)	16	30	51	20	16	9	36	4	13

Table 4.10: Indicator values of each species in the 2004 field count for each treatment rounded to the nearest whole percentage. Values with an "*" are maximum values where $P < 0.05$ as determined by a Monte Carlo test with 1000 permutations

	CT	MT	NT	CC	C(S)	S(C)	C(OH)	O(HC)	H(CO)
Abutilon theophrasti Medicus (ABUTH)	1	15	0	0	0	44 *	0	0	0
Acalypha virginica L. (ACCVI)	0	0	11	0	0	0	0	22	0
Amaranthus retroflexus L. (AMARE)	20	34	24	20 *	37	3	8	14	7
Ambrosia artemisiifolia L. (AMBEL)	2	4	0	4	0	0	0	0	7
Arctium minus (Hill) Bernh. (ARFMI)	0	6	0	0	0	0	0	0	11
Capsella bursa-pastoris (L.) Med. (CAPBP)	4	13	1	1	0	3	0	3	26 *
Cardamine hirsuta L. (CARHI)	3	16	37	9	8	3	7	8	11
Cerastium vulgatum L. (CERVU)	1	0	23 *	0	1	0	2	6	10
Chenopodium album L. (CHEAL)	41 *	37	2	25	8	3	2	30 *	10
Cirsium arvense (L.) Scop. (CIRAR)	0	0	17	0	0	0	0	11	6
Conyza canadensis (L.) Cronq. (ERICA)	0	0	6	0	0	0	0	0	11
Cyperus esculentus L. (CYPES)	7	9	0	0	0	0	0	22 *	7
Digitaria Spp.	15	52 *	16	5	3	0	26	14	35
Echinochloa crus-galli (L.) Beauv. (ECHCG)	1	15	8	2	0	0	1	13	24
Erigeron annuus (L.) Pers. (ERIAN)	2	7	29	8	20	0	6	5	0
Euphorbia humistrata Eng. ex Gray (EPHHT)	3	10	1	3	0	0	2	6	6
Festuca Spp.	0	0	11	0	0	0	0	1	10
Geranium Spp.	0	6	0	0	0	0	0	11	0
Glechoma hederacea L. (GLEHE)	0	6	0	0	0	0	11	0	0
Impatiens pallida	0	0	6	0	0	0	0	11	0
Juncus Spp.	0	0	6	0	0	0	0	11	0
Lactuca serriola L. (LACSE)	0	4	2	7	0	0	0	4	0
Lamium purpureum L. (LAMPU)	3	19	4	2	5	0	8	5	4
Lobelia inflata L. (LOBIN)	0	17	20	1	6	1	3	11	3
Muhlenbergia frondosa (Poir.) Fern. (MUHFR)	1	0	10	0	0	0	0	33 *	0
Oxalis stricta L. (OXAST)	16	22	11	0	0	0	4	76 *	16
Panicum capillare L. (PANCA)	8	22	12	1	5	2	3	29	11
Panicum dichotomiflorum Michx. (PANDI)	38	32	9	21	11	0	5	34	15
Phytolacca americana L. (PHTAM)	3	14	1	8	0	1	1	6	1
Plantago Spp.	24	22	3	0	1	0	12	44 *	16
Poa annua L. (POAAN)	23	34	5	2	25	10	2	4	17
Polygonum aviculare L. (POLAV)	3	13	2	0	1	0	1	1	46 *
Polygonum convolvulus L. (POLCO)	4	8	3	2	0	0	0	28 *	8
Polygonum pensylvanicum L. (POLPY)	12	20	13	1	3	0	1	73 *	4
Portulaca oleracea L. (POROL)	11	20	0	1	38 *	6	0	0	1
Rumex Spp.	4	3	1	0	0	0	1	29	0
Senecio vulgaris L. (SENVU)	5	6	7	21	2	1	1	1	3
Setaria Spp.	17	38	9	8	16	2	0	12	35 *
Solanum ptycanthum Dun. (SOLPT)	4	27	3	0	3	0	2	52	0
Sonchus oleraceus L. (SONOL)	2	17	1	1	0	0	2	28 *	2
Stellaria media (L.) Vill. (STEME)	17	73 *	0	10	9	16	5	14	7
Taraxacum officinale Web. in Wig. (TAROF)	28	42	28	28	10	13	11	22	14
Thlaspi arvense L. (THLAR)	16	0	0	0	0	0	0	44 *	0
Tree Spp.	1	8	12	1	0	12	0	20	1
Trifolium pratense L. (TRFPR)	0	0	22	0	0	0	2	0	28 *
Trifolium repens L. (TRFRE)	5	2	2	0	0	0	40 *	0	1
Veronica Spp.	5	17	34	1	11	4	8	18	4

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CHAPTER 5

VARIATION IN *ABUTILON THEOPHRASTI* MEDICUS (VELVETLEAF) POPULATIONS.

Lynn M. Sosnoskie, Sajal Sthapit, David M. Francis and John Cardina

INTRODUCTION

Abutilon theophrasti Medicus (Malvaceae, velvetleaf) is an important weed of corn (*Zea may* L.), soybean (*Glycine max* L. (Merr.)), cotton (*Gossypium hirsutum* L.), and sorghum (*Sorghum bicolor* (L.) Moench), plus many other crops worldwide (Spencer 1984, Hartzler 1997, Baranpour and Abdollahi 2000, Traore *et al.* 2003, Werner *et al.* 2004). *Abutilon theophrasti* is a summer annual, native to central China, with erect, hirsute, green- or purple-colored stems that branch at higher leaf axils (Warwick and Black 1988). Leaves are alternate, petiolate, toothed, heart-shaped, and covered in soft hairs (Warwick and Black 1988). Pale yellow- to yellow-orange-colored flowers are borne singly in the axils of leaves or in small clusters on short branches in the plant canopy (Warwick and Black 1988). The species is self-compatible, although cross-fertilization can take place by way of insect-mediated pollen transmission (Andersen 1988). Vegetative reproduction and inter-specific hybridization are not known to occur (Warwick and Black 1986). Seed capsules are cup-shaped, hairy,

brown- or yellow-colored, and composed of 12 to 15 radially-arranged, beaked carpels (Warwick and Black 1986, Kurokawa *et al.* 2003a). One to three kidney-shaped, grey- to black-colored seeds are produced in each carpel (Warwick and Black 1986). Seeds may or may not be dormant at maturity (Warwick and Black 1986, Kurokawa *et al.* 2003a). Morphological and phenological traits vary with respect to genotype, environment, and the genotyope-environment interaction (Warwick and Black 1988, Jasieński *et al.* 1997, Kurokawa *et al.* 2003a). *Abutilon theophrasti* is an allohexaploid (2n = 6X = 42), although the diploid and tetraploid progenitors remain unknown (Stegink and Spencer 1988).

Abutilon theophrasti, like kenaf (*Hibiscus cannabinus* L.), beach hibiscus (*Hibiscus tiliaceus* L.), roselle (*Hibiscus sabdariffa* L.), and other members of the Malvaceae, has been, and still is cultivated for its stem fibers, which are used in the manufacture of rope, twine, and other materials (Dempsey, 1975). *Abutilon theophrasti* was domesticated in central China, where it was grown for its soft and lustrous stem fibers (Dempsey 1975). Stem tissue has been used alone to produce twine, paper, sacking, netting, and coarse cloth, or blended with silk to make satin and brocades (Dempsey 1975, Spencer 1984). It has been suggested that *A. theophrasti* was introduced purposely to colonial America to serve as a fiber source for the manufacture of cordage and other necessities (Dempsey 1975, Spencer 1984).

Preliminary observations made in our lab indicate that a substantial amount of variability exists among *A. theophrasti* accessions with respect to physical appearance, flowering phenology, and capsule color. We hypothesize that biotypes can be distinguished phenetically that represent stages of domestication, ranging from crop to

weed. Identifying these biotypes will help us understand crop-weed evolution in *A. theophrasti*. The purpose of this study was to characterize the morphological and phenological variation present among 80 *A. theophrasti* accessions, and to determine if crop and weedy biotypes exist and can be differentiated based on phenotype.

Morphological markers, while easily scored, are subject to environmental influences that alter their expression. Therefore we conducted a pilot study to develop markers for single and low-copy genes in the Malvaceae based on two collections of tomato (*Lycopersicon esculentum* L.) conserved ortholog set (COS) genes. Genetic markers based on random polymorphisms in genomic DNA may become disassociated from phenotypic traits through recombination. The application of codominant markers derived from Mendelian loci to the *A. theophrasti* crop-weed complex will allow us to make inferences about various population processes such as allele frequency, mating system, genetic drift, and natural selection.

MATERIALS AND METHODS

Plant materials:

Seeds of 80 *A. theophrasti* accessions, collected from 21 countries, were provided by Dr. R.N. Andersen (USDA-ARS, retired) and the U.S. National Plant Germplasm System (NPGS) coordinated by the United States Department of Agriculture-Agricultural Research Service (USDA-ARS), for use in this study (Table 5.1). The accessions provided by Dr. Andersen were collected between 1979 and 1985. The samples obtained through the NPGS were collected by the N.I. Vavilov Institute of Plant Industry between 1916 and 1940 (Kurokawa *et al.* 2003a). To mitigate the effects

of maternal environment on gross morphology and phenology, the accessions were increased, once, in a common greenhouse environment before measurements were taken, with the goal of obtaining a minimum of seven "parent plants" per accession (Table 5.1). Samples of this size should provide a 99% probability of seeing a homozygous recessive individual for a trait, assuming that the allele frequency for the attribute is 0.50 (N = ln (1-*P*)/ln (1-f), where *P* = probability and f = allele frequency).

In May 2003, a single seed from each parent plant from every accession was scarified and sown individually in 7.57 L plastic pots filled with a steam-sterilized 1:1 (v:v) mixture of Wooster silt loam soil and ProMix BX (Premier Horticulture, Quakertown, PA). All morphological and phenological measurements were made on these individuals. Pots were arranged randomly on benches in a greenhouse at the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH. The average daily temperature over the course of the study was 25 °C. To ensure a 16-hour photoperiod, natural light was supplemented with metal halide (1000 watt) and high pressure sodium (1000 watt) lamps. Plants were watered as needed and fertilized weekly with a 20:20:20 (N:P:K) solution at a concentration of 100 ppm.

Morphology and phenology measurements:

The morphological and phenological characteristics measured in this study were similar to those used by Warwick and Black (1986) and Kurokawa *et al.* (2003a). Attributes measured for each plant included: initial 50-seed weight (g); stem height (mm) at 4, 7 and 10 weeks; length (mm), width (mm), and petiole length of largest leaf at 4, 7 and 10 weeks; number of days from sowing to flowering; stem height (mm) at

flowering; duration of flowering (days); number of days from sowing to harvest; final height (cm) at harvest; number of nodes at harvest; number of capsules (seed pods) per plant at harvest; mean number of carpels per capsule, for three capsules; mean diameter (mm) and height (mm) of three capsules; and capsule color. Duration of flowering was defined as the number of days between first flowering and harvest. Capsules were harvested from a plant when the last capsule had matured and no new flowers were initiated.

Statistical analysis:

Data for each characteristic were averaged within accessions. Variables were subjected to principal components analysis (PCA), which reduced the quantitative traits into a compact set of factors preserving a significant proportion of the information inherent in the original traits. Mood's median test, a nonparametric alternative to one-way ANOVA, was used (Minitab, release 13.1) to identify variables that differed with respect to capsule color.

Primer development:

In order to develop neutral genetic markers for population level analysis, conserved ortholog set markers were developed *in silico* to study genetic variation in *A. theophrasti*. The tomato COS markers described by Fulton *et al.* (2002) are single- or low-copy genes that, when compared to a database of all translated proteins in the fully sequenced *Arabadopsis thaliana* (L.) Heynh genome, had a score of e-15 and no close second match. Because COS markers represent low- or single-copy genes,

polymorphism can be interpreted as alleles of a single locus, thus maximizing information content as compared to random amplified polymorphic DNA (RAPD) or amplified fragment-length polymorphism (AFLP) markers, without sacrificing the ability to perform high throughput PCR-based analyses.

Ninety-two gene sequences were randomly selected from, but equally divided between, two tomato COS marker collections made available by the Solanaceae Genomics Network (SGN) (http://www.sgn.cornell.edu/) and the Compositae Genome Project (CGP) (http://www.cgpdb.ucdavis.edu/). These sequences were screened against the Cotton Gene Index (CGI, release 6.0), a database of tentative consensus (TC) sequences and expressed sequence tags (EST) maintained by The Institute for Genomic Research (TIGR) (http://www.tigr.org/), to identify corresponding COS genes in cotton. When multiple sequence matches were recovered in a search, the sequence with the lowest e-value was selected for further analysis. Except for two instances, best matches with e-values greater than e-15 were not evaluated further. Cotton was a substitute in the initial screening process because sequence data are lacking for *A. theophrasti*, and there is a sufficient collection of cotton DNA sequences maintained in public databases. Cotton and *A. theophrasti* are members of the family Malvaceae, subfamily Malvoideae.

Cotton EST sequences were screened against *A. thaliana* genomic sequences from the Arabidopsis Genome Initiative (AGI) maintained by The Arabidopsis Information Resource (TAIR) (http://www.arabidopsis.org). The positioning of *A. thaliana* introns was used to predict the locations of putative introns in the cotton COS sequences. Best match sequences with e-values of less than e-15, and sequences with no introns, large introns (> 1 kb), or multiple introns separated by islands of coding regions

that measured less than 100 bp in length, were not selected for further use. Primer pairs amplifying across the expected position of cotton introns were designed using the Primer 3 software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi).

DNA extraction:

Preliminary work with *A. theophrasti* suggested that unidentified polysaccharide and phenolic products can inhibit the successful extraction of amplifiable, high molecular weight DNA. This problem has been reported for cotton, cashew (*Anarcardium occidentale* L.), and epiphytic cacti of the genera *Hylocereus* and *Selenicereus* (Chaudhry *et al.* 1999, Tel-Zur *et al.* 1999, Rout *et al.* 2002). Following Tel-Zur *et al.* (1999), we adapted the protocol used by Kabelka *et al.* (2002) to include a centrifugation step following tissue maceration.

Samples of young etiolated leaf tissue (0.1 – 0.25 g) were frozen at -20 °C for 24 hours prior to extraction. Samples were ground on ice in 600 µl extraction buffer (0.35 M sorbitol, 1M Tris, 5mM EDTA, 0.4 M Na-bisulfite at pH 7.5) with a pestle in sterile 1.5 ml micro-centrifuge tubes, and spun at 3500 rpm (1200 g) in a Marathon 16km centrifuge with a 24 sample fixed-angle rotor (Fisher Scientific, Pittsburg, PA) for 10 minutes to pellet the tissue. The supernatant was decanted and the pellets washed with 600 µl of extraction buffer to remove polysaccharides. The pellets, enriched for nuclei, were re-suspended once in 300 µl extraction buffer, to which 300 µl nuclei lysis buffer (0.2 M Tris, 0.5 M EDTA, 2.0 M NaCL, 2% CTAB at pH 7.5) and 150 µl 5% *N*-lauryl-sarcosine were added.

Samples were mixed by inverting, and incubated in a water bath set at 65 °C for 90 minutes. Sample tubes were mixed every 15-25 minutes by inverting. Samples were cooled to room temperature before adding 600 µl chloroform:isoamyl alcohol (24:1 v:v) to each. Samples were mixed by inverting followed by centrifugation at 13,500 rpm (16,700 g) to separate the phases. The aqueous phases were transferred to sterile 1.5 ml micro-centrifuge tubes and the DNA precipitated with one volume isopropanol. Tubes were placed in a -20 °C freezer for 20 minutes before being spun at 13,500 rpm (16,700 g)for 15 minutes. The supernatant was decanted and the DNA pellets were re-suspended in 100 µl T 1/10 E buffer (10 mM Tris, 1mM EDTA at pH. 7.5). DNA was diluted to PCR concentrations in sterile distilled water.

PCR protocols and primer screens:

Each PCR reaction was run in a total volume of 40 µl and contained 50 ng DNA template, 10 µM each forward and reverse primer, 0.2 mM each dNTP, 1 unit of *Taq* polymerase in a buffer containing 100 mM Tris (pH 8.3), 500 mM KCl, 1.5 mM $MgCl_2$, and 0.01% gelatin (w:v). Reactions were carried out in a thermocycler (PTC 100, M.J. Research Inc., Waltham, MA) set to the following conditions: 2 minutes at 94 °C for initial denaturation followed by 39 cycles of 1 minute at 94 °C, 1 minute at 45 °C to facilitate binding of the cotton-derived primers to the *A. theophrasti* template and 3 minutes at 72 V for extension, with a final extension at 72 °C for 5 minutes. Twenty-µl samples of the amplified products were run in 4% agarose, stained with ethidium

bromide and photographed using the Syngene BioImaging System (Syngene, Frederick, MA). Fragment sizes were estimated against the migration of a 100 bp ladder.

Sequencing:

All single band PCR products were sequenced, using forward primers, at the Molecular Cellular Imaging Center at The OARDC using a 64 lane Perkin-Elmer ABI on a 36 cm gel with 3'-dye dideoxynucleotide triphosphates labeling (http://www.oardc.ohio-state.edu/mcic/genomics/sequencing/seq.html).

Identifying single nucleotide polymorphisms:

Sequenced products from each genotype for each primer pair were aligned using BLAST 2 Sequences software maintained by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Single cut Type II restriction enzyme cleavage sites specific to the putative single nucleotide polymorphisms (SNPs) were detected using Nebcutter V2.0 (http://tools.neb.com/NEBcutter2/index.php).

RESULTS AND DISCUSSION

Seventy-seven of 80 *A. theophrasti* accessions were successfully increased in the greenhouse. Morphological and phenological measurements were made on 586 individual plants propagated from the increased seed. Data for each characteristic were averaged within accessions. Twelve accessions were mixtures of plants with yellow- and brown-colored capsules; therefore, data were averaged within capsule color and evaluated as separate observations. It is not known at this time if this variation is the

result of seedlot contamination or if it represents a source of diversity within accessions because of co-localization of biotypes..

Morphology and phenology:

Morphological and phenological traits varied among accessions (Figure 5.1). Initial 50-seed mass, number of seed capsules/plant, stem height at 4, 7, and 10 weeks, stem height at flowering, and stem height at harvest were the most variable as indicated by the coefficients of variation (Table 5.2). Observed mean, minimum and maximum values for 50-seed mass, plant height at 4 weeks, plant height at 10 weeks, plant height at first flowering, duration of flowering, number of valves per capsule, and diameter and height of capsule are similar to observations made by Warwick and Black (1986), who evaluated intra-specific variation in *A theophrasti* populations collected from southern Ohio to southern Ontario, and Kurokawa *et al.* (2003a).

An analysis of the scree plot indicated that all principal components beyond the third accounted only for random error variance; therefore, only the first three were extracted (data not shown). Principal components analysis, based on 23 quantitative characters, indicated that the first, second, and third principal components accounted for 75% of the total variation (Figure 5.2). Yellow- and brown-colored-capsule accessions separated primarily along the first axis, which was associated with initial 50-seed mass and measures of adult plant size, such as stem height at 10 weeks and at flowering (Table 5.3). Capsule size, as determined by height and diameter, was also associated with the first axis (Table 5.3). The second principal component related to seedling biomass, including length and width of largest leaf at 4 weeks and stem height at 7

weeks (Table 5.3). Life span and number of nodes were also associated with the second principal component (Table 5.3). The third principal component was associated with the number of valves per capsule, and the petiole length of largest leaf at 7 and 10 weeks (Table 5.3). The biological and ecological relevance of the third axis is uncertain.

Results from univariate analyses indicate that plants with yellow and brown capsules differed in morphology and phenology for 20 of 23 variables measured (Table 5.4). In general, plants producing yellow capsules germinated from heavier seeds; had bigger largest-leaves at 4, 7, and 10 weeks; were taller at 4, 7 and 10 weeks and at harvest; flowered later and for a shorter period of time; and produced fewer but larger seed capsules as compared to plants producing brown capsules (Table 5.4). Our results are in agreement with Kurokawa *et al.* (2003a), who performed similar analyses and reported that the accessions could be divided into two biotypes, "crop" and "weedy", based on morphological and phenological characteristics. According to Kurokawa *et al.* (2003a), crop biotypes possessed yellow-colored seed capsules, were taller at all observation dates, were minimally branched, and had a longer vegetative phase than plants with brown capsules. Weedy biotypes were shorter, more branched, and produced greater numbers of seeds in brown-colored pods (Kurokawa *et al.* 2003a).

Our results differ from Kurokawa *et al.* (2003a) with respect to the amount and total length of branching per plant, and reproductive output. We did not observe substantial amounts of branching at any time during the study, whereas Kurokawa *et al.* (2003a) and Warwick and Black (1986) indicated that *A. theophrasti* could produce numerable branches at three and four months. Additionally, we recorded a mean of 15 capsules per plant (range 8 to 29), where as Warwick and Black (1986) and Kurokawa *et*

al. (2003a) reported means of 103 (range 51 to 144) and 276 (range 38 to 839), respectively. This disparity probably results from differences in light quantity and quality among the studies. Both Warwick and Black (1986) and Kurokawa *et al.* (2003a) conducted their studies outdoors, in natural light and daylength conditions. Changes in light quality and quantity have been shown to elicit modifications in branch development (Huber and Stuefer 1997, Heraut-Bron *et al.* 2001).

Differences between the biotypes highlight differences in the selection pressures that acted upon them previously. An upright, minimally branched form of *A. theophrasti* would allow for the easier harvest of stem fibers (Kurokawa *et al.* 2003a). *Abutilon theophrasti* appears to be determinant with respect to growth; therefore, an increase in the duration of the vegetative phase would result in greater stem height, which is directly related to fiber yield (Kurokawa *et al.* 2003a). A rapid transition from vegetative to reproductive phase, and a longer reproductive phase that allows for the maximal production of propagules, is an advantageous trait for weeds (Baker 1974, Patterson 1985). Increased branching is associated with increased capsule production as *A.theophrasti* flowers are produced on short branches that develop from leaf axils in the canopy of the plant (Warwick and Black 1988).

Genetic markers:

We were able to identify 68 COS genes in cotton from 92 gene sequences selected from COS marker collections developed by the SGN and the CGP (Table 5.5). Thirty-seven (80 %) and 31 (67 %) cotton gene sequences were found to be homologous to tomato COS marker genes from the SGN and CGP collections, respectively (Table

5.5). From the 68 cotton sequences, we developed 34 primer pairs, 21 and 13 from SGN and CGP derived sequences, respectively, that were intended to flank putative introns as determined by a screen of *A. thaliana* genomic sequences (Tables 5.5 and 5.6).

Two accessions, each representing one of the purported biotypes, were screned with the sets of primers pairs to evaluate underlying genetic variation. Andersen (And) 13 is a smaller (1.1 m) accession with a shorter life span (102 days from sowing to harvest) that produces seed in brown capsules. Plant introduction (PI) 499252 is a taller (2.5 m) and slightly longer-lived (120 days) accession that produces mostly yellow-colored capsules. Genomic DNA for PCR was isolated from seven one- to two-week old seedlings per accession using a modified mini-prep CTAB extraction protocol (Kabelka *et al.* 2002).

Eight primer pairs failed to amplify *A. theophrasti* genomic DNA. Of the successful primer pairs, 15 yielded products with multiple bands, whereas 11 produced single bands. A conservative estimate suggests that the two accessions shared at least 45 bands. Many bands were difficult to resolve electrophoretically and several single bands were shown to be mixtures of products when sequenced. One primer pair (VL11f and VL11r) gave a polymorphic product that was detected consistently (Figure 5.3). Seven candidate SNPs were detected by comparative sequence analysis of the two phenotypes; four were products of the same marker (Table 5.7). Five putative SNPs were associated with unique Type II restriction enzyme cleavage sites (Table 5.7). Continuing work in our lab will be aimed at verifying the candidate SNPs, developing and screening additional primers, and characterizing the genetic diversity among *A. theophrasti* populations.

Jasieński *et al.* (1997) measured genetic similarity in 27 co-occurring *A. theophrasti* genotypes using 20 RAPD primers that yielded, on average, 415 scoreable bands. Although diversity was estimated to be less than 5%, genetically similar plants performed equally well across a temperature and moisture gradient, suggesting a genetic basis for phenotypic plasticity (Jasieński *et al.* 1997). Results from Kurokawa *et al.* (2003b) indicated that 111 polymorphic bands derived from 28 custom ISSR markers differentiated 45 *A. theophrasti* accessions into two main groups that were defined by capsule color and growth habit. Kurokawa *et al.* (2004) screened 93 *A. theophrasti* samples with a set of 19 universal primer pairs specific to partial regions of the chloroplast DNA. Kurokawa *et al.* (2004) reported that two chloroplast DNA haplotypes (A, B) could be determined and when combined with capsule color, three genotypes (Types I, II, and III) emerged. Type I plants have cpDNA haplotype A and yellow capsules, whereas Types II and III have brown capsules but are of haplotypes A and B, respectively (Kurokawa *et al.* 2004). Kuokawa *et al.* (2003a, 2004) reported that the biotypes are sexually compatible and suggested that Type II might be the result of crosses between the other two.

Considering that weeds account for the most losses in yield as compared to all other crop pests, the area of weed genomics remains underdeveloped (Basu *et al.* 2004). Despite the fact that "weedy characteristics" have been investigated and described with respect to morphology, physiology and biochemistry, relatively few studies have been undertaken to characterize the genes, transcription factors, and regulatory pathways that govern the traits of dormancy, growth rate, fecundity, competitiveness, and plasticity, among others, in economically damaging species (Basu *et al.* 2004). The selection of

weed model species is crucial to the advancement of weed functional genomics (Basu *et al.* 2004). We suggest that that *A. theophrasti* should serve as a candidate owing to the existence of crop and weedy biotypes that are discernable phenetically, as has been shown by our work and others, and genetically (Kurokawa *et al.* 2003a, 2003b, 2004). Additionally, biotypes can overlap with respect to flowering times and geographic ranges, suggesting that gene flow within the crop-weed complex can occur in the presence of a common pollinator.

This study characterized the morphological and phenological variation present in 77 velvetleaf accessions from Asia, Japan, India, Europe, Eastern Africa and North America. Analyses indicate that accessions producing yellow-colored seed capsules are significantly taller, flower later and were longer-lived than their brown-colored counterparts were. This finding supports previous assertions that the yellow-colored-capsule varieties were domesticated for use as a fiber crop. In contrast to Kurokawa *et al.* (2003a), we did not observe substantial amounts of branching or flowering in the weedy biotypes, affirming that morphological markers can vary with respect to environment. The altered expression of traits can complicate analyses and interpretation. Preliminary genetic analyses using COS markers derived from single- and low-copy genes, which are stable in sequence and conserved across families, are still ongoing. Andersen and Lübberstedt (2003) encouraged the use of DNA markers derived from sequence motifs with known, functional products over random markers in future genetics investigatons. Conserved ortholog set markers have been assigned to putative functional categories with respect to basic metabolic processes, and could be useful in

comparative studies in crop-weed complexes, especially if markers can be developed that correspond to genes differentially expressed between biotypes (Fulton *et al.*2002).

Identity	Country	Local provenance	Biotype	Identity	Country	Local Provenance	Biotype
Andersen 2	USA	Mississippi, Merigold	weedy	499215	Africa	Not reported	mix
Andersen 3	USA	Arkansas, Fayetteville	weedy	499216	China	Not reported	mix
Andersen 4	USA	Missouri, Columbia	weedy	499217	China	Not reported	crop
Andersen 5	USA	Illinois, Urbana	weedy	499218	China	Not reported	mix
Andersen 6	USA	Illinois, Urbana	weedy	499219	Russia	North Caucasus Region	weedy
Andersen 7	USA	Nebraska, Lincoln	weedy	499220	Germany	Not reported	weedy
Andersen 8	USA	Iowa, Kelley	weedy	499221	Ukraine	Not reported	mix
Andersen 9	Canada	Ontario, Ridgetown	weedy	499222	Portugal	Not reported	weedy
Andersen 11	Canada	Ontario, Guelph	weedy	499223	India	Not reported	weedy
Andersen 12	USA	Minnesota, Lemond	weedy	499224	Romania	Not reported	mix
Andersen 13	USA	Minnesota, Waseca	weedy	499225	Switzerland	Not reported	weedy
Andersen 14	USA	Minnesota, Rosemont	weedy	499226	U K	Not reported	weedy
Andersen 15	USA	Kansas, Manhattan	weedy	499227	Russia	North Caucasus Region	mix
Andersen 16	USA	Minnesota, Wolverton	weedy	499228	Ukraine	Not reported	mix
Andersen 18	USA	Michigan, Benton Harbor	weedy	499231	Poland	Not reported	mix
Andersen 19	USA	Michigan, East Lansing	weedy	499232	Sweden	Not reported	weedy
Andersen 20	USA	Kentucky, Lexington	weedy	499233	France	Not reported	weedy
Andersen 21	USA	Indiana, Greenfield	weedy	499234	Italy	Not reported	weedy
Andersen 22	Israel	Ein-Shemer	weedy	499235	India	Not reported	mix
Andersen 23	Israel	Atlith	weedy	499236	Portugal	Not reported	weedy
Andersen 24	China	Pan-Shan	weedy	499237	Denmark	Not reported	weedy
Andersen 25	India	Srinagor	crop	499238	Ukraine	Not reported	weedy
Andersen 29	China	Nanjing	weedy	499239	Netherlands	Not reported	weedy
Andersen 33	USA	California, Shafter	weedy	499240	Russia	North Caucasus Region	weedy
Andersen 34	India	Combatore	weedy	499241	Ukraine	Not reported	weedy
Andersen 35	China	Beijing	weedy	499242	Russia	Not reported	weedy
Andersen 36	Italy	Padova	weedy	499243	Middle Asia	Not reported	crop
Andersen 38	India	Bangalore	weedy	499244	China	Not reported	weedy
Andersen 39	USA	Maryland, Westminster	weedy	499245	France	Not reported	crop
Andersen 40	China	Wu-han	weedy	499246	Italy	Not reported	crop
Andersen 42	China	Nanjing	weedy	499247	Russia	Not reported	mix
Andersen 43	China	Huhehot/Mongolia	weedy	499248	China	Not reported	crop
Compton	USA	Ohio, Mt. Liberty	weedy	499249	Poland	Not reported	weedy
499208	Ethiopia	Eritrea	crop	499250	India	Not reported	crop
499209	Kazakhstan	Not reported	mix	499251	China	Not reported	crop
499210	Japan	Not reported	crop	499252	Ethiopia	Not reported	mix
499211	China	North China	crop	499253	Russia	Not reported	crop
499212	Italy	Not reported	weedy	499254	USA	Not reported	mix
499213	Japan	Not reported	mix	499255	Russia	North Caucasus Region	crop
499214	Russia	North Caucasus Region	crop	499256	China	Not reported	weedy

Table 5.1: Accession number, country of origin, local provenance, and biotype designation with respect to capsule color for *A. theophrasti* accessions used in the study. Accessions designated as AndersenXX, where XX = Arabic numbers in the identity were donated by Dr. R.N. Andersen (USDA-ARS, retired). Accessions designated 499XXX were obtained from the U.S. National Plant Germplasm System (NPGS) coordinated by the United States Department of Agriculture-Agricultural Research Service (USDA-ARS). Information about the individual plant introductions (PI) maintained by the NPGS can be found in the Germplasm Resources Information Network (GRIN, www.ars-grin.gov) by searching for accessions using the moniker PI499XXX, where XXX = last three Arabic numbers in the identity.

Figure 5.1: An example of the variation in height and leaf area among *A. theophrasti* accessions. Photograph by J. Cardina.

Traits	Mean	Min.	Max.	CV
50-seed weight (g)	0.58	0.43	0.88	20.31
Four Weeks				
Stem height (cm)	8.08	4.27	12.50	19.94
Largest leaf length (cm)	6.89	4.80	8.83	13.11
Largest leaf width (cm)	7.84	5.45	10.15	13.07
Petiole length of largest leaf (cm)	3.59	1.80	4.88	16.76
Seven Weeks				
Stem height (cm)	43.96	21.97	77.73	22.45
Largest leaf length (cm)	10.83	9.22	13.01	7.68
Largest leaf width (cm)	11.69	9.98	14.11	7.45
Petiole length of largest leaf (cm)	7.912	5.80	10.27	12.24
Ten Weeks				
Stem height (cm)	113.83	82.25	182.17	19.92
Largest leaf length (cm)	11.90	8.83	22.80	19.38
Largest leaf width (cm)	12.33	9.80	18.67	15.87
Petiole length of largest leaf (cm)	10.37	6.30	18.13	17.45
Flowering and Harvest				
Days to flowering	55.60	39.71	92.17	18.38
Height of stem at flowering (cm)	86.75	41.90	229.87	47.83
Duration of Flowering (days)	52.81	27.86	68.29	16.81
Days to harvest	108.41	93.00	126.00	7.67
Height of stem at harvest (cm)	144.63	96.00	254.75	27.50
Number of nodes at harvest	26.847	23.28	32.60	7.40
Number of capsules at harvest	15.37	7.60	29.00	25.72
Number of valves per capsule	14.19	12.00	15.50	4.02
Diameter of capsule (mm)	20.93	19.97	25.35	9.54
Height of capsule (mm)	15.96	13.60	19.65	10.03

Table 5.2: Overall mean, minimum and maximum values, and coefficient of variation (CV) for each of 23 morphological and phenological variables scored in the study on 80 accessions of *A. theophrasti.*

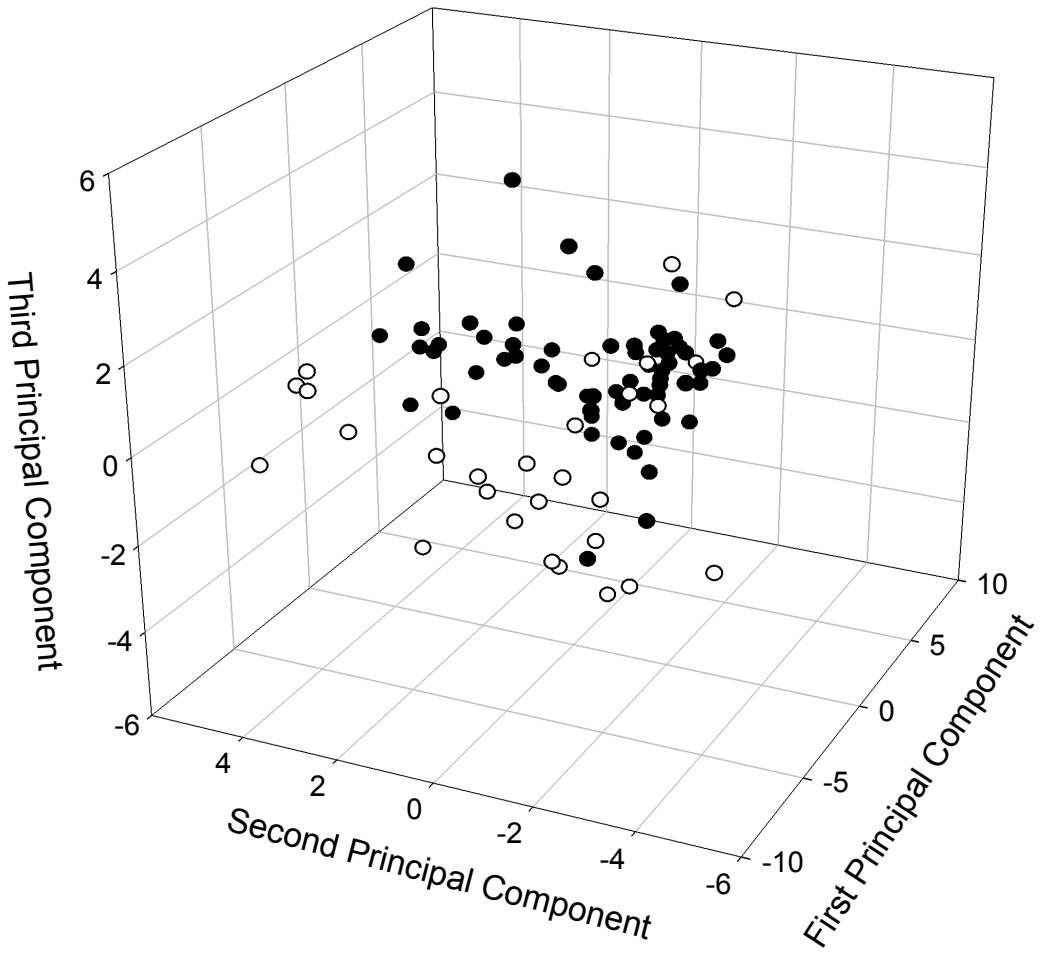

Figure 5.2: Principal components analysis for intra-specific variation in *A. theophrasti*. Open circles correspond to accessions that produced yellow-colored capsules (crop biotypes); closed circles correspond to accessions that produced brown-colored capsules (weedy biotypes). The first, second, and third principal components explain 51 %, 16 %, and 9 % of the total variation, respectively.

Traits	PC1	PC2	PC3
50-seed weight (g)	-0.248	0.032	-0.223
Four Weeks			
Stem height (cm)	-0.214	-0.154	-0.196
Largest leaf length (cm)	-0.160	-0.305	0.234
Largest leaf width (cm)	-0.137	-0.308	0.271
Petiole length of largest leaf (cm)	0.156	-0.135	0.055
Seven Weeks			
Stem height (cm)	-0.200	-0.249	0.009
Largest leaf length (cm)	-0.193	-0.172	0.125
Largest leaf width (cm)	-0.213	-0.185	0.166
Petiole length of largest leaf (cm)	0.152	-0.198	0.352
Ten Weeks			
Stem height (cm)	-0.258	-0.069	0.034
Largest leaf length (cm)	-0.216	0.113	0.113
Largest leaf width (cm)	-0.217	0.176	0.215
Petiole length of largest leaf (cm)	0.008	0.319	0.401
Flowering and Harvest			
Days to flowering	-0.215	0.264	0.092
Height of stem at flowering (cm)	-0.261	0.096	0.056
Duration of Flowering (days)	0.181	0.069	-0.223
Days to harvest	-0.072	0.398	-0.125
Height of stem at harvest (cm)	-0.23	0.231	0.085
Number of nodes at harvest	0.078	0.362	0.135
Number of capsules at harvest	0.229	0.079	0.160
Number of valves per capsule	0.003	0.092	0.451
Diameter of capsule (mm)	-0.256	0.043	-0.121
Height of capsule (mm)	-0.254	0.019	-0.123

Table 5.3: Eigenvectors of 20 morphological and phenological characteristics for the first, second, and third principal components.

Traits	X^2	*P* value	Crop (n = 27) Mean	1 SE	Weedy (n = 64) Mean	1 SE
50-seed weight (g)	33.70	≤ 0.001	0.73	0.02	0.52	0.01
Four Weeks						
Stem height (cm)	28.59	≤ 0.001	9.75	0.25	7.38	0.14
Largest leaf length (cm)	14.35	≤ 0.001	7.51	0.12	6.63	0.11
Largest leaf width (cm)	15.76	≤ 0.001	8.44	0.14	7.59	0.13
Petiole length of largest leaf (cm)	17.29	≤ 0.001	3.10	0.11	3.79	0.06
Seven Weeks						
Stem height (cm)	28.59	≤ 0.001	52.59	1.33	40.32	1.07
Largest leaf length (cm)	19.61	≤ 0.001	11.50	0.15	10.55	0.09
Largest leaf width (cm)	33.70	≤ 0.001	12.55	0.12	11.32	0.09
Petiole length of largest leaf (cm)	13.68	≤ 0.001	7.13	0.18	8.25	0.10
Ten Weeks						
Stem height (cm)	28.59	≤ 0.001	139.20	3.14	103.12	1.90
Largest leaf length (cm)	15.76	≤ 0.001	13.96	0.54	11.03	0.17
Largest leaf width (cm)	20.86	≤ 0.001	14.05	0.44	11.61	0.16
Petiole length of largest leaf (cm)	1.17	0.280	9.73	0.29	10.64	0.23
Flowering and Harvest						
Days to flowering	19.61	≤ 0.001	65	2.56	52	0.62
Height of stem at flowering (cm)	33.70	≤ 0.001	133.39	8.49	67.08	2.27
Duration of Flowering (days)	8.50	0.004	47.39	1.97	55.10	0.90
Days to harvest	12.32	≤ 0.001	112	1.13	106	1.09
Height of stem at harvest (cm)	28.59	≤ 0.001	181.66	7.46	129.01	3.54
Number of nodes at harvest	0.38	0.535	26.03	0.29	27.20	0.26
Number of capsules at harvest	30.65	≤ 0.001	11.11	0.75	17.17	0.28
Number of valves per capsule	1.17	0.280	14.09	0.14	14.24	0.06
Diameter of capsule (mm)	33.70	≤ 0.001	23.55	0.24	19.84	0.12
Height of capsule (mm)	33.70	≤ 0.001	18.00	0.22	15.10	0.10

Table 5.4: Results of Mood's median test, means, and standard errors for crop and weedy biotypes of *A. theophrasti* for each of 23 morphological and phenological variables scored in the study.

	SGN	Success rate	CGP	Success rate
Tomato COS markers surveyed	46		46	
Cotton TC/EST sequences identified	37	80%	31	67%
Primer pairs developed based on the location of introns in *A. thaliana*	21	57%	13	42%

Table 5.5: Summary of COS-primer design for *A. theophrasti*. Ninety-two gene sequences randomly selected from two tomato COS marker collections made available by the Solanaceae Genomics Network (SGN) and the Compositae Genome Project (CGP) were screened against the Cotton Gene Index to identify corresponding COS markers in cotton. Cotton sequences were screened against *A. thaliana* genomic DNA available from the Arabidopsis Genome Initiative. Success rate is measured as the percent of successful sequence matches between databases.

COS Source	COS Sequence identifier	GUDB Sequence Identifier	e-value	ATH1 Sequence Identifier and putative function	e-value	Primer name	Primer sequence
Cornell	SGN-E203106	TC21700	Expect = 2.7e-61	At2g22780 68409.t02424 Malate dehydrogenase	Expect = 1e-027	VL1f VL1r	GGATAGGGCAACCGTTATCA CATCCCTTGTCATTCCAGGT
Cornell	SGN-E291385	TC21357	Expect = 1.2e-39	At3g 01500 68410 t00062 Carbonic anhydrase	Expect = 8e-020	VL2f VL2r	GGTGGAATCAAGGGGCTTAT GGGTTTTCTTCACCAATCCA
Cornell	SGN-E280268	TC23975	Expect = 9.9e-53	At2g 30490 68409 t03319 Trans-cinnamate 4-monooxygenase	Expect = 3e-023	VL3f VL3r	TCAGGGACCATTTCCTTGAG GATGGTTCACAAGCTCAGCA
Cornell	SGN-E238151	TC23602	Expect = 4.7e-33	At2g17975 68415.t01980 Zinc finger (Ran-binding) family protein	Expect = 8e-09	VL4f VL4r	GAAGGAGACTGGGAGTGCAG GGCAATTGTTGCAATCTCCT
Cornell	SGN-E314505	BF276963	Expect = 0.52	At3g28710 68416.t03254 H+-transporting two-sector ATPase	Expect = 8e-15	VL5f VL5r	GGAGAGAGCCAGATGCTTGA TTTTGGTTTTGAGCCACACA
Cornell	SGN-E308321	TC24360	Expect = 2.30e-67	At3g02090 68416.t00157 Mitochondrial processing peptidase	Expect = 9e-30	VL6f VL6r	CATCCGGTGCTGTTAAGCAT AAGCTCCCTCAAAAGCAACA
Cornell	SGN-E310596	TC21509	Expect = 8.6e-51	At5g52840 68418.t06092 NADH-ubiquinone oxidoreductase-related	Expect = 2e-22	VL7f VL7r	TACCGTAAGGCCGTTGAGAG GCTTGGGAATAGGAGCATCA
Cornell	SGN-E238449	AI730854	Expect = 2.0e-57	At3g62560 68416.t06492 GTP-binding protein	Expect = 3e-27	VL8f VL8r	TTGGTTCAACATCAGCCAAC AGGGCATCCAGTTCCTTCTT

Table 5.6: Tomato COS sources and sequence identifiers, homologous cotton sequences and expectation values, Arabidopsis transcription unit identifiers and expectation values, and primers pairs developed to amplify putative ortholog counterparts in *A. theophrasti*. Cornell and Davis refer to the SGN and CGP, respectively.

Table 5.6 Cont

Cornell	SGN-E231643	AI725543	Expect = 1.5e-56	At1g11890 68414.t01217 Vesicle transport protein	Expect = 2e-31	VL9f VL9r	ATGTGCGACCGATCTTATCC CGGGTGTCCTGGTACAACTT
Cornell	SGN-E231235	TC24441	Expect = 4.9e-45	At4g29040 68417.t03854 26S Proteasome AAA-ATPase subunit	Expect = 6e-43	VL10f VL10r	AATTGGATGGCTTTGATTCG TCAGCCAATGTCATCCTTGA
Cornell	SGN-E201182	TC23947	Expect = 2.7e-47	At2g36530 68415.t04137 Enolase	Expect = 4e-26	VL11f VL11r	GCTAACCTTGCCGGTAACAG CACCTTCATCACCGACATTG
Cornell	SGN-E282297	TC24268	Expect = 1.9e-67	At1g20630 68414.t02309 Catalase 1	Expect = e-130	VL12f VL12r	CCCTGTTATCGTGCGATTTT GGGAATTTCATTCCATCACG
Cornell	SGN-E202830	TC23958	Expect = 5.7e-99	At2g22240 68415.t02482 Inositol-3-phosphate synthase	Expect = 1e-53	VL13f VL13r	AAGGGGACCAAGAAAGAACAA TGCCATTGATGAAAGGAACA
Cornell	SGN-E203134	TC20861	Expect = 1.1e-53	At1g16700 68414.t01783 NADH-ubiquinone oxidoreductase	Expect = 1e-15	VL14f VL14r	ATCCATTTGAGAAGGGTCCA CCTCTCGTTCCTCAGCCTCT
Cornell	SGN-E311649	BG440119	Expect = 1.7e-10	At2g22420 68415.t02499 Peroxidase 17	Expect = 2e-07	VL15f VL15r	TCATTCGAGGTTGTGGATGA TTGGGCTTGGCATTATGTT
Cornell	SGN-E235400	TC24804	Expect = 1.2e-86	At1g08360 68414.t00824 Ribosomal protein L10A	Expect = 4e-36	VL16f VL16r	TGATCCCCAAAAAGACAAGC AAGAGACGGGGGATCTGTTT
Cornell	SGN-E240166	TC25080	Expect = 1.0e-48	At4g02930 68417.t00364 Elongation factor Tu	Expect = 1e-24	VL17f VL17r	TCTGGTTGTGTCTGCTCCAG TTTCATCCCCAGGAAACTTG
Cornell	SGN-E237706	TC21617	Expect = 1.6e-22	At4g12420 68417.t01866 Multi-copper oxidase	Expect = 2e-25	VL18f VL18r	TGCTTCTCCTTTGGGTGTTC TGCCAAGAACTCCATCTTGC
Cornell	SGN-E292167	TC24381	Expect = 4.8e-74	At5g42740 68418.t04849 Glucose-6-phosphate isomerase	Expect = 1e-21	VL19f VL19r	CATTCAACAGGTCAGCATGG GGTTGCTGGCTCTTCACAAT

Table 5.6 Cont

Cornell	SGN-E315013	AI728515	Expect = 1.3e-27	At1g59750 68414.t06162 Auxin-responsive factor	Expect = 6e-19	VL20f VL20r	CCCATGGTGGATTCTCAGTT CTCCACCCAGTAGTGAGCAAG
Cornell	SGN-C122665	TC22339	Expect = 2.9e-69	At3g61620 68416.t06379 Exonuclease RRP41	Expect = 3e-09	VL21f VL21r	CCAAAGTCATTGCAGCTGTG TAAAGTTGCAGCGTTGATGC
Davis	18260284	CA99278	Expect = 3.4e-77	At1g44575 68414.t04678 Photosystem II 22 K-da	Expect = 5e-20	VL22f VL22r	AGCTGAGCCAATCTTCCAAC TGAAGCTGAACCCCTTCTTC
Davis	16250370	TC24627	Expect = 1.3e-88	At5g05010 68418.t00490 Clathrin adaptor complex	Expect = 1e-38	VL23f VL23r	TGAGACTGAGAATGTGCGATATG CTTGTGCCCAAGACAAATGA
Davis	16248369	TC21610	Expect = 1.5e-90	At1g80460 68414.t08596 Glycerol kinase	Expect = 5e-18	VL24f VL24r	GCAAGTCTCACATTGCTCGT CAACAGCTTTGCACCAAGAA
Davis	16247765	BQ404572	Expect = 6.0e-68	At5g37510 68418.t04192 NADH-ubiquinone dehydrogenase	Expect = 2e-44	VL25f VL25r	ATCGCTGGAGTTGACATTCC CTTCTTTGCCAATGGCGTAT
Davis	16247338	TC24990	Expect = 9.2e-74	At1g27530 68414.t03016 Expressed protein similar to gb|AF151884	Expect 4e-61	VL26f VL26r	TACTTATCCGGCCACTGCTC GGCTGCATCATCCTTATGCT
Davis	16246206	TC21476	Expect = 3.8e-86	At5g21070 68418.t02273 Expressed protein	Expect = 3e-50	VL27f VL27r	AAAGGTGGGCACAGTCATTC GCTGGCTAGCACCGTAGAAC
Davis	15811889	TC24972	Expect = 3.2e-46	At2g30410 68415.t03440 Tubulin folding cofactor A	Expect = 4e-14	VL28f VL28r	TAACCAATGGCAACAGTTCG ATCCTTGATTCAGCCAGCAC
Davis	13777428	TC24383	Expect = 7.3e-100	At1g43710 68414.t04583 Serine decarboxylase	Expect = 2e-49	VL29f VL29r	TGGACTCATGATGCCTTTTG GAGCATGACCATTCCGACTT
Davis	12627983	BF268698	Expect = 9.0e-70	At3g56940 68416.t05865 Dicarboxylate di-iron protein	Expect = 4e-39	VL30f VL30r	TTCGTTGAGTTCTTGGAGAGG GAAAATGAACTTTGGCTTGAAAA

Table 5.6 Cont

Davis	12627225	TC26441	Expect = 2.4e-96	At1g61620 68414.t06362 Expressed protein	Expect = 8e-13	VL31f VL31r	CCATGTCTTGCCAAAAAGGT CGTGGAAACCATTCTTGTCTC
Davis	12626638	AW187541	Expect = 1.8e-49	At5g40660 68418.t04597 ATP12 protein-related	Expect = 8e-43	VL32f VL32r	TGATGATGGAGTTGGATGGA ATGATCTTCGTGCGTGTGAG
Davis	9434057	TC24745	Expect = 1.0e-91	At3g57610 68416.t05942 Adenylosuccinate synthetase	Expect = 1e-27	VL33f VL33r	GAGGTTGGAGCCTTTCATTG CCAGAGCCAACTCTTGTGGT
Davis	9291142	TC25617	Expect = 1.7e-81	At3g20740 68416.t02345 Fertilization-independent endosperm protein	Expect = 5e-08	VL34f VL34r	TGCATTCTGATATTTGCTGGA TGTCCATGTGAATGATTTCTCC

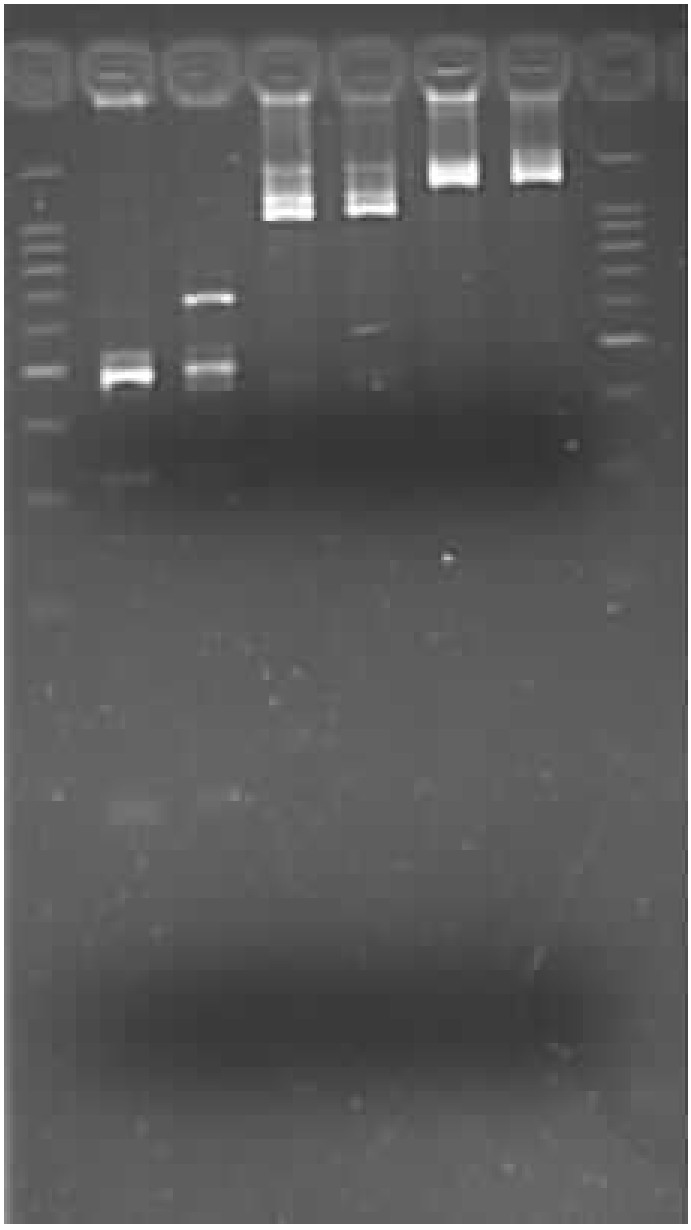

Figure 5.3: Observed polymorphism between weedy (And 13) and crop (PI 499252) biotypes for primers VL11f and VL11r. Lane one is a 100 bp size standeard. Lane two is And 13. Lane 3 is PI 499252. Following lanes correspond to non-polymorphic markers, and are not discussed.

Genotype	Primer	Product size	SNP	Type II RE / one cutter
And 13	VL6f	275 bp	TTTGA	Tfi I, Hinf I, Taq I
499252	VL6f	275 bp	TTCGA	
And 13	VL9f	275 bp	GGAC	Hha I, Alu I, Hae I
499252	VL9f	275 bp	GGCC	
And 13	VL18f	550 bp	TTACT	Cvi JI, Alu I, Mae III
499252	VL18f	550 bp	TTAGC	
And 13	VL24f	900 bp	GTATT	Tsp509 I
499252	VL24f	900 bp	GAATT	
And 13	VL24f	900 bp	CCCGA	Sau96 I, Stu I, Hae III
499252	VL24f	900 bp	CCTGA	
And 13	VL24f	900 bp	ACACT	No restriction sites
499252	VL24f	900 bp	ACATT	
And 13	VL24f	900 bp	AAAAA	No restriction sites
499252	VL24f	900 bp	AAGAA	

Table 5.7: Summary of single nucleotide polymorphisms (SNPs).

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