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POPULATION DIVERSITY AND MANAGEMENT OF COMMON DANDELION (Taraxacum officinale Weber) IN NO-TILLAGE CROPPING SYSTEMS

Ву

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ABSTRACT

POPULATION DIVERSITY AND MANAGEMENT OF COMMON DANDELION (*Taraxacum officinale* Weber) IN NO-TILLAGE CROPPING SYSTEMS

By

Aaron Scott Franssen

Common dandelion has developed into a troublesome agronomic weed in Michigan and the North Central Region. Widespread adoption of no-tillage cropping practices and use of glyphosate-resistant crops is likely to have contributed to the proliferation of this weed. This research was conducted to evaluate population diversity of common dandelion and to identify herbicide programs to control this weed in no-tillage cropping systems. Population diversity was examined using morphological characteristics and genetic analysis to determine if common dandelion collected from different geographical regions exhibit phenotypic and genetic variability. Common dandelion was collected from 16 counties in Michigan and 11 additional states. A southern and northern field nursery was established in Michigan near East Lansing and Chatham, respectively. Overall, common dandelion grown at the East Lansing nursery were larger and produced more seed than those at the Chatham nursery. Individual collections that were larger and produced more seed at the East Lansing nursery were also among the largest and most prolific at the Chatham nursery. Genetic diversity of common dandelion collections established in the field nurseries was evaluated using randomly amplified polymorphic DNA (RAPD) analysis. The diversity of RAPD banding patterns observed suggest that there is a high level of genetic diversity; however there was no apparent relation between genetic similarity and geographical location. There does not appear to be a relation between morphological characteristics and genetic similarity in the collections examined. Field research trials were conducted on established populations of common dandelion in no-tillage soybean and corn to identify strategies that effectively control this weed. Glyphosate and 2,4-D ester were applied at typical use rates at different preplant timings in the fall and spring, followed by postemergence applications of glyphosate in glyphosate-resistant soybean. When common dandelion control was evaluated at crop planting, glyphosate was more effective than 2,4-D ester regardless of application timing. In addition, fall applications of either herbicide were more effective than spring applications. A sequential application of glyphosate either at the V3 or V6 soybean crop stage was necessary to provide effective common dandelion control through soybean harvest. Additional field trials were conducted to evaluate postemergence corn herbicides for common dandelion control. Glufosinate and mesotrione applied alone and in combination with atrazine were the most effective 28 days after treatment. However, late-season plant regrowth was observed with these treatments. By 56 days after treatment, dicamba + diflufenzopyr was the most effective treatment. In the absence of tillage, effective management of common dandelion will include a combination of fall herbicide applications and sequential treatment with postemergence herbicides. In addition, the high level of variability observed with this species will make population specific management of common dandelion unlikely.

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CHAPTER 1

EVALUATING COMMON DANDELION (*Taraxacum officinale* Weber) POPULATION DIVERSITY USING MORPHOLOGICAL CHARACTERISTICS AND DNA BASED GENETIC ANALYSIS.

Abstract: Population diversity of common dandelion was examined using morphological characteristics and genetic analysis. Seed from individual common dandelion plants were collected from multiple counties in Michigan and several states. Subsequent plants were established in field nurseries at Michigan State University research stations near East Lansing and Chatham to determine if common dandelion collected from different geographical regions exhibit phenotypic variability. Overall, plants at the East Lansing nursery tended to be larger and produce more seeds than those at the Chatham nursery. Individual collections that were larger and produced more seed at the East Lansing nursery were also the largest and most prolific at the Chatham nursery. Genetic diversity of common dandelion collections established in the field nurseries were also evaluated using randomly amplified polymorphic DNA analysis. Nine random primers amplified a total of 44 fragments that were polymorphic. Of the 26 populations screened, 24 were distinguishable from each other using the RAPD analysis. The diversity of polymorphic banding patterns observed suggest that there is a high level of genetic diversity in common dandelion in Michigan and the other states. Genetic similarity coefficients for all the populations evaluated ranged from 0.25 to 1.00. There was no discrete separation between common

dandelion from Michigan and the other collections. A survey of plants collected from a single no-tillage field in Michigan also revealed a high level of diversity. An additional study was conducted to verify the apomictic reproductive nature of this species. All progeny that were tested using the given random primers were genetically similar to the maternal plant. There does not appear to be a visible relation between morphological characteristics and the genetic similarity examined here. A greenhouse study was conducted to determine if differences in plant size would be observed between selected collections of common dandelion. Nine collections of common dandelion were grown in the greenhouse and the size of the plants compared 60 days after planting using leaf area and dry weight. Differences in plant size were observed; however collections that were genetically similar did not necessarily similar in size. With the high level of diversity documented in this study one could expect diversity in common dandelion response to certain herbicides.

Nomenclature: common dandelion, *Taraxacum officinale* Weber; red seeded dandelion *Taraxacum laevigatum* L.

Index words: apomictic, phenotypic variation, RAPD analysis, genetic variation, genetic similarity.

INTRODUCTION

Common dandelion (*Taraxacum officinale* Weber) has developed into a troublesome agronomic weed, especially in no-tillage crop production in Michigan and parts of the United States. Putatively originating in west central Asia (Richards 1970), this species can be found world-wide, primarily concentrated in temperate and cold regions (Solbrig and Simpson 1974). It was proposed that common dandelion was originally introduced to the Americas via the Alaskan ice bridge following the most recent ice age (Richards 1973). It is also presumed that early European settlers reintroduced common dandelion as an ornamental used to seed the roofs of sod houses to make them stand out on the prairie (Solbrig 1971; Stubbendieck et al. 1995).

Common dandelion, as it is collectively classified in the United States, is comprised of two similar *Taraxacum* species. Red seeded dandelion (*T. laevigatum*) is virtually indistinguishable from *T. officinale* except for the red coloration of the achene (GPFA 1996). Morphological and biochemical analysis comparing these two species showed no clear differentiation between them (Taylor 1987). A more comprehensive genetic analysis of ribosomal DNA (rDNA) and chloroplast DNA (cpDNA) supported this lack of a definite separation between these species (King 1993). Furthermore, it has been argued that morphological variation is by and large a response to the local environment (Taylor 1987). For these reasons, *T. laevigatum* and *T. officinale* are collectively considered common dandelion here.

The genus *Taraxacum* is comprised of both sexual and asexual species. *Taraxacum* species that reproduce sexually are diploid (2n = 16), whereas the asexual species are triploid (3n = 24) and reproduce via agamospermous apomictic seed production. The asexual species, which include *T. laevigatum* and *T. officinale*, are found primarily in North America where it is accepted that they reproduce via apomixis. The result of this apomictic mode of reproduction is progeny that are clones of the maternal parent. Despite the potential for populations in a given area to be genetically identical, differences in overall fitness and isozyme characterization have been documented (Solbrig and Simpson 1974). Additional studies of genotypic variation in rDNA have been reported in asexual lineages of common dandelion thought to be brought about by somatic mutations (King and Schaal 1990).

DNA-based molecular markers, such as randomly amplified polymorphic DNA (RAPD), are a powerful tool to examine genetic variation within a species (Williams et al. 1990). An advantage of using RAPD markers over other DNA-based markers (ie. SSR and AFLP) is that no prior knowledge of the species genome is required. Single oligonucleotide primers of arbitrary sequence are used to randomly amplify segments of template DNA. The simple presence or absence of an amplified DNA fragment represents a difference in the genome that can be used to compare individuals. The use of RAPD analysis has been utilized to examine genetic diversity in such weed species as leafy spurge (Euphorbia esula L.) (Rowe et al. 1997), wild mustard (Sinapsis arvensis L.) (Moodie et al. 1997), and hemp dogbane (Apocyanum cannabinum L.) (Ransom

et al. 1998), as well as economically important species such as tea plant (*Camellia sinensis* L.) (Jorge et al. 2003) and walnuts (*Juglan spp.*) (Orel et al. 2003).

How genetic variation affects common dandelion management is currently unknown. Therefore studies were conducted to understand genetic variation of this species to aid in future management strategies for this weed. The objectives of this research were to utilize RAPD analysis to 1) determine the amount of genetic diversity of common dandelion in Michigan and the United States, and 2) identify unique populations of common dandelion.

MATERIALS AND METHODS

Plant material

To examine the population diversity of common dandelion, mature seed (as indicated by the presence of white pappus) was collected from individual plants from selected sites in 2001 (Table 1). Seeds were removed from the flower receptacle and stored at 4 C until planting. Seed were planted in 1000 ml pots filled with commercial potting soil and maintained in the greenhouse. Seedlings were transplanted to individual 1000 ml pots filled with Spinks loamy sand (sand, mixed mesic Psammentic Hapludalfs) with pH of 6.8 and 2.4% organic matter. Plants were maintained in the greenhouse until they were transplanted to a field nursery.

Phenotypic variation among collections

Phenotypic variation of common dandelion was examined by establishing plants in field nurseries and observing a number of different morphological and reproductive characteristics. Common dandelion field nurseries were established at two sites in Michigan. A southern and northern nursery was established at the Michigan State University Agronomy Farm at East Lansing (42° N latitude) and the Michigan State University Upper Peninsula Research Station near Chatham (47° N latitude), respectively. Morphological differences were compared among common dandelion collections from 12 counties in Michigan, 11 states, and a collection obtained from the Beal Botanical Garden at Michigan State University that originated in Germany (Table 1). Single plants were randomly selected to represent that population in which it was growing. Common dandelion plants from agronomic fields and residential areas were selected for this analysis.

Common dandelion seedlings were established in the greenhouse and transplanted into 0.6 by 0.6 m plots at the nurseries in the spring of 2002. Plants were irrigated weekly for the first month and with natural rainfall for the remainder of the experiment. Plants grew free of competition by hand weeding around established common dandelion plants. Characteristics observed at each of the nurseries included winter survival, plant diameter, leaf shape, leaf pubescence, flowering date, growing degree days to flowering, total number of flowers produced, and seeds produced per flower. Winter survival was determined by observing plants in the spring of the year following establishment in the field nurseries. Plant diameter was recorded as the average of two perpendicular

measurements of the common dandelion rosette. Leaf shape was determined using a scale from 1-5, where 1 represented a leaf with deeply lobed leaf margins and 5 represented an entire leaf margin. Flowering date was recorded as the day in which the first yellow flower was present on the individual plant. Growing degree days were calculated using a 10 C base beginning on March 1, 2003. Total flower production was monitored weekly starting in May and continuing until flower production declined approximately one month later. The total number of flowers produced each week was recorded, the mature flowers were removed, and the seeds placed in paper envelopes. The number of seeds produced per flower was determined by randomly collecting four individual mature flowers per plant prior to seed dissemination. Mature flowers were dried at 70 C for 24 h and stored at room temperature until the seeds were counted.

Genetic variation among collections

Genetic analysis of common dandelion using RAPD analysis was conducted to assess the amount of genetic variation in this species. Population genetic diversity was examined for common dandelion collected from 16 counties in Michigan, 9 states, and the collection from Germany (Table 1). Genomic DNA was extracted from established plants in the common dandelion nursery at East Lansing.

Within-field genetic variation

The genetic diversity of common dandelion within a field population was examined using eight established plants that were collected from a 0.5 ha area of a no-tillage production field. Two plants were collected from each of four 3 m by 9 m plots from a field near Elsie, Michigan that had been in a no-tillage cornsoybean rotation for 10 years. Entire plants (above and below ground biomass) were randomly collected from each of the four plots and maintained in the greenhouse. Genetic analysis was conducted using the original plant collected from the field.

Among-progeny genetic variation

The apomictic nature of this species was examined by collecting mature seeds from a single flower and conducting the RAPD analysis on 10 sibling progeny and the maternal plant. Common dandelion seedlings were established and maintained in the greenhouse for this analysis. Collections selected for this evaluation included common dandelion from Michigan, Oregon, and Germany.

DNA extraction and RAPD analysis

Genomic DNA was extracted from the newest leaf material emerging from plants growing either in the greenhouse or field nursery. DNA was extracted from four 10 mm diameter leaf disks (approx. 45 mg fresh leaf tissue) using the protocol described with the PUREGENETM DNA isolation kit² (Appendix A1) and stored in Tris-EDTA (TE) buffer (pH 7.0). DNA concentration was determined by

visual comparison with a known quantity of DNA mass ladder³ on an agarose gel stained with 0.1 µg ml⁻¹ ethidium bromide. The presence of an unidentified PCR inhibitor required a 1:20 dilution (concentrated DNA:TE) of DNA be conducted prior to PCR amplification. This resulted in a DNA concentration of less than 20 ng ul⁻¹. The PCR primers utilized were 10-base pair (bp) random oligonucleotides from primer kit A4. Each PCR reaction was carried out in a 25 µl reaction volume consisting of 50 ng genomic DNA, 2.5 µg bovine serum albumin (BSA), 5.0 mM MaCl₂, 3.2 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.2 mM each deoxynucleotide triphosphate (dNTP), 1.2 pmol 10 base pair (bp) oligonucleutide primer, and 0.1 units Tag DNA polymerase⁵. PCR reactions for each random primer were conducted at least twice for each plant sample in a heated-bonnet thermal cvcler⁶ programmed for an initial denaturation temperature of 94 C for 5 min followed by 35 cycles of 1 min 15 sec at 94 C, 1 min 15 sec at 40 C, and 2 min at 72 C. The final cycle was followed by 3 min at 72 C, after which the temperature was held at 4 C until gel electrophoresis. A 10 µl aliquot of the PCR product was loaded with DNA loading dye [50% glycerol, 0.25% bromophenol blue, 10 mM Tris HCl (pH 8.0), 1 mM EDTAl onto a 2.0% (w/v) agarose⁷ gel stained with 0.1 µg ml⁻¹ EtBr. Amplified products were resolved at 80 volts for 3 hr in a 1X Trisacetate (TAE) buffer (40 mM Tri-acetate, 1 mM EDTA). A 100 bp DNA ladder8 was used as a size reference. The gel was viewed and photographed on an ultraviolet light box to confirm product amplification. Polymorphic PCR fragments were scored as either present (1) or absent (0). Only those fragment length polymorphisms that were repeatable and intensely amplified were scored.

Comparison of plant size

A greenhouse experiment was conducted to compare plant sizes of selected common dandelion collections. Common dandelion from eight counties in Michigan and one county in Illinois were selected for this experiment. Collections were selected based on the results from the RAPD analysis. Plant collections were selected to represent both genetically similar and dissimilar collections. Mature seed was collected from the respective collections in the field nursery and stored at 4 C until planted in the greenhouse. Common dandelion seeds were planted 0.25 cm deep and seedlings individually transplanted to 1000 ml pots containing commercial potting mixture approximately 2 weeks later. Greenhouse temperatures were maintained at 30/25 ± 3 C (day/night) with 14:10 h (day:night) photoperiod. Supplemental light intensity from sodium vapor lamps provided a total midday light intensity of 1.000 µmol m⁻² s⁻¹ photosynthetic photon flux at plant height. Common dandelion plants were watered as needed and fertilized with 50 ml of N, P₂O₅, K₂O (20%-20%-20%) at 20 ppm to promote optimum plant growth.

Comparison of plant size for 9 common dandelion collections was conducted 60 days after planting. Plant collections were compared by measuring total leaf area and plant dry weight. Leaf area was measured with a transparent belt conveyor accessory for a portable leaf area meter⁹. Dry weight was determined for the above ground biomass; harvested plant material was dried at 70 C for 24 h.

Statistical analysis

Common dandelion collections were established in the field nurseries in a randomized complete block design and each collection was replicated four times at each of the nurseries. Data were subjected to analysis of variance with SAS¹⁰ and means separated using Fisher's Protected LSD (α = 0.05). Nursery by collection interactions were significant; therefore data from each nursery location were analyzed and presented separately.

Genetic similarity coefficients between common dandelion collections were determined using Nei and Li's (1979) calculation for qualitative data. Dendograms for genetic distance were created using the unweighted pair group method with arithmetic averages (UPGMA) cluster analysis. Genetic similarity calculations and dendograms were made using NTSYS-pc version 2.11L software¹¹ (Rohlf 2002). Collections were compared using calculated genetic similarity coefficients where 0.00 indicated no similarity and 1.00 indicated that the collections were identical.

The experiment to compare plant size was conducted as a completely randomized design. Each plant collection was replicated four times and the experiment was conducted twice. Data were subjected to analysis of variance with SAS and means were separated using Fisher's Protected LSD (α = 0.05). Variances were determined to be homogenous, thus the experiments combined.

RESULTS AND DISCUSSION

Phenotypic variation among collections

Following the winter of 2002-03 it was observed that all of the plants established in the Chatham nursery survived, whereas mortality was observed for some collections in East Lansing. However, mortality was no more than one plant from any collection. The one exception was the common dandelion collection from Oceana Co. Michigan. As a result it was dropped from the analysis of the East Lansing nursery. At the Chatham station in the Upper Peninsula of Michigan, the mean annual snow fall is 380 cm. This snow cover insulated the nursery, allowing these plants to survive the winter. The lack of snow fall and extreme cold temperature at the East Lansing nursery in 2002-03 may explain the common dandelion mortality and lower plant vigor in the 2003 growing season.

Some of the characteristics measured, such as leaf shape and the presences of pubescence on the leaf were variable between plants as well as on an individual plant. Leaf shape on an individual plant was highly variable, resulting in difficulty identifying differences in leaf shape between collections (data not shown). Previous research using common dandelion leaf morphology not only found leaf shape to be highly variable but also found that it was influenced by the environment and even varied between seasons (Sturtevant 1886; Taylor 1987). The presence of pubescence on the leaf surfaces appeared to be related to the age of the leaf. Newly emerging leaf material for all of the

collections was typically pubescent. In contrast, the older leaf tissue lacked pubescence, regardless of the collection (data not shown).

The common dandelion plants at the Chatham nursery were much smaller in diameter compared with East Lansing (Table 2). A common dandelion collected from Tolland Co. Connecticut was the largest in diameter at both East Lansing and Chatham with 55 cm and 22 cm, respectively. At Chatham, common dandelion from Hall Co. Nebraska was among the largest; however it was one of the smaller collections in East Lansing. The common dandelion collection from Germany was the smallest in diameter at both of the nurseries.

The date at which common dandelion began to flower was determined to be different at each of the nurseries; however differences within the nurseries were not apparent. The common dandelion collections at the East Lansing nursery initiated flowering within 1 week of each other beginning on May 1, 2003 at an accumulation of 239 growing degree days (data not shown). This coincides with previous research that classified common dandelion as a day-neutral plant (Gray et al. 1973). At Chatham, flower initiation commenced approximately 3 weeks later.

There were no significant differences observed in the total number of flowers produced or the total number of seeds produced per collection (Appendix A2). However, there were differences observed in the number of seeds produced per flower among collections (Table 2). Common dandelion in the East Lansing nursery tended to produce more seeds per flower than at Chatham. Seed production ranged from 106 to 230 seed per flower at Chatham and 123 to 304

seeds per flower in East Lansing. This difference in productivity is likely due to the greater accumulation of growing degree days at the East Lansing nursery as compared to the Chatham nursery, which was located at a more Northern latitude. At both nurseries, common dandelion from Baker Co. Oregon and Cache Co. Utah were the most prolific producers of seeds. The Alger Co. collection, which was collected on the Chatham station itself, was one of the more prolific plant collections at both of the nurseries. The common dandelion collections from Hall Co. Nebraska and Germany were the least prolific at each nursery.

Genetic variation among collections

Successful amplification of PCR products was dependent on the random primer used. Of the 20 primers screened, 9 primers resulted in the amplification of a DNA fragment. The nine random primers amplified a total of 71 repeatable DNA fragments, of which 44 were polymorphic (Table 3). The number of polymorphic fragments amplified per random primer ranged from 1 to 12. Of the 26 populations screened, 24 were distinguishable from each other using the RAPD analysis. Common dandelion from Berrien and Calhoun counties in Michigan were indistinguishable from each other.

The diversity of RAPD banding patterns observed suggest that there is a high level of genetic diversity in common dandelion in Michigan and the other states. Genetic similarity coefficients among all collections ranged from 0.25 to 1.00 (Table 4). Within Michigan, genetic similarity ranged from 0.27 to 1.00.

There was no discrete separation among common dandelion collections from Michigan and those collected from other states or Germany. However, common dandelion from Michigan tended to be more similar to other Michigan collections than with collections from the other states (Figure 1). Most of the Michigan collections were grouped together in the dendogram, with a few of the counties appearing more closely related to common dandelion from other states. Clustering of collections within the dendogram indicate more similarity among those collections than others outside the cluster. The amount of genetic variation observed here is similar to that observed for other weed species. For example, RAPD analysis of wild mustard (Sinapis arvensis) was found to be highly variable (Moodie et al. 1997). In addition, analysis of wild mustard plants sampled over two consecutive seasons showed different levels of population diversity, suggesting the influence of environmental variability. Isozyme analysis of two perennial species of snakeweed (Gutierrezia spp.) indicated a high level of diversity both within species and between species (Sterling and Hou 1997).

Common dandelion from three Michigan counties and two states were identifiable with unique DNA banding patterns (data not shown). The presence of a single unique DNA fragment is associated with the collections from Stafford Co. Kansas, Benton Co. Indiana, and Presque Isle and Clinton Counties in Michigan using the random primers OPA-8, 9, 11, and 18, respectively. The absence of the 675 bp fragment amplified by OPA-18 was unique to the Luce Co. collection. Several additional collections shared either the presence or the absence of two random DNA fragments (data not shown). A single 1000 bp random DNA

fragment amplified using OPA-18 was exclusive only to the collections from Berrien, Calhoun, Hillsdale, and Ingham Counties in Michigan.

Geographical location and genetic similarity did not appear to be related when comparing common dandelion collections. Many of the Michigan collections, which are in relatively close geographic proximity, tended to be genetically similar. However, an exception to this trend included the Ingham Co. collection that was genetically more similar to Adams Co. Colorado (0.69) and Benton Co. Indiana (0.69) than to any collection from Michigan.

The common dandelion from Presque Isle Co. Michigan, Stafford Co. Kansas and Germany were identified from their seed color as red seeded dandelion. No RAPD polymorphisms were identified that were unique to red seeded dandelion. Single polymorphic fragments from different random primers were amplified that were unique to the Presque Isle Co. and Stafford Co. collections. Random primer OPA-09 amplified a 450 bp fragment that was unique to Stafford Co., whereas OPA-18 amplified a 650 bp fragment in the Presque Isle Co. collection only. Random primer OPA-07 failed to amplify an 875 bp fragment in either the Stafford Co. or Presque Isle Co. collections but did amplify a 1550 bp fragment in the Stafford Co. and Germany collections. The Stafford Co. collection was genetically more similar to the collection from Germany than to Presque Isle Co., 0.54 and 0.37, respectively. Common dandelion collected from Stafford Co. and Germany were grouped together in the dendogram (Figure 1), indicating they shared more unique DNA fragment length polymorphisms. The

Presque Isle Co. collection was more similar to other Michigan collections of *T. officinale* then the other two *T. laevigatum* collections.

Within-field genetic variation

The common dandelions that were collected from the no-tillage production field near Elsie, Michigan demonstrated a broad range of genetic similarity. Coefficients of genetic similarity for the eight plants collected ranged from 0.30 to 1.00 (data not shown). Two of the plants were indistinguishable from each other. A coefficient of genetic similarity of approximately 0.80 was calculated for three of the collected plants from across the area. Plants that were collected from the same 3 m by 9 m plots were not necessarily more related to each other. The level of diversity observed here is similar to that previously reported. Using isozymes and plant growth competitiveness, Solbrig and Simpson (1974) identified the presence of at least four common dandelion biotypes within an area of 0.01 ha.

Among-progeny genetic variation

RAPD analysis conducted on ten progeny from each of the three collections selected did not reveal any genetic variation between the progeny and the maternal plant using the 9 random primers. This observation supports that common dandelion is apomictic, at least with the individuals tested. King and Schaal (1990) screened the rDNA of over 700 progeny from 26 different parental genotypes and observed 42 plants with nonparental rDNA. This rate is higher

than what would be expected by mutation alone. Results from this study indicate that a large number of individuals are needed to find genetic differences.

Comparison of plant size

The nine collections in this experiment varied in their respective rate of growth after 60 days. Overall the dry weights of the 9 common dandelion collections ranged from 0.84 to 2.0 g per plant (Table 5). Total leaf area ranged from 216 to 432 cm². The Michigan collections from Alger, Monroe, and Shiawassee Counties had the greatest leaf area and dry weights at the end of the experiment. Conversely, the collections from Berrien, Luce, and Newaygo Counties in Michigan were the lowest in terms of leaf area and dry weight. The collection from Alger Co. demonstrated the greatest growth rate as measured by leaf area and dry weight. This collection was genetically most similar to the Ingham Co. collection with a coefficient of 0.61 (Table 4). These two collections had similar leaf areas but were different in terms of dry weight. The collection from Alger Co. was the least genetically similar to the common dandelion from St. Clair Co. and had a higher leaf area and dry weight (Table 5). The Shiawassee Co. and Berrien Co. collections were genetically the most similar (0.87) but differed in their growth rates. Conversely, the Vermillion Co. and Ingham Co. collections were the least genetically similar (0.35) but were similar in size. The lack of an obvious relation between genetic similarity and plant size indicate that the polymorphisms identified were not associated with traits influencing plant development.

The common dandelion collections in this analysis demonstrated a high level of morphological and genetic variability. From the RAPD analysis we did not identify distinctly unique biotypes of common dandelion. Similarity was observed between collections within a geographical region but there were no discrete boundaries. Genetic similarity did not appear to be related to similarity in morphological characteristics. These characteristics measured in the common dandelion nurseries are likely to be quantitatively inherited traits that are controlled by more than one gene. In addition, the numbers of polymorphisms used here to quantify the genetic diversity were insufficient to identify a relation between phenotype and genotype.

The high level of variability in common dandelion observed in this research could possibly be a result of the method of seed dissemination in this species. Mature seeds attached to white pappus are capable of long distance travel, spreading the genetic diversity across a large area. In addition, common dandelion become established across a wide range of climates and geographical regions, as is evident from its distribution throughout the world. And finally, the high level of genetic and morphological diversity observed will make population-specific management of common dandelion unlikely.

Source of Materials

¹ Baccto, Michigan Peat Co, P.O. Box 98029 Houston TX, 77098

² Puregene DNA Isolation Kit, Gentra Systems, Minneapolis, MN 55441.

³ Low DNA Mass Ladder, Invitrogen Life Technologies, Carlsbad, CA 92008.

⁴ Primer Kit A, Operon Technologies, Inc., Alameda, CA 94501.

⁵ Taq DNA polymerase, Invitrogen Life Technologies, Carlsbad, CA 92008.

⁶ PTC-225 Peltier Thermal Cycler, MJ Research Inc., Waltham, MA 02451.

⁷ Agarose, Invitrogen Life Technologies, Carlsbad, CA 92008.

⁸ 100 base pair ladder, Invitrogen Life Technologies, Carlsbad, CA 92008.

⁹ Portable leaf area meter, Li-Cor Inc., Lincoln, NE 68504.

¹⁰ SAS version 8.2, SAS Institute, SAS Circle, Box 8000, Cary, NC 27512-8000.

¹¹ NTSYS-pc ver. 2.11L software, Exeter Software, Setauket, NY 11733-2870.

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Table 1. Location and site description of common dandelion collections included in the field nurseries for RAPD analysis.

Collection	Site	Site Description ^a	Included in field nurseries	Included in genetic analysis
1	Alger Co. MI	dairy pasture	X	X
2	Berrien Co. MI	NT agriculture	X	X
3	Calhoun Co. MI	NT agriculture	X	X
5	Clinton Co. MI	NT agriculture		X
6	Hillsdale Co. MI	NT agriculture	X	X
7	Ingham Co. MI	residential	X	X
9	Ionia Co. MI	NT agriculture	X	X
12	losco Co. MI	state park	X	×
13	Leelanau Co. MI	state park		X
14	Monroe Co. MI	NT agriculture	X	X
15	Newaygo Co. MI	CT agriculture	X	X
17	Oceana Co. MI	fruit orchard	X	×
18	Presque Isle Co. MI ^b	state park		×
20	Shiawassee Co. MI	CT agriculture	X	X
21	St. Clair Co. MI	CT agriculture		X
22	Luce Co. MI	state park	X	X
23	Yolo Co. CA	fruit orchard	X	×
26	Adams Co. CO	sod farm	X	×
27	Tolland Co. CT	dairy pasture	X	X
28	Champaign Co. IL	wooded area	×	
29	Vermillion Co. IL	CT agriculture	X	×
30	Benton Co. IN	CT agriculture		×
31	Riley Co. KS	CT agriculture	X	
32	Stafford Co. KS ^b	CT agriculture		X
33	Hall Co. NE	residential	X	X
36	Baker Co. OR	pasture	X	
37	Elk Co. PA	road side	×	
39	Cache Co. UT	residential	×	×
40	Brazos Co. TX	residential	X	×
42	Germany⁵	unknown	X	X

^a Abbreviations: NT = no-tillage; CT = conventional tillage.

^b Plants collected from these sites were identified as red seeded dandelion (*T. laevigatum*).

Table 2. Seed production and plant diameter for common dandelion at East Lansing and Chatham field nurseries.

	Number of seeds		Rosette diameter	
Collection	East Lansing	Chatham	East Lansing	Chatham
	seeds pe	seeds per flower		<u>1 ————</u>
Alger Co. MI	275	200	42.2	15.6
Berrien Co. MI	232	208	37.8	20.6
Calhoun Co. MI	230	209	47.0	19.7
Hillsdale Co. MI	234	179	41.4	13.4
Ingham Co. MI	230	202	37.4	24.1
Ionia Co. MI	207	153	38.1	14.6
losco Co. MI	206	148	52.1	19.1
Leelanau Co. Mi	223	198	44.5	17.8
Monroe Co. MI	187	153	31.8	19.4
Newaygo Co. MI	152	153	40.3	14.3
Oceana Co. MIª	-	185	-	10.8
Shiawassee Co. MI	168	153	33.4	21.3
Luce Co. MI	183	142	36.2	11.5
Yolo Co. CO	173	132	31.7	17.8
Adams Co. CO	293	180	38.9	15.0
Tolland Co. CT	245	214	54.6	24.9
Champaign Co. IL	163	166	32.2	20.0
Riley Co. KS	212	230	38.8	16.5
Hall Co. NE	145	131	26.0	22.3
Baker Co. OR	304	222	38.5	19.4
Elk Co. PA	178	199	42.2	21.2
Cache Co. UT	261	215	35.6	14.9
Brazos Co. TX	168	145	33.0	21.3
Germany	123	106	22.0	12.2
LSD(0.05)	56	29	13.9	6.1

^a Oceana County collection dropped from East Lansing nursery due to winter mortality

Table 3. RAPD primers used to evaluate the genetic diversity between common dandelion collections.

RAPD Primer	Sequence 5' to 3'	No. of bands	No. polymorphic
OPA-03	AGTCAGCCAC	4	1
OPA-04	AATCGGGCTG	14	8
OPA-07	GAAACGGGTG	10	8
OPA-08	GTGACGTAGG	12	11
OPA-09	GGGTAACGCC	5	2
OPA-10	GTGATCGCAG	6	2
OPA-11	CAATCGCCGT	3	2
OPA-18	AGGTGACCGT	10	6
OPA-19	CAAACGTCGG	7	4
Total		71	44

Table 4. Matrix of genetic similarity coefficients for RAPD analysis of 26 common dandelion collections.

							Commo	n dande	Common dandelion collections	lections						
Collection	1	2	3	2	9	7	6	12	13	14	15	17	18	20	21	22
01 Alger Co. MI	1.00															
02 Berrian Co. MI	0.56	1.00														
03 Calhoun Co. MI	0.56	1.00	1.00													
04 Clinton Co. MI	0.50	0.61	0.61	1.00												
06 Hillsdale Co. MI	0.73	0.67	0.67	0.41	1.00											
07 Ingham Co. MI	0.61	0.67	0.67	0.38	0.65	1.00										
09 Ionia Co. MI	0.58	0.84	0.84	0.69	0.62	0.56	1.00									
12 losco Co. MI	0.42	0.50	0.50	0.29	0.45	0.40	0.52	1.00								
13 Leelanau Co. Mi	0.93	0.60	0.60	0.53	0.71	0.58	0.62	0.36	1.00							
14 Monroe Co. MI	0.59	0.59	0.59	0.83	0.46	0.47	0.67	0.34	0.63	1.00						
15 Newaygo Co. MI	0.58	0.58	0.58	0.63	0.55	0.56	0.53	0.43	0.62	0.61	1.00					
17 Oceana Co. Mi	0.90	0.58	0.58	0.57	0.69	0.56	0.60	0.35	0.97	0.67	0.60	1.00				
18 Presque Isle Co. MI	0.58	0.58	0.58	0.34	0.55	0.56	0.53	0.35	0.62	0.39	0.47	0.60	1.00			
20 Shiawassee Co. MI	0.60	0.87	0.87	0.65	0.64	0.58	0.97	0.55	0.64	0.63	0.55	0.62	0.55	1.00		
21 St. Clair Co. MI	0.50	0.57	0.57	0.56	0.46	0.55	0.67	0.50	0.54	0.61	0.52	0.52	0.44	69.0	1.00	
22 Luce Co. MI	0.53	0.53	0.53	0.47	0.43	0.52	0.48	0.27	0.57	0.51	69.0	0.55	0.48	0.50	0.54	1.00
23 Yolo Co. CA	0.55	0.69	0.69	0.55	0.52	0.53	0.71	0.57	0.59	0.65	0.64	0.57	0.50	0.74	0.64	0.59
26 Adams Co. CO	0.50	0.57	0.57	44.0	0.62	69.0	0.59	0.40	0.46	0.42	0.52	0. 4	0.44	0.62	0.42	0.31
27 Tolland Co. CT	0.63	0.56	0.56	4 .0	0.53	0.61	0.58	0.67	0.60	0.59	0.52	0.58	0.52	0.60	0.50	0.33
29 Vermillion Co. IL	0.56	0.51	0.51	0.79	0.49	0.35	0.58	0.39	0.59	0.86	0.58	0.63	0.42	0.54	0.57	0.43
30 Benton Co. IN	0.58	0.52	0.52	0.40	0.48	69.0	0.47	0.35	0.55	0.39	0.73	0.53	0.47	0.48	0.44	0.62
32 Stafford Co. KS	0.50	0.57	0.57	0.50	0.38	0.41	0.59	0.30	0.54	0.48	0.44	0.52	0.37	0.62	0.50	0.46
33 Hall Co. NE	0.61	0.56	0.56	0.80	0.53	0.38	0.63	0.36	0.65	0.88	0.63	0.63	0.40	0.59	0.63	0.47
39 Cache Co. UT	0.47	0.67	0.67	0.47	0.57	0.65	0.69	0.45	0.43	0.40	0.48	0.41	0.34	0.71	0.46	0.36
40 Brazos Co. TX	0.58	0.53	0.53	0.76	0.50	0.41	0.59	0.40	0.61	0.88	0.59	0.59	0.43	0.56	0.59	0.44
42 Germany	0.53	0.53	0.53	0.47	0.36	0.58	0.48	0.36	0.57	0.51	0.48	0.62	0.41	0.50	0.54	0.57

Table 4 (cont'd). Matrix of genetic similarity coefficients for RAPD analysis of 26 common dandelion collections.

				Commo	n dande	elion col	Common dandelion collections	,		
Collection	23	26	27	59	30	32	33	39	40	42
01 Alger Co. MI										
02 Berrian Co. MI										
03 Calhoun Co. MI										
04 Clinton Co. MI										
06 Hillsdale Co. MI										
07 Ingham Co. MI										
09 Ionia Co. MI										
12 losco Co. MI										
13 Leelanau Co. MI										
14 Monroe Co. MI										
15 Newaygo Co. MI										
17 Oceana Co. Mi										
18 Presque Isle Co. MI										
20 Shiawassee Co. MI										
21 St. Clair Co. MI										
22 Luce Co. MI										
23 Yolo Co. CA	1.00									
26 Adams Co. CO	0.48	1.00								
27 Tolland Co. CT	0.62	0.50	1.00							
29 Vermillion Co. IL	0.50	0.34	0.56	1.00						
30 Benton Co. IN	0.50	0.52	0.52	0.37	1.00					
32 Stafford Co. KS	0.48	0.25	0.50	0.46	0. 4	1.00				
33 Hall Co. NE	0.55	0.38	0.56	0.93	0.40	0.50	1.00			
39 Cache Co. UT	0.52	0.85	0.47	0.38	0.48	0.38	0.35	1.00		
40 Brazos Co. TX	0.57	0.35	0.63	0.93	0.38	0.47	0.95	0.33	1.00	
42 Germany	0.59	0.31	0.40	0.43	0.48	0.54	0.41	0.36	0.39	1.00

Table 5. Comparison of leaf area and dry weight for 9 collections of common dandelion grown in the greenhouse.

Collection	Leaf area	Dry weight ^a
	cm ³	g
Alger Co. MI	432 a	2.02 a
Berrien Co. MI	244 cd	0.84 c
Ingham Co. MI	394 ab	1.56 b
Luce Co. MI	216 d	0.87 c
Monroe Co. MI	387 ab	1.79 ab
Newaygo Co. MI	266 cd	0.97 c
Shiawassee Co. MI	409 a	1.74 ab
St. Clair Co. MI	319 bc	1.57 b
Vermillion Co. IL	310 bc	1.51 b

^a Means followed by the same letter within column are not significantly different according to Fisher's Protected LSD (α =0.05).

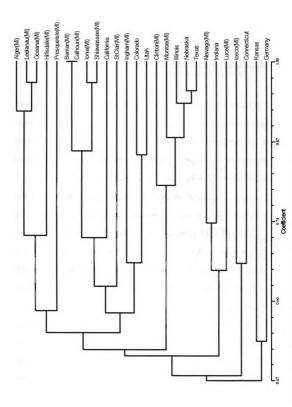


Figure 1. Dendogram for RAPD analysis for 26 collections of common dandelion.

CHAPTER 2

CONTROL STRATEGIES FOR COMMON DANDELION (*Taraxacum officinale* Weber) IN NO-TILLAGE CROPPING SYSTEMS

Abstract: Common dandelion has developed into a troublesome agronomic weed for no-tillage corn and soybean producers in Michigan and throughout the North Central region. Field experiments were conducted on established populations of common dandelion in 2001-02 and 2002-03 to evaluate the effect of preplant herbicide applications and sequential herbicide applications for efficacy on established populations of common dandelion. Preplant treatments of glyphosate or 2,4-D ester were applied early fall, late fall, early spring, and late spring. Glyphosate was applied at 420 g ae ha⁻¹ or 840 kg ae ha⁻¹; 2,4-D ester was applied at 560 g ai ha⁻¹ or 1120 g ai ha⁻¹. A tank mixture of glyphosate plus 2,4-D ester was also evaluated at each of the preplant timings. Common dandelion control was evaluated at the time of crop planting. For both glyphosate and 2,4-D ester, the fall applications were more effective than the spring applications. The late fall application of glyphosate at 840 g ae was the most effective treatment, with 80 percent control of common dandelion. A single application of glyphosate or 2,4-D ester applied either in the fall or spring was not sufficient in providing season long control of common dandelion. A subsequent field experiment was conducted to evaluate the effectiveness of sequential applications of glyphosate to provide season long control of common dandelion.

Sequential treatments of glyphosate following either glyphosate or 2,4-D ester were effective in providing season-long control of common dandelion.

Nomenclature: *Taraxacum officinale*, TAROF, common dandelion; glyphosate, *N*-(phosphonomethyl) glycine; 2,4-D ester, (2,4-dichlorophenoxy) acetic acid.

Key words: application timing, preplant treatment, sequential treatment.

INTRODUCTION

Common dandelion (*Taraxacum officinale* Weber) has developed into a troublesome agronomic weed in Michigan and throughout the North Central region of the U.S. Typically considered a problematic weed unique to forage production and turf grass, the occurrence of common dandelion in no-tillage corn and soybean production is becoming more common. The increased use of herbicide-resistant crops in conjunction with the adoption of no-tillage cropping practices has resulted in an environment conducive to the establishment of common dandelion (Triplett and Lytle 1972).

Common dandelion is a simple perennial that possesses a large fragile taproot that is used for carbohydrate storage and to acquire needed resources. Tillage operations associated with conventional-tillage crop production are an effective method of controlling perennial weeds such common dandelion because tillage disrupts the establishment and development of the taproot (Triplett 1985). Adoption of no-tillage cropping practices by crop producers has occurred in response to environmental and economic incentives. Soil conservation and reduced input costs are the primary drivers for this adoption (Jasa et al. 1991). However, no-tillage cropping systems have a higher reliance on herbicides for weed control (Koskinen and McWhorter 1986), often requiring multiple herbicide applications to manage perennials (Buhler and Mercurio 1988; Buhler and Proost 1990).

Non-selective herbicides, such as glyphosate, are widely used for vegetation management prior to planting and postemergence in no-tillage

glyphosate-resistant cropping systems. A disadvantage of the use of glyphosate as a preplant and postemergence treatment is the lack of residual soil activity (Sprankle et al. 1975a, 1975b). A consequence of the exclusive use of glyphosate without the addition of soil applied residual herbicides is the potential for weeds, including common dandelion seedlings, to emerge following the glyphosate application. Glyphosate is effective in controlling many troublesome perennial weeds (Davison 1972). However, common dandelion is often the one weed not completely controlled. Furthermore, as common dandelion seedlings become established, they become more difficult to control (Triplett et al. 1977).

The objectives of this research were to determine the effect of herbicide, application timing, and sequential applications on control of established populations of common dandelion in no-tillage cropping systems.

MATERIALS AND METHODS

Field experiments were conducted to evaluate control strategies for common dandelion using glyphosate¹ and 2,4-D ester². Preplant treatments of glyphosate and/or 2,4-D ester were applied at four application timings. Additional experiments were conducted to evaluate sequential applications of glyphosate following either glyphosate or 2,4-D ester. Initial applications of glyphosate or 2,4-D ester were applied preplant either in the fall or spring. Sequential applications of glyphosate were applied postemergence in glyphosate-resistant soybean.

Effect of herbicide and application timing

Experiments to evaluate preplant applications of glyphosate or 2,4-D ester were conducted in 2001-02 and 2002-03 at the Michigan State University Clarksville Experiment Station. Two identical experiments were established on adjacent sites in both 2001-02 and 2002-03. Experiments were conducted on sites with established populations of common dandelion that had been in a notillage corn-soybean rotation for 3 years. The soil at the experimental site was a loam with pH 6.8 and 1.8% organic matter. Experimental sites were prepared by removing the previous corn crop as silage approximately one month before the initial fall applications. Plots measured 3 m wide by 9 m long. Herbicide treatments were applied with a tractor mounted, compressed-air sprayer calibrated to deliver 187 L ha⁻¹ at 207 kPa through 8003 flat fan nozzles³.

Treatments of glyphosate or 2,4-D ester were applied at four application timings prior to crop planting; early fall (EFALL), late fall (LFALL), early spring (ESPRING), and late spring (LSPRING) (Table 1). Glyphosate and 2,4-D ester were applied at typical use rates to evaluate common dandelion control. Glyphosate was applied at 420 g ae ha⁻¹ and 840 g ae ha⁻¹; 2,4-D ester was applied at 560 g ai ha⁻¹ and 1120 g ai ha⁻¹. A tank mixture of glyphosate plus 2,4-D ester at 420 g ae ha⁻¹ and 560 g ae ha⁻¹, respectively, was also applied at each of the four preplant timings. All treatments containing glyphosate were applied with 2% (w/v) ammonium sulfate. Common dandelion control was evaluated visually at crop planting and was recorded as percent control as

compared to the untreated; where 0 = no control and 100 = complete common dandelion death.

Effect of sequential applications

Field experiments were conducted in glyphosate-resistant soybean to evaluate the effectiveness of sequential applications of glyphosate following initial applications of either glyphosate or 2,4-D ester. Experiments were conducted in 2001-02 and 2002-03 at the Michigan State University Clarksville Experiment Station as described above. Glyphosate-resistant soybean⁴ were planted at a population of 494,000 seeds ha⁻¹ in 19-cm rows, approximately 3 weeks after the initial spring application. In 2002-03, s-metolachlor⁵ was applied preemergence over the entire study at 1424 g ai ha⁻¹ for annual weed control. A postemergence application of quizalofop-P-ethyl⁶ at 49 g ai ha⁻¹ was applied with non-ionic surfactant⁷ at 0.25% (v/v) for grass control in both 2001-02 and 2002-03.

Initial treatments of either glyphosate or 2,4-D ester at 840 g ae and 1120 g ae, respectively, were applied at two preplant application timings; fall and spring. These application timings correspond with the LFALL and ESPRING timings described above in Table 1. Sequential treatments of glyphosate at 840 g ae were applied postemergence to glyphosate-resistant soybean at the V3 or V6 crop stage. The sequential application at the V6 stage of soybean was evaluated in 2002-03 only. Common dandelion control was evaluated visually and common dandelion plant density recorded at soybean harvest. Common dandelion density

was recorded as the number of plants per square meter. Soybean yield was determined 2001-02 by hand-harvesting the middle 1.5 m of each 4.5 m long plot. In 2002-03, the middle 1.5 m of each 9 m long plot was mechanically harvested with a research plot harvester.

Statistical analysis

The herbicide and application timing experiment was conducted as a randomized complete block design. Treatments were replicated four times for each treatment and the experiment was conducted four times. Data were subjected to analysis of variance with SAS⁸ and means separated using Fisher's Protected LSD (α = 0.05). Variances were determined to be homogenous and the experiments combined.

The sequential application experiment was established as a split block with four replications in 2001-02; the whole plot was the initial application and the sub-plot was the sequential application. In 2002-03, the experiment was conducted as a randomized complete block design with four replications. Data were subjected to analysis of variance with SAS and means separated using Fisher's Protected LSD (α = 0.05). Data collected in 2001-02 and 2002-03 are presented separately.

RESULTS AND DISCUSSION

Effect of herbicide and application timing

Significant differences in common dandelion control were observed between herbicides and herbicide rates. Both glyphosate or 2,4-D ester at higher rates were more effective than at lower rates at each of the four application timings (Figure 1). Glyphosate applied at 840 g ae was usually more effective than 2,4-D ester at 1120 g ae, regardless of application timing (Figure 2). The most effective herbicide treatment to control common dandelion was the LFALL application of glyphosate applied at 840 g ae, resulting in 80 percent control. The effectiveness of fall applications of glyphosate to control common dandelion has been consistently demonstrated (Buhler and Mercurio 1988; Buhler and Proost 1990). At the same LFALL timing, 2,4-D ester at 1120 g ae provided 58 percent common dandelion control. The most effective application timing for 2,4-D ester was the EFALL application timing which resulted in 60 percent control of common dandelion. Glyphosate applied at the same timing was more effective with 74 percent control of common dandelion. Glyphosate at the lower rate was consistently more effective than 2,4-D ester at the lower rate at all application timings (Figure 1).

Timing of the preplant application was as critical as the herbicide and herbicide rate applied. For both glyphosate and 2,4-D ester, the preplant applications in the fall were more effective than the spring applications (Figure 2), despite the fact that temperatures at the time of application were lower in the fall versus the spring, especially in 2001-02 (Table 1). Glyphosate applied at 840 g

ae provided 80 and 74 percent control at the EFALL and LFALL timings, respectively. This same treatment at the ESPRING and LSPRING timings resulted in only 65 and 55 percent control, respectively. A similar trend of reduced control in the spring was also observed with 2,4-D ester (Figure 2). Reduced control of common dandelion by spring treatments may relate to growth patterns of the plant in the spring (Mann 1981; Rutherford and Deacon 1974). Root tissue of common dandelion has the ability to generate new shoots (Mann and Cavers 1979). Carbohydrates stored in the taproot are mobilized to the above ground biomass of the plant during rapid vegetative growth in the spring. Applications at this time result in insufficient herbicide translocation to the roots for complete control.

Glyphosate or 2,4-D ester applied at the lower rates in the fall tended to be more effective than the higher herbicide rates applied in the spring. Applications of 2,4-D ester at 560 g ae at the EFALL and LFALL timings provided 40 and 43 percent control, respectively (Figure 1). These treatments were more effective than the ESPRING and LSPRING applications of 2,4-D ester at 1120 g ae with 34 and 31 percent control, respectively. A similar trend was observed with glyphosate for the spring applications. The LFALL application of glyphosate at 420 g ae was more effective than the LSPRING application of glyphosate at 840 g ae.

Depending on application timing, a tank-mixture of glyphosate plus 2,4-D ester at reduced rates was effective in controlling common dandelion. Regardless of application timing, glyphosate applied at 840 g ae was more

effective than the tank-mixture (Figure 1). At the LFALL application timing, the tank-mixture was more effective than 420 g ae of glyphosate. Common dandelion control with the tank-mixture was more effective than 2,4-D ester at 1120 g ae at the LFALL, ESPRING, and LSPRING application timings. The tank-mixture was as effective as 1120 g ae of 2,4-D ester at the EFALL application timing. The addition of 2,4-D ester to glyphosate did not antagonize common dandelion control when applied at the EFALL, LFALL, and LSPRING application timing. However, at the ESPRING application timing, the tank-mixture was less effective than 420 g ae of glyphosate.

Effect of sequential applications

A single application of glyphosate or 2,4-D ester either in the fall or spring did not provide season-long control of common dandelion (Figure 3). However, the addition of a sequential application of glyphosate following either glyphosate or 2,4-D ester was effective in providing season-long control of common dandelion. In both 2001-02 and 2002-03, glyphosate applied in the fall followed by the sequential application at the V3 stage of soybean provided greater than 80 percent control. In 2002-03, similar control was observed with the fall application followed by the sequential application at the V6 stage of soybean with 87 percent control.

In 2001-02, the spring application of glyphosate followed by the V3 stage of soybean provided 54 percent control. This was significantly lower than the same treatment in 2002-03 with 97 percent control. This discrepancy in control

between years could be attributed to the lack of significant rainfall in 2002-03 following the spring application timing (Table 2). In 2002, the experimental site received over 160 mm of precipitation from January thru April. This was approximately 100 mm more than in 2003. The dry weather pattern reduced new seedling germination and plant regrowth. The lack of new plant growth in 2002-03 is evident from the spring-only treatment of glyphosate that provided 75 percent control at harvest. This same treatment in 2001-02 provided only 20 percent control.

Treatment with 2,4-D ester followed by a sequential application of glyphosate was also effective in controlling common dandelion, depending on the timing of the initial application (Figure 3). In 2001-02, the fall application of 2,4-D ester followed by glyphosate at the V3 stage of soybean provided 81 percent control of common dandelion. However, the spring application of 2,4-D ester followed by glyphosate at the V3 stage of soybean provided only 44 percent control in 2001-02. Fall treatments were again more effective in controlling established common dandelion. In 2001-02, the sequential application of glyphosate at the V3 stage of soybean controlled newly emerged common dandelion from the fall treatment. The initial spring treatment of glyphosate was less effective, resulting in more established plants at the sequential application at the V3 stage of soybean. These mature plants are more difficult to control than seedling common dandelion (Triplett et al. 1977).

Similar to the sequential treatments of glyphosate in 2001-02 and 2002-03, a sequential treatment of glyphosate following 2,4-D ester in the fall

provided 81 and 80 percent control of common dandelion, respectively. Likewise, the spring application of 2,4-D ester followed by glyphosate at the V3 stage of soybean was less effective with only 44 percent control in 2001-02. This same treatment in 2002-03 provided 80 percent control of common dandelion at harvest. This differential response is likely a result of the extended period without rainfall in 2002-03. Delaying the sequential application until the V6 stage of soybean resulted in common dandelion control similar to the timing at the V3 stage of soybean regardless of timing of the initial application (Figure 3).

A single application of either glyphosate or 2,4-D ester applied in the fall or spring was not effective in reducing plant densities as compared to the untreated (Figure 4). However, the addition of a sequential application of glyphosate was effective in reducing common dandelion plant densities. In 2001-02, the addition of the sequential application of glyphosate at the V3 stage of soybean reduced common dandelion plant densities as compared to the fall only treatment of glyphosate. In 2002-03, delaying the sequential application until the V6 stage of soybean significantly reduced common dandelion densities as compared to the fall treatment of glyphosate. A sequential application of glyphosate following a spring treatment of glyphosate did not reduce common dandelion densities in either 2001-02 or 2002-03. A sequential application of glyphosate following 2,4-D ester applied in the fall or spring was effective in reducing common dandelion densities as compared to the initial treatment alone.

Timing of herbicide application had a significant effect on soybean yield. In 2001-02, the fall and spring applications of either glyphosate or 2,4-D ester did

not result in soybean yield greater than the untreated (Figure 5). In 2002-03, the spring application of either glyphosate or 2,4-D ester resulted in soybean yield greater than the untreated or the fall application of either herbicide. It is likely that the lack of moisture that reduced weed seedling germination and regrowth in 2003 contributed to this observation. Soybean yields resulting from a fall application of either glyphosate or 2,4-D ester followed by the sequential application at the V3 stage of soybean were consistently among the highest in both 2001-02 and 2002-03. Delaying the sequential application of glyphosate until the V6 stage of soybean following the spring application of either glyphosate or 2,4-D ester did not affect soybean yield as compared with the sequential application at the V3 stage of sovbean in 2002-03. However, delaying the sequential application following the initial fall application of either glyphosate or 2,4-D ester did result in reduced soybean yield. Reduction of soybean yield associated with the initial fall followed by the sequential application at the V6 stage of soybean is likely due to early-season competition from annual weeds not controlled by the preemergence application of s-metolachlor.

It is apparent that established populations of common dandelion pose a significant challenge to no-tillage crop producers. Common dandelion, when left uncontrolled, has the potential to negatively impact soybean production. An effective management strategy to control this weed includes field monitoring and multiple herbicide applications. The lack of residual control associated with glyphosate and 2,4-D ester makes sequential herbicide applications necessary to achieve season-long control of common dandelion. An effective strategy to

control common dandelion in no-tillage crop production will include fall applications of herbicides such as glyphosate followed by a sequential postemergence application of glyphosate in glyphosate-resistant crops. The timing of this postemergence application will depend on environmental conditions and the presence of emerged weeds.

Source of Materials

¹ Roundup UltraMAX, Monsanto Company, St. Louis, MO 63167.

² 2,4-D ester, Tenkoz Inc., Alpharetta, GA 30202.

³ Flat-fan spray nozzle, Spraying Systems Company, Wheaton, IL 60188.

⁴ DK 23-51, Dekalb Genetics Corp., Monsanto Company, St. Louis MO 63167

⁵ Dual II Magnum, Syngenta Crop Protection, Inc. Greensboro, NC 27409.

⁶ Assure II, E. I. du Pont de Nemours and Company, Wilmington, DE 19898.

⁷ Activator 90, Loveland Industries Inc., Greeley, CO 80632.

⁸ SAS version 8.2, SAS Institute, SAS Circle, Box 8000, Cary, NC 27512-8000.

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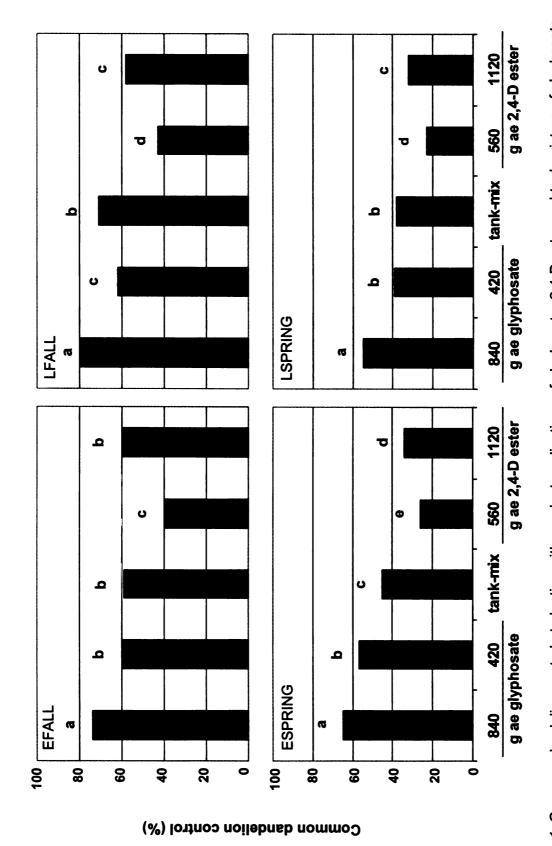
Table 1. Application timing, application date, and average air temperature for glyphosate and 2,4-D ester applications for common dandelion control.

Ę	7007-1007	2002			7007	2002-2003		
Timina	Date of application	Air ten	Air temperature ^a (C)	re ^a (C)	Date of application	Air ter	Air temperature ^a (C)	e _a (C)
9		-	0	+		-1	0	+
Early fall Oc	October 18, 2001	5	ω	10	October 9, 2002	11	14	13
Late fall No	November 5, 2001	œ	2	ω	November 9, 2002	13	12	13
Early spring Ap	April 16, 2002	23	24	20	April 15, 2003	16	23	7
Late spring Ap	April 30, 2002	9	∞	O	April 29, 2003	17	12	12

^a Average air temperature on the day before (-1), the day of (0), and the day after (+1) herbicide application.

Table 2. Yearly accumulation of precipitation at the Michigan State University Clarksville Experiment Station from 2001 thru 2003.

	Α	nnual precipita	tion
Month	2001	2002	2003
		mm	
January	19	7	3
February	64	34	8
March	14	49	35
April	67	78	22
May	136	104	122
June	67	66	50
July	23	47	60
August	103	71	92
September	78	25	45
October	143	45	29
November	48	53	173
December	34	22	21
Total	796	608	670



plus 2,4-D ester at 420 g ae and 560 g ae, respectively, as affected by application timing. All treatments containing glyphosate were Figure 1. Common dandelion control at planting with preplant applications of glyphosate, 2,4-D ester and tank mixture of glyphosate applied with 2% (w/v) ammonium sulfate. Means within application timing with the same letter are not significantly different ($\alpha = 0.05$).

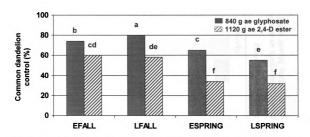
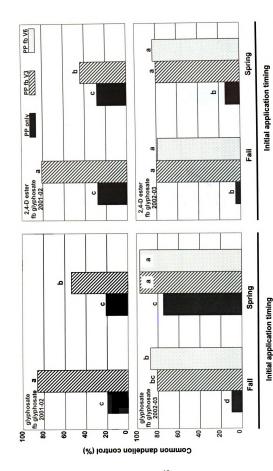


Figure 2. Common dandelion control at planting with glyphosate and 2,4-D ester as affected by application timing. Glyphosate applied with 2% (w/v) ammonium sulfate. Means with the same letter are not significantly different (a = 0.05)



application timing. All treatments containing glyphosate were applied with 2% (w/v) ammonium sulfate. Means within each Figure 3. Common dandelion control at soybean harvest with single and sequential herbicide applications as affected by graph with the same letter are not significantly different (α = 0.05).

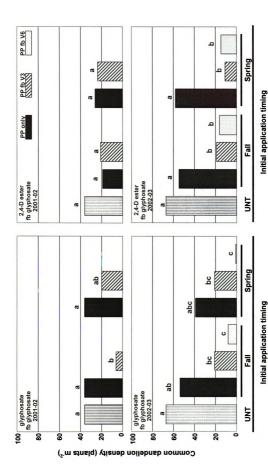


Figure 4. Common dandelion plant densities at soybean harvest with single and sequential herbicide applications as affected by application timing. All treatments containing glyphosate were applied with 2% (w/v) ammonium sulfate. Means within each graph with the same letter are not significantly different ($\alpha = 0.05$).

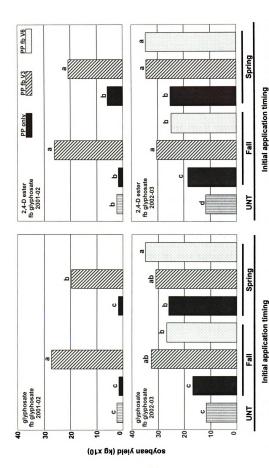


Figure 5. Soybean yield as affected by common dandelion control with single and sequential herbicide applications as affected by application timing. All treatments containing glyphosate were applied with 2% (w/v) ammonium sulfate. Means within each graph with the same letter are not significantly different ($\alpha = 0.05$).

CHAPTER 3

COMMON DANDELION (*Taraxacum officinale* Weber) CONTROL WITH POSTEMERGENCE HERBICIDES IN NO-TILLAGE GLUFOSINATE-RESISTANT CORN (*Zea mays* L.)

Abstract: Common dandelion has developed into a troublesome agronomic weed for no-tillage corn producers. Measures to control common dandelion prior to crop planting are not always effective. As a result, a postemergence herbicide application is often required to reduce common dandelion competition with the crop. Field experiments were conducted in 2002 and 2003 to evaluate 22 postemergence herbicide treatments for efficacy on established populations of common dandelion in no-tillage corn. Postemergence herbicides were applied when the corn was 5-6 collar. All herbicides were applied at labeled rates with recommended adjuvants. At 28 days after treatment (DAT) the most effective treatments included glufosinate, glufosinate + atrazine, mesotrione, and mesotrione + atrazine, providing at least 76 percent control of common dandelion. All other herbicide treatments at this time provided less than 40 percent common dandelion control. Common dandelion control was evaluated 56 DAT when regrowth of treated plants was observed for some herbicide treatments. By 56 DAT, dicamba + diflufenzopyr was the most effective treatment providing 83 percent control of common dandelion. In 2002, all herbicide treatments, with the exception of flumiclorac, resulted in corn yields greater than the untreated. Treatments that provided the greatest control of common dandelion at 28 DAT also resulted in the greatest corn yield. None of the postemergence herbicide treatments that were evaluated completely controlled common dandelion. However; specific treatments were identified that effectively reduced common dandelion competition with the corn crop.

Nomenclature: 2,4-D amine; 2,4-D ester; atrazine; bentazon; bromoxynil; carfentrazone; clopyralid; dicamba; diflufenzopyr; flumetsulam; glufosinate; halosulfuron; mesotrione; metolachlor; nicosulfuron; paraquat; primisulfuron; rimsulfuron; sulfentrazone; thifensulfuron; *Taraxacum officinale*, TAROF, common dandelion.

Abbreviations: AMS, ammonium sulfate; COC, crop oil concentrate; NIS, nonionic surfactant; UAN, 28% urea-ammonium nitrate; EPP, early preplant; PRE, preemergence; POST, postemergence.

INTRODUCTION

Conservation tillage has become a widely accepted practice for both environmental and economic reasons. By eliminating tillage, plant residue remains intact on the soil surface, effectively reducing soil erosion by wind and water movement. Economic benefits include reduced fuel and labor requirements (Phillips et al. 1980; Jasa et al. 1991). Removal of tillage from the cropping system also impacts the dynamics of weed populations present in the field. More specifically, perennial weed species typically become more prevalent than annuals (Triplett and Lytle 1972; Buhler et al. 1994). Populations of perennial species are likely to increase in these systems because the lack of tillage allows the plants to become established (Triplett 1985). And furthermore, as seedlings of perennial species become established, they become more difficult to control (Triplett et al. 1977). Effective weed control is the primary consideration for adopting no-tillage (Koskinen and McWhorter 1986).

Common dandelion (*Taraxacum officinale* Weber) is a perennial weed species that has developed into a troublesome weed problem that is unique to no-tillage cropping systems. Common dandelion has been a concern primarily associated with forage production, where it may contribute up to 30% of total dry matter yield (Moyer 1989). Fortunately for forage producers, the presence of common dandelion does not appear to be detrimental. In fact, common dandelion appears to be high in forage quality (Dutt et al. 1977; Scheaffer and Wyse 1982). However, common dandelion is not so benign in no-tillage corn production. We

have observed significant crop stress and yield reduction from common dandelion competition in no-tillage corn (Franssen, unpublished).

The non-selective herbicide, glyphosate, is effective in providing common dandelion control in no-tillage cropping systems. However, a postemergence application with glyphosate is restricted to use in glyphosate-resistant comnybrids. Glufosinate is another non-selective herbicide that provides control of many troublesome weeds when applied postemergence to glufosinate-resistant corn hybrids. There are many additional conventional herbicides that provide effective weed control in non-herbicide resistant corn production. As the prevalence of common dandelion continues to increase in no-tillage crop production, these herbicides need to be evaluated for common dandelion efficacy. The objective of this research was to evaluate glufosinate as well as several conventional postemergence corn herbicides for efficacy on established populations of common dandelion in no-tillage corn.

MATERIALS AND METHODS

Field experiments were conducted on no-tillage crop production sites at the Michigan State University Clarksville Experiment Station and at a commercial production field near Elsie, MI in 2002 and 2003, respectively. The soil at the Clarksville Experiment Station was a loam with 1.8 % organic matter and pH 6.8. This site was in a no-tillage corn-soybean rotation for 3 years. The soil at the Elsie experimental site was a sandy loam with 2.8 % organic matter and pH 6.8. This site was in a no-tillage corn-soybean rotation for 10 years. The previous

year's crop for the Clarksville and Elsie experimental sites were corn and soybean, respectively. Descriptions of the trial sites are shown in Table 1.

Glufosinate-resistant corn hybrids^{1,2} were planted at 69,200 seeds ha⁻¹ in four row plots measuring 3 m wide by 9 m long. In both 2001 and 2002, paraguat at 525 g ai ha-1 was applied early preplant (EPP) 7 days prior to planting to remove common dandelion above-ground biomass and control winter annual weeds. Annual weeds were controlled at the experimental sites using typical application rates of the herbicides included in accordance with the commercial herbicide label and current commercial practices. A preemergence (PRE) treatment of s-metolochlor at 1424 g ai ha⁻¹ was applied at planting. Common dandelion control was evaluated using the postemergence (POST) herbicide treatments listed in Table 2. All postemergence herbicides were applied when corn reached the 5-6 collar stage. Treatments were applied at the Clarksville and Elsie experimental site on June 12, 2002 and June 22, 2003, respectively. At this time, common dandelion above-ground biomass had fully recovered from the EPP paraguat treatment with an average diameter of 30-35 cm. Common dandelion plant densities at the time of the postemergence applications were 6 and 3 plants m⁻² in 2002 and 2003, respectively. Treatments were applied with a tractor mounted, compressed-air sprayer calibrated to deliver 187 L ha⁻¹ at 207 kPa through 8003 flat fan nozzles³.

Common dandelion control from postemergence herbicides was evaluated visually at 28 and 56 d after treatment (DAT). Common dandelion control was recorded as percent control compared to the untreated; where 0 = no control and

100 = complete common dandelion death. In 2002 only, the middle two rows of corn were harvested and the yields adjusted to 15.5 percent moisture. Due to adverse dry weather conditions and competition from annual weeds, yield data was not collected in 2003.

Statistical analysis

Twenty-two herbicide treatments plus an untreated control were arranged in a randomized complete block design. Treatments were replicated four times and the experiment was conducted twice. Data were subjected to analysis of variance with SAS⁴ and means separated using Fisher's Protected LSD (α = 0.05). Variances were determined to be homogenous and the experiments combined.

RESULTS AND DISCUSSION

There was a difference in the rate at which common dandelion responded to the herbicide treatments evaluated. Regrowth of treated plants reduced the overall efficacy of some herbicide treatments that initially provided good control of common dandelion. Also, herbicides that did not appear effective at earlier ratings were more effective later in the season.

The most effective herbicide treatments at 28 DAT were glufosinate + atrazine, mesotrione, mesotrione + atrazine, and glufosinate with 80, 77, 76, and 76 percent control, respectively (Table 2). Loux and Dobbels (2003) also found mesotrione + atrazine effective for control of common dandelion when applied

preemergence in no-tillage corn. Atrazine applied alone provided only 23 percent control. The pre-mixture of dicamba + diffufenzopyr provided 49 percent control. All other treatments provided between 9 and 41 percent control of common dandelion (Table 2).

By 56 DAT, significant regrowth of treated common dandelion was observed for some treatments. Treatments of glufosinate, glufosinate + atrazine, mesotrione + atrazine, and mesotrione, which were the most effective at 28 DAT, provided only 63, 57, 57, and 54 percent control, respectively. At 56 DAT, dicamba + diflufenzopyr provided the most effective control of common dandelion with 83 percent control. The pre-mixture of atrazine plus dicamba provided 70 percent control. This treatment was more effective than either atrazine or dicamba applied alone.

Herbicides with the same mode of action often provided similar control of common dandelion. Treatments including the acetolactate synthase (ALS)-inhibiting herbicides primisulfuron, rimsulfuron + thifensulfuron, nicosulfuron, and halosulfuron provided similar control with 36, 34, 32, and 30 percent control, respectively at 28 DAT (Table 2). At 56 DAT, nicosulfuron was the most effective ALS-inhibiting herbicide with 55 percent control. The other ALS-inhibiting herbicides were less effective. Similarities were also observed between treatments that contained growth regulator herbicides. Dicamba, clopyralid, and 2,4-D ester provided similar control at 28 DAT. Common dandelion control with 2,4-D amine was as effective as 2,4-D amine and clopyralid. By 56 DAT, common dandelion control had increased slightly and 2,4-D amine was as

effective as dicamba and 2,4-D ester. Clopyralid was the most effective growth regulator herbicide at 56 DAT with 51 percent control. The protoporphyrinogen oxidase (PPO)-inhibitors carfentrazone and flumiclorac were the least effective in controlling common dandelion at both 28 and 56 DAT, neither providing more than 13 percent control.

With a few exceptions, common dandelion control with pre-mixtures and tank mixtures was generally similar to that of the individual herbicide components alone. However, dicamba + diffufenzopyr was more effective than dicamba alone at both 28 and 56 DAT (Table 2). Common dandelion control with primisulfuron + dicamba at 28 DAT was as effective as primisulfuron or dicamba applied alone. By 56 DAT, primisulfuron + dicamba was more effective than primisulfuron alone. At both 28 and 56 DAT, clopyralid + flumetsulam was as effective as clopyralid alone. The addition of atrazine in a tank mixture did not reduce common dandelion control and in some instances it improved control (Table 2). At 28 DAT, dicamba + atrazine was as effective as dicamba alone. However, by 56 DAT, dicamba + atrazine was more effective than dicamba alone. Atrazine + 2,4-D ester was as effective as either 2,4-D ester or atrazine applied alone at both 28 and 56 DAT.

All herbicide treatments, with the exception of flumiclorac, resulted in corn yields greater than the untreated in 2002 (Table 2). Treatments that provided effective control of common dandelion also resulted in the greatest corn yield. Treatments that resulted in the highest corn yield include mesotrione + atrazine, glufosinate, and glufosinate + atrazine with grain yield greater than

10,000 kg ha⁻¹. Similar yield was observed with mesotrione, dicamba + atrazine, and dicamba + diflufenzopyr with 9313, 9171, and 9133 kg ha⁻¹, respectively.

Common dandelion can be managed in no-tillage corn with properly selected postemergence herbicide. Although none of the treatments examined here were effective in completely eliminating common dandelion, herbicide treatments such as glufosinate, mesotrione, and dicamba + diflufenzopyr are effective in suppressing common dandelion competition in no-tillage corn. No-tillage producers that intensively manage this weed over several seasons will ultimately reduce the presence of common dandelion in the soil seed bank. As with many other perennial species, common dandelion seed has limited longevity in the soil (Burnside et al. 1996). Effective management strategies for common dandelion will require careful monitoring of production fields and combination of herbicide applications. Weed control programs that include fall applications of herbicides such as glyphosate or 2,4-D ester (Chapter 2) followed by postemergence herbicide applications will likely be successful in reducing common dandelion competition with no-tillage corn.

Source of Materials

¹ NK 3030 corn hybrid, Syngenta Seeds Inc., Golden Valley, MN 55427.

² N35-B8 corn hybrid, Syngenta Seeds Inc., Golden Valley, MN 55427.

³ Flat-fan spray nozzles, Spraying Systems Company, Wheaton, IL 60188.

⁴ SAS version 8.2, SAS Institute, SAS Circle, Box 8000, Cary, NC 27512-8000.

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Table 1. Trial location, site description, and date of herbicide application for common dandelion control with postemergence corn herbicides.

	2002	2003
Location	Clarksville	Elsie
Years in no-tillage crop production	3	10
Previous crop	corn	soybean
Soil texture	loam	sandy-loam
Soil organic matter	1.8%	2.8%
Soil pH	6.8	6.8
Early preplant application	May 14	May 14
Preemergence application	May 22	May 22
Postemergence application	June 12	June 22
Corn height (cm) ^a	28	30
Corn stage (collars) ^a	5-6	5-6
Common dandelion height (cm) ^a	15	15
Common dandelion diameter (cm) ^a	30	35
Common dandelion density (plants m ⁻²) ^a	6	3

^a measurements taken at the postemergence application timing.

Table 2. Control of treated common dandelion 21 DAT and 56 DAT with postemergence corn herbicides and corn yields.

		Cor	ntrol ^g	Yield
Herbicide treatment ^{ab}	Rate	28 DAT	56 DAT	2002
	g ae ha ⁻¹		%	kg ha ⁻¹
Untreated control		0	0	2913
2,4-D amine	561	28	39	6920
2,4-D ester	561	28	31	7114
clopyralid	91	34	51	7467
dicamba+NIS+UAN	281	35	43	7708
halosulfuron+NIS+UAN ^c	35	30	26	6942
nicosulfuron+COC	35	32	55	7720
primisulfuron+COC	40	36	37	7684
carfentrazone+NIS	9	9	13	5284
flumiclorac+COC ^d	30	11	12	3809
bromoxynil	421	20	26	5785
atrazine+COC ^e	244	23	31	6300
bentazon	1121	15	17	6940
mesotrione+COC+UAN	105	77	54	9313
mesotrione+atrazine+COC+UAN	105+281	76	57	10613
glufosinate+AMS	351	76	63	10524
glufosinate+atrazine + AMS	351+1122	80	57	10310
atrazine+2,4-D ester	628+280	28	32	6029
atrazine+dicamba	1121+560	41	70	9171
primisulfuron+dicamba+NIS+UAN	26+126	39	58	8362
dicamba+diflufenzopyr+NIS+UAN ^f	213+106	49	83	9133
clopyralid+flumetsulam+NIS+UAN	101+34	32	43	6823
rimsulfuron+thifensulfuron+COC+UAN	12+6	34	19	8421
LSD(0.05)		7	17	1804

^a Abbreviations; AMS, ammonium sulfate; NIS, non-ionic surfactant; UAN, urea-ammonia nitrate; COC, crop oil concentrate.

^b Unless otherwise noted, adjuvants rates were; NIS at 0.25% (v/v); UAN at 2.5% (v/v); COC at

^{1% (}v/v); AMS at 1.8% (w/v).

^c UAN applied at 5% (v/v).

d COC applied at 0.6% (v/v).

[°]COC applied at 1.2 % (v/v).

^fUAN applied at 1.25% (v/v).

⁹ Data combined from 2002 and 2003.

APPENDIX

Appendix A1. Protocol for DNA extraction from common dandelion fresh leaf tissue.

Cell Lysis

- 1. Add 30 mg fresh leaf tissue (4 leaf disks) to a 1.5 ml microfuge tube. Grind tissue to a fine powder with liquid nitrogen.
- 2. Add 300 µl Cell Lysis Solution + PVP. Vortex to wet the tissue.
- 3. Incubate cell lysate at 65 C for 60 minutes. After 30 and 60 minutes invert tubes 10 times.

RNase Treatment

- 1. Add 1.5 µl RNase A Solution to the cell lysate.
- 2. Mix the sample by inverting the tubes 25 times and incubate at 37 C for 15-60 minutes.

Protein Precipitation

- 1. Cool sample to room temperature.
- 2. Add 100 ul Protein Precipitation Solution to the cell lysate.
- 3. Mix the Protein Precipitation Solution uniformly with the cell lysate by vortexing each tube at high speed for 20 seconds. Place sample on ice for 15-60 minutes.
- 4. Centrifuge at 16,000 x g for 3 minutes (14,000 rpm on EPPENDORF 5415C). The proteins should form a tight, green pellet. If the pellet is not tight, incubate on ice for 5 minutes and repeat Step 4.

DNA Precipitation

- 1. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5 ml microfuge tube containing 300 µl 100% Isopropanol (2-proponal).
- 2. Mix the sample by inverting gently 50 times.
- 3. Centrifuge at 16,000 x g for 1 minute. The DNA will be visible as a pellet that ranges in color from off-white to light green.
- 4. Pour off supernatant and drain tube briefly on clean absorbent paper. Add 300 µl 70% Ethanol and invert tube several times to wash the DNA pellet.
- 5. Centrifuge at 16,000 x g for 1 minute. Carefully pour off the ethanol. *Pellet may be loose so pour slowly and watch pellet*.
- 6. Invert and drain the tube on clean absorbent paper and allow to air dry for 10-15 minutes.

DNA Hydration

- 1. Add 50 µl DNA Hydration Solution.
- 2. Rehydrate DNA by incubating sample for 1 hour at 65 C or overnight at room temperature.

Appendix A1. (cont'd) Protocol for DNA extraction from common dandelion fresh leaf tissue.

Purifying Protein-Contaminated Samples

- 1. Add 250 µl Cell Lysate Solution. Pipet up and down to mix. Be sure that the sample in completely dissolved.
- 2. Add 100 µl Protein Precipitation Solution and vortex vigorously at high speed for 20 seconds. Place sample on ice for 5 minutes.
- 3. Centrifuge at 16,000 x g for 3 minutes to pellet the protein. Repeat if necessary.
- 4. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5 ml microfuge tube containing 300 μl 100% Isopropanol.
- 5. Centrifuge at 16,000 x g for 1 minute to pellet the DNA. The DNA will be visible as a small white pellet.
- 6. Pour off supernatant. Add 300 µl 70% Ethanol and invert tube several times to wash the DNA pellet.
- 7. Centrifuge at 16,000 x g for 1 minute. Carefully pour off the ethanol.
- 8. Invert and drain the tube on clean absorbent paper and allow to air dry for 10-15 minutes.
- 9. Add 100ul DNA Hydration Solution. Allow DNA to hydrate at 65 C for 1 hour.

Note: Protocol modified from GENTRA Systems for isolating DNA from common dandelion.

Cell Lysis Solution + PVP, 10 ml

- 1. Add 200 mg (0.2 g) Polyvinylpyrrolidone (Sigma PVP-40) to 10 ml Cell Lysis Solution (final concentration 20 mg/ml).
- 2. Incubate at 65°C for 5-10 minutes inverting occasionally until the PVP is dissolved.
- 3. Cool Cell Lysis Solution + PVP to room temperature before using.
- 4. Store at room temperature.

Appendix A2. Reproductive characteristics for common dandelion at East Lansing and Chatham field nurseries in 2003.

_	Number o	f flowers	Total seeds produced		
Collection	East Lansing	Chatham	East Lansing	Chatham	
	— no. per	plant —	no. per	plant ——	
Alger Co. MI	62	10	16920	2015	
Berrien Co. MI	56	7	12888	1534	
Calhoun Co. MI	118	8	27245	1718	
Hillsdale Co. MI	37	6	8649	1088	
Ingham Co. MI	32	6	8342	1256	
Ionia Co. MI	64	8	13036	1192	
losco Co. MI	28	5	7646	700	
Leelanau Co. MI	66	12	14598	2381	
Monroe Co. MI	33	9	6148	1408	
Newaygo Co. MI	136	6	21147	895	
Oceana Co. Ml ^a	•	5	•	925	
Shiawassee Co. MI	52	15	9474	2328	
Luce Co. MI	86	9	15731	1307	
Yolo Co. CO	43	13	7797	1674	
Adams Co. CO	29	6	8499	1057	
Tolland Co. CT	69	8	16939	1659	
Champaign Co. IL	59	12	9423	1991	
Riley Co. KS	26	7	5029	1604	
Hall Co. NE	28	9	4893	1244	
Baker Co. OR	57	12	17049	2572	
Elk Co. PA	71	10	12254	1982	
Cache Co. UT	37	11	9326	2324	
Brazos Co. TX	46	8	7609	1333	
Germany	96	16	13333	1703	
LSD(0.05)	48	5	10524	880	
C.V.	50	35	54	40	

^a Oceana County collection dropped from East Lansing nursery due to winter mortality

