UNDERSTANDING 2,4-D RESISTANCE IN PRICKLY LETTUCE (*Lactuca serriola* L.) AND EVALUATING CHEMICAL FALLOW SYSTEMS FOR THE INLAND PNW

By

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To the Faculty of Washington State University:

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UNDERSTANDING 2,4-D RESISTANCE IN PRICKLY LETTUCE (*Lactuca serriola* L.) AND EVALUATING CHEMICAL FALLOW SYSTEMS FOR THE INLAND PNW

Abstract

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The objectives of this research were to: (1) compare reduced and no-tillage fallow systems with conventional fallow systems; (2) evaluate weed control efficacy of Light-Activated Sensor-Controlled (LASC) sprayer systems compared to conventional broadcast application in chemical fallow; and (3) determine mechanism and inheritance of 2,4-D resistance in prickly lettuce (*Lactuca serriola* L.); and (4) develop EST-SSR's to study genetic diversity among *Lactuca* accessions.

Average soil moisture in 150cm and seed-zone profile during summer fallow was similar for reduced and conventional tillage treatments across locations. Soil moisture content in all tillage treatments was lower at Helix compared to Davenport and a deep-furrow type drill was needed to successfully seed wheat into the soil moisture and obtain an adequate seedling stand. The deep-furrow drill was required to seed wheat could not be used in no-tillage treatments due to greater crop residue and resulted in emergence and yield penalty. The winter wheat yield in undercutter treatments followed by rodweeder or broadcast application of herbicide was similar to conventional tillage treatments but had 7 to 38% greater surface crop residue compared to conventional tillage. The herbicide use by LASC sprayer technology was 45 to 72% less compared to conventional broadcast application system. However, detection of very small weeds by LASC sprayer reduced in dusty and high residue conditions prevalent in sweep

undercutter and no-tillage systems, respectively. Under no-tillage system, glyphosate alone and in mixture with 2,4-D or pyrasulfotole plus bromoxynil controlled weeds (≥95%) similar to conventional broadcast application of glyphosate.

Prickly lettuce is an important weed of Pacific Northwest (PNW) and has developed resistance to 2,4-D. Reduced uptake followed by reduced translocation to the crown in resistant prickly lettuce biotypes could be a mechanism or partial mechanism for 2,4-D resistance in prickly lettuce. Inheritance of 2,4-D resistance in prickly lettuce is governed by one major gene and few modifier-genes.

A total of 15,970 simple sequence repeats (SSRs) were identified from 57,126 expressed sequence tag (EST) assemblies belonging to five different species of genus *Lactuca*. Primer pairs were synthesized for 45 prickly lettuce EST-SSRs and were used to group *Lactuca* accessions based on genetic dissimilarity.

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Dissertation Outline

The dissertation has five chapters. The first chapter includes the literature review and objectives. The research goals, based on the review of literature, are described in chapters two, three, four, and five. Each chapter is formatted differently due to different requirements of the respective journal to which it will be submitted. Chapter two compares conventional and reduced tillage fallow systems and will be submitted to *Agronomy Journal*. Chapter three is written in the format of *Weed Technology* and compares the efficacy of postemergence herbicides applied a with Light-Activated Sensor Controlled (LASC) sprayer in fallow. Chapter four will be submitted to *Weed Science* and describes the mechanism and inheritance of 2,4-D resistance in prickly lettuce (*Lactuca serriola* L.). Chapter five will be submitted to *Euphytica* and it illustrates the development of EST-SSRs and their use in the study of genetic diversity among *L. serriola* biotypes.

Dedication

This dissertation is dedicated to my parents – S. Narinderjit Singh Riar and Mrs. Surinder Kaur Riar

Chapter 1

Introduction

Background

The dryland farming regions of inland Pacific Northwest (PNW) are divided into three precipitation zones: low (<300 mm of precipitation), intermediate (300 to 450 mm of precipitation), and high (450 to 600 mm of precipitation). The intermediate precipitation zone of PNW comprises 620000, 323000 and 25000 hectares of dryland cropping in Washington, Oregon and Idaho, respectively (Schillinger et al. 2003). Winter wheat/summer fallow or winter wheat/spring wheat/summer fallow rotations are the main cropping system of this area (Dhuyvetter et al. 1996 and Juergens et al. 2004). Generally, winter wheat is grown following a year of fallow, which is necessary to recharge soil with sufficient moisture for crop growth and development (Papendick 1998). In spite of long-term efforts, researchers could not develop economically viable annual-cropping system to replace summer fallow cropping systems in this region (Nail et al. 2005). In the absence of tillage, a major portion of soil moisture recharge from fall and winter precipitation is lost during the summer due to capillary flow/evaporation from soil or evapotranspiration by weeds. A study in Australia by Freebairn et al. (1986) showed evaporation caused up to 65% loss of stored soil moisture during the summer fallow period without tillage. Summer fallow tillage systems have been developed to break the capillary continuity between subsoil and surface soil by creating a dust mulch barrier on the surface soil to reduce evaporation losses and conserve moisture in the seed-zone (Lyon et al. 2007). However, these tillage practices degrade soil physical properties and pose a threat of increased soil erosion from wind and water. Moreover, air-borne soil particles generated by fallow tillage and subsequent wind driven soil erosion can endanger human health (Papendick 1998, 2004) and

decrease soil productivity (Tanaka 1989, Saxton 1995, Larney 1998, Sorenson 2000 and Saxton et al. 2000).

In dryland farming of the PNW, conservation tillage practices, particularly minimum tillage and delayed minimum tillage, has been shown to be profitable and environment friendly (Schillinger 2001). Conservation tillage is defined as a management practice that leaves greater than 30% of the previous crop's residues on the soil surface after planting by omitting or reducing the intensity of at least one major tillage operation (Locke and Bryson 1997). Both minimum tillage and delayed minimum tillage systems substitute secondary tillage with non-selective herbicides, if feasible, and use non-inversion implements such as an undercutter cultivator to reduce the intensity of soil movement in primary spring tillage operations. Delayed minimum tillage is similar to minimum tillage but the use of undercutter cultivator as the primary spring tillage is delayed until at least mid May. The undercutter cultivator, comprised of wide (~75 cm) V-shaped blades or sweeps, is an important component of conservation tillage summer fallow systems and is usually followed by two or more rod weeding operations later in the fallow period for weed control or residue management. Undercutter cultivator operations, as part of a conservation tillage program, reduce seed-zone soil moisture loss (Griffith et al. 1986, Aase and Pikul 1995, Uri 1998) and production costs (Weersink et al., 1992) while increasing soil surface residue cover and surface roughness compared to conventional mechanical dust mulch summer fallow systems (Schillinger 2001). The greater residue cover and surface roughness in turn reduce soil erosion due to wind and water. Zaikin et al. (2007) and Nail et al. (2007) reported the undercutter cultivator or sweep tillage fallow system to be more profitable than conventional dust mulch fallow systems, providing similar wheat yields at reduced cost of production. Moreover, under current economic conditions, conservation tillage is more

profitable than conventional tillage systems for winter wheat/summer fallow due to greater fuel (Nail et al. 2007; Janosky et al. 2002) and farm labor expenses with additional restrictions in the availability of farm labor (Young et al. 2008) for the conventional system.

Chemical fallow is form of conservation tillage in which all tillage operations are omitted and the crop is direct-seeded following weed control with non-selective herbicides such as glyphosate and paraquat. Bennett and Pannell (1998) reported that the sparse, patchy nature of weed distribution often results in deposition of most of the broadcast herbicide application on bare soil rather than on weed foliage. Thus, effective spot treatments of herbicides in chemical fallow, even using greater per hectare rates, could result in substantial cost savings, reduced herbicide use and possibly improved weed control compared to broadcast applications. Efficient spot applications of herbicides to fields have not been practical due to the lack of automated equipment or the great amount of technical expertise needed by the sprayer operator. However, the recent introduction of real time light-activated, sensor controlled (LASC) sprayers has resulted in more accurate and precise spot applications of herbicides (Biller 1998) and could be used in chemical fallow systems to reduce the amount and area of herbicide applications. LASC sprayers are comprised of light sensors for red and near infrared (NIR) wavelengths (Felton and McCloy 1992). At red and NIR wavelengths, soil and green plants reflect ambient light at ratios of 1.1:1.5 and 6:15, respectively. The differential reflection allows LASC sprayers to detect plants and activate a solenoid switch above a spray nozzle for a set period of time (Biller 1998). LASC sprayers used for selective postemergence weed control in crop have shown reductions in herbicide cost of nearly 25% compared to broadcast sprayer, with no reduction in crop yield (Dammer and Wartenberg 2007). Other researchers have shown that LASC sprayers reduced herbicide use 30% to 70 % compared to broadcast applications in chemical fallow (Ahrens 1994;

Biller 1998; Blackshaw et al. 1998) and row crops (Hanks and Beck 1998). Recently, Young et al. (2008) reported similar postharvest Russian thistle control with herbicide reductions of 42% using LASC sprayers compared to broadcast sprayer, resulting in savings of $6.68 to $18.21/ha. Even with these positive results, growers have yet to fully utilize LASC applicators for effective no-tillage and conservation tillage systems due to high equipment investment and a lack of efficacy information in various cropping systems. Additionally, herbicide resistant weeds have become a serious problem in the PNW and may need greater rates of herbicides or alternative herbicides for their control. Regardless of the approach to control herbicide resistant weeds, the result will be greater cost to the growers. Ultimately, weeds that are resistant to commonly used herbicides along with the introduction of fewer herbicides of alternative modes of action may limit weed control options in the very near future. One weed that has become a problem in wheat production systems in the PNW is prickly lettuce (*Lactuca serriola* L.).

Prickly lettuce ($2n = 2x = 18$; estimated genome size ~1785Mb) is a major weed species of the genus, has wide ecological amplitude and is native to an arid summer Mediterranean climate (primarily between latitudes of 30-55° in the northern hemisphere with growing degree days more than 3000; Gallardo et al. 1996; Prince et al. 1978). Prickly lettuce occurs throughout the United States, except northern Maine and southern Florida (Uva et al. 1997, United States Department of Agriculture 1970). More recently, prickly lettuce has emerged as a problematic weed in the dry-land farming regions of the Pacific Northwest (PNW; ~3.6 million acres in Washington and Oregon alone; Schillinger and Papendick 2008). Prickly lettuce is a biennial or winter/spring annual weed with a deep tap-root system (Jackson 1995), which grows as a rosette of basal leaves during vegetative phase and produces one or more flowering stems at maturity. Prickly lettuce has vertically oriented leaves with lamina perpendicular to the east and west

intersects. The vertical orientation of leaves allows the plant to reduce water loss without substantial decrease in photosynthesis and also enhances reproduction by positively influencing floral transition and seed set (Werk and Ehleringer 1984, 1986). Prickly lettuce is a long-day plant with high reproductive capacity and can produce up to 200,000 seed per plant (Weaver and Downs 2003). The seed have parachute-like appendage (pappus), which facilitates in anemochory. Collectively, all these characteristics help prickly lettuce to endure the harsh environmental conditions and to invade new landscapes. In a recent study in Canada, prickly lettuce (at densities of ≥50 plants m^{-2}) was reported to cause severe yield losses (60% to 80%) in soybean (Weaver et al. 2006). Conversely, no such reduction in cereal or legume yield was reported in Australia, however, Amor (1986) reported unfavorable effects on grain quality and harvesting efficiency. Fall emerged prickly lettuce usually flowers at the time of crop harvest in the PNW. Consequently, prickly lettuce floral-buds are frequently harvested with cereal grains and are difficult to separate. The latex present in prickly lettuce stems cause economical losses, by clogging the harvest machinery and by raising the moisture content of the harvested grains to unacceptable levels, resulting in a price penalty.

Prickly lettuce can be controlled using various selective and non-selective herbicides. Control becomes more difficult following internode elongation (Yenish and Eaton 2002; Amor 1986). Generally, auxinic and acetolactate synthase inhibitor (ALS) herbicides provide good control of prickly lettuce. Prickly lettuce biotypes have been reported to be resistant to these herbicide groups. ALS-herbicide resistant prickly lettuce was first discovered in Idaho in 1987 (Mallory-Smith et al. 1990a; Alcocer-Ruthling et al. 1992). An inheritance study of ALS-herbicide resistant prickly lettuce indicated that resistance is due to a single nuclear gene with incomplete dominance (Mallory-Smith et al. 1990b). Auxinic herbicides (phenoxycarboxylic acid-, benzoic

acid-, pyridinecarboxylic acid-, and quinolinecarboxylic acid-type herbicides) usually control prickly lettuce by partially mimicking indole-3-acetic acid (IAA), a key natural auxin in higher plants (Sterling and Hall 1997). Generally, auxinic herbicide resistant populations are thought to require more generations to become the dominant type compared to resistant biotypes of ALS and acetyl-coenzyme A carboxylase (ACCase) inhibitor herbicides (Zheng and Hall 2001). Only 27 weed species have been reported worldwide to be resistant to auxin herbicides even though these herbicides were commercially introduced in 1947 and are widely used in cereal production and for noncrop weed control, compared to 101 ALS inhibitor and 68 photosystem II inhibitor herbicide resistant weed species (Heap 2009).

Most auxinic herbicide-resistant weeds discovered to date show cross-resistance to at least one additional auxinic herbicide. Yellow starthistle (*Centaurea solstitialis*) biotypes have been found that are resistant to 2,4-D, picloram, clorpyralid, dicamba and fluroxypyr (Callihan and Schirman 1991; Fuerst et al. 1996). Kochia (*Kochia scoparia*) biotypes have been discovered with resistance to 2,4-D and dicamba (Nandula and Manthey 2002). In another study, Walsh et al. (2004) found multiple herbicide resistance in a wild radish (*Raphanus raphanistrum*) biotype to auxinic, ALS inhibitor and phytoene desaturase inhibitor herbicides. Currently, researchers have identified and used auxinic herbicide resistant wild mustard (*Sinapis arvensis*) as a model plant to determine physiological, biochemical and molecular aspects of auxinic herbicide resistance (Zheng and Hall 2001). Inheritance studies of wild mustard have found a single dominant gene accounting for resistance to picloram and 2,4-D (Jugulam et al. 2005) and dicamba (Jasieniuk et al. 1995). Quinclorac resistance in false cleavers (*Galium spurium*) has found to be a single recessive trait. Conversely, Weinberg et al. (2006) reported MCPA resistance in hemp-nettle as quantitative trait and indicated that resistance is governed by

two or more nuclear genes with additive effects. So far, genetic studies have not agreed on a common inheritance model for auxin-herbicide resistance and different results of various inheritance studies have made it difficult to predict exact mechanisms of auxin-herbicide resistance.

Although 2,4-D, an auxinic herbicide, was the first synthetic herbicide available. The exact mechanism of auxinic herbicide action is still unknown. In order for an herbicide to be effective, it must accumulate at its site of action at a lethal concentration. The site of action of 2,4-D, is meristematic tissue and possible mechanisms of resistance include reduced absorption and translocation to the site of action or greater metabolism of 2,4-D in resistant biotypes compared to susceptible biotypes. Kohler et al. (2004) found 37% more 2,4-D absorption in susceptible ground ivy (*Glechoma hederacea*) biotypes compared to resistant biotypes. Although, percent translocation was found to be similar in both biotypes, susceptible ground ivy biotypes accumulated 42% more herbicide at apical meristem due to the greater absorption of herbicide. Weinberg et al. (2006) found no difference in absorption of MCPA, but detected a lower rate of translocation and higher rate of metabolism in resistant hemp-nettle (*Galeopsis tetrahit*) biotypes compared to susceptible biotypes. Conversely, Zawierucha and Penner (2000) found no difference between absorption, translocation and metabolism among quinclorac tolerant *Eleucine indica* and quinclorac sensitive *Digitaria sanguinalis* suggesting that quinclorac tolerance may be due to an altered target site. Also, differential absorption, translocation and metabolism was found not to be the basis for dicamba resistant kochia (Cranston et al. 2001), picloram resistant yellow star thistle (Fuerst et al. 1996), along with dicamba or picloram resistant wild mustard biotypes (Penuik et al. 1993) and quinclorac resistant false cleavers biotypes (Van Eerd et al.

2005). These studies suggest that the mechanism of auxinic herbicide resistance could be differential absorption, translocation or metabolism, or an altered target site.

Following a picloram application to wild mustard, enhanced ACC (1-aminocyclopropane-1-carboxylic acid) synthase and ethylene biosynthesis were observed in picloram susceptible biotypes with no enhancement observed in resistant biotypes (Hall et al. 1993). In plants, ACC synthase catalyzes the biosynthesis of ACC, which further gets oxidized to ethylene. The evolution of ethylene triggers a cascade of events, ultimately leading to plant death. Webb and Hall (1995) suggested that differences in dicamba sensitivity between wild mustard biotypes may be due to differential interaction of the herbicide with auxin binding proteins or other receptors. Sterling and Hall (1997) postulated that interactions among auxinic herbicides and presumed auxin receptors might induce Ca^{+} and H^{+} fluxes. Calcium is a well-accepted secondary messenger in plant cells and plays an important role in signaling during stress conditions (Pooviah and Reddy, 1993). Flash induced-light scattering signals from protoplast of picloram resistant and susceptible wild mustard biotypes showed hindrance of Mg-ATP dependent ion flux in susceptible biotypes while there is no affect on resistant biotypes (Deshpande and Hall, 1995). The ion flux in the protoplast of resistant wild mustard biotypes had changed only by the simultaneous incubation with picloram and verapamil (a calcium ion channel blocker), suggesting a positive role of calcium signaling in auxin herbicide resistance. All physiological and biochemical studies point toward multiple mechanisms of auxinic herbicide resistance.

Objectives

The main objectives of this study were to (1) investigate methods of conservation tillage in summer fallow which utilize undercutter cultivation, chemical fallow, or combinations of minimum tillage with herbicides compared to conventional dust-mulch systems, (2) evaluate weed control efficacy of LASC sprayer compared to broadcast sprayer applications in chemical fallow systems, (3) determine whether differential absorption or translocation was a mechanism of resistance in resistant and susceptible prickly lettuce biotypes, (4) determine the inheritance of 2,4-D resistance in prickly lettuce (5) develop EST-SSRs from five *Lactuca* species and, (6) study the genetic diversity among *L. serriola* accessions at phenotypic and molecular levels.

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Chapter 2

Comparison of Fallow Tillage Methods in the Intermediate Rainfall Inland Pacific Northwest Environments

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Abstract

Winter wheat is usually grown in the PNW intermediate rainfall zone (300-450 mm annual rainfall) following a year of summer fallow to recharge soil with sufficient moisture for satisfactory seed emergence. A study was conducted near Davenport, WA and Helix, OR over two separate fallow-winter wheat cycles (2007-2008 and 2008-2009) to evaluate the efficacy of reduced tillage systems compared to conventional tillage for weed control, soil profile moisture retention and seed-zone soil moisture, crop residue retention, and winter wheat yield and quality. An additional objective was to evaluate light-activated sensor-controlled (LASC) herbicide application technology for weed control efficacy and herbicide use in fallow. Treatments included conventional tillage (CTRW), undercutter sweep tillage followed by (fb) rodweeder (STRW), undercutter sweep tillage fb by broadcast sprayer (STB) or LASC sprayer (STL), and no-tillage fb broadcast sprayer (NTB) or LASC sprayer (NTL). The LASC sprayer provided similar weed control as the broadcast sprayer in no-tillage systems with 45 to 70% less herbicide use. All no-tillage and sweep undercutter fb herbicide treatments retained 7 to 38% more surface

crop residue compared to the conventional tillage system. Following summer fallow treatments but prior to planting wheat, the average soil moisture content with reduced tillage systems were similar to conventional tillage across locations. Seed-zone moisture conditions at Helix, a lower precipitation location, necessitated the use of a deep-furrow type drill. This drill type was inappropriate for no-till seeding, and affected time of planting, wheat emergence, and subsequent yield. The conventional tillage systems had similar wheat grain yields as STRW and STB but yielded 10 to 21 percentage points more compared to STL and no-tillage sytems across years and locations. Conventional tillage and STB had 3 to 5 percentage points greater thousand kernel weight (TKW) compared to NTL at Davenport across years but all treatments had similar TKW at Helix each year. Reduced tillage systems, in particular the STRW and STB can be equally productive as conventional tillage systems while reducing soil erosion and dust pollution.

Nomenclature: glyphosate; winter wheat, *Triticum aestivum* L.

Keywords: conservation tillage, conventional tillage, fallow, LASC sprayer, no tillage, reduced tillage, residue, rodweeder, soil moisture, undercutter

Abbreviations: CTRW, conventional tillage followed by rodweeder; LASC; light-activated sensor controlled sprayer; NTB; no tillage followed by broadcast sprayer; NTL, no tillage followed by LASC sprayer; STB, undercutter sweep tillagefollowed by broadcast sprayer; STL, undercutter sweep tillagefollowed by LASC; STRW, undercutter sweep tillage followed by rodweeder; UAN, urea ammonium nitrate

Introduction

The intermediate rainfall zone of the inland Pacific Northwest (PNW) comprises 620000, 323000, and 25000 hectares of dryland cropping in Washington, Oregon and Idaho, respectively (Schillinger et al. 2003). The area is typified by an average annual rainfall of 300-450 mm. Winter wheat/summer fallow or winter wheat/spring wheat/summer fallow rotations are the main cropping system of this area (Dhuyvetter et al. 1996; Juergens et al. 2004). Generally, winter wheat is grown following a year of fallow, which is necessary to recharge soil with sufficient moisture for crop growth and development (Papendick 1998). In spite of long-term efforts, researchers have not developed sustainable annual-cropping systems to replace summer fallow cropping systems in this region (Nail et al. 2005). In the absence of tillage, a major portion of soil moisture recharge from fall and winter precipitation is lost during the summer due to capillary flow/evaporation from soil or transpiration by weeds. A study in Australia by Freebairn et al. (1986) showed evaporation caused up to 65% loss of stored soil moisture during the summer fallow period without tillage. Summer fallow tillage systems have been developed to break the capillary continuity between subsoil and surface soil by creating a dust mulch barrier on the surface soil to reduce evaporation losses and conserve moisture in the seed-zone (Lyon et al. 2007). However, these tillage practices degrade soil physical properties and pose a threat of increased soil erosion from wind and water. Moreover, air-borne soil particles generated by fallow tillage and subsequent wind driven soil erosion can endanger human health (Papendick 1998, 2004) and decrease soil productivity (Tanaka 1989, Saxton 1995, Larney 1998, Sorenson 2000 and Saxton et al. 2000).

In dryland farming of the PNW, conservation tillage practices, particularly minimum tillage and delayed minimum tillage, has been shown to be profitable and environmentally friendly

(Schillinger 2001). Conservation tillage is defined as a management practice that leaves greater than 30% of the previous crop's residues on the soil surface after planting by omitting or reducing the intensity of at least one major tillage operation (Locke and Bryson 1997). Both minimum tillage and delayed minimum tillage systems use non-selective herbicides rather than secondary tillage for weed control, if feasible, and use non-inversion implements such as an undercutter cultivator to reduce the intensity of soil movement in primary spring tillage operations. Delayed minimum tillage is similar to minimum tillage but the use of undercutter cultivator as the primary spring tillage is delayed until at least mid May. The undercutter cultivator, comprised of wide (~75 cm) V-shaped blades or sweeps, is an important component of conservation tillage summer fallow systems and is usually followed by two or more rod weeding operations later in the fallow period for weed control or residue management. Undercutter cultivator operations, as part of a conservation tillage program, reduce seed-zone soil moisture loss (Griffith et al. 1986; Aase and Pikul 1995; Uri 1998) and production costs (Weersink et al., 1992) while increasing soil surface residue cover and surface roughness compared to conventional mechanical dust mulch summer fallow systems (Schillinger 2001). The greater residue cover and surface roughness in turn reduce soil erosion due to wind and water. Zaikin et al. (2007) and Nail et al. (2007) reported the undercutter cultivator or sweep tillage fallow system to be more profitable than conventional dust mulch fallow systems, providing similar wheat yields at reduced cost of production. Moreover, under current economic conditions, conservation tillage is more profitable than conventional tillage systems for winter wheat/summer fallow due to greater fuel (Nail et al. 2007; Janosky et al. 2002) and farm labor expenses with additional restrictions in the availability of farm labor (Young et al. 2008) for the conventional system.

Chemical fallow is one form of conservation tillage in which all tillage operations are omitted and the crop is direct-seeded following weed control with non-selective herbicides such as glyphosate or paraquat. Bennett and Pannell (1998) reported that the sparse, patchy nature of weed distribution often results in deposition of most of the broadcast herbicide application on bare soil rather than on weed foliage. Thus, effective spot treatments of herbicides in chemical fallow, even using greater per hectare rates, could result in substantial cost savings, reduced herbicide use and possibly improved weed control compared to broadcast applications. Efficient spot applications of herbicides to fields have not been practical due to the lack of automated equipment or the great amount of technical expertise needed by the sprayer operator. However, the introduction of real-time light-activated, sensor controlled (LASC) sprayers has resulted in more accurate and precise spot applications of herbicides (Biller 1998) and could be used in chemical fallow systems to reduce the amount and area of herbicide applications. LASC sprayers are comprised of light sensors for red and near infrared (NIR) wavelengths (Felton and McCloy 1992). At red and NIR wavelengths, soil and green plants reflect ambient light at ratios of 1.1:1.5 and 6:15, respectively. The differential reflection allows LASC sprayers to detect plants and activate a solenoid switch above a spray nozzle for a set period of time (Biller 1998). LASC sprayers used for selective postemergence weed control in crop have shown reductions in herbicide cost of nearly 25% compared to broadcast sprayer, with no reduction in crop yield (Dammer and Wartenberg 2007). Other researchers have shown that LASC sprayers reduced herbicide use 30% to 70 % compared to broadcast applications in chemical fallow (Ahrens 1994; Biller 1998; Blackshaw et al. 1998) and row crops (Hanks and Beck 1998). Recently, Young et al. (2008) reported similar post-harvest Russian thistle control with herbicide reductions of 42% using LASC sprayers compared to broadcast sprayer, resulting in savings of $6.68 to $18.21/ha.

Even with these positive results, growers have yet to fully utilize LASC applicators for effective no-tillage and conservation tillage systems due to high equipment investment costs and a lack of efficacy information in various cropping systems.

To compare summer fallow systems for the PNW intermediate rainfall area, a study was conducted with the following objectives; (1) investigate methods of conservation tillage in summer fallow which utilize undercutter cultivation, chemical fallow, or combinations of minimum tillage with herbicides compared to conventional dust-mulch systems and (2) evaluate weed control efficacy of a LASC sprayer compared to broadcast sprayer applications in chemical fallow systems.

Materials and Methods

Research was conducted for two fallow-winter wheat cropping cycles (2007-2008 and 2008-2009) at the Washington State University Wilke Research and Extension Farm, Davenport, WA (N 47˚ 39.0' W 118˚ 07.5', 756 m altitude) and on a commercial dryland wheat farm, near Helix, OR (N 45^{o} 57.3' W 118^{o} 47.4', 540 m altitude). Both Davenport and Helix locations had a cereal/summer fallow rotation in place for several years preceding the current study and had long-term annual precipitations of 445 and 415 mm, respectively. Soil types were a Broadax silt loam (fine-silty, mixed, superactive, mesic calcic Argixerollsmesic) and Walla Walla silt loam (coarse-silty, mixed, superactive, mesic Typic Haploxeroll) at the same respective locations. The soil properties across locations and years are provided in Table 1. Monthly and annual precipitation recorded at nearby weather stations over the period of the study at both locations is reported in Table 2.

Experimental Plot Establishment and Tillage Treatments. Each year, all experiment sites received a broadcast application of glyphosate at 840 g ae/ha (April and May at Helix and

Davenport, respectively) to kill weeds and volunteer wheat, which is a standard operation in the area prior to establishing summer fallow. Experiments were laid out in a randomized complete block design with four replications and six fallow treatments. The six fallow treatments included (1) undercutter sweep tillage followed by broadcast herbicide application (STB), (2) undercutter sweep tillage followed by LASC herbicide application (STL), (3) no tillage followed by broadcast herbicide application (NTB), (4) no tillage followed by LASC herbicide application (NTL), (5) undercutter sweep tillage followed by rodweeding (STRW), and (6) conventional tillage with disc or chisel followed by rodweeding (CTRW). Experimental plots were 4.5 m wide by 31.0 m long at Davenport during 2007-2008. However, due to problems in rodweeding operations, the plot width was increased to 9.0 m for 2008-2009 to better accommodate rodweeding. At Helix, experimental plots were 12.0 m wide by 99.0 m long each year. A detailed list of field operations and their timing for individual treatments is shown in Table 3. The specifications of the implements used are listed in Table 4. Plots were mechanically packed following undercutter sweep cultivation in STB and STL treatments at both locations. A roller (30 cm diameter) and a coil packer (45 cm wheel diameter) were used for soil packing at Davenport and Helix, respectively. Fertilizer was applied at 85 kg N/ha to all treatments in the manner described in Table 3.

Herbicide Treatments. In June at both locations, glyphosate at 1680 g ae/ha[i] was applied using a broadcast or LASC sprayer to the respective treatments under the conditions described in Table 3. At Davenport, the LASC sprayer system consisted of 15 individual LASC units[ii] (fitted with Teejet 6504 flat-fan nozzles), 30 cm apart and operated at a height of 60 cm, and two 11.3-L spray solution tanks mounted on 4.5 m-wide boom. Using the continuous spray or flush setting, the same sprayer and setup was used for site-specific and broadcast applications. The LASC

sprayer was calibrated to deliver 187 L ha^{-1} at 260 kPa on a broadcast basis. At Helix, the LASC sprayer system consisted 10 individual LASC units (fitted with Teejet 6504 flat-fan nozzles); spaced 30 cm apart and operated at a height of 60 cm, and six 11.3-L spray solution tanks mounted on 3 m-wide boom. The LASC sprayer at Helix was calibrated to deliver 224 L ha^{-1} at 240 kPa on a broadcast basis. At both locations, the LASC spray system was connected to a tractor-battery operated control box (set at medium sensitivity) and regulated CO_2 supply tank for system pressure. A separate conventional tractor-mounted broadcast sprayer with a 2.7 m-wide boom and XR-8002 flat fan nozzles (45 cm spacing and operated at a height of 60 cm) calibrated to deliver 150 L ha^{-1} at 260 kPa was used for broadcast applications at Helix. All pressure and sprayer volumes described above were consistent over both years within a location. Application dates and treatment conditions are listed in Table 1. For LASC sprayer application in STL and NTL treatments, 5.68 and 3 liters spray solution were mixed separately for individual plots at Davenport and Helix, respectively. The amount of spray solution remaining following the application to an individual plot was measured and subtracted from the original mix amount to determine the spray volume and amount of herbicide applied to the respective plot. The data from four replications was averaged to calculate actual spray volume and herbicide used by LASC sprayer in each treatment. The process of calibration is critical to LASC sprayer operation and herbicide use. The LASC sprayer will not detect and spray over weeds if calibrated over green plants. Moreover, the LASC sprayer can false spray over residue or soil if control box is set at high sensitivity. Therefore, prior to spraying individual plots, the LASC sprayer control box was set at medium sensitivity and sensors were calibrated for the background base of soil and crop residue to reduce application errors. The glyphosate at 1680 g/ha was applied to STB and NTB plots by broadcast applications. The actual amounts of herbicide used in the STL and

NTL were used for comparison between these and the broadcast treatments. At Davenport, weed densities were recorded by species from four random 0.25 m^2 quadrates per plot in each treatment prior to herbicide application or rodweeding. Additionally, weed density and biomass were recorded from random 5 and 1-m^2 areas per plot in July and August at Helix and Davenport, respectively, each year to determine treatment efficacy prior to the preplant herbicide application or tillage.

Soil Sampling. Three soil cores for 150 cm deep soil profile and one core for seed-zone soil profile were collected from random locations in each plot to determine soil moisture content before winter wheat planting. LASC and broadcast herbicide applications were assumed to be the same. Consequently, soil moisture samples were only collected from no-tillage and sweep undercutter treatments that had received broadcast herbicide applications during the fallow rotation. For soil moisture in 150 cm deep soil profile, individual cores were separated into 5 30 cm sections to a depth of 150 cm. At Davenport in 2007, soil cores were taken to depths of 120 cm due to problems getting a consistent sample of deeper soil. The sampling tube used at both locations had 5 and 4.7 cm outer diameter (o.d.) and inner diameter (i.d.), respectively, and 4.0 cm diam cutting edge. Additionally, one core per treatment was taken to a depth of 30 cm at random locations in each plot to determine seed-zone soil moisture. The soil sampling probe for seed-zone soil moisture had 5.1 and 4.6 cm o.d. and i.d., respectively, along with 4.5 cm i.d cutting edge. At Davenport in 2008 and Helix in both years, cores were divided into increments of 0 to 3, 3 to 5, 5 to 7, 7 to 9, 9 to 11, 11 to 13, 13 to15, 15 to 20, 20 to 25, and 25 to 30 cm depth increments using a small-increment electric soil sampler (Wuest and Schillinger 2008) for a total of 10 sampling increments per plot. At Davenport during 2007, a sampling probe with 1.9 cm i.d was used to take three random 30-cm deep seed-zone soil cores per plot. These cores

were divided into 0-5, 5-10, 10-15, 15-20, 20-25, and 25-30 cm depth increments. The fresh weight of soil at each increment was recorded individually for each of the three cores taken per plot. Soil was dried at 105 C for 48 hours to determine dry weight of soil increments. Percent soil moisture on gravimetric basis (% soil moisture) was determined using equation 1.

$$\% \text{ SM} = (\text{fwt} - \text{dwt})/\text{dwt} \times 100 \quad [1]$$

Where, % SM is percent gravimetric soil moisture on dry wt. basis, fwt is fresh weight of soil and dwt is dry weight of soil. For statistical analysis, the soil moisture contents of the three cores taken from same plot were averaged for individual increments.

Winter Wheat Planting and Emergence, and Previous Crop Residue Cover. Following soil moisture sampling, winter wheat was sown in September in each year and location, with a few exceptions. In 2007 at Helix, several plots that received the no-till fallow treatment had to be reseeded due to poor initial wheat plant emergence. In addition, because of dry soil conditions in the fall of 2008, winter wheat sowing in no-tillage treatments at Helix was delayed until November when seed-zone moisture was adequate for germination. At Davenport, 'ORCF 102' soft white winter wheat was planted at 80 kg/ha to a depth of 5 cm. Row spacing was 25 cm. At Helix, a mixture of 'Boundary' and 'Elious' hard red winter wheat was planted at 100 kg/ha to a depth of 5 cm each year (when measured after soil displacement from the deep-furrow planter units). At Helix, conventional and undercutter tillage treatments were seeded in 40 cm row spacings with a deep-furrow type drill, and no-tillage treatments (NTL and NTB) had row spacings of 25 cm. and were seeded with a hoe-type, no-till drill. Crop emergence was measured in October or November of each year at both locations by counting the number of plants within five random one-meter row lengths in each plot. The emergence count was converted from plants/ meter row length to plants/m^2 based on the row spacing of individual treatments. Percent

crop residue cover was measured in November or December of each year following wheat emergence. At Davenport, the residue cover measurement for the 2007-2008 fallow-winter wheat cycle was delayed until April 8, 2008 because of prolonged snow cover on the study site. Digital photographs of three random 0.25 m^2 quadrates per plot were taken from a constant height of 1 meter. The area in each photograph within the quadrate was superimposed onto a 100-point grid and percent crop residue was calculated by counting the number of grid points that intersected crop residue in the photograph.

Weed Control in Winter Wheat. Weed species composition and density were determined in the winter wheat crop in May of 2008 and 2009 by counting the number of weeds of each species within four random 0.25 quadrates per plot. At Davenport, weeds were completely controlled following these counts with imazamox at 35 g ae/ha mixed with pyrasulfotole plus bromoxynil at 310 + 250 g ai/ha, UAN (2.5% v/v) and non-ionic surfactant (NIS, 0.25%, v/v). At Helix, broadleaf and grass weed control in winter wheat was attained with a mixture of metsulfuron plus 2,4-D at 4.2 + 380 g ae/ha and a mixture of propoxycarbazone plus mesosulfuron 17 + 110 g ai/ha, respectively. NIS (0.25% v/v) was added to all herbicide solutions used for weed control in winter wheat at Helix.

Winter Wheat Yield and Quality Parameters. Wheat grain yield (kg/ha) was determined by harvesting two 1.5-m swathes across the entire length of each plot at Davenport using a small plot harvester and harvesting entire plots at Helix using a commercial-size combine. At Davenport, the harvested grain from each plot was collected in bags labeled for respective plot, cleaned with a Clipper mill and weighed to determine yield (kg/ha). At Helix, wheat yield (kg/ha) of individual plots was calculated by combine harvesting all grain in each plot, and transferring to a stationary wagon equipped for weighing. Subsamples of wheat grain were used

to determine test weight (kg/hL), thousand-kernel weight (TKW) and percent protein content for both locations. The protein content was determined by the AACC International (2000) approved near-infrared spectroscopy (NIRS) method 39-11 (Davies and Berzonsky 2003).

Statistical Analyses. For statistical analysis, data were subjected to ANOVA using PROC MIXED in SAS[iii] (SAS Institute 2005) and sum of squares were partitioned to evaluate the effect of treatment, year and location on weed density, weed biomass, percent gravimetric soil moisture, winter wheat emergence, crop residue, grain yield, test weight, TKW, grain protein and moisture content. Prior to analysis, data were tested for normality and homogenous variance using PROC UNIVARIATE. Year, location, and replication were considered random variables, and main effects and interactions were tested based on the randomized complete block design used in the study (McIntosh 1983). Mean separations were performed on data using Fisher's protected least significant difference (LSD) test at p-values ≤ 0.05. Data showed significant treatment by year and treatment by location interactions, thus data is presented by years and locations.

Results and Discussion

Weed Density and Biomass. During the summer fallow period, different weed species emerged at Davenport and Helix (Table 1) and interactions existed among years and treatments for weed density and biomass. Therefore data are presented separately for years and locations. In all locations and years, CTRW and STRW systems were kept weed-free with timely rodweedings after each new flush of weeds and weeds were completely controlled at the end of the fallow period (Tables 5 and 6). At Davenport in 2007, tumble pigweed densities were similar in all non-rodweeded systems (Table 5) but STL had greater biomass compared to NTB (Table 6).

Similarly, the greatest tumble pigweed densities and biomass were observed in STB and STL, respectively, at Davenport in 2008 (Tables 5 and 6). In contrast to tumble pigweed control, the LASC sprayer provided better control of wild oat and downy brome in undercutter systems compared to no-tillage systems at Davenport in 2007 (Tables 5 and 6), which was likely due to the failure of the LASC sprayer to detect very small weeds that were fully or partially obscured under heavy surface crop residue of no-tillage systems (Figure 1A). All treatments controlled grass weeds similarly at Davenport in 2008 (data not shown) because of no difference in residue cover among no-tillage and undercutter systems. Similar results were observed at Helix, where Russian thistle densities were greater in the STL systems compared to other systems during each year (Table 5).

Airborne dust churned up by the LASC and broadcast spray tractors resulted in reduced control and increased densities of tumble pigweed and Russian thistle in the tire tracks compared to elsewhere at both locations (authors' personal observation). Other studies (Mathiassen and Kudsk 1999; Zhou et al. 2006) have reported reduced efficacy of glyphosate in dry and dusty conditions. Young et al. 2008 and Blackshaw et al. 1998 also expressed concerns about reduced weed control efficacy of the LASC sprayer in dusty conditions. The reduced detection of very small weeds by LASC sprayer in dusty conditions is probably due to the dust accumulation on the light sensors and/or dispersion of light due to airborne dust particles. Moreover, sweep tillage resulted in a visible increase in tumble mustard and Russian thistle density compared to no-till, due to probable, but unexplained, seedbed micro-environment difference between fallow tillage systems (authors' personal observation). Therefore, each year at Davenport and Helix, the reduced tumble pigweed and Russian thistle control, respectively, in STL compared to no-tillage systems was due to the greater weed densities before spray in undercutter systems, reduced

tumble pigweed control by glyphosate in dry and dusty conditions and reduced detection of very small weeds by LASC sprayers under dry dusty conditions (Mathiassen and Kudsk 1999; Young et al. 2008; Zhou et al. 2006).

At Helix, LASC and broadcast application treatments had similar prickly lettuce densities in all treatments during 2007 (data not shown). However, greater prickly lettuce emergence in no-tillage systems compared to sweep undercutter (authors' personal observation) and reduced ability for the LASC sprayer to detect very small weeds during dry and dusty conditions resulted in greater prickly lettuce densities in NTL than NTB systems at Helix during 2008 (Table 5). At Helix, weed biomass was not recorded in 2007 and was recorded as total of all species in 2008 (Table 6). Total weed biomass at Helix in 2008 was greater in STL system compared to other systems because of greater Russian thistle densities. The broadcast and LASC systems had similar total weed biomass under no-tillage systems at Helix in 2008 (Table 6). Weeds in the standing winter wheat were equally controlled in all the systems across years and locations and therefore data are not presented.

Herbicide Use: LASC applications used 45 and 55% less glyphosate than broadcast applications at Davenport in year 1 and year 2, respectively, and 60 and 72% less at Helix in the same respective years. Previous research has shown similar herbicide use reductions with LASC sprayer technology compared to broadcast herbicide applications (Ahrens, 1994; Biller, 1998; Blackshaw et al. 1998, Young et al. 2009). With the 45 to 70 % reduction in herbicide loading per unit area by LASC sprayers as measured in this and other studies, increasing herbicide rates applied through the LASC sprayers is a possible method to improve the efficiency of LASC sprayers in dusty and dry conditions. For the more effective weed control, LASC sprayers can

be used in future to apply restricted use chemicals at higher rate because of less herbicide loading per unit area.

Crop Residue. Percent crop residue cover in summer fallow systems ranged from 67 to 93 % and 30 to 53% at Davenport and Helix, respectively, during 2007-2008 and 2008-2009 fallow-winter wheat cycles (Figure 1 A-B). At Davenport, there was substantial residue remaining from the previous spring wheat and spring barley crops during 2007-2008 and 2008-2009 cropping years, respectively. At Davenport in 2007, crop residue cover was recorded after wheat planting and conventional tillage had residue cover similar to undercutter systems and NTB due to residue drag by seed drill (Figure 1A). However in 2008 at Davenport conventional tillage had lower residue compared to all other systems. In general, conventional tillage had less crop residue cover compared to no-tillage and undercutter systems after fallow period in both years at Davenport. The previous crop residue in conventional tillage systems was finely divided by the disk/chisel and more rapidly degraded compared to the intact above ground residue remaining following undercutter sweep and no-tillage systems (authors' personal observation). Tanaka (1986) also reported more rapid degradation of winter wheat and spring wheat surface residue in conventional tillage compared to chemical fallow. In both years, there was less soil residue cover at Helix partly due to wider row spacing in the previous cereal crop and lower crop productivity (because of less clay and organic matter content; Table 1) compared to Davenport, although the residue was from winter wheat with greater biomass production potential (Figure 1 A-B). No-tillage systems had greater residue cover compared to conventional and undercutter sweep systems at Helix in 2007-2008 (Figure 1B). Undercutter sweep tillage system followed by rodweeding retained less residue cover compared to STL but all other systems were similar during 2008-2009 at Helix. Overall, conventional tillage resulted in reduced residue cover

compared to sweep undercutter and no-tillage systems at all locations and years. Greater crop residue coverage in conservation tillage systems can help reduce soil water evaporation (Schillinger and Bolton 1993), increase soil water storage and infiltration (Hatfield et al. 2001) and reduce soil erosion (Ramig and Ekin 1987).

Soil Moisture. The percent gravimetric soil moisture content in 30 cm (seed-zone) and 150 cm soil profiles varied across locations, so data is presented as years and locations because of significant soil moisture by year and/or location interactions. At Davenport in 2007, there were no significant differences in fallow soil moisture among systems down to depths of 90 cm (Figure 2A). However, NTB had 23 percentage points less moisture than STB in the 90 to 120 cm soil profile. During 2008 at Davenport, there were no significant differences in soil moisture among systems at any depth (Figure 2B). Rainfall in the summer of 2008 (May to August) was 30 percentage points lower than 2007 (Table 2). Therefore, soil moisture between fallow systems in the deeper profiles did not differ in 2008 (Figure 2B). The difference in seed-zone soil moisture among tillage systems at Davenport existed only for the top 5 cm soil profile in each year (Figures 3A and B). Rainfall two weeks prior to sampling confounded the results in 2007, where top 5cm soil profile of CTRW showed greater moisture compared to STB. However, because of the loosening of the top soil by the rodweeder, CTRW and STRW contained less moisture in upper 3 cm soil compared to STB and NTB during 2008. Never-the-less, seed-zone moisture at depth of planting (around 9-11cm) was similar in all systems at Davenport in 2007 and 2008.

At Helix, a lower-rainfall site compared to Davenport, both 150 cm and seed-zone soil profiles had less moisture compared to Davenport (Figures 2 and 3). At Helix in 2007, the top 60 cm soil profile had very low moisture in all the systems due to extremely dry conditions at the

time of wheat planting (Figure 2C). The seed-zone moisture at Helix in 2007 was less than needed for optimum wheat seed germination, therefore, a deep-furrow type of drill was needed to place seed into moisture in conventional and sweep tillage systems. The deep-furrow drill used in this study (JD HZ) does not work well in high residue seedbeds such as found in no-till. Therefore, no-till plots were seeded using an appropriate no-till drill, and resulted in poor initial crop emergence in 2007 (data not shown). This poor initial crop emergence in 2007 no-till systems necessitated reseeding at a later date in the NTB and NTL systems. At Helix in 2008, the total annual precipitation was even lower than 2007 (Table 2), thus both 150 cm soil profile and seed-zone soil profile were very dry. In 2008 at Helix, no-tillage had less moisture content in top 30 cm soil profile compared to STB systems (Figure 2D). Schillinger and Bolton (1993) also found that no-tillage systems lose more moisture in the top layer of soil due to upward capillary flow of water. Because of the very low seed-zone moisture at Helix in 2008, the deep-furrow drill was again used in conventional and sweep tillage plots in September, but the no-till plots were not seeded until November since seed could not be placed into moisture with the no-till drill until that time.

Wheat Emergence, Yield and Quality. Wheat seedling establishment was similar for all the systems at Davenport in both years (Table 7) due to similar seed-zone soil moisture in all systems. At Helix in the 2007-2008 crop year, NTB had greater wheat stand counts than CTRW and STRW (Table 8). In 2007 at Helix, due to poor initial wheat plant emergence (data not shown), the no-till plots were reseeded after rainfall with no-till drill at narrow row spacing thus resulted in higher total stand counts (Table 8). Similarly, during the 2008-2009 fallow-winter wheat cycle at Helix, no-tillage systems had greater wheat stand counts compared to other systems, but this was due to a later seeding date with different planting equipment, with a higher

seeding rate. At Helix in 2008, dry soil moisture conditions in 3 to 7 cm seed-zone soil profile resulted in non-significant less emergence of winter wheat in STB and STL systems (Figure 3 and Table 8). Collectively, seed-zone soil moisture governed appropriate seeding time and subsequent wheat emergence at both locations. Systems that could be seeded into moisture resulted in better wheat stand counts.

Differential wheat crop emergence and slight differences in soil moisture content among systems were only partly reflected in the wheat grain yield in either year of this study (Tables 7 and 8). Wheat grain yields were similar in all systems at Davenport in the 2007-2008 crop year, but rodweeded systems tended to have numerically higher yields compared to other systems (Table 7). In general, wheat grain yields in all the systems at Davenport were lower in 2007-2008 compared to 2008-2009 crop cycle due to very cold winters. During 2008-2009 at Davenport, winter wheat grain yield in conventional tillage was significantly greater than no-tillage and STL systems (Table 7). Rasmussen et al. (1997) reported heavy crop residue, low soil temperatures and high pathogen activity as one reason for low winter wheat yield in no-tillage systems. Lower yield in STL systems were due to reduced tumble pigweed control. Winter wheat yield with conventional tillage system was similar to STRW and STB systems that had greater soil residue cover than conventional tillage. The higher yield in CTRW, STRW and STB systems compared to no tillage and STL system might be due to better weed control during the fallow period or high soil temperatures at the time of seedling establishment, or both (Camara et al. 2003; Rasmussen et al. 1997).

Similary, at Helix, STRW produced greater wheat yield than STL during 2007-2008 (Table 8). However, all other systems were similar in that year. The reduced yield of STL systems was possibly due to unreliable Russian thistle control during the fallow period, which reduced soil

moisture available for the cropping year. During the 2008-2009 fallow-winter wheat cycle at Helix, sweep undercutter and conventional tillage systems provided similar wheat grain yield. Nevertheless, CTRW and STB had greater yields than NTL. Reduced yields of no-tillage systems were due to later planting of wheat.

The STRW system had 1.8 and 0.6 percentage points greater test weight compared to NTL system at Davenport during 2007-2008 and 2008-2009, respectively, but all other systems had similar test weight. At Helix, there was no difference in test weight among systems during 2007-2008 but STRW system had 0.8 to 1.5 percentage points lower test weight compared to STB, NTB and NTL systems. CTRW and STB had 6 and 4 percentage points, respectively, greater thousand-kernel weight (TKW) compared to no-tillage systems in 2007-2008 and 2008-2009, respectively. However, the TKW was similar among all systems at Helix each year. At Davenport, grain protein content was similar among all systems for 2007-2008. The months of May and July were very dry during 2008 at Davenport. No-tillage systems tend to lose more moisture due to upward capillary flow during dry summers (Schillinger and Bolton 1993). Moreover, the 150 cm soil profile showed less soil moisture in the deeper soil layers of no-tillage systems compared to all other systems, ultimately leaving less moisture for upward flow during dry summer period. Drought or temperature stress tends to increase protein content of grains (Gooding et al. 2003). Greater stress to wheat plants because of drought stress, possibly increased protein content in no-tillage systems compared to undercutter systems during 2008-2009 (Table 8). Similarly, protein content was similar among all systems at Helix during 2007-2008 but drought stress increased protein content in no-tillage systems compared to conventional tillage systems. Stress increased protein content in no-tillage systems but the increase was not sufficient to cover yield loss.

In summary, tillage systems affected the weed species make up during fallow period. The densities of Russian thistle and tumble pigweed were greater with undercutter systems. Prickly lettuce density was greater in no-tillage systems compared to other systems each year. Therefore, control of these common weeds could be planned in advance based on the summer-fallow system used. After the fallow period, soil moisture did not vary greatly between systems across years and locations. Moreover, all reduced tillage systems retained greater surface crop residue compared to conventional tillage. Sweep undercutter tillage systems including STRW and STB resulted in similar wheat grain yield and quality as conventional tillage. However, winter wheat yield decreased in no-tillage systems compared to conventional tillage. Although, the yield of sweep undercutter systems was similar to conventional tillage, sweep undercutter followed by rodweeding or herbicide applications can provide a sustainable and environment friendly cropping system. This can be achieved by reducing production cost due to less tillage, decreased soil erosion, and soil moisture losses due to greater surface residue cover and decreased dust pollution due to greater soil surface roughness compared to conventional tillage. In the present study, LASC herbicide applications used 45-72% less herbicide than the broadcast sprayer and provided equally effective weed control as broadcast sprayer in chemical fallow systems. However, more research is required to evaluate the efficacy of the LASC sprayer in dry and dusty fallow systems with higher rates of herbicides. Moreover, the failure to detect very small weeds by LASC sprayer is a problem. In future, LASC sprayers with improved efficacy due to greater herbicide rates and proper timing of application can provide a useful tool for conservation tillage fallow systems of the PNW.

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Sources of Materials

[i] Roundup Original Max®. Monsanto Company, 800 N. Lindbergh Blvd.St. Louis, MO 63167

[ii] Weed Seeker™ NTech Industries, INC., 740 South State Street, Ukiah, CA 95482.

[iii] SAS software, version 9.1. SAS Institute Inc., Box 8000, SAS Circle, Cary, NC 27513

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Table 1. Soil properties, application dates and conditions for herbicide treatments during 2007 and 2008 fallow period at Davenport, WA and Helix, OR.

		Davenport, WA		Helix, OR	
		2007	2008	2007	2008
Soil properties	pH	5.2	5.0	5.7	6.3
	Organic matter (%)	3.0	2.9	1.3	1.4
	CEC (cmol kg^{-1})	21.0	19.0	13.3	12.7
Soil texture	Sand (%)	30.0	28.8	31.9	29.3
	Silt (%)	58.8	61.2	62.1	60.9
	Clay (%)	11.2	10.0	6.0	9.8
Crop stubble		Spring wheat	Spring barley	Winter wheat	Winter wheat
LASC/Broadcast application dates		3-Jul-07	9-Jul-08	28-Jun-07	2-Jul-08
Application conditions during LASC/Broadcast herbicide treatments	Air temperatures (°C)	31	29	NA	27
	Relative humidity (%)	21	24	NA	46
	Wind (km h^{-1})	6	1.6	NA	3
	Cloud cover (%)	10	5	NA	30
	Soil temperature (°C at 10 cm depth)	22	20	NA	21
Weed height at the time of treatment	Tumble pigweed	2-12 cm	10-15 cm	NP	NP
	Prickly lettuce	NP	NP	10-30 cm	10-50 cm
	Russian thistle	12-25 cm	NP	10-25 cm	7-30 cm

(NA) Data not available; (NP) weed species not present

Table 2. Monthly and annual precipitation during study period at Davenport, WA and Helix, OR.

	Davenport, WA			Helix, OR		
Month	2006-07	2007-08	2008-09	2006-07	2007-08	2008-09
	Precipitation (mm)					
Sep-Feb	252.1	235.5	113.5	185	133.9	107.7
Mar	23	35.3	33.3	25.2	20.7	71.1
Apr	18.9	23.7	17.8	16.8	6.7	20.7
May	33.1	17	29.2	7.2	36.1	22
Jun	17.7	25.9	26.7	22	13.3	7.2
Jul	6.1	0	10.4	3.2	0	0
Aug	18.1	9.4	18.3	6.2	6.9	18.5
Annual	369	346.9	249.2	265.5	217.6	247.2

Table 3. Timeline of field operations and applications of individual treatments for each fallow-winter wheat crop cycle at Davenport, WA and Helix, OR[a].

Month	Treatment					
	STB	**STL**	**NTB**	**NTL**	**STRW**	**CTRW**
Apr	----------Broadcast application of glyphosate at 840 g ae/ha to kill weeds and volunteer wheat----------					
May	Primary tillage with sweep undercutter at 30 cm depth + fertilization with injection of UAN solution at 85 kg N/ha + roller packer at Davenport and coil packer at Helix				Primary tillage with sweep undercutter at 30 cm depth+ fertilization with injection of UAN (32%) at 85 kg N/ha	Primary tillage with tandem disk (two passes) at 15 cm depth, followed by fertilization with broadcast application of urea at 85 kg N/ha. At Davenport in 2007-08, this treatment was fall chisel plowed to a depth of 20 cm with single disking in the spring.
Jun	Application of glyphosate at 1680 g ae/ha with LASC or broadcast sprayer as appropriate (see Table 2)				First rod weeding at 10 cm depth. Not required In 2008 at Davenport, so first rodweeding was delayed till July.	
Jul	Follow-up rod weeding at 10 cm depth					
Aug	Recorded weed density and biomass					
	Applied glyphosate at 1680 g ae/ha if necessary prior to winter wheat planting				Preplant rod weeding at 10 cm depth	
	Wheat harvesting for the crop following fallow treatments.					
Sep[b]	Soil moisture sampling; seed-zone and 1.5 m deep soil profile					
	Winter wheat seeding + fertilization in NTB and NTL with seed planter (urea at 85 kg N/ha)					
Oct	Winter wheat emergence measured					
Nov	Soil surface residue cover measurement					

(a) Abbreviations: CTRW, conventional tillage; LASC; light-activated sensor controlled sprayer; NTB; no tillage followed by broadcast sprayer; NTL, no tillage followed by LASC sprayer; STB, undercutter followed by broadcast sprayer; STL, undercutter followed by LASC; STRW, undercutter followed by rodweeder; UAN, urea ammonium nitrate

(b) Winter wheat in no-tillage (NTB, NTL) treatments at Helix was planted in November during 2008.

Table 4. Equipment used in studies at Davenport, WA and Helix, OR.

Equipment	Location	
	Davenport, WA	Helix, OR
Sweep undercutter	Haybuster with overlapping 80 cm wide V-shape blades	Sunflower 3672 with 66 cm wide V-shape blades
Rodweeder	Calkins centre drive (4.5 m wide)	Calkins trashmaster
Additioaln tillage tools	Tandem disk cultivator (60 cm diamter)	Calkins cultivator used for applying fertilizer in tilled plots
LASC sprayer	15 WeedSeeker™ (model PhD 600) units mounted on 4.5 m wide boom at spacing of 30 cm and height 60 cm.	10 WeedSeeker™ (model PhD 600) units mounted on 3 m wide boom at spacing of 30 cm and height 60 cm.
Broadcast sprayer	WeedSeeker™ sprayer assembley with flush setting	Conventional broadcast sprayer with XR-8002 flat fan nozzles mounted on 2.7 m wide boom at 45 cm spacing
Planter	Direct seed plot drill (180 cm drill width with 7 openers)	Great Plains 4010 for planting in no-tillage treatments and JD HZ616 for planting in sweep undercutter and conventional tillage treatments
Soil Packer	Cultipacker roller packer (30 cm diameter)	Flexi-coil spiral packer (45 cm wheel diameter)
Combine	KINCAID small plot combine	JD 9770

Table 5. Weed density after herbicide or rodweeding applications in different tillage treatments at Davenport, WA and Helix, OR during fallow period of years 2007-08 and 2008-2009[a].

	Davenport, WA			Helix, OR		
	2007		2008	2007	2008	
Trt	AMAAL	AVEFA+ BROTE	AMAAL	SASKR	LACSE	SASKR
	plants/m^2					
CTRW	0.00	0.00	0.00	0.00	0.00	0.00
STRW	0.00	0.00	0.00	0.02	0.00	0.00
STB	2.01	0.36	0.12	0.09	0.02	0.10
STL	1.80	0.11	0.09	2.50	0.27	1.20
NTB	1.84	0.36	0.04	0.05	0.73	0.02
NTL	1.32	0.53	0.04	0.50	1.45	0.32
LSD[b] (0.05)	1.59	0.34	0.05	0.65	0.41	0.69

(a) Abbreviations: AMAAL, tumble pigweed; AVEFA, wild oat; BROTE, downy brome; CTRW, conventional tillage; LASC; light-activated sensor controlled sprayer; LACSE, prickly lettuce; SASKR, Russian thistle; NTB; no tillage followed by broadcast sprayer; NTL, no tillage followed by LASC sprayer; STB, undercutter followed by broadcast sprayer; STL, undercutter followed by LASC; STRW, undercutter followed by rodweeder

(b) LSD is Fisher's protected least significant difference among treatments at $P \leq 0.05$

Table 6. Weed dry biomass in different tillage treatments after herbicide or rodweeding treatments at Davenport, WA and Helix, OR during fallow period of 2007-08 and 2008-09[a].

	Davenport, WA			Helix, OR
	15-Aug-07		19-Aug-08	17-Jul-08
Trt	AMAAL	AVEFA+BROTE	AMAAL	Total Biomass[c]
	g/m^2			
CTRW	0.00	0.00	0.00	0.00
STRW	0.00	0.00	0.00	0.00
STB	11.52	0.54	0.41	8.28
STL	17.67	0.14	1.06	37.68
NTB	5.87	0.32	0.12	4.48
NTL	10.92	2.66	0.24	9.37
LSD[b] (0.05)	10.21	1.72	0.65	23.12

(a) Abbreviations: AMAAL, tumble pigweed; AVEFA, wild oat; BROTE, downy brome; CTRW, conventional tillage; LASC; light-activated sensor controlled sprayer; NTB; no tillage followed by broadcast sprayer; NTL, no tillage followed by LASC sprayer; STB, undercutter followed by broadcast sprayer; STL, undercutter followed by LASC; STRW, undercutter followed by rodweeder

(b) LSD is Fisher's protected least significant difference among treatments at $P \leq 0.05$.

(c) Weeds in total biomass estimates at Helix included Russian thistle and prickly lettuce.

Table 7. Wheat crop emergence, yield, test weight, thousand kernal weight (TKW) and protein content in different tillage treatments at Davenport, WA during 2007-08 and 2008-09[a].

Trt	Wheat Emergence		Yield		Test Weight		TKW		Protein Content	
	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09
	plants/m^2		kg/ha		kg/hL		g		%	
CTRW	170	157	2800	5750	72.66	74.90	38.89	40.65	11.93	11.60
STRW	158	156	2900	5480	72.95	75.30	37.76	40.45	11.80	11.00
STB	164	168	2470	5220	71.91	75.08	38.76	41.25	12.35	10.98
STL	153	158	2260	5140	71.72	75.10	37.00	39.93	12.45	11.53
NTB	175	146	2520	5070	72.31	75.13	36.59	40.10	12.73	11.70
NTL	154	152	2290	5090	71.61	74.85	36.58	39.78	12.43	11.30
LSD[b] (0.05)	NS	NS	NS	550	1.24	0.42	2.31	1.46	NS	0.66

(a) Abbreviations: CTRW, conventional tillage; LASC; light-activated sensor controlled sprayer; NTB; no tillage followed by broadcast sprayer; NTL, no tillage followed by LASC sprayer; STB, undercutter followed by broadcast sprayer; STL, undercutter followed by LASC; STRW, undercutter followed by rodweeder

(b) LSD is Fisher's protected least significant difference among treatments at $P \leq 0.05$.

Table 8. Wheat crop emergence, yield, test weight, thousand kernal weight (TKW) and protein content in different tillage treatments at Helix, OR during 2007-08 and 2008-09[a].

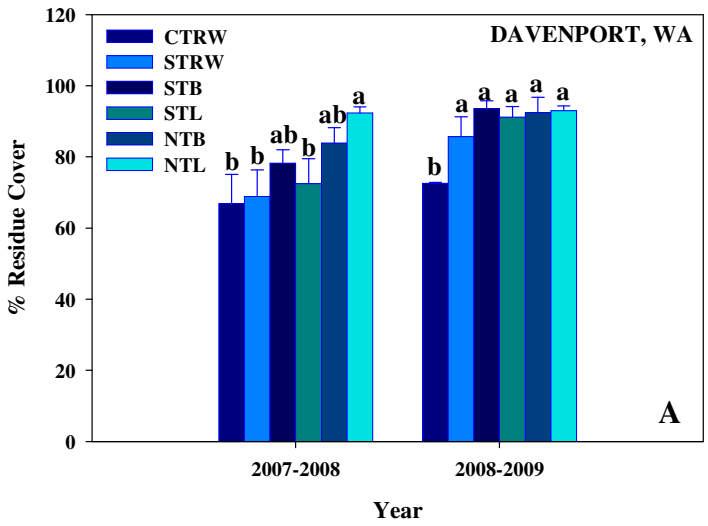
Trt	Wheat Emergence		Yield		Test Weight		TKW		Protein Content	
	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09
	plants/m2		kg/ha		kg/hL		g		%	
CTRW	86	70	3950	3390	75.63	75.87	37.85	34.23	12.68	10.95
STRW	87	74	4000	3070	75.63	75.55	38.31	34.53	12.58	11.75
STB	95	46	3830	3440	75.63	76.70	38.50	35.63	12.60	11.78
STL	109	61	3380	2820	74.38	76.33	38.31	35.53	12.78	11.90
NTB [c]	177	166	3630	2810	75.94	76.12	38.44	34.23	12.73	11.45
NTL [c]	170	152	3550	2650	75.00	76.40	37.50	34.53	12.70	11.53
LSD [b] (0.05)	29	33	580	650	NS	0.84	NS	NS	NS	0.52

(a) Abbreviations: CTRW, conventional tillage; LASC; light-activated sensor controlled sprayer; NTB; no tillage followed by broadcast sprayer; NTL, no tillage followed by LASC sprayer; STB, undercutter followed by broadcast sprayer; STL, undercutter followed by LASC; STRW, undercutter followed by rodweeder

(b) LSD is Fisher's protected least significant difference among treatments at $P \leq 0.05$.

(c) NTB and NTL treatments required reseeding (2007-2008) or were seeded at a later date (2008-2009) due to dry seed-zone moisture conditions. Therefore emergence counts were made after these operations.

Figure 1 A-B. Mean percent crop residue (with standard error bars) in different tillage treatments during two winter wheat - fallow cycles at, Davenport, WA and Helix, OR. Within years, each treatment followed by different letter is significantly different at P< 0.05. Abbreviations: CTRW, conventional tillage; LASC; light-activated sensor controlled sprayer; NTB; no tillage followed by broadcast sprayer; NTL, no tillage followed by LASC sprayer; STB, undercutter followed by broadcast sprayer; STL, undercutter followed by LASC; STRW, undercutter followed by rodweeder



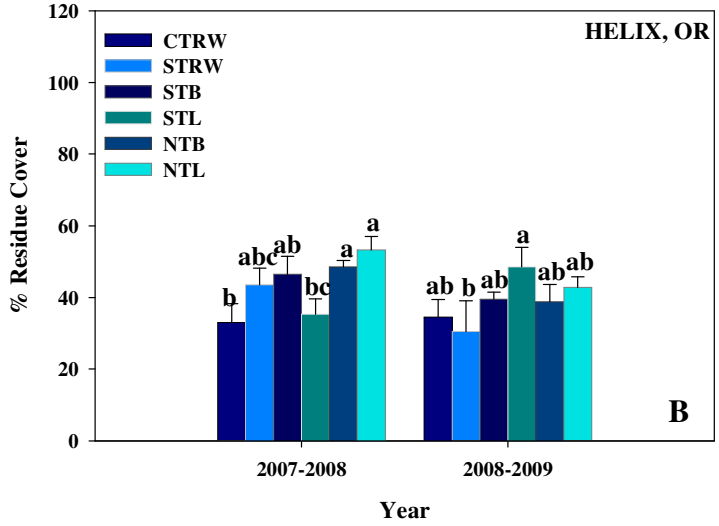


Figure 2 (A-D). Gravimetric soil moisture (% dry wt. basis) content in 150 cm deep soil profile at (A) Davenport, WA-2007 (B) Davenport, WA-2008 (C) Helix, OR -2007 and (D) Helix, OR -2008 fallow periods. '*' respresents significant difference ($P<0.05$) between one or more treatments at respective depths. Abbreviations: CTRW, conventional tillage; NTB, no tillage followed by broadcast sprayer; STB, undercutter followed by broadcast sprayer; STRW, undercutter followed by rodweeder.

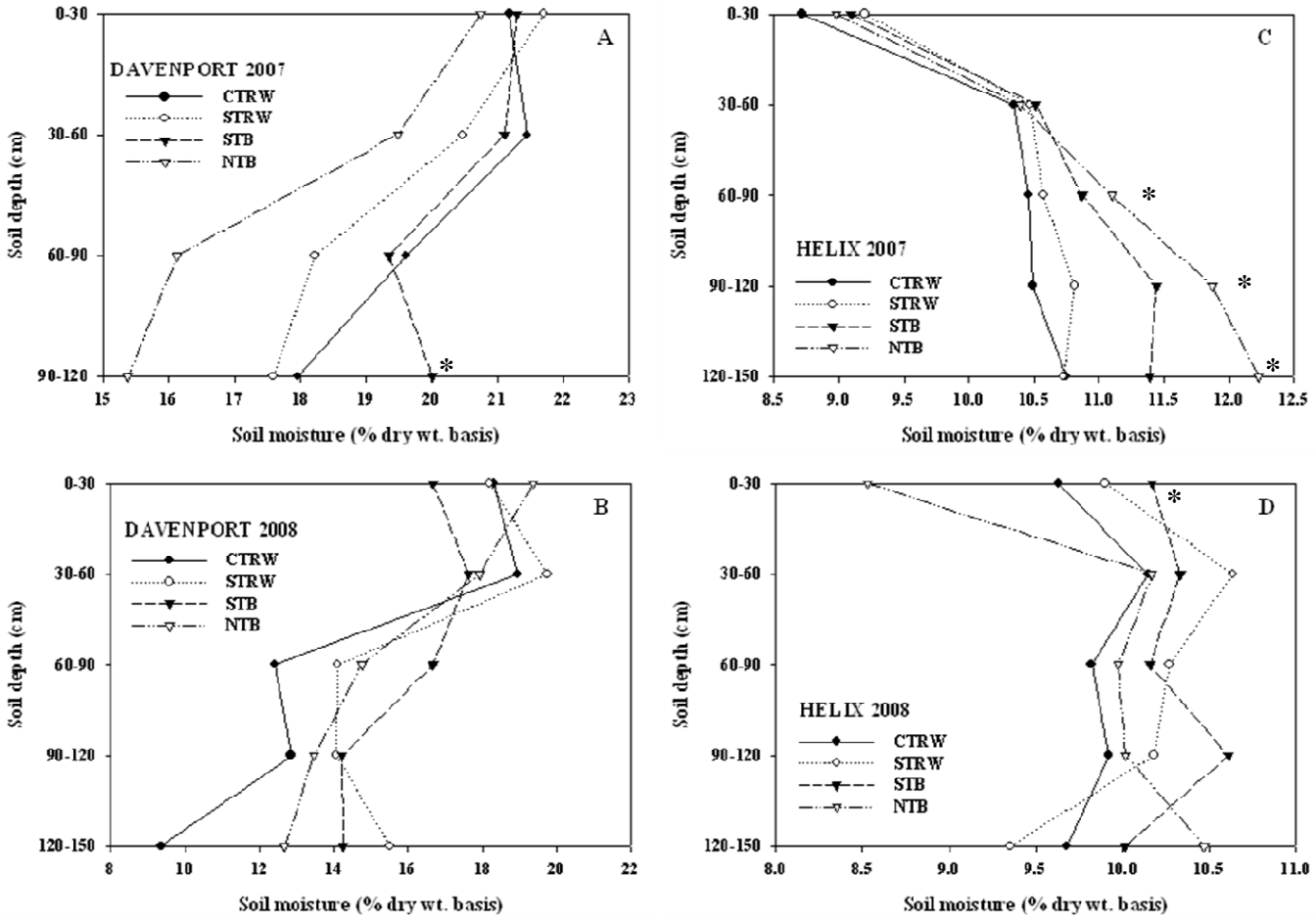
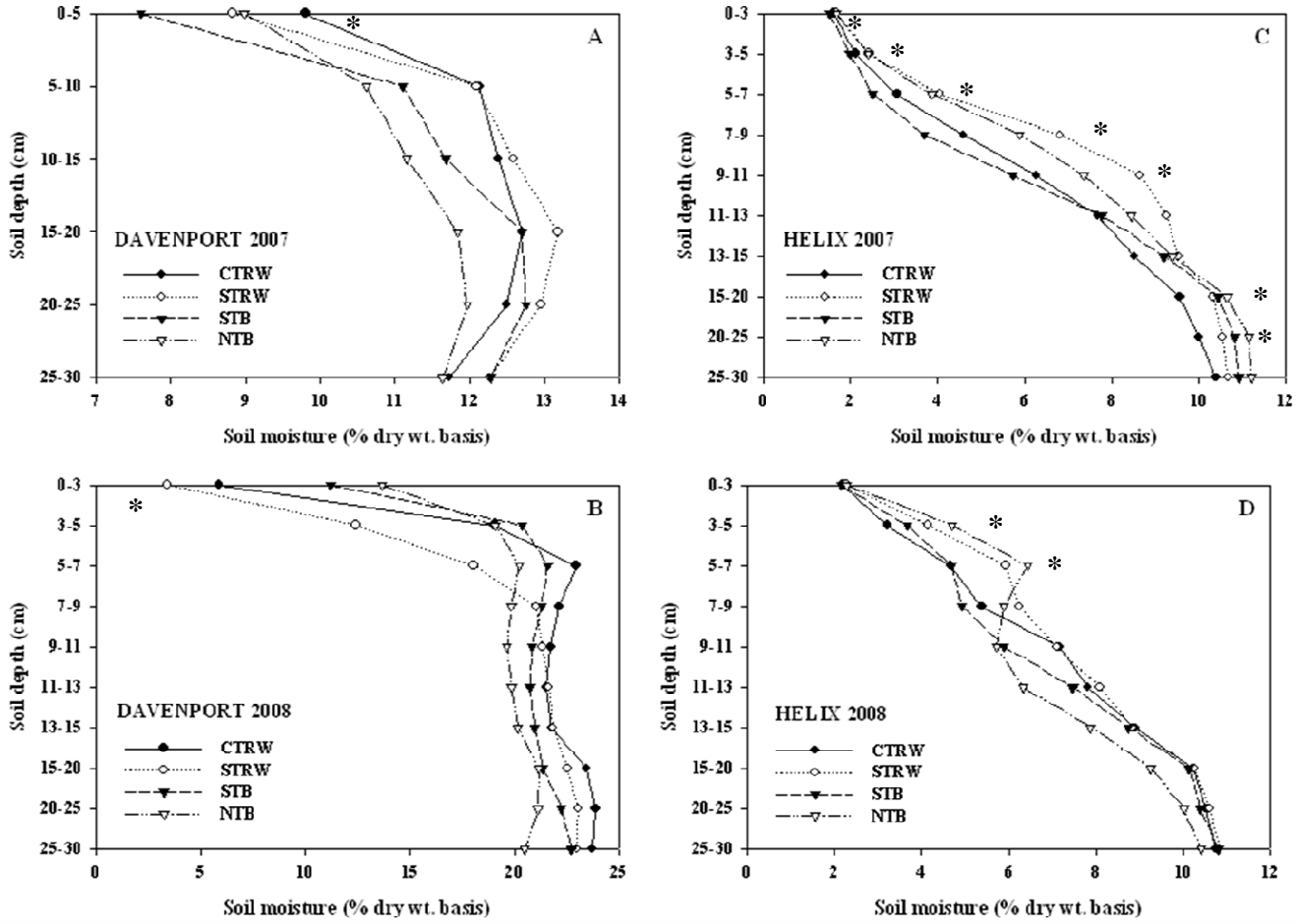


Figure 3 (A-D). Gravimetric soil moisture (% dry wt. basis) content in seed-zone soil profile at (A) Davenport, WA-2007 (B) Davenport, WA-2008 (C) Helix, OR -2007 and (D) Helix, OR -2008 fallow periods. '*' respresents significant difference ($P<0.05$) between one or more treatments at respective depths. Abbreviations: CTRW, conventional tillage; NTB, no tillage followed by broadcast sprayer; STB, undercutter followed by broadcast sprayer; STRW, undercutter followed by rodweeder.



Chapter 3

Efficacy of Postemergence Herbicides with a Reduced Herbicide Applicator in Fallow

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Abstract

A study was conducted in summer fallow fields near Davenport, WA and Pendleton, OR in 2007 and 2008 to compare the postemergence weed control efficacy of herbicide treatments applied with a light activated, sensor-controlled (LASC) sprayer to the conventional broadcast application of glyphosate at 1680 g ae/ha. The LASC sprayer applied treatments were glyphosate at 840, 1680 and 3360 g/ha applied alone, paraquat at 840 g ai/ha applied alone, or treatments of pyrasulfotole at 35 g ai/ha plus bromoxynil at 290 g ai/ha; bromoxynil at 560 g ai/ha; 2, 4-D at 970 g ae/ha; or dicamba at 280 g ae/ha plus carfentrazone at 35 g ae/ha applied alone or in mixture with glyphosate at 840 g/ha. LASC application of glyphosate alone (at 840, 1680 and 3360 g/ha) and in mixture with pyrasulfotole plus bromoxynil or 2,4-D had weed control (≥ 95%) and biomass (≤14 g/m^2) similar to the broadcast application of glyphosate across locations and years. LASC applications of carfentrazone plus dicamba or 2,4-D without glyphosate reduced tumble pigweed and prickly lettuce control by 36 and 14 percentage points, respectively, compared to broadcast application of glyphosate. Bromoxynil had minimum weed

control (≤ 67%, averaged across species) and maximum total weed biomass (68 g/m^2) of all treatments across years and locations.

Nomenclature: 2,4-D; bromoxynil; carfentrazone; dicamba; glyphosate; paraquat; downy brome, *Bromus tectorum* L. #[iv] BROTE; prickly lettuce, *Lactuca serriola* L. # LACSE; pyrasulfotole; Russian thistle, *Salsola tragus* L. # SASKR; tumble mustard, *Sisymbrium altissimum* L. # SSYAL; tumble pigweed, *Amaranthus albus* L. # AMAAL; wild oat, *Avena fatua* L. # AVEFA

Keywords: chemical fallow, herbicide efficacy.

Additional index words: Herbicide resistance, synthetic auxins, winter wheat.

Abbreviations: DAT, days after treatment; NIS, nonionic surfactant; WAT, weeks after treatment.

[iv] Letters followed by this symbol are a WSSA-approved computer code from Composite List of weeds, Revised 1989. Available only on computer disk from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897

Introduction

Summer fallow is a common practice to conserve moisture in the dryland wheat production systems of the low and intermediate rainfall zones of the inland Pacific Northwest (PNW) of the USA. Conventional fallow methods utilize a soil dust mulch tillage system that conserves soil moisture within the seed-zone by establishing a dry layer of soil over subsurface moisture (Schillinger and Papendick 2008). However, intensive tillage operations for weed control in conventional dust mulch fallow systems result in decreased soil organic matter (Rasmussen and Parton 1994) and increased wind and water erosion of soil (Papendick 1998). Current alternatives to dust mulch fallow systems rely heavily on the non-selective herbicide glyphosate due to its low cost, broad spectrum of control, and lack of soil activity (Jemmett et al. 2008). Generally, multiple applications of glyphosate at 840 to 1680 g/ha are made during fallow period to keep field weed free. Lower rates of glyphosate (840 g/ha) in spring effectively control volunteer wheat and winter annuals because of sufficient soil moisture. However, Tanpipat et al. (1997) found that glyphosate efficacy was severely reduced when applied under hot, dry conditions; a common phenomenon during summer in the PNW. Therefore, higher rates of glyphosate are needed for effective weed control during summer. Additionally, over-reliance on a single herbicide has resulted in the development of herbicide resistant weed populations that require alternative weed control options for efficient control. These alternative control measures may include mixtures of herbicides that may be cost prohibitive compared to rod-weeding and other forms of mechanical control.

Herbicides are used on 87 million ha of cropland in the USA (Gianessi and Reigner 2007). Moreover, herbicides represent 60% of the volume and 65% of the expenditure of pesticides in the USA (Donaldson et al. 2002). The PNW has the greatest per ha dryland wheat production in

the world (Young 2004). Herbicides comprise a major input cost in the wheat production regions of PNW and are applied to 92 % of the winter wheat crop area annually (NASS 2009). Bennett and Pannell (1998) reported that the sparse, patchy nature of weed distribution often results in deposition of most of the broadcast herbicide application on bare soil rather than on weed foliage. Thus, effective spot treatments of herbicides in chemical fallow, even using greater per hectare rates, could result in substantial cost savings, reduced herbicide use and possibly improved weed control compared to broadcast applications. Efficient spot applications of herbicides to fields have not been practical due to the high cost, the lack of automated equipment or the great amount of technical expertise needed by the sprayer operator. However, the introduction of real time LASC sprayers has resulted in more accurate and precise spot applications of herbicides (Biller 1998) and could be used in chemical fallow systems to reduce the amount and area of herbicide applications.

Light-activated sensor-controlled (LASC) sprayers can be used in chemical fallow systems to reduce the amount and area of herbicide applications. LASC technology operates on differential red and near infrared light absorption by green plant material relative to bare soil or residues of the previous crop to detect a plant and activate a solenoid switch above a spray nozzle for a set period of time (Biller 1998; Felton and McCloy 1992). The use of a LASC sprayer for selective weed control in a crop resulted in herbicide saving of nearly 25% compared to broadcast sprayer without reducing crop yield (Dammer and Wartenberg 2007). Reductions of 30% to 70 % in herbicide use have been achieved with LASC relative to conventional broadcast applications in chemical fallow (Ahrens 1994; Biller 1998; Blackshaw et al. 1998) and row crops (Hanks and Beck 1998). Most recently in the PNW, Young et al. (2008) found similar Russian thistle

control with the LASC sprayer compared to a broadcast sprayer, but saw a large reduction in herbicide use (42%) and cost ($13.27/ha).

The objectives of this study were to evaluate alternative herbicides for the replacement or the enhancement of glyphosate in chemical fallow systems in the PNW and to evaluate the efficacy of the LASC sprayer applied herbicide treatments compared to the conventional broadcast application of glyphosate in chemical fallow systems.

Materials and Methods

A two year study was conducted at the Washington State University Wilke Research and Extension Farm near Davenport, WA (N 47˚ 39.02' W 118˚ 07.48' and N 47˚ 39.03' W 118˚ 07.30' in summer 2007 and 2008, respectively, at an altitude of 756m) and at the Columbia Basin Agricultural Research Center near Pendleton, OR, (N 45˚ 43.38' W 118˚ 37.77' and N 45˚ 43.33' W 118 37.63' in summer 2007 and 2008, respectively, at an altitude of 450m). Davenport and Pendleton have average annual precipitation of 450 and 415 mm, respectively, and growers at both locations commonly include summer fallow within crop rotations. Each year, treatments were established on Broadax silt loam (fine-silty, mixed, superactive, mesic calcic Argixerollsmesic) and on Walla Walla silt loam (coarse-silty, mixed, superactive, mesic Typic Haploxeroll) at Davenport and Pendleton, respectively. The soil properties and texture across locations and years are provided in Table 1. The average monthly temperature and total rainfall during the study periods at each location are shown in Figure 1 (A-D).

At each location, experiments were arranged at separate but adjacent areas each year in a randomized complete block design with fourteen herbicide treatments and four replications. In both years, experimental plots were 4.6 m wide by 12.2 m long at Davenport and 3.0 m wide by 21.3 m long at Pendleton. Treatments included broadcast application of glyphosate at 1680 g

ae/ha as a control along with LASC sprayer[v] applied herbicide combinations. LASC sprayer applied treatments were glyphosate at 840, 1680 and 3360 g/ha applied alone, paraquat at 840 g ai/ha applied alone, or treatments of pyrasulfotole at 35 g ai/ha plus bromoxynil at 290 g ai/ha; bromoxynil at 560 g ai/ha; 2, 4-D at 970 g ae/ha (applied as an isooctyl ester); or dicamba at 280 g ae/ha plus carfentrazone at 35 g ae/ha applied alone or in mixture with glyphosate at 840 g/ha. The treatment with 2,4-D at 970 g/ha applied alone included 1.0 % v/v of a water conditioning agent that contained ammonium sulfate[vi] at 430 g/L. All other treatments included 0.5% v/v non-ionic surfactant (NIS)[vii] along with 1.0 % v/v water conditioning agent. Exception to these treatments during year 2007 was the exclusion of glyphosate at 1680 g/ha with LASC sprayer treatment at both locations.

At Davenport, the LASC sprayer assembly consisted of 15 individual LASC units mounted on 4.5 m-wide boom at 30 cm nozzle (TeeJet 6502 flat-fan) spacing and 60 cm height. The same sprayer and setup was used for broadcast applications using continuous spray. The sprayer was calibrated to deliver 187 L/ha at 260 kPa, on a broadcast basis. At Pendleton, the LASC sprayer assembly consisted of 10 individual LASC units mounted on 3 m-wide boom at 30 cm nozzle (TeeJet 6502 flat-fan) spacing and 60 cm height. The control box (set at medium sensitivity) and electronics of LASC sprayer assembly were operated from the tractor-battery and regulated CO_2 tanks were used to pressurize the system at both locations. The LASC sprayer was calibrated to deliver 224 L/ha at 240 kPa, on a broadcast basis. A separate broadcast sprayer with 2.7 m-wide boom and XR-8002 nozzles (45 cm nozzle spacings operated at a height of 60 cm) was used at Pendleton. The tractor-mounted sprayer was calibrated to deliver 150 L/ha at 260 kPa. All pressure and sprayer volumes were consistent across locations and years. Dates of treatment applications and conditions for treatments are listed in Table 1.

Experimental areas were broadcast sprayed with glyphosate at 840 g/ha during April and May at Pendleton and Davenport, respectively, to control volunteer wheat and weeds prior to the establishment of treatments. Surviving and later emerging grass weeds were controlled with a broadcast application of quizalofop at 93 g ai/ha over the entire experimental area to prevent confounding of the experiments due to differential grass weed control (Table 1). Percent broadleaf weed control was visually estimated on a scale of 0 (no-control) to 100% (complete plant death) at 7, 14 and 28 days after treatment (DAT) (Frans et al. 1986). Weed density for each weed species and total weed biomass were evaluated 28 DAT by sampling all weeds within four 0.25-square meter quadrats in each plot. Weeds were harvested by cutting plants at the soil surface. They were then hand separated by species, weighed for fresh biomass, dried at 60 C for 48 hr, and weighed again for dry biomass.

The data were tested for homogeneity of variance by using PROC UNIVARIATE procedure in SAS[viii] (SAS Institute 2005). To improve variance homogeneity, the percentage weed control data were subjected to arcsine square root percent transformation, and weed biomass and density data were subjected to log + 1 transformation. The data from the nontreated control were removed before analysis to stabilize variance. Transformed data were subjected to ANOVA using the PROC MIXED procedure in SAS and sum of squares were partitioned to evaluate the effect of herbicide treatments on percent weed control and biomass. Year, location and treatment replication were considered random variables, and main effects and interactions were tested on the basis of randomized complete block design (McIntosh 1983). Fisher's protected least significant difference (LSD) was used to perform mean separations when p-values for the F-test were ≤ 0.05 (Steel et al. 1997).

Results and Discussion

Analyses of data indicated that there was a significant treatment by year and/or location interaction for total weed biomass, and percent control and density of each weed species (data not shown). Treatment means were plotted to see if interactions affected treatment rankings across years and locations (data not shown). Plots of interactions indicated that treatment by location and year interactions were minimal and trends were not affected if treatments were pooled across years and locations. Treatment by year and/or location interaction was attributed to the order of magnitude changes caused by climatic variations (Jemmett 2006). Tumble pigweed was present at both locations every year. However, prickly lettuce and tumble mustard were present at both locations only during 2008. Thus, the data for prickly lettuce and tumble mustard were pooled across locations (for year 2008) and the data for tumble pigweed and total weed dry biomass were pooled across years and locations. The mean values are presented in the original (nontransformed) scale.

Tumble Pigweed Control. Paraquat and all glyphosate treatments controlled 90% or more tumble pigweed (Table 2). The tumble pigweed control by LASC applications of glyphosate alone at all rates and in mixture with 2,4-D or pyrasulfotole plus bromoxynil (≥ 97%) was similar to the conventional broadcast application of glyphosate across locations and years. Glyphosate is reported to be an effective herbicide treatment for the control of pigweed species (Corbett et al. 2004; Culpepper and York 1999; Krausz et al. 1996). However, there was 5 to 10 percentage points decrease in tumble pigweed control by LASC applications of paraquat and glyphosate in mixture with bromoxynil or carfentrazone plus dicamba compared to broadcast application of glyphosate. O'Sullivan and O'Donovan (1980) also observed antagonism among glyphosate and bromoxynil, and reported reduced weed control with glyphosate in mixture with bromoxynil. At both locations, field conditions were dry and dusty due to the low rainfall and

high air temperatures during the month of July each year (Figure 1). Reduced tumble pigweed control by paraquat was due to the decrease in efficacy of paraquat under dusty conditions (Rytwo and Tavasi 2003). Additionally, the efficacy of LASC sprayers to detect very small plants decreases under dusty conditions. The reduced detection of very small weeds by LASC sprayer in dusty conditions is probably due to the dust accumulation on light sensors and/or dispersion of light by airborne dust particles. Young et al. 2008 and Blackshaw et al. 1998 also found reduced weed control efficacy of the LASC sprayer in dusty conditions. Tumble pigweed control by pyrasulfotole plus bromoxynil (85%) was similar to paraquat and glyphosate in mixture with bromoxynil or carfentrazone plus dicamba. Auxinic herbicides applied without glyphosate were not effective for tumble pigweed control. The tumble pigweed control by 2,4-D and carfentrazone plus dicamba was only 64 and 67%, respectively. Bromoxynil was least effective of all treatments and controlled only 30% tumble pigweed across years and locations. Mickelson et al. (2004) also found inconsistent kochia (*Kochia scoparia*) control by bromoxynil, carfentrazone, or 2,4-D under the drought stress conditions of summer fallow. Corbett et al. (2004) found reduced efficacy of bromoxynil for the control of pigweed species taller than 8 cm under summer field conditions.

There was a general agreement between visual estimates of tumble pigweed control and density measurements for the ranking of the efficacy of the various treatments. Bromoxynil, 2,4-D or carfentrazone plus dicamba had maximum tumble pigweed densities (10 to 20 plants/m^2) across years and locations (Table 3). All other treatments had 4 plants/m^2 or less tumble pigweed densities. The LASC applications of glyphosate alone (at all rates) or in mixture with 2,4-D or pyrasulfotole plus bromoxynil had tumble densities similar to conventional broadcast spray of glyphosate.

Prickly Lettuce Control. The LASC applications of paraquat, pyrasulfotole plus bromoxynil, and all glyphosate treatments except glyphosate in mixture with bromoxynil controlled prickly lettuce (≥94%) similar to conventional broadcast application of glyphosate (Table 2). Generally, paraquat, pyrasulfotole plus bromoxynil and glyphosate are considered effective treatments for prickly lettuce control (Welker and Smith 1972; Burke et al. 2009). Prickly lettuce control was reduced to 88% with glyphosate in mixture with bromoxynil treatment. O'Sullivan and O'Donovan (1980) also reported that when applied in mixture, bromoxynil antagonizes the weed control efficacy of glyphosate. LASC sprayer applied 2,4-D and carfentrazone plus dicamba controlled prickly lettuce (85 and 84%, respectively) similar to glyphosate in mixture with bromoxynil. Reduced prickly lettuce control with 2,4-D and carfentrazone plus dicamba was due to regrowth of prickly lettuce. Burke et al. (2009) showed regrowth of prickly lettuce after the application of phenoxyalkanoic acid herbicides. Burke et al. (2009) reported 98% prickly lettuce control by bromoxynil in the green house studies. However, in the present study, bromoxynil alone controlled only 27% of prickly lettuce across years and locations and proved to be the least effective treatment for prickly lettuce control. Other studies have reported reduced weed control with bromoxynil under drought stress conditions of summer and larger weed size (Mickelson et al. 2004; Corbett et al. 2004). Therefore, reduced prickly lettuce control by bromoxynil might be due to dry field conditions and larger prickly lettuce size (Table 1 and Figure 1).

Prickly lettuce densities across years and locations in all the LASC applied treatments except bromoxynil alone and glyphosate in mixture with carfentrazone plus dicamba or bromoxynil were < 1 plant/m^2, and were similar to the broadcast application of glyphosate (Table 3). Prickly lettuce densities in LASC applied 2,4-D or carfentrazone plus dicamba treatments were similar to

broadcast glyphosate application and indicated that the reduced prickly lettuce control was due to the regrowth of prickly lettuce plants. Bromoxynil treatments had more prickly lettuce densities (≥ 2 plants/m^2) compared to the glyphosate applied alone at all rates (with either herbicide applicator) and carfentrazone plus dicamba treatments because of reduced prickly lettuce control, but densities were similar to all other treatments. Prickly lettuce densities in LASC applied glyphosate in mixture with carfentrazone plus dicamba were greater than glyphosate applied alone with either herbicide applicator but were similar to all other treatments.

Tumble Mustard Control. Tumble mustard control by LASC applications of 2,4-D, pyrasulfotole plus bromoxynil and all glyphosate treatments except glyphosate in mixture with bromoxynil was similar (≥98%) to the broadcast treatments of glyphosate (Table 2). However, tumble mustard control reduced to 95% in glyphosate plus bromoxynil or carfentrazone plus dicamba. Tumble mustard control by paraquat further reduced to 90%. As illustrated by O'Sullivan and O'Donovan (1980), the reduced tumble mustard control by glyphosate in mixture with bromoxynil was due to antagonism of glyphosate by bromoxynil. Reduced tumble mustard control by paraquat or carfentrazone plus dicamba at Pendleton was likely due to the advanced plant growth at the time of treatment application (Table 1). Tumble mustard control by bromoxynil alone (64%) was less than all other treatments. Corbett et al. (2004) showed that weed control by bromoxynil application usually decreases if plant height is greater than 8 cm. Similar to visual control, tumble mustard densities in all LASC treatments except bromoxynil alone or carfentrazone plus dicamba were similar (≤1.38 plants/m^2) to broadcast treatment of glyphosate. Bromoxynil treatment had maximum tumble mustard densities (3.63 plants/m^2) because of reduced tumble mustard control compared to other treatments.

Total Weed Biomass. As with the visual estimate of control, treatments with LASC applications of glyphosate alone or in mixture with 2,4-D or pyrasulfotole plus bromoxynil had total weed dry biomass ($\leq$ 14 g/m^2) similar to the broadcast application of glyphosate (Table 3). Young et al. (2008) also saw no differences in weed control regardless of whether herbicides were applied with a broadcast or LASC sprayer. LASC applications of glyphosate in mixture with bromoxynil or carfentrazone plus dicamba resulted in greater weed biomass (25 and 21 g/m^2, respectively) compared to broadcast application of glyphosate because of reduced control of tumble pigweed and/or prickly lettuce. Bromoxynil or carfentrazone plus dicamba probably antagonized the weed control efficacy of glyphosate. Efficacy of 2,4-D, carfentrazone plus dicamba or bromoxynil significantly reduced without glyphosate. Bromoxynil treatment had maximum weed biomass (68 g/m^2) of all treatments. Carfentrazone plus dicamba or 2,4-D treatments also had greater weed biomass (38 and 44 g/m^2) than all treatments except bromoxynil. Weed biomass in pyrasulfotole plus bromoxynil and paraquat treatments was less than broadcast or LASC treatments of glyphosate alone or in combination with 2,4-D or pyrasulfotole plus bromoxynil. Overall, glyphosate alone and in combination with pyrasulfotole plus bromoxynil or 2,4-D were the most effective treatments and had least weed biomass, while bromoxynil, 2,4-D, or carfentrazone plus dicamba were the least effective treatments and had maximum weed biomass.

In summary, tumble pigweed, prickly lettuce and tumble mustard were the major weeds in fallow period across locations and years in this study. Bromoxynil, 2,4-D, or carfentrazone plus dicamba did not controlled weeds effectively at either location. Tumble pigweed control by paraquat and pyrasulfotole plus bromoxynil treatments reduced compared to broadcast application of glyphosate in dry conditions. Therefore, greater rates of paraquat or pyrasulfotole

plus bromoxynil may be needed in intermediate rainfall inland PNW environments to achieve reliable and consistent weed control by LASC applicator. LASC sprayer applications of glyphosate alone at different rates (840, 1680 and 3360 g/ha) and in combination with pyrasulfotole plus bromoxynil or 2,4-D controlled weeds similar to broadcast applications of glyphosate. LASC application of none of the tested residual herbicides was as effective as broadcast applications of glyphosate. However, pyrasulfotole plus bromoxynil or 2,4-D have more soil activity and residual effect for weed control compared to glyphosate, and can supplement glyphosate for comparatively longer term weed control compared to glyphosate alone in chemical fallow systems in future. Additionally, previous research has shown herbicide use reduction of 30-70% through the use of LASC sprayer technology (Ahrens, 1994; Biller, 1998; Blackshaw et al 1998, Young et al. 2008). Thus, LASC sprayer technology may allow greater application rates to individual plants with possibly lower per area herbicide loading. In view of increasing diesel prices, environmental concerns, and costly (if available) farm labor, chemical fallow with LASC application of herbicide could be an effective alternative to dust mulch fallow practices in the intermediate rainfall zone of inland PNW.

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Sources of Materials

[v] Weed Seeker^{TM} Ntech Industries, Inc., 740 South State Street, Ukiah, CA 95482.

[vi] Bronc® Max. A mixture of ammonium sulfate, ammonium alkyl aryl sulfonate, polycarboxylic acid and silicone. Wilbur-Ellis Company. PO Box 1286. Fresno, CA 93715.

[vii] R-11®. Non Ionic Surfactant. A mixture of alkylphenol ethoxylate, butyl alcohol, dimethylpolysiloxane. Wilbur-Ellis Company. PO Box 1286. Fresno, CA 93715.

[viii] Statistical Analysis Systems (SAS) software, Version 9.1. SAS Institute Inc., Box 8000, SAS Circle, Cary, NC 27513.

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Table 1. Dates of different operations and application conditions in chemical fallow experiments conducted in 2007 and 2008.

		Davenport, WA		Pendleton, OR	
Parameter		2007	2008	2007	2008
Grass weed control	Timing	21-Jun-07	12-Jun-08	NA[b]	NA
Weed height at the time of treatment	Tumble pigweed	2-12 cm	10-15 cm	10-15 cm	1.5-10 cm
	Prickly lettuce	NP[a]	10 leaf to bolting	NP	5-30 cm
	Tumble mustard	NP	30 cm to flowering	NP	Flowering
Stubble type		Spring wheat	Spring wheat	Winter wheat	Winter wheat
Treatment application dates	Timing	3-Jul-07	9-Jul-08	26-Jun-07	25-Jun-08
Application conditions for treatments	Air temperatures (°C)	31	29	14	36
	Relative humidity (%)	21	24	62	31
	Wind (km/h)	6	1.6	1.6	4.8
	Cloud cover (%)	10	5	0	10
	Soil temperature (°C at 10 cm depth)	22	20	11	28
Soil properties	pH	5.1	4.9	6.2	5.5
	Organic matter (%)	3.2	3	2.4	2.5
	CEC (cmol kg^{-1})	20	21	16.9	16.3
Soil texture	Sand (%)	28.8	28.8	29.8	25.7
	Silt (%)	61.2	61.2	56.4	63.6
	Clay (%)	10	10	13.8	10.7

(a) Weed species not present. (b) Date not available

Table 2. Percent weed control with broadcast and LASC applied treatments at 15DAT in chemical fallow, averaged across Davenport, WA and Pendleton, OR and years 2007 and 2008[a].

Treatment	Rate	AMAAL	LACSE	SSYAL
	g/ha		% control	
Glyphosate*	1680	100	98	100
Glyphosate	840	97	97	100
Glyphosate	1680	100	99	100
Glyphosate	3360	97	100	100
Glyphosate + 2,4-D	840 + 970	97	95	100
2,4-D	970	65	85	100
Glyphosate + pyrasulfotole + bromoxynil	840 + 35 + 290	98	97	98
Pyrasulfotole + bromoxynil	36 + 290	85	97	99
Glyphosate + bromoxynil	840 + 560	90	88	95
Bromoxynil	560	30	27	64
Glyphosate + carfentrazone + dicamba	840 + 35 + 280	92	97	100
Carfentrazone + dicamba	35 + 280	67	84	95
Paraquat	840	95	94	90
LSD (0.05)[b]		3	4	2

(a) Abbreviations: AMAAL, tumble pigweed; LACSE, prickly lettuce; SSYAL, tumble mustard; DAT, days after postemergence treatments; LSD, least significant difference

(b) Fisher's protected LSD among treatments at $P \leq 0.05$ after arcsine square root percent transformation of data with the nontransformed means shown.

(*) Treatments were sprayed with conventional broadcast sprayer. All other treatments were sprayed with LASC sprayer.

Table 3. Weed density by species and total dry biomass in broadcast and LASC applied treatments at 28 DAT in chemical fallow, averaged across Davenport, WA and Pendleton, OR and years 2007 and 2008[a].

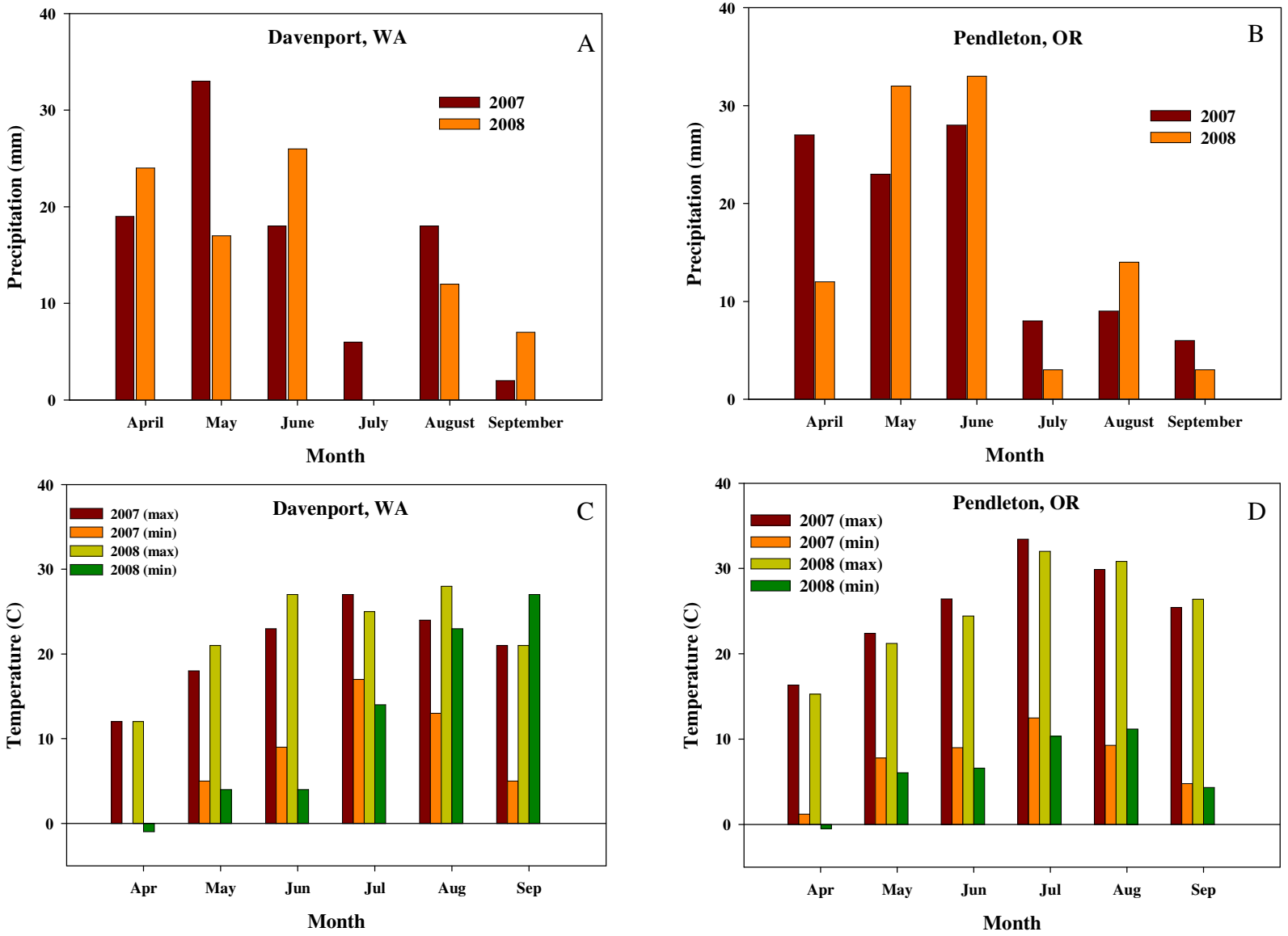
Treatment	Rate	AMAAL	LACSE	SSYAL	Total Weed Bioamss[c]
	g/ha	——— plants/m^2 ———			g/m^2
Glyphosate*	1680	0.25	0.04	0.00	7.14
Glyphosate	840	0.84	0.21	0.38	14.16
Glyphosate	1680	0.00	0.08	0.01	2.38
Glyphosate	3360	2.00	0.08	0.06	9.82
Glyphosate + 2,4-D	840 + 970	2.44	0.54	0.13	12.89
2,4-D	970	9.68	0.88	0.01	44.03
Glyphosate + pyrasulfotole + bromoxynil	840 + 35 + 290	1.21	0.92	0.13	11.11
Pyrasulfotole + bromoxynil	35 + 290	2.76	0.50	1.13	26.91
Glyphosate + bromoxynil	840 + 560	4.08	2.00	1.31	24.97
Bromoxynil	560	11.86	2.19	3.63	67.74
Glyphosate + carfentrazone + dicamba	840 + 35 + 280	3.98	1.29	0.00	21.31
Carfentrazone + dicamba	35 + 280	19.98	0.42	2.38	38.34
Paraquat	840	3.48	0.71	0.88	23.86
LSD (0.05)[b]		2.50	1.07	1.99	8.30

(a) Abbreviations: AMAAL, tumble pigweed; LACSE, prickly lettuce; SSYAL, tumble mustard; DAT, days after postemergence treatments; LSD, least significant difference

(b) Fisher's protected LSD among treatments at $P \leq 0.05$ after log+1 transformation of data with the nontransformed means shown.

(*) Treatments were sprayed with conventional broadcast sprayer. All other treatments were sprayed with LASC sprayer.

Figure 1. A-D. Mean monthly precipitation (A and B) and mean monthly maximum and minimum air temperatures (C and D) at Davenport, WA and Pendleton, OR during study period.



Chapter 4

2,4-D Resistance in Prickly Lettuce (*Lactuca serriola*)

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Abstract

Prickly lettuce has evolved resistance to the synthetic auxin herbicide 2,4-D. To determine the mechanism of resistance, absorption and translocation studies were conducted using ^{14}C-2,4-D applied to 2,4-D resistant and susceptible biotypes. Peak absorption was observed 96 hours after treatment (HAT) in each biotype. At 96 HAT, resistant biotype absorbed 21percentage points less of applied ^{14}C than susceptible biotypes. Of the total ^{14}C recovered at 96 HAT, resistant prickly lettuce plants retained 20 percentage points more radioactivity in the middle portion of treated leaf and translocated 20 percentage points less radioactivity out of treated leaf compared to susceptible plants. Moreover, the resistant biotype translocated 26 and 22 percentage points less recovered ^{14}C to the leaves above treated leaf and crown, respectively, compared to the susceptible biotype. In accordance with the absorption and translocation studies, genetic studies revealed that 2,4-D resistance in prickly lettuce is an oligogenic trait governed by one major gene and one or two modifying genes. Re-growth of the resistant prickly lettuce biotype commonly occurs from apical or lateral meristems located in the crown. Reduced herbicide uptake and translocation to the crown in resistant biotype could be a mechanism or partial mechanism for 2,4-D resistance in prickly lettuce. Metabolism study is required in future to validate the results

of translocation study and to determine the form of radioactivity translocated to the crown of resistant and susceptible biotypes.

Nomenclature: 2,4-D; prickly lettuce*, Lactuca serriola* L. #^ LACSE.

Abbreviations: Days after treatment, DAT; hours after treatment, HAT.

Keywords: Auxinic herbicides, herbicide resistance, radiolabeled herbicide, autoradiography, uptake and translocation, inheritance.

Introduction

Prickly lettuce is a well adapted, winter/spring annual weed of the Pacific Northwest (PNW) region of the United States. Prickly lettuce survives the drought conditions during dry summers of PNW due to its deep taproot system (Jackson 1995). Additionally, prickly lettuce has vertically oriented leaves with lamina perpendicular to the east and west intersects. The vertical orientation of leaves allows the plant to reduce water loss without substantial decrease in photosynthesis and also enhances reproduction by positively influencing floral transition and seed set (Werk and Ehleringer 1984, 1986). Prickly lettuce is invading new landscapes at fast pace because of high reproductive capacity (number of achenes ranging from 2,200 to 87,000 per plant; Weaver et al. 2006) and anemochorous seed with little or no dormancy (Marks and Prince 1982). In a recent study in Canada, *L. serriola* (at densities of ≥50 plants m^{-2}) was reported to cause severe yield losses (60% to 80%) in soybean (Weaver et al. 2006). Besides competing with crops for moisture, space and nutrients, it also interferes with the harvest of many crops (Amor 1986). Usually, prickly lettuce flowers at the time of crop harvest in the PNW. Consequently, prickly lettuce floral-buds are frequently harvested with cereal grains and are difficult to separate. The latex present in prickly lettuce stems cause economical losses, by clogging the harvest machinery and by raising the moisture content of the harvested grains to unacceptable levels, resulting in a price penalty.

Prickly lettuce can be controlled using various selective and non-selective herbicides. Control becomes more difficult following internode elongation (Yenish and Eaton 2002; Amor 1986). Generally, auxinic and acetolactate synthase inhibitor (ALS) herbicides provide good control of prickly lettuce. Prickly lettuce biotypes have been reported to be resistant to these herbicide groups. ALS-herbicide resistant prickly lettuce was first discovered in Idaho in 1987 (Mallory-

Smith et al. 1990a; Alcocer-Ruthling et al. 1992). An inheritance study of ALS-herbicide resistant prickly lettuce indicated that resistance is due to a single nuclear gene with incomplete dominance (Mallory-Smith et al. 1990b). Auxinic herbicides (phenoxycarboxylic acid-, benzoic acid-, pyridinecarboxylic acid-, and quinolinecarboxylic acid-type herbicides) usually control prickly lettuce by partially mimicking indole-3-acetic acid (IAA), a key natural auxin in higher plants (Sterling and Hall 1997). Generally, auxinic herbicide resistant populations are thought to require more generations to become the dominant type compared to resistant biotypes of ALS and acetyl-coenzyme A carboxylase (ACCase) inhibitor herbicides (Zheng and Hall 2001). Only 27 weed species have been reported worldwide to be resistant to auxin herbicides even though these herbicides were commercially introduced in 1947 and are widely used in cereal production and for noncrop weed control, compared to 101 ALS inhibitor and 68 photosystem II inhibitor herbicide resistant weed species (Heap 2009).

Most auxinic herbicide-resistant weeds discovered to date show cross-resistance to at least one additional auxinic herbicide. Yellow starthistle (*Centaurea solstitialis*) biotypes have been found that are resistant to 2,4-D, picloram, clorpyralid, dicamba and fluroxypyr (Callihan and Schirman 1991; Fuerst et al. 1996). Kochia (*Kochia scoparia*) biotypes have been discovered with resistance to 2,4-D and dicamba (Nandula and Manthey 2002). In another study, Walsh et al. (2004) found multiple herbicide resistance in a wild radish (*Raphanus raphanistrum*) biotype to auxinic, ALS inhibitor and phytoene desaturase inhibitor herbicides. Currently, researchers have identified and used auxinic herbicide resistant wild mustard (*Sinapis arvensis*) as a model plant to determine physiological, biochemical and molecular aspects of auxinic herbicide resistance (Zheng and Hall 2001). Inheritance studies of wild mustard have found a single dominant gene accounting for resistance to picloram and 2,4-D (Jugulam et al.

2005) and dicamba (Jasieniuk et al. 1995). Quinclorac resistance in false cleavers (*Galium spurium*) has found to be a single recessive trait. Conversely, Weinberg et al. (2006) reported MCPA resistance in hemp-nettle as quantitative trait and indicated that resistance is governed by two or more nuclear genes with additive effects. So far, genetic studies have not agreed on a common inheritance model for auxin-herbicide resistance and different results of various inheritance studies have made it difficult to predict exact mechanisms of auxin-herbicide resistance.

Although 2,4-D, an auxinic herbicide, was the first synthetic herbicide available. The exact mechanism of auxinic herbicide action is still unknown. In order for an herbicide to be effective, it must accumulate at its site of action at a lethal concentration. The site of action of 2,4-D, is meristematic tissue and possible mechanisms of resistance include reduced absorption and translocation to the site of action or greater metabolism of 2,4-D in resistant biotypes compared to susceptible biotypes. Kohler et al. (2004) found 37% more 2,4-D absorption in susceptible ground ivy (*Glechoma hederacea*) biotypes compared to resistant biotypes. Although, percent translocation was found to be similar in both biotypes, susceptible ground ivy biotypes accumulated 42% more herbicide at apical meristem due to the greater absorption of herbicide. Weinberg et al. (2006) found no difference in absorption of MCPA, but detected a lower rate of translocation and higher rate of metabolism in resistant hemp-nettle (*Galeopsis tetrahit*) biotypes compared to susceptible biotypes. Conversely, Zawierucha and Penner (2000) found no difference between absorption, translocation and metabolism among quinclorac tolerant *Eleucine indica* and quinclorac sensitive *Digitaria sanguinalis* suggesting that quinclorac tolerance may be due to an altered target site. Also, differential absorption, translocation and metabolism was found not to be the basis for dicamba resistant kochia (Cranston et al. 2001), picloram resistant

yellow star thistle (Fuerst et al. 1996), along with dicamba or picloram resistant wild mustard biotypes (Penuik et al. 1993) and quinclorac resistant false cleavers biotypes (Van Eerd et al. 2005). These studies suggest that the mechanism of auxinic herbicide resistance could be differential absorption, translocation or metabolism, or an altered target site.

Following a picloram application to wild mustard, enhanced ACC (1-aminocyclopropane-1-carboxylic acid) synthase and ethylene biosynthesis were observed in picloram susceptible biotypes with no enhancement observed in resistant biotypes (Hall et al. 1993). In plants, ACC synthase catalyzes the biosynthesis of ACC, which further gets oxidized to ethylene. The evolution of ethylene triggers a cascade of events, ultimately leading to plant death. Webb and Hall (1995) suggested that differences in dicamba sensitivity between wild mustard biotypes may be due to differential interaction of the herbicide with auxin binding proteins or other receptors. Sterling and Hall (1997) postulated that interactions among auxinic herbicides and presumed auxin receptors might induce Ca^{+} and H^{+} fluxes. Calcium is a well-accepted secondary messenger in plant cells and plays an important role in signaling during stress conditions (Pooviah and Reddy 1993). Flash induced-light scattering signals from protoplast of picloram resistant and susceptible wild mustard biotypes showed hindrance of Mg-ATP dependent ion flux in susceptible biotypes while there is no affect on resistant biotypes (Deshpande and Hall, 1995). The ion flux in the protoplast of resistant wild mustard biotypes had changed only by the simultaneous incubation with picloram and verapamil (a calcium ion channel blocker), suggesting a positive role of calcium signaling in auxin herbicide resistance. All physiological and biochemical studies point toward multiple mechanisms of auxinic herbicide resistance.

Recently, Burke et al. (2009) identified 2,4-D resistant prickly lettuce biotype near Pullman, WA. Based on earlier auxin herbicide resistance research, a study was conducted to determine

the mechanism of 2,4-D resistance in prickly lettuce. The objectives of the study were to (1) determine whether differential absorption or translocation was a mechanism of resistance in these biotypes and (2) determine the inheritance of 2,4-D resistance in prickly lettuce.

Materials and Methods

Plant Material and Growth Conditions. Resistant and susceptible prickly lettuce biotypes used by Burke et al. (2009) were self-pollinated for three generations by means of single seed descent to ensure homozygous plants. Third generation resistant and susceptible seed were used for uptake, translocation, metabolism and inheritance studies. Plants for all experiments were grown under controlled environment glasshouse conditions of 32/25 ± 3 C day/night temperature and 14-hr photoperiod consisting of natural light supplemented with light from sodium vapor lamps. For all experiments, ten seed were planted in 0.5L volume plastic pots filled to capacity with commercial potting media[1]. Following emergence, plants were thinned to one plant per pot. Pots were sub-irrigated as necessary and received no additional fertilizer other than that initially in the potting media.

For the inheritance study, plants were grown in three sets with intervals of two weeks between sowing of sets to synchronize flowering of resistant and susceptible biotypes to ensure successful crossing. Susceptible and resistant plants were kept in different rooms of a glasshouse before and after crossing. Plants were transplanted to 4L volume pots when they reached 5 to 8 leaf stage of growth.

Radiolabeled 2,4-D Experiments. Non-formulated 2,4-D with ^{14}C labeled benzene ring (specific activity of 9250 MBq) and non-radiolabeled formulated 2,4-D amine (Formula 40) were obtained from Sigma-Aldrich® and Nufarm Americas Inc., respectively. For uptake and

translocation studies, labeled 2,4-D was dissolved in a water:7M dimethylamine (13:1, v/v) solution to make a stock solution with specific activity of 0.33 kBq μl^{-1}. All chemicals and reagents used were of technical grade (greater than 99% pure) except the formulated non-radiolabeled 2,4-D.

Uptake and Translocation of [^{14}C] 2,4-D. The experiment was arranged in a split-split plot design with six harvest timings (1, 4, 8, 24, 48 and 96 hours after treatment (HAT) as main plots, two prickly lettuce biotypes (resistant and susceptible) as sub-plots and seven different plant portions (leaf portion treated with radioactive 2,4-D (S), portion of leaf outside the treated area towards leaf tip (ST), portion of leaf outside the treated area towards leaf base (SB), all leaves above treated leaf (UL), all leaves below treated leaf (LL), crown (C), and roots (R) as sub-sub plots. Treatments were replicated four times. The experiment was conducted twice.

A 2-cm portion of the second fully expanded leaf of individual prickly lettuce plants was delineated across the entire width of leaf roughly ½ way between the stem and leaf tip. To ensure realistic absorption and translocation, plants were over-sprayed with 0.514 kg non-radiolabeled 2,4-D amine ae ha^{-1} at the five to six leaf stage. The delineated portion of the second leaf was covered with a 2 cm wide plastic stake during this application. As soon as plants dried, (10 to 15 minutes) 10-µl of a herbicide solution containing 3.33 kBq of [^{14}C] 2,4-D was applied evenly across the delineated portion of each appropriate leaf on the marked portion of leaves on the adaxial side. A 25-µl microsyringe delivering 0.5-µl droplets equipped with a repeating dispenser was used to apply the [^{14}C] 2,4-D solution. Plants were harvested 1, 4, 8, 24, 48 and 96 HAT by cutting them at the soil surface and dissected into the seven plant components described above.

The treated leaf was the first plant component processed at each time interval. Only the treated portion of this leaf was rinsed for 15 sec with 1ml of a methanol:water (1:1 v/v) solution to remove unabsorbed 2,4-D. Rinse solution was collected in a 25-ml scintillation vial, mixed with 20 ml of scintillation fluid and radio-assayed by liquid scintillation spectrometry (LSS) to determine the amount of nonabsorbed ^{14}C.

Following rinsing of the treated leaf portion and dissection, all plant parts were dried for 48 hr at 40 C, weighed and oxidized using a biological sample oxidizer. The evolved CO_2 was trapped in 15 ml of scintillation fluid and radio-assayed by LSS.

Autoradiography. A portion of the second fully expanded leaves of resistant and susceptible prickly lettuce biotypes was delineated and treated with 3.33 kBq of radioactive herbicide solution as described in the absorption and translocation experiment. Plants were harvested at 96 and 168 HAT along with roots. Soil was removed from roots by washing with distilled water. Non-absorbed ^{14}C was collected by rinsing the treated leaf with 10 ml of methanol:water (1:1, v/v) solution. Plants were mounted on a 22 × 28 cm^2 sheet of plain copier/printer paper, spread carefully to avoid contact of treated leaf with other plant parts, covered with another sheet of paper and pressed in wooden press boards (Nandula et al. 2007). The order of stacking in the press from bottom to top was wooden plate, cardboard, copy/printer paper, fully spread plant, copy/printer paper, cardboard and second wooden plate. The press was held together by large metal binder clips on all four sides and stored in -20 C. After one week, plants were taken out of the press and wrapped in plastic cling wrap avoiding contact of the treated leaf with other plant parts. The wrapped plants were exposed to 35 × 43 cm Kodak Scientific X-OMAT Blue XB-1 imaging films (Perkin Elmer Life Sciences) for 4 weeks. After exposure, the imaging films were developed in a dark room (facilities of department of Crop and Soil Science, Washington State

University, Pullman, WA) by rinsing films for 3 minutes in 20% Kodak GBX developer and replenisher solution (Eastman Kodak Company, NY, USA), followed by 2 seconds in water and 30 second in 20% Kodak GBX fixer and replenisher solution (Sigma-Aldrich Inc., Mo, USA). Films were dried for half an hour and scanned with a flatbed scanner. The experiment was conducted a single time with two replications of each treatment. Non-treated plants were used as control.

Inheritance of 2,4-D Resistance in Prickly Lettuce. Four resistant (R13, R18, R21 and R23) and two susceptible (S11 and S33) prickly lettuce plants were crossed in all combinations for a total of 100 crosses. Crosses using susceptible biotypes as female (S × R; designated as F_1 crosses) and resistant biotype as female (R × S; designated as reciprocal F_1 (RF_1) crosses) were made for each pairing of resistant/susceptible biotypes. Crosses were accomplished using the clip-and-wash method of emasculation (Nagata 1992). Following crossing, female parent plants were covered with transparent cloth bags and individual flowers were tagged with the respective cross information. Seed produced from F_1 and RF_1 crosses were collected, kept separated for the individual crosses, and stored under dry conditions until needed for further studies.

Prickly lettuce is an obligate self-fertilizing plant (Ryder, 1986). Therefore, it is crucial to determine the success of the crossing procedure. Since there is no morphological marker for 2,4-D resistance in prickly lettuce to evaluate success of crossing and homozygosity of parents, 15 F_1 and 15 RF_1 hybrid plants from different crosses along with ten resistant and eight susceptible plants as controls were grown in growth chamber set to provide 16/8 hrs light/dark,and 22/15 C day/night temperature. All plants were sprayed with 0.43 kg 2,4-D ae ha^{-1} at the 5 to 8 leaf stage to determine susceptibility of the plants. The herbicide dose was based on GR_{50} values calculated by Burke et al. (2009) for resistant and susceptible prickly lettuce biotypes. Plants were sprayed

using air-pressurized indoor spray chamber calibrated to deliver 186 L ha^{-1} at 190 k Pa with an 8002E flat-fan nozzle. F_1 plants from S11×R23 crosses were self pollinated to produce F_2 seed. F_2 plants were grown in growth chamber under same conditions as F_1 plants and sprayed with the same rate of 2,4-D when plants reached 5 to 8 leaf stage. The 2,4-D injury symptoms expressed in F_2 plants were a continuum from highly responsive to no response. Therefore, individual F_2 plant responses to 2,4-D were evaluated on a visual injury rating scale of 1 to10 with representative injury ratings shown in Figure 1. Accordingly, the frequency of F_2 plants within each visual score rating was calculated.

Statistical Analyses

Inheritance of 2,4-D Resistance. Chi square analysis of 2,4-D treated F_2 populations was performed to find the best fit for segregation of 2,4-D resistance. Chi-square was calculated by:

$$\chi^2 = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}} \qquad [1]$$

There was no distinction of resistant and susceptible plants following the 2,4-D application. Therefore, resistance to 2,4-D within F2 populations was qualitatively evaluated on a designated scale of 1to10 with 1 showing no response to 2,4-D (resistant) and 10 showing complete plant mortality (susceptible). Goodness-of-fit for the null hypothesis of 1:3, 1:15, 9:3:3:1 and 1:64 phenotype ratios was determined by chi square (χ^2) test ($\alpha = 0.05$).

Uptake and Translocation of [^{14}C] 2,4-D. The absorption data are expressed as percent of [^{14}C] 2,4-D applied. However, translocation data are expressed as a percent of radioactivity (^{14}C) recovered in plants. Data were tested for normality using PROC UNIVARIATE procedure in SAS[2]. Data were subjected to arcsine square root transformation and outliers were excluded from the data prior to ANOVA to normalize data and improve homogeneity of variance. Transformation improved homogeneity of all data except SB, which were analyzed using non-

transformed data. The sums of squares were partitioned to reflect a split-split plot treatment structure and trial effects using the mixed procedure of SAS. Since the repeated runs of absorption and translocation studies were not significantly different (data not shown) the data from the two trials of each experiment were pooled for each respective study. Trial effects were considered random and statistically significant if p-values ≤ 0.05. Fisher's protected least significant difference (LSD) was used to perform mean separations as appropriate (Steel and Torrie 1980).

Additionally, sum of squares were partitioned to test trial replication and linear, quadratic or higher order polynomial effects of uptake and translocation of ^{14}C in different plant parts for resistant and susceptible plants over time (Draper and Smith 1981). Data were averaged over trial runs because trial-by-trial and trial-by treatment interactions were non-significant. There was a technical problem in biological oxidizer during analysis of translocation data for 48 HAT and accordingly those data were removed from the analysis. Regression analysis was performed and ANOVA indicated higher order polynomial effects for uptake and translocation of ^{14}C among resistant and susceptible plants and their parts over time. Correspondingly, nonlinear regression analysis was executed using PROC NLIN to test correlation of uptake and translocation data with Gompertz three parameter function (Berger 1981):

$$y= a*\exp(-b*\exp(-k*hi)) \qquad [2]$$

Where, y is the response to harvest interval (hi) with a (upper limit of y), b (lower limit of y) and k (rate) as sigmoidal curve variables. If data fit to Gompertz function, an adjusted R^2 value was obtained as in equation 3:

$$R^2 = (CSS-RSS)/CSS \qquad [3]$$

Where, CSS is total corrected sum of squares and RSS is residual sum of squares. Graphs for absorption and translocation response curves were made using SigmaPlot 8.0® (SPSS Inc. 2002)

Results and Discussion

Absorption of [^{14}C] 2,4-D. Greater than 90% of the total ^{14}C applied was recovered from both resistant and susceptible prickly lettuce biotypes at each harvest interval (data not shown). Uptake, expressed as percent of applied [^{14}C] 2,4-D, increased from 11.5 and 9.8% at 1 HAT to 33.8 and 42.8% at 96 HAT in resistant and susceptible prickly lettuce biotypes, respectively (Table 1). Absorption of 2,4-D has previously been reported to range from 18 to 70% depending upon plant species, study duration and environmental conditions (Lym and Moxness 1989; Kohler et al. 2004; Mayeux and Scifres 1980 and Hart et al. 1991). The gradual increase in the absorption of radioactivity over time in each biotype was in accordance with studies conducted by Mayeux and Scifres (1980) and Hart et al. (1991). Both biotypes absorbed similar radioactivity during early harvest intervals. However, statistical differences were observed at 96 HAT, when susceptible biotype absorbed 21 percentage points more ^{14}C compared to resistant biotype. Similar rates of absorption during early harvest intervals suggest that there are no morphological, physical or chemical differences in biotype tissues. Nonetheless, increase in the size of leaf cuticular fissures (due to greater leaf tissue injury) of susceptible biotype in response to the herbicide at the later harvest intervals may have resulted in greater ^{14}C absorption by susceptible biotype compared to resistant biotype. Additionally, the rate of osmosis/penetration of auxin herbicides (including 2,4-D) through leaf surface layers is proportional to their concentration gradient across the layers. Greater radioactivity was translocated out of the treated leaf and less was retained in the treated portion (S) of the leaf at 96 HAT in susceptible biotype

compared to resistant biotype (Table 1). Therefore, greater translocation of radioactivity out of the treated leaf in susceptible biotypes compared to resistant biotype might have decreased the concentration gradient across surface layers which added to differential absorption.

Using the Gompertz equation, regression parameters were determined for percent absorption over time by susceptible and resistant biotypes (Table 2 and Figure 2A). The greater values for upper asymptote (a) in susceptible (36.0%) compared to resistant (47.8%) biotype also suggested greater absorption by susceptible plants over time. Consideration of auxin herbicide absorption as mechanism of resistance is very conflicting in previously reported studies. Kohler et al. (2004) theorized differential absorption as probable mechanism of resistance in 2,4-D resistant ground ivy. In contrast, Hart et al. (1991) rejected differential absorption as a mechanism of 2,4-D resistance in perennial *Glycine* species. Williams et al. (1960) also excluded differential absorption as a reason for 2,4-D tolerance in cocklebur (*Xanthium commune*) and jimsonweed (*Datura stramonium*), and 2,4-D susceptibility in bur cucumber (*Sicyos angulatus*). However, the present study shows that lower absorption of ^{14}C by resistant biotype compared to susceptible biotype could be a reason for reduced response to 2,4-D in the resistant prickly lettuce biotype.

Translocation of [^{14}C] 2,4-D. Analysis of variance indicated no trial by biotype or harvest interval interaction (data not shown); thus data were pooled for the two trial runs (Table 1). Data for 48 HAT gave unreliable results due to technical problem (clogging of filter) in the biological oxidizer at the time of oxidation of samples and therefore, were omitted from the analysis. The ^{14}C translocation out of the treated leaf increased over time for both biotypes (6.4 and 7.8% at 1 HAT to 25.5 and 31.9% at 96 HAT in resistant and susceptible biotypes, respectively), however the rate of translocation out of treated leaf decreased following 24 HAT. Conversely, radioactivity present in treated leaf decreased from 90.0 and 91.8% at 1 HAT to 74.5 and 70.1%

in susceptible and resistant biotypes, respectively. Lym and Moxness (1989) reported a similar rate of ^{14}C translocation when radioactive 2,4-D was applied to leafy spurge. However, Van Eerd et al. (2005) and Weinberg et al. (2006) reported up to 62 and 58% ^{14}C translocation out of treated leaf and up to 6 and 55% ^{14}C accumulation in the treated leaf of resistant and susceptible biotypes, respectively. Susceptible biotype translocated 31 (24 HAT) and 20 (96 HAT) percentage points greater radioactivity out of treated leaf compared to resistant biotype (Table 1). Weinberg et al. (2006) also showed greater ^{14}C translocation from the treated leaf of susceptible compared to resistant hemp-nettle biotypes. The treated leaf was divided into three parts including the leaf portion treated with radioactive 2,4-D (S), portion of leaf outside the treated area towards leaf tip (ST), portion of leaf outside the treated area towards leaf base (SB). The major portion of absorbed radioactivity remained in the 'S' leaf part for each biotype, however radioactivity decreased with time due to translocation. In accordance with the total translocation out of treated leaf, susceptible biotype retained 20 percentage points less radioactivity in 'S' leaf portion compared to resistant biotype at 96 HAT. The portion of treated leaf towards tip showed no difference of radioactivity content between biotypes. However, the 'SB' contained 41 percentage points greater radioactivity in susceptible compared to resistant biotype at 96 HAT. The 'SB' portion of treated leaf was a channel for translocation of radioactivity from 'S' to other plant parts. Therefore, the 'SB' of susceptible biotype temporarily accumulated radioactivity at 96 HAT due to greater absorption into and translocation out of 'S' leaf parts in susceptible biotype compared to resistant biotype at 96 HAT. Treated leaf as a whole did not show statistical difference in the radioactivity between susceptible and resistant biotypes at 96 HAT due to the confounded results in SB. However, if the temporal samplings were prolonged, the excessive radioactivity would likely have translocated out of 'SB'. Bhan et al. (1970) reported continuous

translocation of ^{14}C from the site of application up to 288 HAT, when radioactive 2,4-D was applied to yellow nutsedge. Generally, treated leaves accumulated more and translocated less radioactivity in resistant biotype compared to susceptible biotype.

Most of the absorbed ^{14}C translocated to leaves above the treated leaf and crown for each biotype (Table 1). Nonetheless, the rate of acropetal movement was greater in susceptible compared to resistant biotype. The leaves above treated leaf and crown of resistant biotype accumulated 26 and 22 percentage points less radioactivity, respectively, compared to susceptible biotype at 96 HAT. The crown of the resistant biotype accumulated 53 percentage points less radioactivity compared to susceptible biotype at 24 HAT. Weinberg et al. (2006) also reported 50% less acropetal movement of ^{14}C in resistant compared to susceptible hemp-nettle biotypes. After herbicide treatment, most of the regrowth in resistant prickly lettuce has been reported to occur from lateral or apical meristems located in crown portion of plant (Burke et al. 2009). At 96 HAT, both biotypes showed 2,4-D injury symptoms including epinasty along with cupped and strap shaped leaves, however crowns of the resistant biotype appeared healthier than susceptible biotype (personal observation). Potentially, the healthier crown and greater regrowth of resistant biotype compared to susceptible biotype was due to the less ^{14}C accumulation. Although the crown of resistant biotype had less radioactivity compared to the susceptible biotype, the difference in quantified radioactivity in crown of two biotypes was less prominent compared to the visual crown injury. The greater metabolism of [^{14}C]2,4-D and in turn greater amount of radioactivity in the form of inactive ^{14}C metabolites of 2,4-D in the crown of resistant biotype might be the reason for healthier crown of resistant compared to the susceptible biotype.

Both biotypes accumulated similar radioactivity in leaves lower to the treated leaf, however, resistant plants tend to have more basipetal movement of radioactivity compared to susceptible

biotype following 24 HAT. A very small portion of absorbed radioactivity was quantified in roots. Nevertheless, roots of susceptible prickly lettuce biotype contained 36 percentage points more ^{14}C compared to resistant biotype. Collectively, resistant biotype translocated lesser radioactivity to leaves above treated leaf, crown and roots compared to susceptible biotype.

Data for plant parts showing differences between susceptible and resistant plants were regressed to solve the Gompertz equation and regression parameters were determined for the percent translocation among susceptible and resistant plants over time for different plant parts (Table 2, Figure 2 B-F). The data for roots failed to meet the criterion for sigmoidal curve and the data for 'SB' showed very low correlation with the Gompertz model. Consequently, regression parameters for neither could be computed with certainty. Regression analysis also showed that resistant biotype have greater accumulation of radioactivity in treated leaf (especially middle treated portion) and less translocation out of treated leaf to leaves above treated leaf and crown compared to susceptible biotype over time. Again, suggesting that less 2,4-D translocation to leaves above treated leaf and crown by resistant biotype compared to susceptible biotype can be a reason for 2,4-D tolerance. Translocation data were presented as percent of absorbed ^{14}C. However, 21 percentage points less absorption at the time of maximum translocation to upper leaves and crown (96 HAT) by resistant biotype compared to susceptible biotype might have augmented the tolerance in resistant biotype. Consequently, absorption and translocation appeared to have additive effect on the tolerance shown by 2,4-D resistant biotype.

Autoradiography. The current study showed maximum translocation and absorption of ^{14}C at 96 HAT. Therefore, X-ray autoradiograms of the susceptible and resistant prickly lettuce biotypes were prepared at 96 and 168 HAT to visualize the translocation of radioactivity (Figure 3A-D). As ascertained by quantification of ^{14}C in the translocation study, radioactive 2,4-D

applied to the second fully opened leaf of prickly lettuce plants, showed little basipetal translocation and ^{14}C accumulated mostly occurred in the treated leaf followed by leaves above the treated leaf and in the crown for each biotype and harvest interval. Moreover, negligible amounts of radioactivity translocated to roots. Interestingly, autoradiograms showed results similar to the quantitative translocation study at 96 HAT, where, resistant biotype tended to retain more radioactivity in the treated leaf with translocation being more even throughout the plant compared to susceptible biotype (Figure 3A-B). The susceptible biotype appeared to have accumulated most of the radioactivity into the leaves above treated leaf and crown portion of the plant with little translocation of radioactivity to other parts. Although susceptible biotype absorbed more radioactive 2,4-D at 96 HAT, the low intensity of the radiograph image may have resulted from leakage of radioactivity from injured crown to soil. At 168 HAT, the intensity of the autoradiograms decreased even more for both biotypes (Figure 3C-D). Again, the resistant plants accumulated greater radioactivity in the treated leaf rather than in the crown (especially in SB) compared to susceptible biotype at 168 HAT. Translocation of radioactivity almost stopped in the susceptible biotype at 168 HAT. Being a weak acid, 2,4-D transportation occurs in the phloem resulting in the free proliferation of parenchyma cells lying in the phloem, which ultimately disrupts phloem transport (Eames 1950). Disruption of phloem might be the reason for reduced translocation in susceptible biotype. The resistant biotype showed active acropetal translocation even at 168 HAT, however, as evident from the intensity of the radiograms, much less radioactivity translocated at 168 HAT compared to 96 HAT. The survival of resistant biotype, even after continuous translocation of 2,4-D to meristematic tissues, may be due to the reduced rate of translocation relative to susceptible biotype or greater metabolism of active 2,4-D

to ineffective metabolites followed by translocation of these ^{14}C containing metabolites to the meristem regions .

Inheritance of 2,4-D Resistance. The phenotypic screening of 15 F_1s and RF_1s each confirmed the homozygosity of the parental genotypes as all F_1s and RF_1s showed 2,4-D resistance equivalent or better to the resistant parent (data not shown). These results also suggested a dominant action of the putative resistant gene(s). As the expected (based on monogenic inheritance) two classes were not observed in F2 segregation for the 2,4-D resistance, an arbitrary scale with injury rating values ranging from 0 to 10 was derived and used to characterize 2,4-D resistance in the F_2 population. The visual injury among F_2 plants ranged from no response to complete mortality (Figure 1). Based on the visual injury rating scale, the susceptible and resistant parents rated between 8 to 10 and 1 to 3, respectively. Therefore, all F_2 plants were divided into three groups: resistant with injury rating 1 to 3, intermediate with injury rating 4 to 7 and susceptible with injury rating 8 to 10. Out of the total 191 F_2 plants, 51 (25%) plants had the injury rating between 1 to 3 (highly resistant), 100 (55%) plants had injury rating between 4 to 7 (intermediate resistance) and 40 (21%) plants had injury rating between 8 to 10 (highly susceptible). The chi square analysis of these phenotypic classes suggested a monogenic inheritance (1:2:1) with co-dominant gene action. The phenotypic screening of F_1s and RF_1s suggested that 2,4-D resistant trait is a dominant but the segregation of F_2 plants showed the decrease in magnitude of resistance of plants grouped in the intermediate class.

The resistance to auxinic herbicides in hemp-nettle was reported to have a polygenic control (Weinberg et al. 2006). The above observation can also be explained in physiological terms, where mutations at several loci were reported to be required for the expression of resistance to group-4 herbicides (including 2,4-D), due to their multiple modes of action (see Jasieniuk et al.

1995; Gressel and Segal 1982 for details). Among all herbicide groups, occurrence of resistance is minimal for auxinic herbicides that also suggest the involvement of multiple genes. The traits showing polygenic inheritance usually follow a normal or bell-shaped distribution. The pattern of inheritance observed in the present study did not follow normal distribution but the response ratings of F_2 plants to 2,4-D was a tetramodal curve (Figure 4). Therefore, 2,4-D resistance in prickly lettuce cannot be categorized as a quantitative trait. Additionally, the presence of high number of resistant plants (25%) in F_2 population argues against the polygenic inheritance of 2,4-D resistance. The tetramodal curve suggested that inheritance of 2,4-D resistance might be di-genic. However, based on chi-square analyses, the data did not fit to the di-genic Mendelian segregation ratios of 1:15 and 9:3:3:1 and rather suggested monogenic inheritance with codominant gene action with segregation ratio of 1:2:1. Previous inheritance studies of 2,4-D and picloram resistance (Jugulam et al. 2005) and dicamba resistance (Jasieniuk et al. 1995) in wild mustard also revealed that auxinic herbicide resistance is governed by single dominant gene. The F_1 data clearly shows a dominant gene action for the 2,4-D resistance as all F_1s and their reciprocals were highly resistance. However, the co-dominant segregation (1:2:1) of F_2 plants with decrease in the magnitude of resistance in the intermediate group plants suggests that one or two genes are modifying the effect of single major gene. Thus, inheritance of 2,4-D resistance in prickly lettuce is oligogenic and is governed by single major gene with one or two modifying genes. Moreover, differences in absorption and translocation among susceptible and resistant biotypes observed in radioactive 2,4-D study also suggested involvement of more than one gene in the inheritance of 2,4-D resistance.

Prickly lettuce biotype resistant to 2,4-D absorbed 21percetage points less ^{14}C-2,4-D than susceptible biotype at 96 HAT. Additionally, resistant biotype accumulated greater radioactivity

in the treated leaf and transported less ^{14}C to the younger leaves above treated leaf, crowns and roots at 96 HAT compared to susceptible biotype. Less 2,4-D translocation to the crown with resistant biotype resulted in a healthier crown capable of greater regrowth than in the susceptible biotype. Absorption and translocation occurred actively through 96 HAT in both biotypes. However, autoradiograms revealed that less translocation occurred at 168 HAT in susceptible biotype compared to resistant biotype. Greater phloem damage compared to resistant biotype may have resulted in reduced translocation in susceptible plants at 168 HAT. A future study with extended harvest intervals may be helpful in quantifying the differential absorption and translocation between these two biotypes. Regrowth and recovery in resistant biotype in spite of continuous translocation towards apical and lateral meristems indicate towards metabolism of 2,4-D at later harvest intervals. A metabolism study is necessary to confirm the results of the translocation study since the exact form of radioactivity (active herbicide or inactive metabolite) translocated to the leaves above treated leaf and crown is not clear. Authenticating the results of radioactive study, the inheritance study also revealed that one major with one or two modifying genes govern the inheritance of 2,4-D resistance.

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Sources of Material

[1] Potting media, LC1 Mix. Sun Gro Horticulture Distribution inc., 15831 N. E. 8th St. Suite 100, Bellevue, WA 98008.

[2] SAS software, Version 9.1. SAS Institute Inc., Box 8000, SAS Circle, Cary, NC 27513

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Table 1. Uptake and translocation of ^{14}C in resistant and susceptible prickly lettuce treated with [^{14}C] 2,4-D[a]

Harvest Interval	Biotype	Uptake	Plant Portions								
			S[c]	SB	ST	LL	UL	C	R	TL	TOTL
Hours		% of applied	% absorbed by plant								
1	R	11.5 (1.3)	83.0 (2.6)	3.5 (0.3)	1.5 (0.2)	2.0 (0.5)	1.4 (0.3)	1.5 (0.2)	*1.5 (0.4)	90.0 (1.8)	6.4 (1.0)
	S	9.8 (0.8)	88.0 (1.5)	2.4 (0.1)	1.5 (0.2)	1.7 (0.3)	2.7 (0.6)	1.1 (0.1)	*2.3 (0.6)	91.8 (1.4)	7.8 (1.1)
4	R	15.7 (2.1)	81.4 (3.1)	6.0 (0.4)	3.8 (2.3)	1.8 (0.2)	2.0 (0.3)	1.7 (0.2)	0.3 (0.0)	94.0 (0.3)	5.8 (0.7)
	S	16.2 (2.3)	83.4 (2.3)	6.8 (0.5)	4.3 (2.2)	1.8 (0.4)	1.8 (0.4)	1.5 (0.2)	0.9 (0.5)	94.0 (0.9)	6.1 (0.8)
8	R	16.0 (1.9)	71.6 (4.6)	10.9 (3.2)	1.9 (0.6)	1.9 (0.3)	5.4 (1.1)	2.6 (0.6)	0.6 (0.1)	88.0 (3.0)	11.4 (2.6)
	S	17.2 (1.0)	71.7 (3.4)	14.9 (3.5)	1.8 (0.4)	3.1 (0.5)	7.3 (1.2)	3.0 (0.5)	0.6 (0.2)	86.6 (0.9)	13.6 (0.8)
24	R	27.4 (1.6)	58.6 (4.0)	15.2 (3.0)	2.3 (0.6)	8.0 (2.5)	10.8 (1.2)	*3.7 (0.6)	*0.3 (0.1)	76.3 (3.1)	*21.7 (3.3)
	S	28.8 (3.2)	64.8 (2.9)	9.0 (1.7)	3.1 (1.5)	5.8 (1.0)	13.9 (2.0)	*7.8 (1.7)	*1.0 (0.1)	73.9 (3.6)	*28.5 (3.8)
48	R	30.3 (1.9)	52.8 (3.2)	16.7 (5.0)	3.3 (0.9)	4.8 (1.4)	12.9 (2.4)	*8.3 (1.3)	*1.2 (0.3)	72.8 (2.2)	*27.2 (2.2)
	S	33.1 (2.4)	59.3 (2.8)	20.7 (4.6)	2.4 (0.4)	3.8 (1.0)	13.7 (2.1)	*1.2 (0.6)	*0.7 (0.2)	82.4 (3.7)	*17.6 (3.7)
96	R	*33.8 (2.3)	*59.0 (2.2)	*11.9 (0.6)	3.6 (0.5)	4.6 (0.5)	*11.8 (0.9)	*7.7 (0.6)	*1.4 (0.2)	74.5 (1.5)	*25.5 (1.5)
	S	*42.8 (2.9)	*47.5 (2.3)	*20.3 (3.7)	2.3 (0.2)	3.5 (0.6)	*16.1 (1.8)	*9.9 (0.6)	*2.2 (0.2)	70.1 (2.3)	*31.9 (1.4)

[a]Data (n=8) are means with SE in parenthesis of two trials that were pooled. [b] Data was tested for normality with PROC UNIVARIATE and because data was not normal, all the data were subjected to arcsine square root transformation prior to ANOVA. However, data presented in the table is nontransformed data. Transfromation did not make SB and C data normal and therefore, were tested as such. * Indicates significant difference between resistant and susceptible plant parts at a particular harvest interval using PROC MIXED ($(\alpha = 0.05)$. [c] Abbreviations: S, treated part of treated leaf; SB, base of treated leaf; ST, top of treated leaf; UL, leaves above treated leaf; LL, leaves below treated leaf; C, crown; R, root; TL, treated leaf; TOTL, translocation out of treated leaf.

Table 2. Regression parameters (and standard errors) for uptake and translocation of ^{14}C-2,4-D in resistant and susceptible prickly lettuce[a].

Biotype	Regression parameters	Uptake	S[c]	UL	C	TOTL	TL
		Mean (Standard error)					
Resistant	y	22.59 (1.45)	71.30 (2.51)	6.50 (0.89)	3.67 (0.49)	14.43 (1.69)	83.47 (2.05)
	a	*36.02 (0.82)	*61.89 (1.53)	11.56 (0.43)	7.85 (0.28)	28.87 (0.57)	*87.05 (2.21)
	b	1.22 (0.03)	-0.66 (0.09)	3.13 (0.14)	2.04 (0.07)	2.35 (0.16)	-0.09 (0.03)
	k	0.08 (0.01)	0.39 (0.11)	0.16 (0.01)	0.09 (0.01)	0.10 (0.01)	0.02 (0.01)
	Adjusted R^2	0.72[b]	0.43	0.85	0.81	0.74	0.42
Susceptible	y	25.41 (2.07)	70.78 (3.18)	7.95 (1.16)	4.45 (0.68)	16.62 (1.99)	82.56 (2.07)
	a	*47.57 (1.94)	*49.25 (1.33)	21.92 (2.14)	8.62 (0.41)	34.58 (2.16)	*61.23 (3.44)
	b	1.49 (0.05)	-1.20 (0.23)	4.44 (0.67)	2.97 (0.23)	1.96 (0.07)	-0.57 (0.10)
	k	0.06 (0.01)	0.13 (0.01)	0.16 (0.02)	0.18 (0.02)	0.12 (0.01)	0.08 (0.02)
	Adjusted R^2	0.79	0.76	0.77	0.76	0.9	0.58

[a] The regression parameters were calculated (means with 48 HAT excluded and standard error in parenthesis) using SAS PROC NLIN to solve the gompertz model at 95% level. *The regression parameters for resistant and susceptible plants (uptake) or plant parts (translocation) were significantly different ($\alpha = 0.05$). [c] Abbreviations: S, treated part of treated leaf; SB, base of treated leaf; UL, leaves above treated leaf; C, crown; TL, treated leaf; TOTL, translocation out of treated leaf.

Figure 1. Visual injury rating scale from 1 to 10 based on 2,4-D response of F_2 population at 14 DAT.

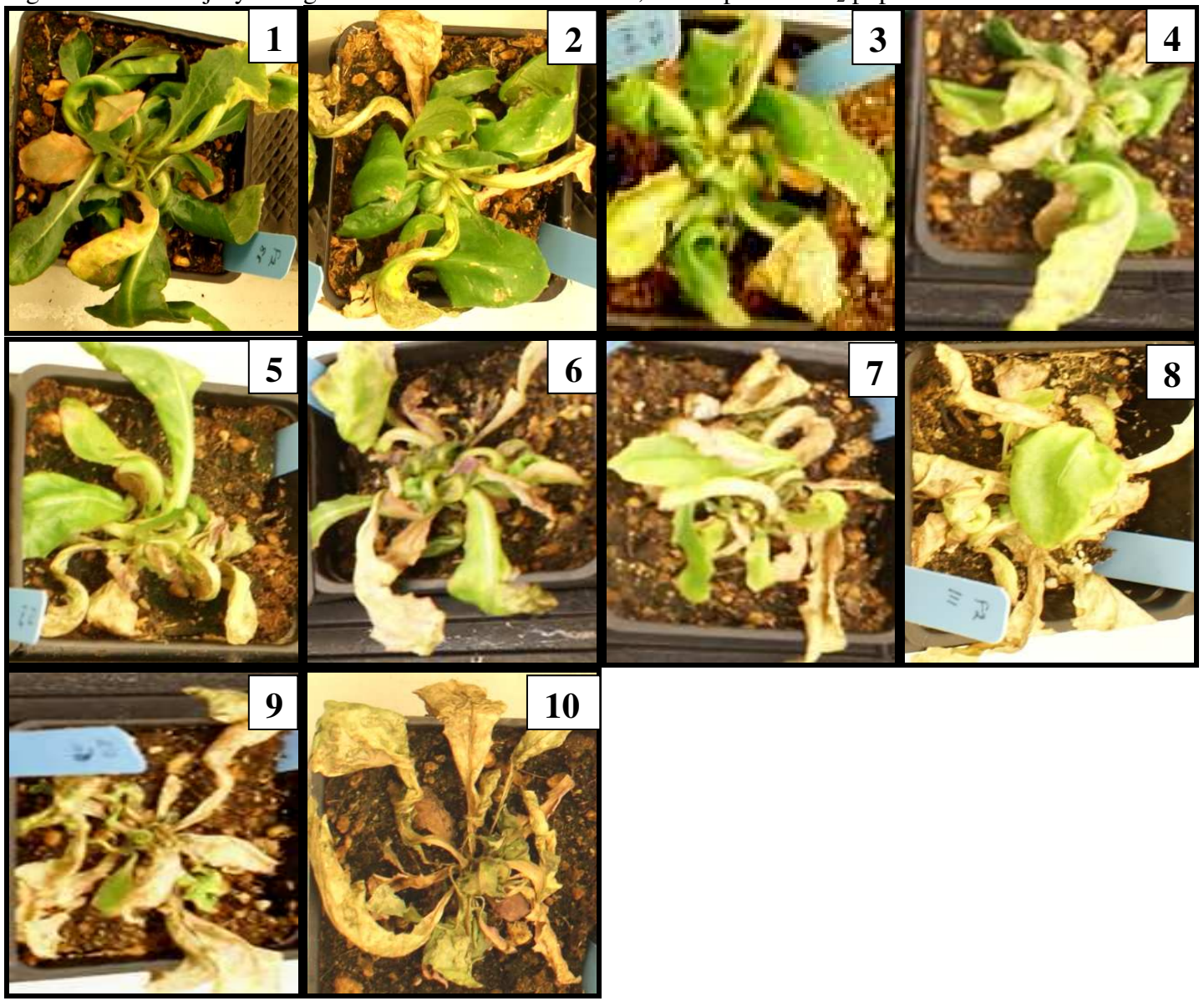


Figure 2(A-G). Uptake and translocation of ^{14}C applied with [^{14}C] 2,4-D in resistant and susceptible prickly lettuce biotypes. (A)Uptake (% of applied). Translocation (B) total out of treated leaf (TOTL), (C) in crown, (D) in leaves above treated leaf (UL) based on % of ^{14}C absorbed. Accumulation in (E) middle treated part of leaf (S) and (F) treated leaf (TL) based on % of [^{14}C] absorbed. Regression curves were fit to gompertz three parameter model excluding 48 HAT treatment.

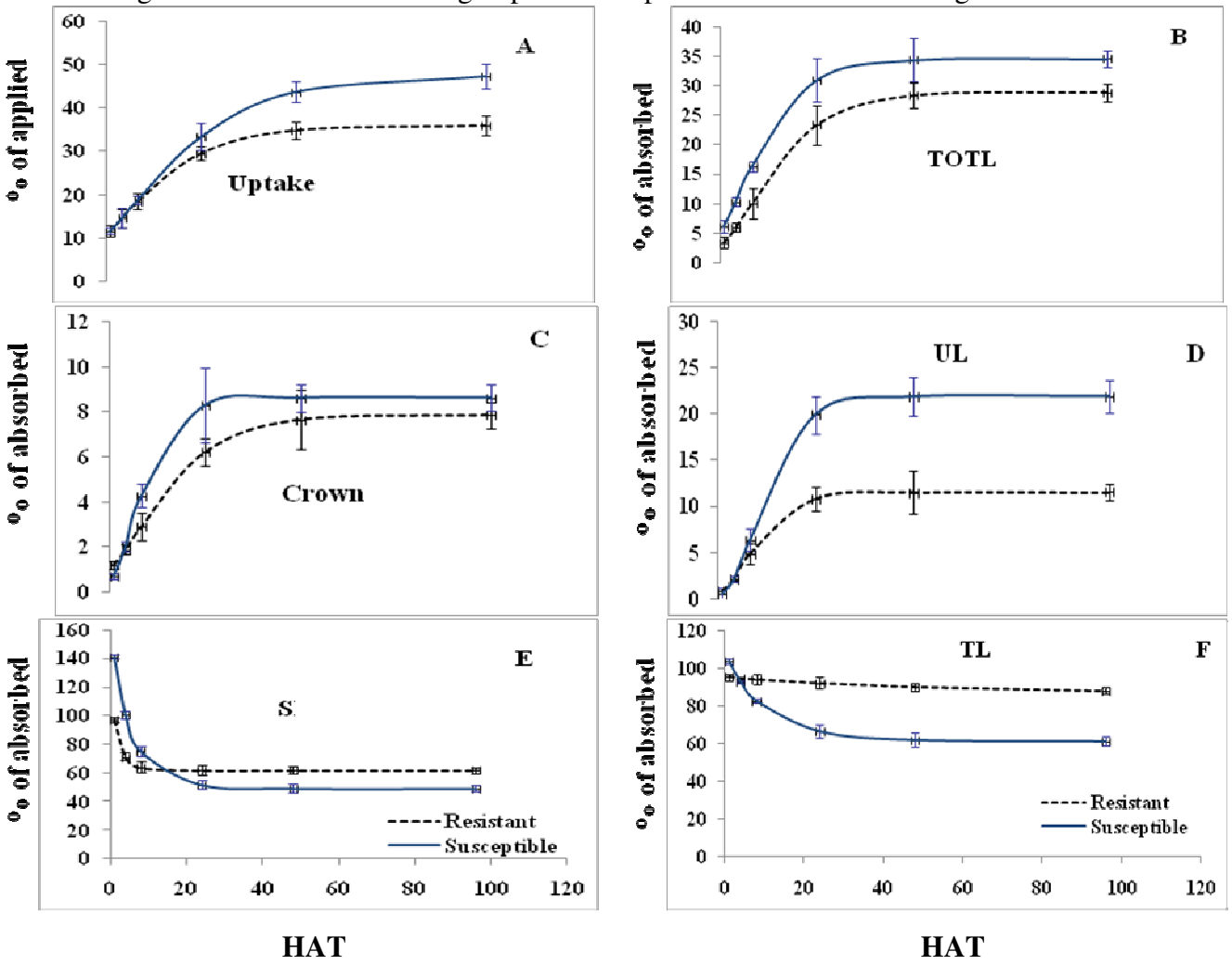


Figure 3 (A-D). Radioautographs showing translocation of [14 C] in susceptible and resistant prickly lettuce biotypes. (A) susceptible biotypes at 96 HAT (B) resistant prickly biotype at 96 HAT (C) susceptible biotypes at 168 HAT (D) resistant biotype at 168 HAT.

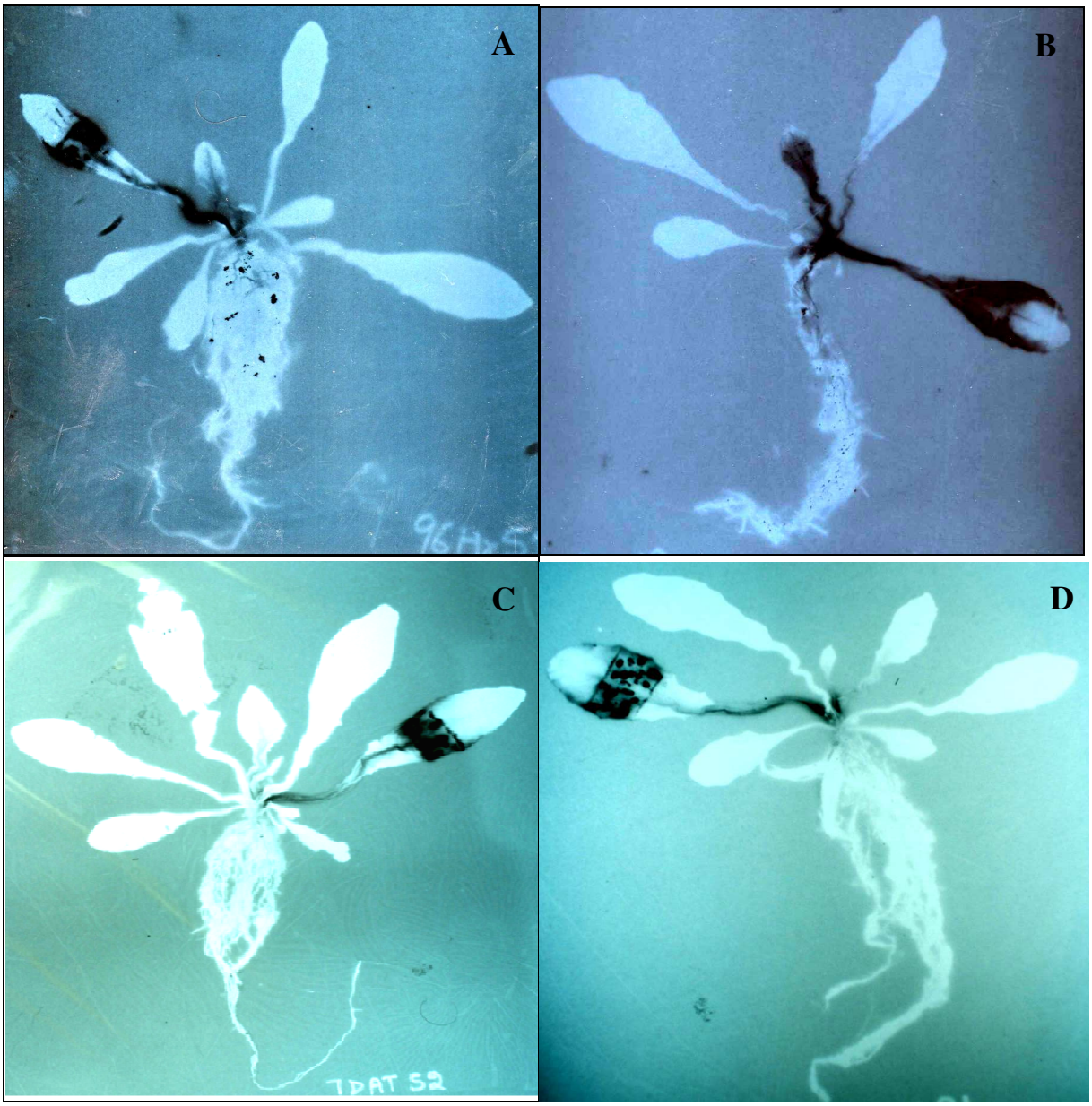
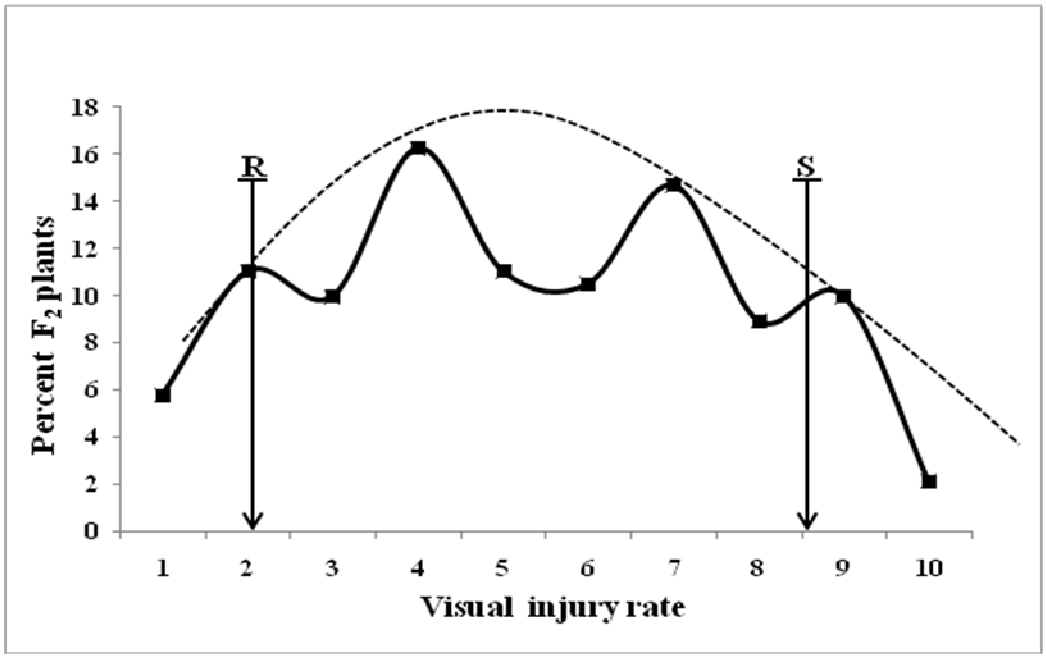


Figure 4. Percent distribution of F_2 plants (n=191) based on visual injury ratings 15 DAT after 2,4-D treatment (0.43 kg ae ha^{-1}). Arrows indicate average injury response of ten resistant (R) and eight susceptible plants (S) at same herbicide rate. Visual injury rate of 1 indicate most resistant and 10 indicate most susceptible plants.



Chapter 5

EST-SSR Development from Five *Lactuca* species and their Use in Studying Genetic Diversity among *L. serriola* Biotypes

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Abstract

Prickly lettuce (*Lactuca serriola* L.) has become a problematic weed throughout Pacific Northwest (PNW) and has recently developed resistance to the synthetic auxin herbicide 2,4-D. In view of non-availability of publically available simple sequence repeat (SSR) markers to tag 2,4-D resistance gene in *L. serriola*, a study was planned to develop SSR markers from expressed sequence tags (ESTs) of five Lactuca species. A total of 15,970 SSRs were identified among 57,126 (26.53 Mbs) EST assemblies belonging to five different species of genus *Lactuca*. The percentage of SSR-containing EST (SSR-ESTs) ranged from 6.23 to 7.87, and SSR densities ranged from 1.28 to 2.51kb^{-1} among the ESTs of five *Lactuca* species. The trinucleotide repeats were the most abundant SSRs detected during the study. As a representative sample, 45 ESTs carrying class I SSRs (≥20 nucleotides) were selected for designing primers, these ESTs were also searched against the dbEST entries for *L. sativa* and *Helianthus annuus* (≤e^{-50} and score ≥100). On the basis of *in silico* analysis of 45 SSR-ESTs, 82% showed conservation across species and 68% across genera. Primer pairs synthesized for the above 45 EST-SSRs were used to study their amplification from an accession each of *L. sativa* and *L. perennis*, and genetic

diversity among a collection of 22 *L. serriola* biotypes. Comparison of the resultant dendrogram to that developed using phenotypic evaluation of the same subset of lines showed only a limited level of correspondence. Taken together this study reports a collection of useful SSR markers for *L. serriola*, confirm usefulness of this approach to quickly isolate SSRs for a species by using EST database, and demonstrate usefulness of these SSRS in various types of genetic studies including genetic diversity.

Abbreviations: AFLP, amplified fragment length polymorphism; ALS, acetolactate synthase; EST, expressed sequence tag; ISSR, inter simple sequence repeat; PNW, Pacific Northwest; QTL, quantitative trait loci; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SFP, single feature polymorphism; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; TRAP, target region amplification polymorphism.

Keywords: EST-SSR, genetic diversity, *Lactuca species*, 2,4-D

Introduction

Lactuca is a genus in the family Asteraceae. There are almost one hundred species in the genus, including economically important weeds and horticulturally important plants. *L. serriola* L. (prickly lettuce; $2n = 2x = 18$; estimated genome size ~1785Mb) is a major weed species of the genus, has wide ecological amplitude and is native to an arid summer Mediterranean climate (primarily between latitudes of 30-55° in the northern hemisphere with growing degree days more than 3000; Gallardo et al. 1996; Prince et al. 1978). *L. serriola* occurs throughout the United States, except northern Maine and southern Florida (Uva et al. 1997, United States Department of Agriculture 1970). More recently, *L. serriola* has emerged as a problematic weed in the dry-land farming regions of the Pacific Northwest (PNW; ~3.6 million acres in Washington and Oregon alone; Schillinger and Papendick 2008). *L. serriola* is a winter/spring annual weed with a deep tap-root system (Jackson 1995), which grows as a rosette of basal leaves during vegetative phase and produces one or more flowering stems at maturity. *L. serriola* has vertically oriented leaves with lamina perpendicular to the east and west intersect. The vertical orientation of leaves allows the plant to reduce water loss without substantial decrease in photosynthesis and also enhances reproduction by positively influencing floral transition and seed set (Werk and Ehleringer 1984, 1986). *L. serriola* is a long-day plant with high reproductive capacity and can produce up to 200,000 seed per plant (Weaver and Downs 2003). The seed have parachute-like appendage (pappus), which facilitates in anemochory. Collectively, all these characteristics help *L. serriola* to endure the harsh environmental conditions and to invade new landscapes. In a recent study in Canada, *L. serriola* (at densities of ≥50 plants m^{-2}) was reported to cause severe yield losses (60% to 80%) in soybean (Weaver et al. 2006). Conversely, no such reduction in cereal or legume yield was reported in Australia, however, Amor (1986) reported

unfavorable effects on grain quality and harvesting efficiency. Fall emerged *L. serriola* usually flowers at the time of crop harvest in the PNW. Consequently, *L. serriola* floral-buds are frequently harvested with cereal grains and are difficult to separate. The latex present in *L. serriola* stems cause economical losses, by clogging the harvest machinery and by raising the moisture content of the harvested grains to unacceptable levels, resulting in a price penalty.

Historically, *L. serriola* has been controlled by a variety of selective or non-selective herbicides. Two major classes of herbicides used for *L. serriola* control are group-2 acetolactate synthase (ALS) inhibiting herbicides (e.g., imidazolinones) and group-4 growth regulator herbicides (e.g., phenoxyacetic herbicides). However, reported resistance to acetolactate synthase (ALS) inhibiting herbicides (Mallory-Smith et al. 1990a; Alcocer-Ruthling et al. 1992) and 2,4-D (Burke et al. 2009) has raised questions about its effective control in the foreseeable future.

L. serriola is a progenitor of *L. sativa* (cultivated lettuce), with the latter being cultivated and consumed throughout the world for its vitamin A and folic acid rich leaves. Significant amounts of genetic/genomic information are now available for cultivated lettuce including a large repertoire of molecular markers, represented by RFLPs (Kesseli et al. 1994), RAPDs (Waycott and Fort 1994), ISSRs (Vicente et al. 2008), genomic/EST-SSRs (van de Wiel et al. 1999; Monteiro et al. 2007; Simko 2009), AFLPs (Koopman et al. 2001; Jeuken et al. 2001; Jansen et al. 2006), TRAPs (Hu et al. 2005), SNPs (Moreno-Vázquez et al. 2003) and SFPs (van Leeuwen et al. 2009). These molecular markers have been effectively used for linkage mapping (Truco et al. 2007), gene-tagging, QTL mapping (Jeuken et al. 2001) and genetic diversity studies (Kuang et al. 2008), which has significantly contributed to our understanding of *L. sativa* genome. Unfortunately, very little genetic/genomic information is currently available, thus making genetic-dissection of complex traits (e.g., herbicide resistance) difficult in *L. serriola*.

Efficient use of wild relatives as a source of desirable genes for cultivated species requires a detailed understanding of their genetic structure and diversity. Understanding the nature, organization, geographical distribution, and differentiation of a wild species is not only critical for their biological conservation but is also important for broadening of the genetic base of the cultivated species (Nevo 1998). For instance, *L. serriola* can serve as the primary gene pool for transferring a number of important traits including resistance for biotic/abiotic stresses and herbicide tolerance to the cultivated lettuce (Mallory-Smith et al. 1990b). A large project of sequencing expressed sequence tags (ESTs) from five lettuce species including *L. serriola*, *L. sativa*, *L. perennis*, *L. virosa* and *L. saligna* was initiated under the auspice of the Compositae Genome Project (CGP) that resulted in more than 200,000 ESTs (CGP; http://compgenomics.ucdavis.edu).

Simple sequence repeats (SSRs) or microsatellites are considered as the markers of choice for most of genetic/genomic application (reviewed in Gupta and Varshney 2000; Ellis and Burke 2007). Availability of a large number of ESTs in the public domain made SSR development even more appealing as EST-derived SSRs (EST-SSRs) or gene-based SSRs (genic-SSRs) are very cost effective, show high reproducibility, and cross-species transferability (Gupta et al. 2003; Gupta and Rustgi 2004). This has made EST-SSRs the best choice for studying genetic diversity and transferability across-species. The objectives of this study were to: (i) screen *Lactuca* ESTs for the presence of SSRs, (ii) study the genetic diversity among *L. serriola* accessions at phenotypic and molecular levels, and (iii) study the transferability of *L. serriola* EST-SSRs to *L. sativa* and *L. perennis*.

Materials and Methods

Plant materials. Seed of 22 *L. serriola* accessions were collected from different locations across eastern Washington during the summers of 2005 to 2007, and *L. sativa* and *L. perennis* seed were procured from Barbara Hellier (USDA-ARS, Washington State University, Pullman). Geographical location and accession number for each *Lactuca* accession are listed in Table 1. A single seed from each of 24 *Lactuca* accessions were planted in the glasshouse maintained at 16 hr light at 400 µmol m^{-2} s^{-1} and day/night temperatures of 22°C and 18°C, respectively, to produce more seed and increase homozygosity. Seed from each accession were harvested separately, and single plants from each accession were grown again in the glasshouse under the same conditions to extract genomic DNA.

Phenotypic data. The experiment was conducted at Cook Research Farm, Washington State University, Pullman (WA) during the growing season of 2007 and 2008. Experimental location is at 765m elevation with an average annual precipitation of ~500 mm. The primary soil type was Palouse silt loam and average length of growing season was 120 to 150 days considering 0˚C as base temperature. The plots were 2 m wide and 10 m long and were arranged in a randomized complete block design with 20 *L. serriola* biotypes as subplots (see Table 1 for collection sites) within 6 whole plot treatments. The study had four replications. Phenotypic characters were recorded on three random plants of each biotype in each of four replications, involving the following 13 traits: herbicide resistance (HR), leaf shape (LS), leaf margin (LM), leaf length (LL in cm), leaf width (LW in cm), leaf pubescence (LP), leaf color (LC), total midrib prickles (TMP), prickles per cm midrib length (PPcm), prickle color (PC), seed color (SC), thousand seed weight (TSW) and plant dry weight at harvest (DWH in gm). The phenotypic data for LS, LM, LP, LC, PC and SC were recorded on the scale of 1 to 10 and for HR were recorded at the scale of 1 to 4 (2,4-D resistance, ALS resistance, resistance to both 2,4-

D and ALS, and resistance for none of the herbicides). The correlations among all the phenotypic traits were calculated and tested at 1% and 5% levels of significance following Mir et al. (2008).

EST analysis to mine SSRs. A total of 175,446 ESTs including 48,748 ESTs from *L. serriola* (assembled into 8,093 contigs and 8,992 singletons), 53,328 ESTs from *L. sativa* (assembled into 7,914 contigs and 13,154 singletons), 25,652 ESTs from *L. saligna* (assembled into 5,225 contigs and 1,266 singletons), 24,257 ESTs from *L. virosa* (assembled into 5,184 contigs and 1,194 singletons), and 23,461 ESTs from *L. perennis* (assembled into 4,876 contigs and 1,322 singletons) were retrieved from the CGP webpage (http://cgpdb.ucdavis.edu/ as on June 9, 2008). The EST assemblies were mined for the presence of all possible combinations of SSR motifs ranging from mono- to heptanucleotide repeats with a minimum length of 12bp using a macro provided by Dr. N.D. Young, University of Minnesota, St Paul (MN). Poly A and poly T repeats present towards the end of EST assemblies were excluded from the further analysis, suspecting those to represent the poly A tails of mRNAs.

Reassembly of SSR-containing ESTs (SSR-ESTs), designing of PCR primers and annotation of selected SSR-ESTs. The SSR-ESTs were reassembled using CAP3 program (http://pbil.univ-lyon1.fr/cap3.php), the redundant entries were excluded from the analysis, and unique entries were selected for designing SSR primers. Out of the selected SSR-ESTs, forty five *L. serriola* sequences carrying the largest di- and trinucleotide SSRs (with ≥20 nucleotides, designated as class I SSRs) were selected for designing SSR primers and putative functions were assigned to these sequences using TBLASTX (score ≥100; E value ≤e-50; http://blast.ncbi.nlm.nih.gov/Blast.cgi) similarity searches. Primers pairs were designed from SSR flanking sequences with the help of PRIMER3 software (http://frodo.wi.mit.edu/), and primers were synthesized from Sigma Aldrich, Inc., USA (Table 2).

DNA extraction and PCR amplification. DNA was extracted from one-month-old seedlings of each of the 24 genotypes following the modified CTAB method (Saghai-Maroof et al. 1984). DNA was treated by RNAase and was purified by phenol extraction (25 phenol: 24 chloroform: 1 isoamyl alcohol, v/v/v) followed by ethanol precipitation (Sambrook et al. 1989). Concentrations of the all DNA samples were adjusted to 50 ng μl^{-1} using *Hind* III digested λ DNA as marker. DNA amplification was carried out using two different assays: the manual sequencing gels with silver staining and the automated ABI 3730XL DNA analyzer. For the first assay technique, PCR reactions were carried out in 10 µl reaction mixtures, each carrying 50 ng template DNA, 0.2 µM SSR primers, 200 µM dNTPs, 1.5 mM $MgCl_2$, 1× PCR buffer and 0.5 U *Taq* DNA polymerase (Life Technologies, New York, USA) using the following PCR profile in a DNA Mastercycler (Eppendorf, Hamburg, Germany): initial denaturation at 95°C for 5 min followed by 35 cycles at 95°C for 30 sec, 60°C for 30 sec, 72°C for 45 sec, and a final extension at 72°C for 10 min. The amplification products were resolved on 10% polyacrylamide denaturing gels (PAGE) followed by silver staining (Tegelstrom 1992). The hundred base pair ladder was used as a size marker (New England BioLabs, Inc., Beverly, USA). The amplified product/allele sizes were determined using Fragment Size Calculator available at http://www.basic.northwestern.edu/biotools/SizeCalc.html.

For the second assay, PCR amplifications were performed in 12 µl reaction mixtures, each carrying 100 ng template DNA, and three different PCR primers [M13F primer (5′-CACGACGTTGTAAAACGAC-3′) labeled with one of the 4 fluorescent dyes (6-FAM, PET, VIC, or NED; Applied Biosystems, Foster City, CA), long forward primer with M13F-tail at its 5′ end and reverse primer]. The PCR mix and cycling conditions were same as described above, except that the PCR mix contained 0.25 µM of each reverse and M13F primers, and 0.0625 µM

of the long forward primer. The PCR products were multiplexed and analyzed using an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA) and GENE MARKER software (Softgenetics, State College, PA).

Polymorphic information content (PIC) and genetic diversity. For each SSR locus, PIC was calculated using equation 1:

$$PIC = 1-\Sigma(Pi)^2 \qquad [1]$$

Where, Pi is the proportion of genotypes carrying the i^{th} allele (Botstein et al. 1980). For dissimilarity analysis, null alleles were scored as zero (0) and other microsatellite alleles (length variants) were each scored in the form of single bands of expected sizes, which were later converted into number of repeat units as allele codes (all modalities were given equal weight during the analysis). Similarly, phenotypic data were recorded at different scales for each of the traits and converted in numerical data to calculate Sokal and Michener dissimilarity indices (di–j) between pairs of accessions (Sokal and Michener 1958): $d_{i-j} = (n11 + n00)/(n11 + n01 + n10 + n00)$, with n11 the number of fragments present in both *i* and *j*, n01 and n10 the number of fragments present in one accession but absent in the other, and n00 the number of fragments absent in both *i* and *j*. From the distance matrix obtained, a non-weighted Neighbor-Joining tree (Saitou and Nei 1987) was computed using the Darwin 5.0 software (Perrier et al. 2003) and branches robustness were tested using 1000 bootstraps. In order to find a minimum set of SSR markers, which can potentially characterize 24 accessions, each marker was successively excluded and patterns of remaining markers were detected. If the remaining markers could still characterize 24 *Lactuca* accessions, the excluded marker was considered redundant. This process was repeated in an iterative manner till a minimum set of SSRs became available (Mir et al. 2008).

Results and Discussion

***In silico* study of EST-SSRs.** The results of in-silico study are listed in Table 3. Only a small fraction of the screened ESTs (ranging from 6.23% in *L. perennis* to 7.87% in *L. sativa*) from the respective species contained SSRs, giving an average density of one SSR every 1.28 kb in *L. sativa* to 2.51 kb in *L. saligna* ESTs (Table 3). The percentage of SSR-containing ESTs (SSR-ESTs) and SSR densities reported in the present study are relatively higher than what was recently reported for *Lactuca* species (4.5% to 4.9% SSR-ESTs and one class I EST-SSR every 14.7kb, Simko 2009; Kumpatla and Mukhopdhyay 2005). However, the values in present study correspond well with that reported by Morgante et al. (2002) where SSR density ranged from $0.74kb^{-1}$ to $2.1kb^{-1}$ for the different plant species including *Arabidopsis* and wheat (*Triticum aestivum*). The observed variations in the frequency of SSR-ESTs and density of EST-SSRs may be attributed to the criteria/parameters used for mining SSRs in different studies (Varshney et al. 2005). In all *Lactuca* species, trinucleotides were the most frequent (ranging from 41.79% in *L. saligna* to 46.13% in *L. sativa*) and heptanucleotides were the least frequent (ranging from 0.95% in *L. virosa* to 1.31% in *L. perennis*) repeat type. The frequencies of the different types of EST-SSRs are summarized in Table 3. A large number of trinucleotide repeats observed during the present study is in conformity with the number of trinucleotide SSRs reported for other plant species (Gupta and Rustgi 2004).

Simple perfect SSRs were classified into two groups: long hypervariable class I (>20 nucleotides) SSRs and potentially variable class II (12–20 nucleotides) SSRs. Number of class I SSRs ranged from 241 in *L. virosa* to 873 in *L. sativa* and class II SSRs from 1501 in *L. perennis* to 4301 in *L. sativa.* In all the five species, most abundant repeat type(s) in class I SSRs were tri- and dinucleotide repeats (frequencies ranged from 28.63% to 36.43% for trinucleotide repeats

and 26.12% to 33.44% for dinucleotide repeats in five *Lactuca* species). Nonetheless, tri-nucleotide repeats solely represented the most abundant repeat type in class II SSRs. The frequency of trinucleotide repeats in the class II SSRs ranged from 46.90% to 52.55% in all five *Lactuca* species. Among the class I SSRs of all five *Lactuca* species, the most and least frequent dinuceotide repeat motif among were AG/TC (42.4%-55.4%) and CA/GT (0.4%-4.0%; Fig. 1a-j), respectively. Nevertheless, several trinucleotide repeat motifs were favored in the class I SSRs of the *Lactuca* species: CTA/GAT (11.3%) in *L. serriola,* CTT/GAA (9.7%) in *L. sativa,* CTT/GAA (12.9%) in *L. perennis,* AGA/TCT (11.1%) in *L. saligna* and ACT/TGA (10.3%) in *L. virosa* (Fig. 1a-j). The least favored motif types were ACG/TGC (0.4%-1.5%) and AGC/TCG (1.4%; Fig. 1a-j). The motif AAG/GAA/AGA represent 60% of all microsatellite motifs of the dicot *Arabidopsis* (Cardle et al. 2000) and are also known to be one of the most frequent motif in the plant genomes (Gupta et al. 1996). The SSRs detected from all five *Lactuca* species also showed a bias towards the A+T-rich repeat motifs (~60.0%) in comparison with the G+C-rich repeat motifs (~40.0%). The above observation is in conformity with the previous reports where, A+T-rich repeat motifs were suggested to be favored in dicotyledon species (Cardle et al. 2000; Mun et al. 2006).

Primer functionality, transferability and polymorphism. The compatibility of marker data obtained from the two genotyping assays was tested using a set of four common markers on both of the systems. No plausible difference was observed in the number and approximate size of alleles among two assays, suggesting that both of the systems can be used in parallel for genotyping. Thus, data from both assays was combined for the analyses. A set of forty-five class I SSRs was selected to design and synthesize SSR primers. When tested with the genomic DNA of twenty-two *L. serriola* biotypes, 40 (88.89%) out of 45 SSR primer pairs amplified products

in the expected size range (±50 bp). The number of functional primers was significantly higher than in a recent study by Simko 2009, where only 71.76% of the tested primers were functional in two *L. sativa* accessions. Out of the forty functional primer pairs, five primers (12.5%; *WSULs1, WSULs25, WSULs43, WSULs158* and *WSULs218*) amplified products larger in size than expected. A possible explanation for this could be the amplification of introns from the genomic DNA (Thiel et al. 2003).

A large number of functional primer pairs also amplified products in the expected range from *L. sativa* (97.5%) and *L. perennis* (85%), suggesting significant amount of cross-species transferability of the EST-SSRs. Very high levels of cross-species transferability was also observed in cereals where 80% of *Hordeum vulgare* EST-SSRs showed transferability to *H. bulbosum* (a wild relative of cultivated barley; Thiel et al. 2003) and 84% of *Triticum aestivum* EST-SSRs showed transferability to 18 species of *Triticum-Aegilops* complex (a group of wild wheat relatives including the progenitors of tetraploid and hexaploid wheats; Bandopadhyay et al. 2004). Transferability of the functional EST-SSRs was also checked at the sequence level by conducting BlastN searches of *L. serriola* SSR-ESTs against the *L. sativa* and *Helianthus annuus* ESTs available in the public domain (total number of ESTs for *L. sativa* = 80,781 and *H. annuus* = 133,682). More than 82% EST-SSRs showed transferability across species and around 68% SSRs showed transferability (based on sequence similarity) even across genera (Table 4; Fig. 2a,b)**.** As expected, the proportion of transferable markers dropped with increase in the phylogenetic distance of *L. serrola* from other species including *L. sativa*, *L. perennis* and *H. annuus* (data not shown).

Relatively greater level of polymorphism compared to other self-pollinating species (in wheat by Gupta et al. 2003; and in Jute by Mir et al. 2008) was observed within (among the *L.*

serriola biotypes) and between species (*L. serriola*, *L. sativa* and *L. perennis*), which is evident from large number of alleles (ranging from 2 to 16 alleles with an average of 7.5 alleles) and high PIC values (ranging from 0.23 to 0.89 with an average of 0.72) recorded for each marker locus (Table 2). A large number of alleles detected could be attributed to the class of SSRs (class I) selected for designing SSR primers. All the functional primer pairs detected variation in length of the SSR tracts (length polymorphism; Fig. 3), whereas five (12.5%) primer pairs also detected variation due to null alleles (+/- polymorphism) in up to 14 of the 22 *L. serriola* biotypes. These cases of null alleles may be attributed either to sequence divergence in the primer binding sites or to an absence of specific SSR loci in respective genotypes (Gupta et al. 2003). Interestingly, all EST-SSRs showed polymorphism within species (discriminated at least two or more *L. serriola* biotypes) and between species (discriminated at least one *L. serriola* biotype from *L. sativa* and/or *L. perennis*). Ninety percent of the functional EST-SSRs also discriminated between *L. sativa* and *L. perennis*. Out of total functional primer pairs, 25% amplified unique alleles from *L. sativa* and *L. perennis*, whereas 75% of primer pairs amplified common alleles shared with *L. serriola* biotypes. Taken together these markers seem quite useful in discriminating *L. serriola* biotypes and other species of the genus *Lactuca*.

Phenotypic variability and interrelationships. Phenotypic data was collected on a number of leaf and seed characteristics on the 20 *L. serriola* biotypes collected from Pacific Northwest of United States. High level of phenotypic variability was observed among the morphological characteristics. Leaf characteristics, including LL, LW, TMP and PPcm varied from 15cm to 28cm (mean 21.5cm), 4.5cm to 13.5cm (mean 8.9cm), 76 to 323 (mean 136) and 4.0 to 17.5 (mean 6.44), respectively. The other visually scored leaf traits, including LM, LP, LC, PC and LS also varied from incised to double serrated, glabrous to very hairy, green to dark bluish green,

white to green/brown and oblanceolate to deep pinnately-lobed, respectively. Similarly, TSW, SC, and DWH varied from 0.25g-0.55g (average 0.38g), sea-green to dark brown and 134g-597g (average 282.6g), respectively. Interestingly, the 20 *L. serriola* biotypes also varied for their response to two herbicide classes. For instance, 45% of the genotypes showed resistance against ALS herbicides, 5% showed resistance against 2,4-D, and 10% showed resistance against both 2,4-D and ALS resistant herbicides. Positive and negative correlations were also observed among a number of phenotypic traits at 1% and/or 5% significance levels (Table 5). Significant positive correlations were observed between the following combinations: TMP-PPcm, HR-LL, HR-LC, and LM-TSW. However, negative correlations were observed between LS-LW, LS-DWH, LL-DWH, LW-SC, and PPcm-TSW. In general, phenotypic traits showed very low level of correlation among each other and biological significance of some correlations is not known yet. All biotypes were grown in an environment different from their natural habitat; therefore, some of the correlations could be due to environmental differences.

Diversity analyses. The genetic relationship among *Lactuca* accessions was evaluated based on the combined profiles of 40 functional EST-SSR markers. The genetic dissimilarity coefficient (GD) values for all possible 276 pairs of genotypes ranged from 0.48 (between accession # 7 and 8) to 0.98 (between accession # 17 and 20) with a mean of 0.78. All 24 accessions were grouped into three clusters (Fig. 4). Each cluster was further subdivided into two sub-clusters. As expected, *L. sativa* and *L. perennis* grouped separately from *L. serriola* accessions in cluster three, but in separate sub-clusters. Interestingly, the clustering pattern observed in the dendrogram corresponded well with the geographical distribution of the *L. serriola* accessions (except for accession 10, 13 and 17). Additionally, nine (# 1, 2, 3, 6, 7, 8, 11, 12, and 22) out of the thirteen accessions in cluster I were resistant to ALS, 2,4-D or both herbicides, whereas,

seven (# 5, 10, 16, 17, 18, 23 and 24) out of eleven accessions in cluster II and III were susceptible to either type of herbicides. Although, most of the *L. serriola* accessions clustered together based on the herbicide resistance trait but none of the phenotypic trait fully explained the clustering of accessions.

Genetic distances among 20 *L. serriola* biotypes were also estimated on the basis of thirteen phenotypic traits (Table 5). The genetic dissimilarity coefficient (GD) values for all possible 190 pairs of genotypes ranged from 0.46 (between accession # 1 and 8) to 1.0 (between 4 different combinations each of accessions 17 and 18 with accessions 2, 4, 11 and 12) with a mean of 0.80. The dendrogram prepared using phenotypic traits grouped twenty biotypes into three clusters (Fig. 5). As in dendrogram based on EST-SSR markers, *L. serriola* accessions partially grouped together based on herbicide resistance or susceptibility. Accessions exhibiting 2,4-D resistance (# 12) and/or resistance against both types of herbicide, ALS and 2,4-D (# 2 and 3) were grouped in cluster I. All the accessions grouped in cluster II (# 1, 6, 7, 8, 19 and 20) exhibited ALS resistance, whereas, all the accessions grouped in cluster III (#14, 16, 17, 18) did not show resistance to any herbicide. However, cluster I included both resistant (resistant to 2,4-D, ALS and both herbicide groups) and susceptible *L. serriola* accessions. The grouping of susceptible accessions (#5, 9, 10 and 15) with 2,4-D and/or ALS herbicide resistant accessions in cluster I indicated that these accessions are morphologically similar to resistant accessions and may evolve resistance to either 2,4-D and/or ALS in future.

The clustering patterns of genotypic and phenotypic dendrograms did not correspond with each other. Additionally, the clustering pattern of the dendrogram prepared using the phenotypic data also deviated from the geographical distribution of the genotypes (Table 1; Fig. 5). Most of the phenotypic traits are influenced by the environmental conditions. Therefore, sole reliance on

the phenotypic traits is not worthwhile for studying plant genetic diversity. Similar conclusions were also reached in other studies, where efforts have been made to compare molecular diversity patterns with those obtained using phenotypic traits (Bar-Hen et al. 1995; Dillmann et al., 1997; Lefebvre et al., 2001; Papa et al., 1998). Markers derived from the expressed regions of the genome such as EST-SSRs/cDNA-SSR, and SRG-RFLPs (stress responsive genes) assay polymorphism that is associated with the coding regions of the genome thus should detect 'true genetic diversity' available inside or adjacent to the genes (Eujayl et al. 2002; Maestri et al. 2002; Thiel et al. 2003). In view of that, the use of molecular markers (especially one derived from the genic-regions of the genome) is more valuable to complement the genetic diversity studies.

Minimum number of SSRs required for assessment of the diversity. While studying the patterns of forty functional SSRs in twenty-four *Lactuca* accessions, we noticed that all of the above genotypes could be discriminated from each other on the basis of these 40 genic-SSRs. It was also noticed that the above 24 accessions could also be discriminated by using as few as four SSRs (*WSULs18, WSULs25, WSULs33* and *WSULs43*). Furthermore, taking the number of alleles for each SSR marker into consideration, these four markers could theoretically give as many as 7290 patterns. Therefore, the number of SSR markers used in the present study was not only adequate, but was rather redundant, since the same information could be derived with a much smaller number of markers. This suggests that the above set of four markers can be used as diagnostic-markers to discriminate 24 *Lactuca* accession belonging to three different *Lactuca* species at the early stages of plant development.

In summary, a large number of EST-SSRs were retrieved from publically available ESTs of five *Lactuca* species. EST-derived SSRs can be easily extracted from EST databases and can be

successfully used along with the other available markers, in *L. serriola* and *L. sativa*. These SSRs are abundant and will have high level of transferability, associated with high intra- and inter-specific polymorphism. Due to the high level of conservation across genera, these markers can be used for comparative mapping and studying phylogenetic relationships among different genera of family Asteraceae. Additionally, EST-SSRs also have a higher probability of being in linkage disequilibrium with genes/QTLs controlling economic traits and can prove relatively more useful for studies involving marker-trait association, QTL mapping and genetic diversity analysis. The genetic diversity analysis using EST-SSRs grouped all *Lactuca* accessions mainly based on species and geographical location, and partially based on herbicide resistance. Common management strategies can be developed in future for the *L. serriola* genotypes with similar genetic makeup.

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Table 1. A list of *Lactuca* genotypes used in the present study.

Sr. no.	Accession no./cultivar	Geographical location	
Lactuca serriola		N	W
1	8-31-06-1*	46.79344	-118.73572
2	9-21-06-1*	46.40518	-117.25440
3	9-21-06-2*	46.44550	-117.43108
4	9-21-06-3*	46.53202	-117.78203
5	9-21-06-5*	46.69213	-117.79233
6	9-21-06-6*	46.78925	-117.62885
7	10-4-06-1*	46.72357	-116.76685
8	10-4-06-2*	46.55337	-116.68963
9	10-4-06-3*	46.52055	-116.66520
10	9-13-06-3*	46.92627	-117.08788
11	9-13-06-4*	46.97620	-117.11992
12	9-13-06-5*	47.08827	-117.16157
13	9-13-06-6*	47.20565	-117.21192
14	10-6-06-1*	46.80349	-119.07489
15	10-6-06-2*	46.72285	-119.03190
16	10-6-06-3*	46.57030	-119.04595
17	10-6-06-4*	46.32177	-119.08208
18	10-6-06-5*	46.21169	-118.96343
19	10-6-06-6*	46.29864	-118.40838
20	10-6-06-7*	46.35657	-117.95143
21	S11	Pullman, WA, USA	
22	R23	Pullman, WA, USA	
Lactuca perennnis			
23	PI 274378	Romania	
Lactuca sativa			
24	Cultivar Baja	Butter head lettuce production regions	

* Biotypes used for phenotypic analyses

Table 2. Details of SSR primers (designed from expressed sequence tags of *Lactuca serriola*) used for studying genetic diversity and transferability among *Lactuca* accessions.

Primer name	Forward primer (5'-3')	Reverse primer (3'-5')	Motif	Product size (bp)	Observed allele size (bp)	Allele no.	PIC value
WSULs1	*AAATCTCCGCCCATTCAGGT	TGGTGTCAAAACCCAATGTCA	$(TC)_{17}$	226	294-326	6	0.78
WSULs4	*ACCCTTTTGCTGCACTCCAC	GAAGCCGCTGTTTCCATCAC	$(TC)_{23}$	226	210-243	10	0.85
WSULs15	*CGGGGCATCACACCTACACT	TTCCAGTGGCACAGACATCAA	$(TTC)_{12}$	204	177-223	9	0.86
WSULs18	*GAAGGTGGTGGGTTGCTGTC	TGGGCAATTGCAGATTGAGA	$(TCT)_{13}$	222	197-226	9	0.84
WSULs25	*GGGGAAGCCAATACAGAATCAA	CCACCAGACCAACTGCTGTC	$(CAA)_{10}$	227	323-390	10	0.85
WSULs33	*TCGGCTTTTCCACGTGATCT	CGGAGGTGTAACCGGACAAA	$(CT)_{13}$	208	192-231	9	0.84
WSULs43	*TATGGTGCCGTGGATTTTCC	CGTCTTTCCATGTTTGCAAGG	$(GAA)_{8}$	232	317-381	9	0.82
WSULs45	*TTGCCACTCGAAGCCTTTACA	CCCTTTGCACCTCCGGTTAT	$(GAT)_{8}$	186	164-191	10	0.87
WSULs49	*AAAGGCTGGACCGATGAATG	TTTGGTTTGGTTTGGCAAGA	$(TCT)_{13}$	202	0-226	8	0.58
WSULs75	*GGATGGTGAGCTGGATCCTTT	AAGTAGCATGGGCGCTGAAG	$(TCT)_{10}$	194	189-233	8	0.74
WSULs76	*GGCCCTGACGAATTGTTGTC	CTCGGCGAGCTTAGCAGTGT	$(CT)_{19}$	206	184-223	11	0.85
WSULs89	*TGCGGTTCTTTTGTGCTGTG	CAGAAACCGATTTCATATCAATACCC	$(TTC)_{11}$	238	234-262	9	0.85
WSULs102	*TCCTTCCTTTGCTTGTTTATTTTGG	TGCAGGGTTTTCAGAACACACATT	$(TC)_{19}$	239	0-285	16	0.89
WSULs103	*GTGGTCAATGCCAACGAATGTA	AGAACAACGCAACGATTGGTCA	$(TC)_{17}$	181	195-226	9	0.84
WSULs120	*CCTAGTGCGTTTTGCTGGGTTT	TTGATCCCACGATCATTCCTGA	$(AG)_{13}$	241	253-286	8	0.86
WSULs126	*GGAGGCCGTGGACGGTAGTA	GAAGAGACCATGCTGCTGCAAA	$(AG)_{11}$	176	195-259	10	0.86
WSULs128	*TTGCATCATCGGTTGTTCTTTG	GTTTTTGCTTCAAAGCCAATTTT	$(TC)_{12}$	233	0-265	7	0.79
WSULs153	*ACCGCTCTCACTCTGAGCACAC	CAGTTTGTGCTAGCGTTTCACCA	$(CT)_{25}$	208	216-253	11	0.87
WSULs155	*GGGGGAGGGGAGACCATTTA	TCAATATGCTGACGTGGCCTGT	$(CT)_{22}$	198	199-238	6	0.73
WSULs156	*ATTGCAAAACCCTGAACCCTCA	AGTAGTTCGGCTCCCCTTCACC	$(TC)_{22}$	219	219-248	8	0.73
WSULs158	*TTGTCCTGTTGGACCTCTGCAC	ATCTGGATCCAACCCATGATCC	$(CT)_{18}$	244	0-477	6	0.79
WSULs159	*GGACTTCACTAGTCGACGACATC	GCTTGTCTTTCCAACCCAAAAG	$(CT)_{17}$	180	196-209	5	0.73
WSULs162	*TTTCTCGCTTTCTCTCCTTTCC	CAAATCTCCACCCCCAAATAGG	$(TC)_{16}$	250	215-294	9	0.79
WSULs163	*ACTTTTGACAGCTTTGAGACTCG	AGGCCATTGATGCCTTTAGTGA	$(GA)_{15}$	194	207-221	6	0.71
WSULs166	*GGCTCTCTGTCCGACTCACG	CCATTTTCCTCTTTCTGCGACAA	$(CT)_{12}$	153	151-188	8	0.77
WSULs167	*CCCTTTGCCTTGTTGATTTTCG	GTAGATCGGAGCAACGGTGGAT	$(GA)_{12}$	185	205-285	10	0.84

WSULs202	*CGGTTTTACCCCTTTGGTGATG	ATCGACCTCGATATGCGACCTT	$(AG)_{17}$	221	197-240	5	0.71
WSULs204	*CAGCAGATAAACGCCACCACTC	CTCCTTGGGAGGTAGCGAAGC	$(CT)_{23}$	198	197-223	9	0.85
WSULs212	*ATGTTGGTGAAGGGATGGAGGA	AAGCCAACCCACCAACAATCTC	$(ATG)_{11}$	246	253-279	7	0.69
WSULs213	*CAGGCTGTTGTGGGTAAACCAG	GCTTTGACTGCAACTCATCTTCA	$(ATG)_{8}$	220	241-250	4	0.66
WSULs214	*GCCTGATGGGTGTTGTCATTTG	CCCAAGACATGATCGCTTAAAAA	$(ATG)_{8}$	177	0-207	5	0.63
WSULs215	*CCTCTTCAAACCTGATCCTCCTG	AAGCGCACCAGTAATCACCACA	$(ATT)_{10}$	237	150-276	7	0.53
WSULs216	*ACGAGCTTCTTGCTTGCGATTT	ATGGACTCCGACTCCTCGTGAT	$(CAT)_{8}$	245	262-272	3	0.35
WSULs217	*TGCCCAATCTCGAGCTTATCCT	TCGCCTCTATTCAACGATTCCA	$(CTT)_{10}$	189	197-232	10	0.82
WSULs218	*GGAGCTGGACCCAAAAGTATGC	AAGCAACAGGAAGAGCGTCTGAA	$(GAT)_{8}$	244	260-271	4	0.23
WSULs219	*ACGGAATGCATCACCACAGAAA	TCCCCATAGTCAAACTGCCTTG	$(GAT)_{8}$	236	300-330	3	0.23
WSULs220	*TCCCTGAAGAAAACGTGAAGGAA	AAACATGACATGGGCTTCCAAA	$(GGT)_{8}$	245	0-272	4	0.61
WSULs302	*CCACCAATTGGGAAGGTTATGG	TTTCCATCAGGAAGAGGGAATG	$(AAC)_{13}$	160	152-184	4	0.55
WSULs303	*CAAACCATCGAAATCCCAAACC	TTGGATCCTGACATATGGGGTCT	$(ATA)_{12}$	153	143-146	2	0.47
WSULs304	*CGGCTCTCTGTGAGTCCAACAT	AGAAAGGCGTCACCTTCCCTTT	$(AAG)_{9}$	196	0-224	6	0.70

*Forward M13 tail, sequence 5′-CACGACGTTGTAAAACGAC-3′

Table 3. A summary of SSRs detected in expressed sequence tags (ESTs) of five *Lactuca* species.

Parameters	Plant species				
	L. serriola	*L. sativa*	*L. perennis*	*L. saligna*	*L. virosa*
Total no. of EST assemblies analyzed	17085	20973	6199	6491	6378
Average size of ESTs (bp)	414.4	334.2	575.9	818.1	558.2
Total no. of SSR containing EST assemblies	3543	4196	1462	1676	1667
Total no. of SSRs	4471	5456	1833	2113	2097
Total no. of simple SSRs	4274 (95.59%)	5175 (94.85%)	1772 (96.67%)	1993 (94.32%)	2011 (95.90%)
Mononucleotide repeats	394 (8.81%)	493 (9.04%)	208 (11.35%)	273 (12.92%)	322 (15.36%)
Dinucleotide repeats	464 (10.38%)	537 (9.84%)	210 (11.46%)	237 (11.22%)	217 (10.35%)
Trinucleotide repeats	2059 (46.05%)	2517 (46.13%)	837 (45.66%)	883 (41.79%)	902 (43.01%)
Tetranucleotide repeats	618 (13.82%)	711 (13.03%)	238 (12.98%)	292 (13.82%)	278 (13.26%)
Pentanucleotide repeats	225 (5.03%)	258 (4.73%)	87 (4.75%)	84 (3.98%)	87 (4.15%)
Hexanucleotide repeats	470 (10.51%)	605 (11.09%)	168 (9.17%)	202 (9.56%)	185 (8.82%)
Heptanucleotide repeats	44 (0.98%)	54 (0.99%)	24 (1.31%)	22 (1.04%)	20 (0.95%)
Total no. of compound SSRs	197 (4.41%)	281 (5.15%)	61 (3.33%)	120 (5.68%)	86 (4.10%)
SSR density (SSRs/Kb)	1.58	1.28	1.95	2.51	1.70

Table 4. A list of *L. serriola* simple sequence repeat-containing expressed sequence tags (SSR-ESTs) showing homology with *L. sativa* and *H. annuus* ESTs.

Primer name	CGP accession #	*L. sativa*			*Helianthus*			Putative function
		EST	e-value	score	EST	e-value	score	
WSULs75	>XLR1_Contig3635	BQ864128	3.00E-115	414	GE505760	9.00E-129	462	Peptide chain release factor 2
WSULs76	>XLR1_Contig1809	DW126962	0	1650	EL513103	0	922	General Regulatory Factor 9
WSULs89	>QGE10H13.yg.ab1	DY984264	0	689	-	-	-	C3HC4-type RING finger
WSULs33	>XLR2_Contig245	DW129161	0	1585	-	-	-	RAB GTPase activator
WSULs45	>XLR2_Contig2336	DY977277	0	1432	-	-	-	Sodium/potassium/calcium exchanger
WSULs18	>CLRX6885.b1_J18.ab1	DW128403	0	1312	EL430276	0	636	DEGP protease 5
WSULs25	>CLRX2404.b1_H01.ab1	DW140690	0	1352	DY932971	6.00E-110	399	DNA cytosine 5-methyltransferase (DRM5)
WSULs1	>CLRX9570.b1_D18.ab1	-	-	-	-	-	-	-
WSULs4	>CLRX5494.b1_L05.ab1	DW136039	0	1541	GE491554	3.00E-148	527	Fragile Fiber 1, microtubule motor
WSULs15	>CLRY3296.b1_O08.ab1	DY975927	0	1397	EL470287	9.00E-79	296	Auxin-regulated protein (IAA8)
WSULs43	>CLRY8337.b2_A22.ab1	-	-	-	-	-	-	-
WSULs49	>QGF25G04.yg.ab1	-	-	-	-	-	-	-
WSULs102	>XLR1_Contig2526	BQ861288	0	713	EL436182	3.00E-107	390	Cyclin D3
WSULs103	>XLR1_Contig3276	DY968805	0	1182	EL430158	4.00E-144	513	Chaperone protein dnaJ
WSULs120	>QGF13N14.yg.ab1	-	-	-	-	-	-	-
WSULs126	>QGF23J15.yg.ab1	BQ849539	4.00E-120	430	-	-	-	-
WSULs128	>QGF10E02.yg.ab1	BQ845954	6.00E-141	500	-	-	-	-
WSULs153	>CLRY6724.b1_G02.ab1	-	-	-	EE634141	7.00E-66	280	Conserved hypothetical protein
WSULs155	>CLRY8316.b2_G16.ab1	DW129433	0	1534	EL470585	0	733	Serine/threonine-protein kinase PBS1
WSULs156	>CLRY3019.b1_F12.ab1	DW132657	0	1503	EL470593	0	874	Conserved hypothetical protein
WSULs158	>CLRY8287.b2_M08.ab1	DW135657	0	1447	DY948718	3.00E-128	461	*Arabidopsis thaliana* homeobox protein 33
WSULs159	>CLRX3832.b1_P22.ab1	DY979245	0	1416	EL463265	0	906	Brix domain-containing protein
WSULs162	>CLRX7506.b1_C06.ab1	DY964450	0	1436	DY939744	5.00E-118	426	Calcium-dependent protein kinase
WSULs163	>XLR2_Contig1938	DY969824	0	1588	EL452860	0	978	Galactose mutarotase-like
WSULs166	>XLR2_Contig2991	DW136617	0	1586	EE645638	0	854	Cellular retinaldehyde binding/alpha-tocopherol transport
WSULs167	>CLRY1587.b1_E13.ab1	-	-	-	-	-	-	-

WSULs202	>QGF10D10.yg.ab1	BQ851086	0	747	EE643810	1.00E-150	535	(E)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase
WSULs204	>XLR1_Contig4364	DY982926	0	783	DY918669	5.00E-61	437	Conserved hypothetical protein
WSULs212	>QGF27H03.yg.ab1	BQ848077	0	760	GE488127	7.00E-102	372	NMDA receptor-regulated protein
WSULs213	>XLR1_Contig565	DW130242	0	913	CD849971	2.00E-135	484	Tubulin alpha-3 chain
WSULs214	>XLR1_Contig3168	BQ862510	0	1211	EE608630	6.00E-131	470	Ran GTPase-activating protein 1
WSULs215	>QGE19M04.yg.ab1	-	-	-	-	-	-	Zinc finger protein
WSULs216	>QGF15N19.yg.ab1	DY981848	0	1124	EL467789	1.00E-74	282	SAUR family protein
WSULs217	>XLR1_Contig3907	DY976755	0	875	EL461667	4.00E-75	284	Zinc finger (C3HC4-type RING finger)
WSULs218	>XLR1_Contig4317	DW129571	0	1380	DY941923	0	930	Ubiquitin conjugating enzyme 9 (UBC9)
WSULs219	>XLR1_Contig4620	DY981274	0	1599	EL457891	0	832	OTU-like cysteine protease family protein
WSULs220	>XLR1_Contig3120	BQ847769	0	814	-	-	-	-
WSULs302	>CLRX4428.b1_G04.ab1	DY969881	0	1570	EE616339	5.00E-106	387	Cyclin-L1, putative
WSULs303	>CLRY7562.b1_C20.ab1	DY978101	0	1388	-	-	-	DNA-binding protein (Transcription factor)
WSULs304	>XLR2_Contig3213	DW139492	0	710	DY948986	3.00E-47	190	-

* CPG = Compositae genome project; http://compgenomics.ucdavis.edu/compositae_index.php

Table 5. Simple correlation coefficients among thirteen phenotypic traits in twenty *L. serriola* accessions.

Trait	LS	LM	LL	LW	LP	LC	TMP	PPcm	PC	SC	TSW	DWH
HR	0.30	0.14	0.53*	-0.11	0.06	0.45*	-0.13	-0.33	-0.36	-0.01	-0.26	-0.38
LS	-	-0.04	0.42	-0.47*	-0.06	-0.07	-0.15	-0.27	-0.41	0.24	-0.21	-0.47*
LM	-	-	-0.18	-0.20	-0.01	-0.27	0.00	0.07	-0.08	0.09	0.52*	-0.12
LL	-	-	-	-0.03	-0.19	0.25	0.19	-0.20	-0.19	0.03	-0.30	-0.51*
LW	-	-	-	-	-0.08	-0.19	0.18	0.19	0.14	-0.49*	-0.10	0.43
LP	-	-	-	-	-	0.17	0.28	0.39	0.22	-0.18	0.24	0.13
LC	-	-	-	-	-	-	-0.01	-0.11	0.21	0.13	-0.18	-0.20
TMP	-	-	-	-	-	-	-	0.92**	0.22	-0.19	0.41	-0.23
PPcm	-	-	-	-	-	-	-	-	0.29	-0.21	0.51*	-0.04
PC	-	-	-	-	-	-	-	-	-	-0.16	0.12	0.37
SC	-	-	-	-	-	-	-	-	-	-	0.21	-0.27
TSW	-	-	-	-	-	-	-	-	-	-	-	-0.12

*P<0.05; **P<0.01

Figure 1a-j. Histograms depicting frequencies of class I genic-SSRs with two different types of repeat motifs (di- and trinucleotide) in (a and b) *L. serriola*, (c and d) *L. sativa*, (e and f) *L. perennis*, (g and h) *L. saligna* and (I and j) *L. virosa*.

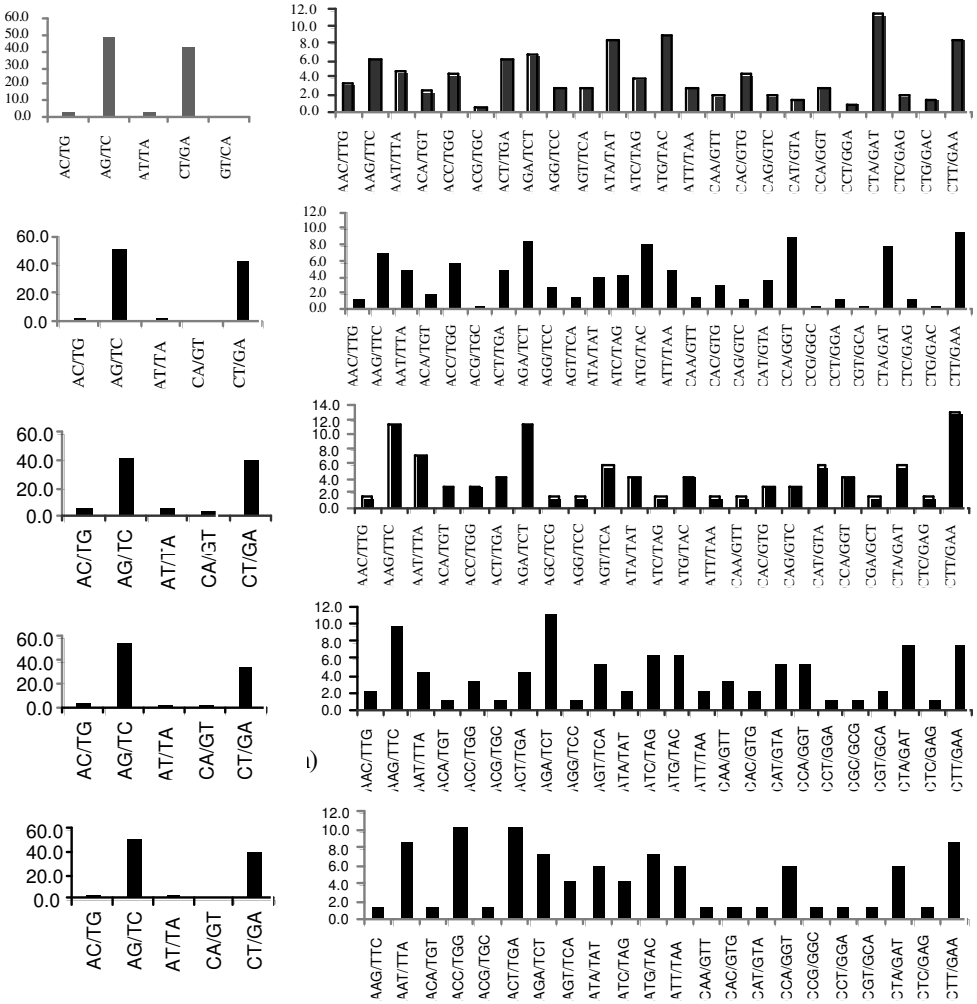

Figure 2a,b. Alignment of sequence among *Lactuca serriola*, *L. sativa* and *Helianthus annuus* (a) *DRM5* and (b) *IAA8* (only a part shown). The red bar indicates SSR containing region in the sequences.

Figure 3. PCR amplification profile of WSULs18 showing a range of alleles in 22 *Lactuca serriola* accessions and transferability to one accession each of *L. sativa* and *L. perennis* (see Table 1 for genotypes details).

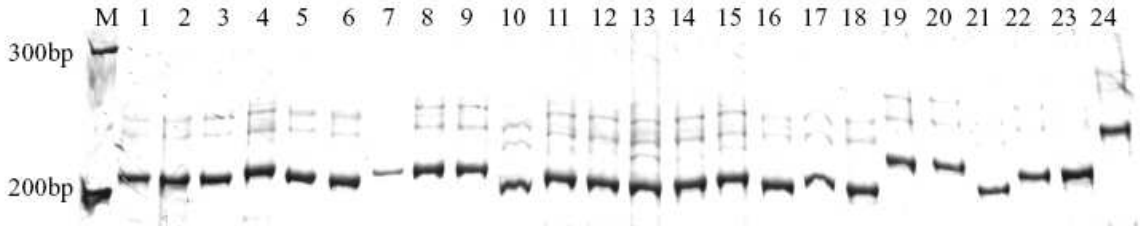

Figure 4. A dendrogram showing clustering pattern of 24 *Lactuca* accessions (see Table 1 for genotype details) based on genetic distances estimated from SSR polymorphism. Bootstrap values (>20) are indicated at different nodes.

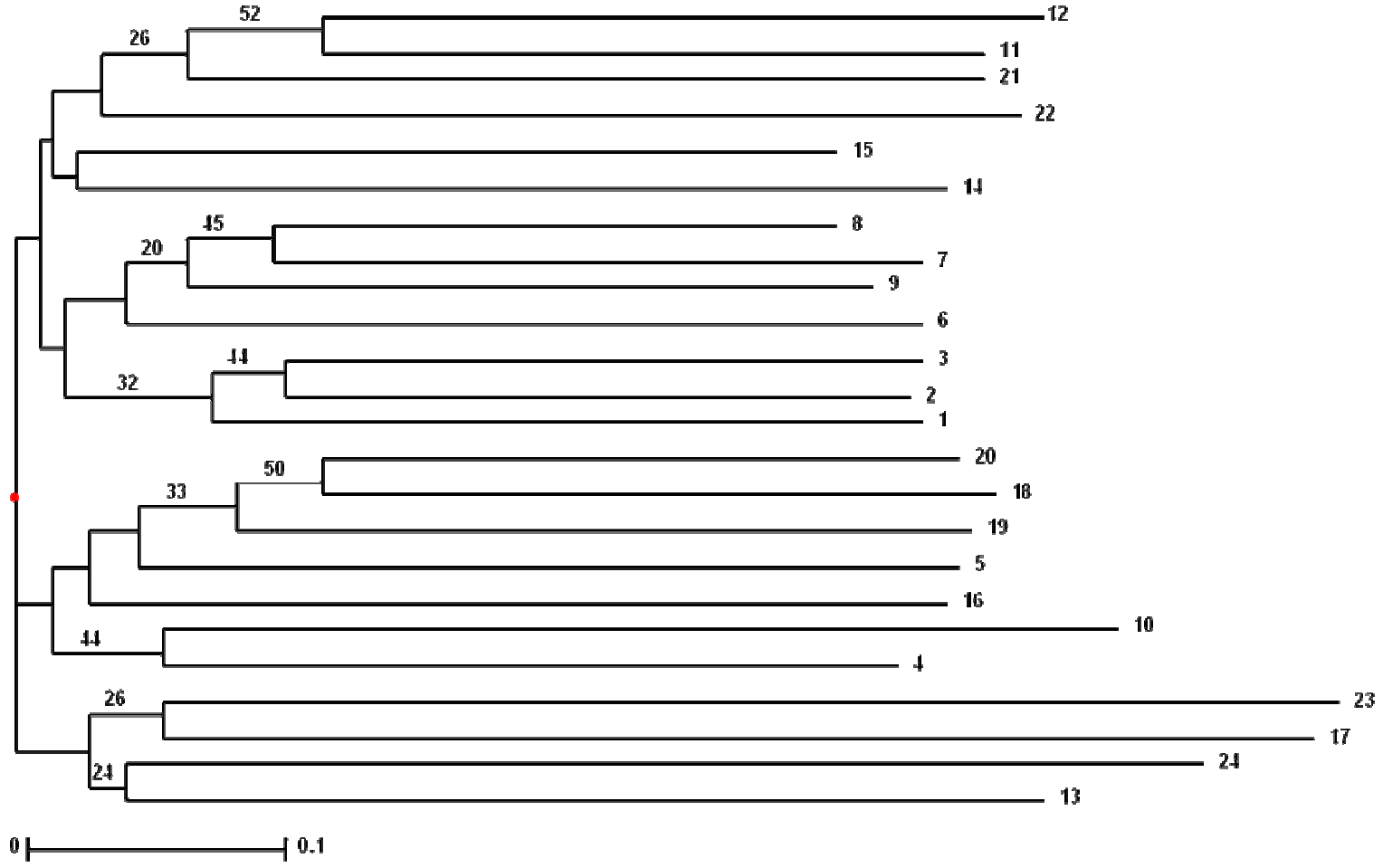

Figure 5. A dendrogram showing clustering pattern of 20 *Lactuca* accessions (see Table 1 for genotype details) based on genetic distances estimated from phenotypic variability. Bootstrap values (>20) are indicated at different nodes.