

AN ABSTRACT OF THE THESIS OF

Blake D. Kerbs for the degree of Master of Science in Crop Science presented on November 17, 2016

Title: EXPANSION OF SCOURINGRUSH (*EQUISETUM* SPP. L.): CROP INTERFERENCE AND CONTROL OPTIONS IN WINTER WHEAT (*TRITICUM AESTIVUM* L.) AND CHEMICAL FALLOW CROPPING SYSTEMS

Abstract approved:

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Scouringrushes (*Equisetum hyemale* L.; *E. xferriisii* Clute; *E. laevigatum* L.) are ancient perennial seedless vascular plants historically associated with wetlands, low-lying roadsides or field margins with more plant available water. There has been little research conducted on scouringrush species in the context of agricultural production because traditional farming practices confined them to field margins and roadside depressions. An increasing amount of dryland winter wheat (*Triticum aestivum* L.) hectares in the inland Pacific Northwest have replaced summer tilled-fallow rotations with chemical fallow. Where chemical fallow rotations have become the standard practice, scouringrush has expanded out of its historical habitat into production fields and established at high enough densities to cause concern from growers. Research was conducted to identify control options that fit chemical fallow cropping systems, evaluate the magnitude of crop interference by scouringrush, and address how soil pH affects scouringrush growth and establishment, as soil acidification is another agronomic issue caused by intensive wheat production in the Pacific Northwest. Field studies located in Reardan, WA, and near The Dalles, OR, were established in commercial wheat production fields that evaluated 10 herbicide treatments for efficacy on scouringrush. An additional factor in the trials was to determine if pre-herbicide mowing affected herbicide efficacy. At both locations pre-herbicide mowing had no effect on efficacy and only chlorsulfuron plus MCPA-ester controlled scouringrush through the subsequent winter wheat rotation. A third herbicide trial determined that triclopyr or increased rates of chlorsulfuron plus 2,4-D and dicamba or asulam were able to effectively control scouringrush seven and 10 months after treatment at a non-crop site in eastern Oregon. Under field conditions wheat yield reductions were correlated with increasing scouringrush density, but in a controlled

study scouringrush density had no effect on winter wheat development or grain yield. Disagreement between these results is hypothesized to be a function of nutrient deficiencies within production fields. Results from three greenhouse studies showed that scouringrush biomass production increased as soil pH increased from ≈ 4.6 to ≈ 8.0 and that scouringrush was able to establish and survive in soil pH conditions that are unsuitable for winter wheat production.

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OPTIONS IN WINTER WHEAT (*TRITICUM AESTIVUM* L.) AND CHEMICAL FALLOW CROPPING
SYSTEMS

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I understand that my thesis will become a part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Blake D. Kerbs, Author

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Dr. Andrew G. Hulting advised all aspects of research completed in this thesis and was the primary contact for all revisions. Dr. Drew Lyon was the lead researcher for the Reardan, WA field trial completed in 2014-2015. Kyle Roerig and Henry Wetzel were involved with establishing and conducting field trials.

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EXPANSION OF SCOURINGRUSH (*EQUISETUM* SPP. L.): CROP INTERFERENCE AND CONTROL OPTIONS IN WINTER WHEAT (*TRITICUM AESTIVUM* L.) AND CHEMICAL FALLOW CROPPING SYSTEMS

CHAPTER 1: Introduction

Biology and Ecology of Equisetum Species

The horsetail family (Equisetaceae) is an ancient group of plants estimated to have been in existence for the last 400 million years. There are 15 remnant horsetail species divided into two subgenera, *Equisetum* L. and *Hippochaete* L. Scouringrush is a perennial *Hippochaete* species that is distinguished by its hollow segmented shoots that remain photosynthetically active through multiple growing seasons. The evergreen, perennial nature and lack of nodal branching are characteristics that separate scouringrush from field horsetail (*Equisetum arvense* L.). Shoots of scouringrush arise from an extensive system of terraced rhizomes, which is unique to the horsetail family. Reproduction is primarily asexual with new shoots emerging as rhizomes grow through the soil, but sexual reproduction is possible with spore bearing cones borne at the ends of fertile shoots (Husby, 2013).

Equisetum species are the only terrestrial plants that require silicon as an essential nutrient (Epstein, 1999), which results in a strong accumulation of silica in *Equisetum* plant tissue. Lignin functions as a binding agent for cell wall components and is the foundation for mechanical strength in most terrestrial plants. A unique characteristic to horsetail species is the mechanical strength of their stems is a result of silica comprising the outer most layer of the epidermis, which does not occur in any other terrestrial plant family (Yamanaka et al., 2012).

Prior to agricultural influences, scouringrush was primarily a wetland species, but the species can become a weed associated with irrigation ditch banks and roadside depressions. Associations with these areas are not surprising as they both may contain a higher amount of plant available water than adjacent agricultural fields (Rutz and Farrar, 1984). Bare soil exposed to sunlight and high water content are requirements for sexual reproductive processes in *Hippochaete* species. Though spores for scouringrush are larger and more robust than their *Equisetum* counterparts, the window for gametophyte formation is narrow. Spores can germinate within 24 hours after being released from the cone and are viable for up to 17 days depending on the water content of the soil where they land (Hauke, 1963). Sexual reproduction

requiring a narrow window of environmental conditions results in the rhizome system being the essential mechanism for scouringrush reproduction, establishment and spread (Husby, 2013).

There is considerable overlap in habitat ranges between *Hippochaete* species. In the Pacific Northwest there are three species typically found in agricultural areas: *Equisetum hyemale* L. (scouringrush), *Equisetum laevigatum* L. (smooth scouringrush) and a sterile hybrid of the two, *Equisetum x ferrissii* Clute (intermediate scouringrush). These species are all perennials, but the growth characteristics of their above ground shoots differ. *Equisetum hyemale* shoots are evergreen with persistent reduced leaves and basal and apical black bands at each node. Of the three species *E. hyemale* is typically larger and found in the wettest sites. *Equisetum laevigatum* is generally smaller with deciduous aerial stems containing only an apical black band at each node, and can be found in drier sites. Where *E. hyemale* and *E. laevigatum* hybridize, the progeny of the two species is a true intermediate in morphology, habitat preference and life cycle characteristics. The lower portions of shoots are evergreen while the top portion is deciduous. Each node has persistent reduced leaves with a basal black band. *E. x ferrissii* is not found at the upper or lower soil moisture limits of the genus. Hybrids produce a cone bearing only nonviable spores (Hitchcock and Cronquist, 1973; Rutz and Farrar, 1984; Husby, 2013).

Though the habitat range for *E. hyemale* and *E. laevigatum* is broad and overlapping, their ecological niche for sexual reproduction is narrow. Both species require bare continually moist soil for fertilized gametophytes to complete their development cycles. For example, Irrigation ditches and streams with cut banks exposing bare soil are sites where sexual reproduction is possible. At sites suitable for sexual reproduction where both species are present, hybridization between the two species is common because the chromosome number is uniform throughout the subgenus ($n=108$) (Husby, 2013). High densities of *E. x ferrissii* have been identified in agricultural production fields near The Dalles, Oregon. An increased abundance of this sterile hybrid species suggests hybridization contributes to the expanded role scouringrush plays as an agricultural weed.

Agricultural Tillage Practices and Scouringrush Expansion

Conventional tillage practices can set the stage for serious problems in modern agriculture. Exposed soil is prone to erosion and compaction. Poor water retention and increased pollution from off-site movement of fertilizers and pesticides are other issues that arise often with conventional tillage regimes. Despite high up-front cost and a demanding integration process, no-till and conservation tillage farming practices have been successful for mitigating soil degradation and pollution issues caused by conventional tillage. Any management decision is going to have trade-offs, and unexpected shifts in weed species composition are a consequence of no-till integration into cropping systems (Huggins and Reganold, 2008). Scouringrush invading production fields from roadsides and ditch banks has been a weed species shift linked to removing tillage from corn and soybean crops in the Midwestern United States (Hartzler, 2009). Similar to the Midwest, an increased density of scouringrush has been in eastern Oregon and eastern Washington dryland winter wheat and chemical fallow cropping systems. Despite scouringrush being a common agricultural weed of increasing concern to growers, quantifiable data are lacking on how scouringrush may interfere with wheat physiological processes and impact grain yield.

While tillage is what likely kept scouringrush confined to field margins, it could also be a contributing factor to its increased presence in some production fields. All horsetails have aerial shoots and rhizomes that are well adapted to reestablish following disturbance. Rhizome fragments as small as 1 cm have the ability to sprout at burial depths up to 30 cm (Cloutier and Watson, 1985). Each aerial node has root and bud promordia that permit rapid reestablishment following partial or complete burial. Adaptation to burial gives scouringrush a competitive advantage not only in roadside depressions and ditch banks where sedimentation rates are high, but in conventionally tilled fields where plant material is fragmented, relocated and buried (Husby, 2013). By monitoring field horsetail growth, Cloutier and Watson (1985) calculated that with two foci containing ten 15 cm rhizome fragments each, only five years was required for the infestation to spread to one hectare. In a cropping system where tillage is removed, years of spreading rhizome fragments throughout fields with tillage implements has likely created potential for dense patches of scouringrush to establish similar to observations of field horsetail (Cloutier and Watson, 1985).

Chemical Control

Herbicides play an essential role in conservation and no-till cropping systems where tillage regimes that control weeds in summer fallow are replaced with herbicide applications (Huggins and Reganold, 2008). Typically three herbicide applications are made through a typical chemical fallow rotation with glyphosate being the most common treatment (Lutcher, 2015). In the Columbia Plateau region of eastern Oregon, 1.8 million hectares of farmland are under a winter wheat followed by summer fallow rotation. Weed control is not only important in the winter wheat crop but also in the summer fallow rotation where winter precipitation and plant essential nutrients are conserved for the next winter wheat planting (Machado, 2004).

In this production system, where herbicides are a key management tool, expanding populations of scouringrush are cause for concern as they are tolerant of herbicide applications and few herbicides with activity on scouringrush are available for use in winter wheat and chemical fallow. For example, chlorsulfuron is a herbicide registered for use in wheat and chemical fallow, but has poor activity on scouringrush at labeled application rates. Scouringrush was controlled by chlorsulfuron for more than one year when applied at 0.07 kg ha^{-1} , which is 10 times the labeled rate in wheat (Bernards et al., 2010; Frasure and Bernards, 2011; Finnerty and Glaser, 1980; Yoder et al., 1983). Studies completed using ^{14}C -glyphosate to describe mechanisms causing poor herbicide efficacy on field horsetail demonstrated glyphosate is slowly absorbed and only small amounts are translocated after absorption (Coupland and Peabody, 1981). It is likely a similar mechanism is present in scouringrush species.

Outside winter wheat fields and chemical fallow there are more herbicides available for scouringrush control. A majority of these options are either plant growth regulators (Group 4) or acetolactate synthase (ALS) inhibiting (Group 2) herbicides. In addition, glyphosate (Group 9) has been found to be effective when a 360 g/L concentrate was wiped onto individual shoots using a sponge. All shoots wiped with glyphosate were controlled with no new shoots emerging 104 days after treatment (Ainsworth et al., 2006). MCPA dimethylamine salt applied at 4.5 mL/L with and without an organosilicone surfactant also controlled all treated shoots with no reemergence 104 days after treatment (Ainsworth et al., 2006). Picloram and a tank mix of chlorsulfuron and triclopyr have also been shown to

provide at least 90% control of scouringrush (Wood and Johnston, 1976; Yoder et al., 1983). Application timing, spray coverage and the use of surfactants are all contributing factors to herbicide efficacy on scouringrush (Yoder et al., 1983).

Soil Acidification

Soil chemical properties likely contribute to scouringrush expansion in winter wheat chemical fallow cropping systems. Nitrogen fertilizers are a known contributor to soil acidification. Depending on the nitrogen fertilizer source, a minimum of one hydrogen ion is released into the soil solution following fertilization. Organic nitrogen, anhydrous ammonia and urea consume one hydrogen ion in the conversion to ammonium increasing soil pH. In the conversion of ammonium to nitrate, two hydrogen ions are released, which over multiple growing seasons will acidify soils. A general assumption for this process in western Oregon is that for every 112 kg of ammonium applied per hectare, a 0.1 unit decrease in soil pH will occur. Eastern Oregon soils can have less buffering capacity thus likely increasing the rate of acidification (Hart et al., 2013). Integration of no-till principles can expedite soil acidification due to increased nitrogen inputs required immediately following adoption (Huggins and Reganold, 2008).

In wheat-fallow crop rotations with reduced tillage, soil acidification is an important factor to consider. Wheat is sensitive to soil pH, and yield loss can occur when soil pH drops below 5.4. Fertilizer is commonly top-dressed in conservation and no-till systems creating stratified pH zones in the soil profile where the top 8 – 15 cm becomes more acidic than the underlying horizons (Hart et al., 2013). A characteristic that has influenced the ability of scouringrush to survive for hundreds of millions of years is its adaptation to thrive in a wide range of environments and soil conditions (Husby, 2013). Schroeder et al. (2012) described soil pH as having no influence on the distribution and abundance of scouringrush on New Mexico canal banks, but data are lacking on the extent that soil pH effects establishment and growth habits of scouringrush. With wheat having a threshold soil pH of 5.4 and scouringrush potentially growing independent of soil pH, it is possible that the influence of agricultural practices on soil pH is contributing to expanding scouringrush populations.

Summary

Scouringrush species are ancient plants native to North America. Their ecological niche, specifically in wetland environments is well understood. In contrast, very little knowledge exists about how scouringrushes fit into cropping systems as a weed species. Foundational work is needed to understand how agricultural practices have created a window for scouringrush to expand its role as a weedy species and what effect, if any, scouringrush plant density has on wheat yield in direct seeded winter wheat-chemical fallow-winter wheat cropping systems. There is also a need to identify herbicides with activity on scouringrush to help growers manage a weed that will likely continue to cause issues in direct seeded winter wheat production. The following research was conducted to address these questions and serve as a starting point to gain a more complete understanding of how scouringrush impacts winter wheat and chemical fallow cropping systems.

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CHAPTER 2: Scouringrush (*Equisetum* spp. L.) Control in Pacific Northwest Direct Seeded Dryland Winter Wheat (*Triticum aestivum* L.) Cropping Systems

ABSTRACT

The adoption of chemical fallow rotations in Pacific Northwest dryland winter wheat (*Triticum aestivum* L.) production has caused a weed species composition shift where smooth and intermediate scouringrush (*Equisetum* spp. L.) have established in production fields. Thus, there has been interest in identifying herbicide treatments that effectively control smooth and intermediate scouringrush in winter wheat-chemical fallow cropping systems. Trials in commercial grower fields were established in Reardan, Washington (2014) and The Dalles, Oregon (2015). Ten herbicide treatments were applied to mowed and non-mowed plots containing naturally established smooth or intermediate scouringrush during chemical fallow rotations. Treatments were evaluated through the subsequent winter wheat rotation. Mowing was applied to evaluate what effect, if any, pre-herbicide application mowing has on herbicide efficacy. Smooth and intermediate scouringrush stem densities m row^{-1} were quantified the following spring and after wheat harvest at both locations. Chlorsulfuron plus MCPA-ester was the only treatment that resulted in nearly 100% control of scouringrush through wheat harvest. Pre-herbicide mowing had no effect on efficacy. All herbicide treatments had no effect on wheat yield at either location; including where chlorsulfuron plus MCPA-ester reduced smooth scouringrush stem density to > 2 stems m row^{-1} from nearly 50 stems m row^{-1} at Reardan, WA, and intermediate scouringrush to 0 stems m row^{-1} from nearly 20 stems m row^{-1} at The Dalles, OR. Results from these trials suggest chlorsulfuron plus MCPA-ester would be a commercially acceptable treatment for smooth and intermediate scouringrush control in winter wheat-chemical fallow cropping systems. However, the apparent lack of positive yield response by controlling smooth and intermediate scouringrush should factor into management decisions as including an additional acetolactate synthase (ALS) inhibiting herbicide in chemical fallow rotations would increase selection pressure for ALS resistance.

INTRODUCTION

The inland Pacific Northwest (PNW) is an important region for dryland agriculture in the western United States. Of the 4,377,500 hectares estimated to be under dryland production in the region, 76% of the hectareage are in the inland PNW (Schillinger et al., 2003). Agronomic practices in the region are largely dictated by precipitation (Leggett, 1959). Prevailing westward winds carry frontal weather systems off of the Pacific Ocean into the Cascade Mountains of western Oregon and Washington, causing a rain shadow effect over the inland PNW. These macro-weather trends result in a semiarid Mediterranean climate where 66% of annual precipitation occurs between October and March, and high-pressure systems create warm and dry conditions during the summer months (Schillinger et al., 2003). Within the region, hectares under dryland production are generally classified into 3 precipitation zones: (i) low, < 300 mm year⁻¹; (ii) intermediate, 300-450 mm year⁻¹; and (iii) high, > 450 mm year⁻¹ (Schillinger and Papendick, 2008).

Growers in low and intermediate precipitation zones have historically relied on a winter wheat (*Triticum aestivum*) followed by summer fallow cropping rotation where annual crop production is limited or poses economic risk (Schillinger et al., 2008). Fallowing production fields provides some economic utility wherein more stable grain yields are achieved on a biennial basis (Juergens et al., 2004) by allowing winter precipitation storage, adequate time for nitrogen and sulfur to mineralize and for effective weed control (Machado et al., 2015). However, benefits of conventional summer fallow can be offset by soil erosion and depletion of soil organic carbon (Camara et al., 2003; Unger et al., 1971; Williams, 2008). Eight or more passes with tillage implements including: cultivators, chisels and disk plows are common during a 13-month conventional summer fallow rotation (Machado et al., 2015; Schillinger et al., 2008). As a result of multiple passes with tillage implements, soil aggregation is reduced and fine soil particles are prone to removal by wind at drier locations, and water through freeze thaw cycles at sites with higher soil moisture (Williams et al., 2014).

Although there has been some resistance to adopting new on-farm practices, conservation/reduced tillage and no-till cropping systems have been gaining acceptance among PNW growers (Huggins and Reganold, 2008). Reducing and/or removing tillage from 2-year wheat-fallow systems has reduced soil erosion and decreased fossil fuel inputs (Veseth, 1988), but has brought substantial changes to weed

management regimes. Intensive tillage practices involved in conventional fallow rotations have offered effective and relatively simple weed control for inland PNW wheat growers. When tillage is removed from the system, weeds are managed through a chemical fallow strategy where successive selective and nonselective herbicide applications replace conventional tillage (Jemmett et al., 2008; Wicks and Smika, 1973). Fall planted crops are then direct-seeded into previous crop residue, omitting all pre-plant tillage (Riar, et al., 2010).

Chemical fallow herbicide treatments most commonly include glyphosate, but paraquat, paraquat plus diuron, 2,4-D, dicamba, and chlorsulfuron also are frequently applied (Moyer et al., 2004). A typical chemical fallow herbicide application schedule involves a first application to control winter annual grasses in March or April, followed by an application in May or June for broadleaf weeds, and an application in July if late flushes of weeds are present. Late summer herbicide applications are commonly made by aircraft as dust generated by self-propelled ground spraying equipment can generate heavy dust, which reduces herbicide efficacy in wheel tracks; specifically when applying glyphosate, as it rapidly adsorbs to soil particles (Lutcher, 2015; Zhou et al., 2006).

Following a 6-year field study evaluating the feasibility of replacing conventional summer fallow with no-till chemical fallow, Machado et al. (2015) found there was no yield penalty between the traditional winter wheat-summer fallow rotation and the winter wheat chemical fallow rotation in the Columbia River Basin of north-central Oregon. Their results also suggest that an intensified flex-cropping approach, where spring wheat or spring barley (*Hordeum vulgare* L.) is added to the crop rotation if soil moisture is at an adequate level, also did not cause a yield penalty for winter wheat when compared to the historical rotation. These long-term field experiments have helped with grower adoption of chemical fallow and annual crop rotations in low precipitation zones like north-central Oregon, where annual precipitation is roughly 280 mm year⁻¹.

Where chemical fallow has been integrated as the standard practice, a weed species composition shift has taken place where scouringrushes (smooth scouringrush: *Equisetum laevigatum* L. and intermediate scouringrush: *Equisetum x ferissii* Clute) have invaded production fields. Scouringrushes are native to the inland PNW (Husby, 2013), but their association with winter wheat-summer fallow cropping systems has traditionally been of little concern to growers because plants were rarely seen growing with

winter wheat crops. All scouringrush species are deep-rooted perennial seedless vascular plants that spread primarily through a terraced rhizome system. Intermediate scouringrush occurs almost exclusively in low-density stands of 1-50 stems m⁻², whereas smooth scouringrush occurs most commonly in medium density stands of 50-200 stems m⁻² (Rutz and Farrar, 1984). See Chapter 1 for a more complete description of scouringrush biology; also see Husby, 2013; Rutz and Farrar, 1984.

While intensive tillage likely prevented smooth and intermediate scouringrush from establishing at high densities in production fields, it is likely that the practice played a role in how the species was able to proliferate to a point of concern for growers. Tillage implements have been shown to increase dispersal of vegetative propagules. Chemical fallow and direct seeding will reduce vegetative propagule dispersal by tillage implements (Guglielmini and Satorre, 2004). However, scouringrush species are naturally tolerant of most herbicides (Rutz and Farrar, 1984; Ainsworth et al., 2006; Frasure and Bernards, 2011), which results in a situation where management tactics are limited. Bernards et al. (2010) evaluated 24 herbicide active ingredients for efficacy on scouringrush (*Equisetum hyemale* L.) and only two (chlorsulfuron and dichlobenil) were found to provide commercially acceptable control. Unfortunately, dichlobenil is not labeled for use in wheat and would not fit a dryland chemical fallow cropping system because mechanical incorporation or irrigation is required for efficacy in that study. Chlorsulfuron is labeled for use in wheat, but was applied at 10 times the labeled rate for winter wheat in the inland PNW. Therefore, herbicide treatments that both have efficacy on scouringrush, and fit winter wheat-chemical fallow cropping systems need to be identified.

Mowing is another option to manage smooth and intermediate scouringrush in chemical fallow rotations. Both scouringrush species of interest in the inland PNW produce a single late spring flush of stems, making them vulnerable to early season cuttings. However, timing and thoroughness of cutting by mowing implements both factor into how effective mowing treatments are for scouringrush control (Rutz and Farrar, 1984). Herbicides can be used in combination with mowing, but research is limited in this area. Field studies conducted in Murdock, Nebraska, resulted in no herbicide by mowing interactions when scouringrush (*Equisetum hyemale*) was mowed following herbicide applications. A split application strategy was used where herbicides were applied on July 6 and August 11, 2007, and mowing took place on July 31 and October 31 of the same year (Bernards et al., 2010). Nice et al. (2010) reported that imazapyr

and aminopyralid applied separately to mowed plots in April and November produced adequate control of scouringrush (*Equisetum hyemale*) 200 days after the November treatments, and efficacy was dependent on pre-herbicide mowing.

Objectives of this study were to evaluate herbicide efficacy on smooth and intermediate scouringrush in grower fields during a chemical fallow rotation, and to quantify any interactions resulting from pre-herbicide mowing. Trials were located in grower fields near Reardan, WA, and The Dalles, OR, in 2014 and 2015, respectively. Both locations are representative of typical direct-seeded winter wheat-chemical fallow cropping systems in intermediate precipitation zones of the inland PNW.

MATERIALS AND METHODS

Site Descriptions

Reardan, Washington: The trial site was located in Lincoln County approximately 11 km northeast of Reardan, WA, on an Athena silt loam soil with 3.3% organic matter and a soil pH of 4.9. Typical crop rotations at the site include a summer chemical fallow, followed by direct seeded winter wheat, followed by multiple spring cereal crops before rotating back to summer fallow depending on available moisture. Smooth scouringrush was the species present at the trial site. Average smooth scouringrush plant density was 167 stems m² with heights between 30.5 and 50.8 cm.

The Dalles, Oregon: The trial site was located in Wasco County approximately 13 km southeast of The Dalles, OR, on a Walla Walla silt loam soil with 2.7% organic matter and a soil pH of 5.86. Plots were located on a north aspect with 20-35% slope. Typical crop rotations at the site are direct seeded winter wheat followed by summer chemical fallow, then planted back to winter wheat. Intermediate scouringrush was the species present at the trial site. Dr. Richard Halse (Oregon State University: Department of Botany and Plant Pathology) was consulted to verify the taxonomy of intermediate scouringrush found at the trial site. Average intermediate scouringrush plant density was 52 stems m² with heights between 23 and 63 cm. Plant density was calculated by taking the average number of stems counted over 12, 0.5 m² quadrats. Intermediate scouringrush height is presented as the upper and lower bound of 24 height measurements taken throughout the trial site. Climate data for both locations is presented in Tables 2.1 and 2.2.

Treatments

At both locations 10 herbicide treatments were applied to mowed and non-mowed smooth or intermediate scouringrush during the chemical fallow rotation prior to seeding winter wheat. Plots were mowed with a flail-mower 24 hours before herbicide applications. Herbicide treatments included: 2,4-D ester (Base Camp LV 6, 0.66 kg L⁻¹ a.e. 2,4-D ester; Wilber-Ellis Company LLC, P.O. Box 16458 Fresno, CA 93755), MCPA ester (Rhonox, 0.45 kg L⁻¹ a.e. MCPA ester; Nufarm Inc., 11901 S. Austin Ave. Alsip, IL 60803), clopyralid plus MCPA-ester (Curtail M, 0.05 kg L⁻¹ a.e. clopyralid and 0.28 kg L⁻¹ a.e. MCPA-ester; Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268), chlorsulfuron (Glean XP, chlorsulfuron 75% by weight; DuPont, 1007 Market Street, Wilmington, DE 19898) plus MCPA-ester (Rhonox 0.45 kg L⁻¹ a.e. MCPA-ester), halosulfuron (Sanda, halosulfuron 75% by weight; Gowan Company LLC., 370 S. Main St. Yuma, AZ 85364) plus MCPA-ester (Rhonox 0.45 kg L⁻¹ a.e. MCPA-ester), glyphosate (Roundup PowerMax, 0.66 kg L⁻¹ glyphosate, Monsanto Company; 800 N. Lindbergh Blvd. St. Louis, Missouri, 63167), glyphosate (Roundup PowerMax, 0.66 kg L⁻¹ glyphosate) plus saflufenacil (Sharpen, 0.34 kg L⁻¹ saflufenacil; BASF, Crop Science Division, 26 Davis Drive, Research Triangle Park, NC 27709), fluroxypyr (Starane Ultra, 0.34 kg L⁻¹ a.e. fluroxypyr; Dow AgroSciences LLC), quinclorac (Paramount, 75% quinclorac by weight; BASF Crop Science Division) and glyphosate (Roundup PowerMax, 0.66 kg L⁻¹ glyphosate) plus glufosinate (Liberty, 0.28 kg L⁻¹ a.e., Bayer Crop Science, 2T.W. Alexander Drive, Research Triangle Park, NC 27709). Increased rates (1.7x) of chlorsulfuron plus MCPA-ester and halosulfuron plus MCPA-ester were applied at The Dalles, OR compared to Reardan, WA. An application error resulted in rate discrepancies between locations. A complete list of treatments and rates are presented in Table 2.3.

Reardan, Washington: Herbicide treatments were applied on July 25, 2014 with a CO₂ powered sprayer with a 4-nozzle boom equipped with TeeJet XR11002 nozzles pressurized at 207 kPa. Treatments were applied at 140 L ha⁻¹ with a ground speed 5.6 kilometers per hour. Environmental conditions during the application were 10% cloud cover, an ambient temperature of 21° C with 36% relative humidity and southwest winds at 10 km per hour. Soil temperature was 16° C.

The Dalles, Oregon: Herbicide treatments were applied on September 9, 2015 with a compressed air powered unicycle sprayer with a 2.3 m boom equipped with 5 TeeJet XR8003 nozzles pressurized at 138 kPa. Treatments were applied at 187 L ha⁻¹ with a ground speed of 6.1 kilometers per hour. Environmental conditions during the application were 0% cloud cover, an ambient temperature of 22° C with 70 % relative humidity and winds out of the west at 3.2 kilometers per hour. Soil temperature was 18° C at a depth of 15 cm.

Planting and Trial Maintenance

Reardan, Washington: Hard red winter wheat ‘Whetstone’ (Syngenta Seeds, Inc., 410 Swing Road, Greensboro, NC 27409) was seeded in 24 cm rows at a rate of 67 kg ha⁻¹ on September 10, 2014, using a Bourgalut 3710 disc drill. A fertilizer application of 95 kg ha⁻¹ nitrogen, 11 kg ha⁻¹ phosphorus and 17 kg ha⁻¹ sulfur was applied at planting. Spring herbicides were applied prior to smooth scouringrush emergence. An application of 17 g ha⁻¹ pyroxsulam (PowerFlex HL, 10.3% by weight pyroxsulam; Dow AgroSciences LLC), 38 g ha⁻¹ pyrasulfotole with 216 g ha⁻¹ bromoxynil (Huskie, 36.8 g L⁻¹ pyrasulfotole and 209 g L⁻¹ bromoxynil; Bayer Crop Science) and 87.4 g ha⁻¹ flurasulam with 4.5 g ha⁻¹ fluroxypyr (Starane Flex, 99.4 g L⁻¹ flurasulam and 5.01 g L⁻¹ a.e. fluroxypyr; Dow AgroSciences LLC) was applied on April 15, 2015, to control grass and broadleaf weeds.

The Dalles, Oregon: Soft white winter wheat ‘ORCF-101’ was seeded on October 7, 2015, in 30 cm paired-rows at a rate of 95 kg ha⁻¹ using a Flexi-Coil 5000 air drill with double shoot attachments. A fertilizer application of 78.4 kg ha⁻¹ nitrogen and 11.2 kg ha⁻¹ sulfur was applied at planting. An application of 17 g ha⁻¹ pyroxsulam (PowerFlex HL, 10.3% by weight pyroxsulam) and 38 g ha⁻¹ pyrasulfotole with 216 g ha⁻¹ bromoxynil (Huskie, 36.8 g L⁻¹ pyrasulfotole and 209 g L⁻¹ bromoxynil) was applied on April 7, 2016, to control grass and broadleaf weeds. Due to an infestation of downy brome (*Bromus tectorum* L.), a second spring herbicide application was made on May 10, 2016. There were no differences in included herbicides or rates between the two spring applications.

Experimental Design

Experimental designs differ between locations due to terrain and smooth or intermediate scouringrush patch dynamics. The Reardan location had less slope influence and the smooth scouringrush patch size, shape and uniformity allowed for more design flexibility. To ensure intermediate scouringrush uniformity within the trial area in The Dalles, plots were placed within a narrow strip of intermediate scouringrush growing parallel to the contour of the slope.

Reardan, Washington: A randomized complete block design with a split-plot arrangement was used. Mowing split the main plots and herbicide treatments were applied to subplots. Mowed and non-mowed herbicide treatments were replicated four times. Plots were 2.4 x 10.6 m. Refer to Figure 2.1.

The Dalles, Oregon: A randomized complete block design was used with a split-block arrangement replicated four times. Herbicide treatments were applied across entire plots with mowing applied as a sub-plot. Plots were 2.4 x 9.14 m. Approximately four rows were not seeded on the southernmost end of all non-mowed plots within the trial. To ensure accuracy of wheat yield data, the area of every non-mowed plot was calculated separately at harvest. A split-block arrangement was used due to equipment limitations from the steep terrain, and only the down-slope side of every plot was mowed. A normal randomization process was used to assign herbicide treatments to plots. To match the slope contour, block 1 and 2 were pivoted at the upslope break from blocks 3 and 4. Refer to Figure 2.2

Data Collection

Visual control of smooth or intermediate scouringrush was estimated at 14 and 26 days after treatment in Reardan, and at 14 and 41 days after treatment in The Dalles. The spring following herbicide application, smooth or intermediate scouringrush density (stems m row⁻¹) was quantified by counting all stems contained on 1 m transects between rows in all mowed and non-mowed plots. Spring stem densities were counted on May 15, 2015, in Reardan, and June 26, 2016, in The Dalles. Spring stem count timings were different due to intermediate scouringrush emerging later in the season at The Dalles. However, spring stem densities were counted roughly 10 months after treatment at both locations. Stem density counts were repeated following wheat harvest on August 10, 2015, in Reardan, and August 3, 2016, in The Dalles. To account for variability in intermediate scouringrush plant density at The Dalles site, three, 1 m

transects were counted per plot in June and August 2016 compared to two, 1 m transects per plot at the Reardan site in May and August 2015. Where plots were not seeded at The Dalles site, stem density was quantified using 0.5 m² quadrats in May and August of 2016. Results for stems 0.5 m² quadrat are presented in Appendix A.

Wheat was harvested on July 21, 2015, in Reardan with a Kincaid 8XP combine. Harvest in The Dalles took place on July 27, 2016, by using a sickle bar mower to cut a 1.2 m strip through the center of each plot. Wheat was then sampled by hand and threshed with a Wintersteiger Nursery Master Elite combine equipped with a pick-up header. Alternative harvest methods were used at the The Dalles due to steep terrain.

Smooth or intermediate scouringrush rhizome fragments were collected at both trial locations during the May/June stem counts to evaluate what effect, if any, herbicide applications have on vegetative propagation. Three 8 to 15 cm rhizome fragments were taken from all mowed and non-mowed plots within a single replication using a small shovel. Rhizomes were only removed from one replication at the Reardan site to reduce the overall sampling impact on the trial. One 8 to 15 cm rhizome fragment was taken from every mowed and non-mowed plot at The Dalles. Each fragment was planted into 1.18 L pots containing potting mix (Sunshine Mix 1 Potting Mix; Sun Gro Horticulture, Inc., 110th Ave. NE, Suite 490, Bellevue, WA 98004) and grown in a greenhouse maintained at 20-25° C under ambient light supplemented by lights to achieve 14-hours of 25 mW cm⁻² light per day. A completely randomized design was used to arrange the pots in the greenhouse. After 200 days in the greenhouse, total above and below ground dry biomass was measured by harvesting stems and separating the root system and rhizomes from potting mix and drying for 48 at 55° C. Results for the rhizome fragmentation study are presented in Appendix B.

Data Analysis

Scouringrush stem density counts were averaged within each subplot for statistical analysis. Analysis of variance (ANOVA) was conducted on visual control ratings, wheat yield, smooth or intermediate scouringrush stem number at spring and postharvest timings, and quadrat data from unseeded sections of not mowed plots at The Dalles using SAS Proc Mixed (SAS version 9.3, 2012). A Satterthwaite

approximation was used to account for heterogeneity of variance introduced by analyzing stem count data. Reardan data were analyzed as a split-plot design and The Dalles data were analyzed as split-block design due to the lack of randomization among mowed subplots. Herbicide and mowing treatments were treated as fixed effects with blocks treated as random effects. Differences between spring and postharvest smooth or intermediate scouringrush stem density were analyzed as split-plots in time. T test statistics were used to analyze differences in treatment means using the LSMEANS function of SAS Proc Mixed. All analysis of variance and T-tests were completed using a 5% significance level.

RESULTS AND DISCUSSION

Visual Control Ratings

14 Days After Treatment: Multiple herbicides with different modes of action induced a color response in treated smooth or intermediate scouringrush where stems became black following application. Black scouringrush within plots were considered to be controlled. There was no significant mowing effect or herbicide by mowing interaction at the Reardan site ($P>0.05$). At The Dalles, there was a treatment by mowing interaction ($P=0.0126$) where MCPA-ester, chlorsulfuron plus MCPA-ester, halosulfuron plus MCPA-ester and glyphosate plus glufosinate treatment had increased control in sub-plots that were not mowed. Clopyralid plus MCPA-ester (75% control), chlorsulfuron plus MCPA-ester (76% control), and halosulfuron plus MCPA-ester (64% control) resulted in the best control of smooth scouringrush at Reardan. Percent control for each treatment is presented as an average across all mowed and non-mowed plots where mowing and interaction terms are not significant. Chlorsulfuron plus MCPA-ester (mowed: 43% control; non-mowed: 89% control) and halosulfuron plus MCPA-ester (mowed: 39% control; non-mowed: 88% control) resulted in the best control at The Dalles. Interactions for results in these studies are only reported between equivalent herbicide treatment levels, as it is expected that any pre-application mowing effects would produce different efficacy responses across herbicide treatments with different modes of action.

26 and 41 Days After Treatment: There was no significant mowing effect or herbicide by mowing interaction at the Reardan site ($P>0.05$). Percent control for each treatment is presented as an

average across all mowed and non-mowed plots where mowing and interaction terms are not significant. Clopyralid plus MCPA-ester (70% control), chlorsulfuron plus MCPA-ester (79% control), and halosulfuron plus MCPA-ester (67% control) resulted in the best control of smooth scouringrush at Reardan. Visual control ratings were estimated after planting at The Dalles. No intermediate scouringrush was present in mowed plots after seeding equipment passed through the trial area; therefore, control ratings were only estimated in non-mowed plots. Chlorsulfuron plus MCPA-ester (83% control) and halosulfuron (75% control) resulted in the best control of intermediate scouringrush 41 days after treatment in The Dalles, OR.

At both locations chlorsulfuron plus MCPA-ester and halosulfuron plus MCPA-ester resulted in the best control of smooth or intermediate scouringrush at 14 days after treatment, and at 26 and 41 days after treatment at Reardan and The Dalles respectively. Across all timings and locations of visual ratings, every treatment providing the highest level of control contained MCPA-ester. A complete summary of visual control ratings is presented in Table 2.4.

Spring Scouringrush Densities: 10 Months After Treatment

Reardan, Washington: No mowing effects or herbicide by mowing interactions were observed for smooth scouringrush stem densities measured between rows at Reardan in May 2015 ($P > 0.05$); therefore, stem densities were averaged across mowed and non-mowed plots. Treatments of 2,4-D-ester, chlorsulfuron plus MCPA-ester, halosulfuron plus MCPA-ester, glyphosate alone, and glyphosate plus saflufenacil reduced smooth scouringrush stem density per meter of row when compared to untreated controls ($P < 0.05$). Chlorsulfuron plus MCPA-ester reduced smooth scouringrush stem density to 1.25 stems m^{-1} , which was better than all other treatments ($P < 0.05$). Untreated control plots averaged 39 stems m^{-1} . All treatments other than chlorsulfuron plus MCPA-ester that reduced smooth scouringrush stem density were all found to have ≈ 20 stems m^{-1} , which would not be acceptable for as a commercial weed control treatment. Clopyralid plus MCPA-ester produced that highest visual control ratings 14 and 26 days after treatment, but spring stem densities were no different than untreated control plots, which suggest using the color response induced by herbicide applications is a poor indicator of long term efficacy on

smooth scouringrush. Complete results for Reardan spring smooth scouringrush density are presented in Figure 2.3.

The Dalles, Oregon: A mowing by herbicide interaction was observed for intermediate scouringrush stem densities measured between rows at The Dalles ($P=0.05$); therefore mowed and non-mowed sub-plots were analyzed separately. However, the interaction was likely due to a gradient where intermediate scouringrush density increased from north to south within the trial area and along the same contour outside of the trial (field observation). Mowing was applied on the north half of each plot and the intermediate scouringrush gradient likely caused a false mowing effect within the data as all treatments had significantly more intermediate scouringrush per meter of row in non-mowed/south subplots than mowed/north subplots (untreated plots and plots treated with 2,4-D-ester did not show mowing/density effects in June 2016).

Mowed and non-mowed control (no herbicide treatment) subplots were found to be nearly equal at The Dalles in June 2016. However, at the post harvest timing, non-mowed/south plots were found to have 3.25 times more intermediate scouringrush per meter of row than mowed/north subplots, suggesting intermediate scouringrush had not yet entirely emerged in June 2016. Mowing treatments in these trials were evaluated to determine how pre-herbicide mowing affects herbicide efficacy, not how summer mowing controls smooth or intermediate scouringrush. Late summer mowing treatments have been shown to have little effect on scouringrush the following season (Bernards, et al., 2010; Rutz and Farrar, 1984), making it unlikely that mowing influenced the overall intermediate scouringrush stem density in the control plots, which supports the hypothesis that significant mowing effects were the result of a plant density gradient.

Glyphosate treatments at both locations provide additional evidence that the mowing effect at The Dalles, OR was in reality an effect of a plant density gradient. Glyphosate has no soil residual activity and poor coverage can decrease herbicide efficacy (Clifford et al., 2004; Lutcher, 2015; Zhou et al., 2006). Coverage is an especially important factor when applying glyphosate to any scouringrush species because it has very little surface area, poor herbicide uptake, and a majority of its biomass is below the soil surface (Ainsworth et al., 2006; Husby, 2013). Mowing smooth and intermediate scouringrush prior to herbicide applications reduced the surface area, and the potential for glyphosate to adsorb to soil particles on plants

increased. These factors suggest that pre-herbicide mowing would reduce glyphosate efficacy on all scouringrush species. Spring and postharvest stem densities at Reardan support that hypothesis. Despite the main effects introduced by mowing being insignificant in the ANOVA, glyphosate applied alone appeared to have little efficacy on smooth scouringrush, while only chlorsulfuron plus MCPA-ester performed better than glyphosate applied to non-mowed plots (Figure 2.3). The opposite effect was observed in glyphosate treated plots in The Dalles; non-mowed plots were found to have ≈ 2.5 times more intermediate scouringrush stems m row^{-1} than mowed plots (Figure 2.3). Smooth and intermediate scouringrush density maps for both trials are presented in Figures 2.5 and 2.6.

Chlorsulfuron plus MCPA-ester was the only treatment that reduced intermediate scouringrush stems m row^{-1} to a density less than untreated controls and all other treatments in both mowed and non-mowed subplots ($P < 0.05$). Mowed and non-mowed subplots treated with chlorsulfuron plus MCPA-ester averaged 0 and 0.25 intermediate scouringrush stems m row^{-1} , respectively, untreated mowed and non-mowed plots averaged 10 and 11 stems m row^{-1} , respectively. Complete results for The Dalles spring intermediate scouringrush density are presented in Figure 2.3.

Postharvest Scouringrush Stem Densities: 1 Year After Treatment

Reardan, Washington: No mowing effects or herbicide by mowing interactions were observed for smooth scouringrush stem densities measured between rows at Reardan in August 2015 ($P > 0.05$); therefore, stem densities were averaged across mowed and non-mowed plots. Chlorsulfuron plus MCPA-ester was the only treatment that reduced smooth scouringrush stem density per meter of row compared to the untreated control and all other herbicide treatments through an entire growing season ($P < 0.05$). Plots treated with chlorsulfuron plus MCPA-ester contained on average > 2 smooth scouringrush stems m row^{-1} , while untreated control plots averaged 38 stems m row^{-1} . Overall stem densities within the trial increased from May to August 2015; however, there were no month by herbicide treatment interactions within or between mowing treatments ($P > 0.05$).

The Dalles, Oregon: A mowing by herbicide interaction was observed for intermediate scouringrush stem densities measured between rows at The Dalles ($P = 0.0124$); therefore mowed and non-

mowed sub-plots were analyzed separately. Overall stem densities within the trial decreased from June to August 2015; however, there were no month by herbicide treatment interactions within or between mowing treatments ($P>0.05$). No differences were detected between any treatments in mowed subplots ($P>0.05$), likely because overall intermediate scouringrush stem densities were too low for differences to be detected. However, no intermediate scouringrush was present in plots treated with chlorsulfuron plus MCPA-ester. All other mowed plots, including untreated controls, averaged ≈ 5 intermediate scouringrush stems m^{-1} . Differences between treatments were detected in non-mowed plots where overall intermediate scouringrush density was greater. Non-mowed plots treated with chlorsulfuron plus MCPA-ester had 0 intermediate scouringrush stems m^{-1} , which was different from all other treatments and the untreated control ($P<0.05$). Average intermediate scouringrush stems m^{-1} ranged between 9 and 19 for other treatments.

Winter Wheat Grain Yield

Reardan, Washington: A mowing by herbicide interaction was observed for wheat grain yield at Reardan, WA ($P=0.0027$); therefore, mowed and non-mowed sub-plots were analyzed separately. Non-mowed plots treated with quinclorac and glyphosate plus glufosinate were found to have greater yields than mowed plots receiving the same treatments. Though non-mowed plots treated with glyphosate and glufosinate produced grain yields higher than any other mowed or non-mowed treatment ($P<0.05$), generally, yields were nearly equal across all treatments including untreated controls (Figure 2.4). Average grain yield at the Reardan site was 4821 ± 590 $kg\ ha^{-1}$ with a range of 3564 to 6231 $kg\ ha^{-1}$.

The Dalles, Oregon: Due to an error resulting in an application of 1.7x rates of halosulfuron plus MCPA-ester and chlorsulfuron plus MCPA-ester, crop injury ratings were visually estimated on May 24, 2016. There was no mowing effect or mowing by herbicide interaction on crop injury ($P>0.05$); therefore, crop injury, as defined by % stand reduction, were averaged across mowed and non-mowed plots. Plots treated with fluroxypyr, halosulfuron plus MCPA-ester and chlorsulfuron plus MCPA-ester were found to have 10%, 30%, and 4% stand reductions respectively. Yield data from mowed and non-mowed subplots treated with halosulfuron plus MCPA-ester were excluded from statistical analysis because substantial crop

injury and lack of intermediate scouringrush control caused the interpretation of yield results to be misleading. Other treatments did not result in any stand reduction.

There was no herbicide treatment effect or mowing by herbicide interaction on wheat grain yield at The Dalles in 2016 ($P > 0.05$). However, there was a mowing effect ($P = 0.01$) where mowed plots produced 28.3% greater yields (kg ha^{-1}) on average than non-mowed plots. Despite greater intermediate scouringrush densities in non-mowed plots, when compared among all mowed or non-mowed plots, there was no difference in yield between untreated control plots and plots treated with chlorsulfuron plus MCPA-ester where intermediate scouringrush was nearly 100% controlled throughout the growing period (Figure 2.4). Additionally, considering wheat yields were nearly equal across mowing and herbicide treatments at Reardan, differences in wheat yield between mowed and non-mowed plots at The Dalles were likely a result of factors not evaluated in the trial. Stand reductions induced by fluroxypyr and chlorsulfuron plus MCPA-ester applications did not result in reduced grain yield compared among mowed and non-mowed subplots (Figure 2.4). Average grain yields for mowed and non-mowed subplots at The Dalles were $2381 \pm 663 \text{ kg ha}^{-1}$ with a range of 976 to 3904 kg ha^{-1} and $1856 \pm 622 \text{ kg ha}^{-1}$ with a range of 637 to 2576 kg ha^{-1} , respectively.

Hard red winter wheat variety testing conducted by Washington State University near Reardan found the variety 'Whetstone' yielded 4054 kg ha^{-1} on average in 2015, which was 767 kg ha^{-1} less than the average yield recorded for the Reardan plots in this study. Soft white winter wheat variety testing conducted by Oregon State University near Moro, OR (53 km east of The Dalles) found the variety 'ORCF-101' yielded 4638 kg ha^{-1} on average in 2016, which was 2257 kg ha^{-1} and 2782 kg ha^{-1} more than mowed and non-mowed plots respectively for The Dalles plots in this study. The 48% and 60% respective yield reduction observed between field plots established in The Dalles grower fields and the Moro experiment station variety trials in the same year is likely a result of a downy brome infestation. Depending on the density of downy brome and competitive ability of the wheat cultivar, yield loss can range anywhere from 10 to 90% (Challaiah et al., 1986; Blackshaw, 1994; Flemming et al., 1988).

Through the development of Clearfield® wheat varieties like 'ORCF-101', growers are able to control downy brome in season by applying the herbicide imazamox in the spring without wheat injury (Colquhoun et al., 2003). Imazamox was applied to the field surrounding the plots in The Dalles. However,

to avoid confounding herbicide treatments applied for intermediate scouringrush control, the trial was excluded from the imazamox treatment. Although trial maintenance herbicides with activity on grasses were applied, the result was an adjacent wheat stand relatively clean of downy brome compared to wheat plots in the trial.

Implications For Management

Fallow applications of chlorsulfuron are not currently labeled in Washington, Oregon, Northern Idaho or California. The maximum winter wheat use rate for chlorsulfuron in these states is 17 g ha⁻¹, while in north central Texas and southern Oklahoma chlorsulfuron can be applied at 26 g ha⁻¹. When applied at 26 and 43 g ha⁻¹ during summer fallow rotations, chlorsulfuron effectively controlled smooth and intermediate scouringrush without affecting wheat grain yields in Reardan and The Dalles in 2015 and 2016, respectively. It should be noted Brewster and Appleby (1983) applied spring applications of chlorsulfuron up to 140 g ha⁻¹ without affecting wheat grain yield, suggesting chlorsulfuron rates applied in these trials would result in adequate crop safety in winter wheat-chemical fallow cropping systems. However, the minimum rotation interval for non-cereal crops is 36 months in Washington, Eastern Oregon and Idaho. Thus, the 26 g ha⁻¹ rate of chlorsulfuron plus 1.12 kg ha⁻¹ MCPA-ester could be a commercially acceptable treatment option for smooth or intermediate scouringrush control in summer fallow before seeding winter wheat in the inland PNW.

Chlorsulfuron is an acetolactate synthase (ALS) inhibiting (group 2) herbicide, which are among the most commonly used in the world and commonly select for resistant weed populations. Currently there are more group 2 resistant weeds than any other herbicide group (Tranel and Wright, 2002). Genetic diversity is greater between scouringrush populations than within populations due to inefficiencies associated with sexual reproduction within the genus *Equisetum* (Korpelainen and Kolkkala, 1996). A lack of gene flow between individuals within a scouringrush population suggests that ALS resistance would be less likely to be selected for than an obligate outcrossing species. However, high levels of genetic divergence between scouringrush populations in relatively small geographic areas make selecting ALS resistance a possibility.

Another aspect to consider is that at both locations where applications of chlorsulfuron plus MCPA-ester resulted in near complete control of smooth and intermediate scouringrush, no differences in wheat grain yields were observed between plots clean of smooth and intermediate scouringrush in plots with up to 50 stems m row⁻¹ and 20 stems m row⁻¹ in Reardan and The Dalles, respectively. At The Dalles, OR site downy brome possibly contributed to a 48-60% yield reduction compared to the same variety grown in the same year at the Columbia River Agricultural Research Center at Moro, OR. Applying an in season imazamox treatment would have likely controlled the downy brome within the trial area. However, imazamox is also an ALS inhibiting herbicide and resistant populations of downy brome have been characterized in the PNW (Campbell et al., 2011). Special considerations should be made to determine if/when smooth or intermediate scouringrush control is worth increasing selection pressure for ALS resistance of other weeds by adding another ALS-inhibiting herbicide into a winter wheat-chemical fallow crop rotation.

CONCLUSIONS

Pre-herbicide mowing likely has little to no effect on smooth or intermediate scouringrush control for the 10 treatments evaluated. Visual control ratings following herbicide applications were largely ineffective for predicting how smooth and intermediate scouringrush responds to herbicide treatments the spring following application. At both locations chlorsulfuron plus MCPA-ester applied during late summer chemical fallow was the only treatment that resulted in commercially acceptable smooth and intermediate scouringrush control through the winter wheat rotation. An application error resulted in a 4% stand reduction in plots treated with chlorsulfuron plus MCPA-ester at The Dalles site, however, no grain yield penalty resulted from chlorsulfuron applied at 43 g ha⁻¹ plus MCPA-ester applied at 1.87 kg⁻¹. No difference in yield was observed between plots treated with chlorsulfuron plus MCPA-ester and untreated weedy controls at either location. Results from these field studies suggest that applying chlorsulfuron plus MCPA-ester during chemical fallow would be an effective treatment for controlling smooth and intermediate scouringrush (*Equisetum leavigatum* and *Equisetum xferriisii*) in winter wheat rotations. However, applying an additional ALS inhibiting herbicide in a winter wheat-chemical fallow cropping

system should be implemented with caution to avoid selecting for resistance, especially if standard weed control practices involve the use Clearfield® production systems.

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Table 2.1. Average monthly climate summary for the region surrounding Reardan, WA from 1948 through 2005. Temperature is presented in °C and precipitation in cm. Regions receiving between 30 and 45 cm of annual precipitation are classified as intermediate.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Maximum Temperature (°C)	-0.6	3.2	8.4	14	19	23.4	28.4	28.2	23.1	15	5.3	0.2	14
Minimum Temperature (°C)	-7.4	-4.8	-2.2	0.56	4.3	7.4	9.9	9.7	5.8	0.78	-2.8	-6.3	1.2
Total Precipitation (mm)	46	32	36	27	35	26	17	14	17	26	48	53	378
Total Snow Fall (mm)	315	152	79	10	2	0	0	0	0	8	127	127	993
Snow Depth (mm)	178	15.2	25	0	0	0	0	0	0	0	25	76	25

*Climate data from the Western Regional Climate Center.

Table 2.2. Average monthly climate summary for the region surrounding The Dalles, OR from 1893 through 2014. Temperature is presented in °C and precipitation in cm. Regions receiving between 30 and 45 cm of annual precipitation are classified as intermediate.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Maximum Temperature (°C)	5.2	9.1	14.2	18.9	23.1	26.1	31	30.8	26.6	19.6	10.9	6.1	18.5
Minimum Temperature (°C)	-2	-0.4	2.2	5.2	8.7	12.2	14.4	14.2	10.2	5.6	1.7	-0.7	6
Total Precipitation (mm)	64	45	31	18	14	13	04	06	13	26	57	65	356
Total Snow Fall (mm)	244	74	18	0	0	0	0	0	0	0	53	114	503
Snow Depth (mm)	25	0	0	0	0	0	0	0	0	0	0	0	0

*Climate data from the Western Regional Climate Center.

Table 2.3. Herbicide treatments, rates and adjuvants for trial locations in Reardan, WA and The Dalles, OR. Herbicide rates are presented in kg a.i* . ha⁻¹ and adjuvants as % v/v.

Herbicides	Rate by location	
	Reardan, WA	The Dalles, OR
untreated control	-	-
2,4-D-ester	1.12 kg ha ⁻¹	1.12 kg ha ⁻¹
NIS*	0.334% v/v	0.25% v/v
MCPA-ester	1.12 kg ha ⁻¹	1.12 kg ha ⁻¹
NIS	0.334% v/v	0.25% v/v
clopyralid	0.14 kg ha ⁻¹	0.14 kg ha ⁻¹
MCPA-ester	0.76 kg ha ⁻¹	0.76 kg ha ⁻¹
NIS	0.334% v/v	0.25% v/v
chlorsulfuron	0.026 kg ha ⁻¹	0.043 kg ha ^{-1**}
MCPA-ester	1.12 kg ha ⁻¹	1.87 kg ha ^{-1**}
NIS	0.334% v/v	0.25% v/v
halosulfuron	0.067 kg ha ⁻¹	0.12 kg ha ^{-1**}
MCPA-ester	1.12 kg ha ⁻¹	1.87 kg ha ^{-1**}
NIS	0.334% v/v	0.25% v/v
glyphosate	1.26 kg ha ⁻¹	1.26 kg ha ⁻¹
NIS	0.334% v/v	0.25% v/v
AMS*	3.5 kg ha ⁻¹	3.5 kg ha ⁻¹
glyphosate	1.26 kg ha ⁻¹	1.26 kg ha ⁻¹
saflufenacil	0.01 kg ha ⁻¹	0.01 kg ha ⁻¹
COC*	1% v/v	1% v/v
AMS	3.5 kg ha ⁻¹	3.5 kg ha ⁻¹
fluroxypyr	0.27 kg ha ⁻¹	0.27 kg ha ⁻¹
NIS	0.334% v/v	0.25% v/v
quinclorac	0.28 kg ha ⁻¹	0.28 kg ha ⁻¹
MSO*	2.34 kg ha ⁻¹	2.34 kg ha ⁻¹
AMS	3.5 kg ha ⁻¹	3.5 kg ha ⁻¹
glyphosate	0.84 kg ha ⁻¹	0.84 kg ha ⁻¹
glufosinate	0.62 kg ha ⁻¹	0.62 kg ha ⁻¹
NIS	0.334% v/v	0.25% v/v
AMS	3.5 kg ha ⁻¹	3.5 kg ha ⁻¹

*NIS: non-ionic surfactant; AMS: ammonium sulfate; COC: crop oil concentrate; MSO: methylated seed oil; a.i.: active ingredient.

**Herbicide rates differ between trial locations.

Table 2.4. Visual ratings of % control of smooth or intermediate scouringrush at both trial locations. Values presented are averages and standard deviations of estimated % control. Different letters signify statistical significance within each column ($\alpha=0.05$).

	Reardan, WA				The Dalles, OR			
	14 DAT**		26 DAT		14 DAT		41 DAT	
	Mowed	Not Mowed	Mowed	Not Mowed	Mowed	Not Mowed	Mowed	Not Mowed
2,4-D-ester	28 ± 10 ^a	30 ± 10 ^a	30 ± 8 ^a	37 ± 6 ^a	0 ^a	8 ± 5 ^a	N/A	4 ± 5 ^a
MCPA-ester	60 ± 14 ^b	50 ± 14 ^b	60 ± 16 ^b	50 ^b	* 3 ± 5 ^a	* 34 ± 18 ^{bc}	N/A	25 ± 13 ^a
clopyralid + MCPA-ester	73 ± 15 ^c	78 ± 3 ^c	65 ± 17 ^b	75 ± 4 ^c	10 ± 14 ^a	25 ± 28 ^{ab}	N/A	18 ± 17 ^a
chlorsulfuron + MCPA-ester	84 ± 10 ^c	68 ± 13 ^c	82.5 ± 10 ^c	75 ± 6 ^c	* 43 ± 49 ^b	* 89 ± 10 ^d	N/A	83 ± 10 ^b
halosulfuron MCPA-ester	66 ± 18 ^{bc}	63 ± 5 ^{bc}	66.25 ± 11 ^b	68 ± 5 ^c	* 39 ± 38 ^b	* 88 ± 19 ^d	N/A	75 ± 31 ^b
glyphosate	18 ± 5 ^a	20 ± 8 ^a	15 ± 6 ^d	20 ± 7 ^d	0 ^a	0 ^a	N/A	13 ± 15 ^a
glyphosate + saflufenacil	18 ± 5 ^a	13 ± 5 ^a	10 ^d	10 ^e	0 ^a	3 ± 5 ^a	N/A	28 ± 30 ^a
fluroxypyr	25 ± 6 ^a	23 ± 5 ^a	31.25 ± 6 ^a	28 ± 5 ^{ac}	0 ^a	15 ± 13 ^{ab}	N/A	15 ± 13 ^a
quinclorac	15 ± 6 ^a	17 ± 5 ^a	20 ± 8 ^{ad}	18 ± 5 ^e	0 ^a	0 ^a	N/A	12.5 ± 5 ^a
glyphosate + glufosinate	51 ± 30 ^d	33 ± 10 ^d	58.5 ± 22 ^b	35 ± 13 ^a	* 15 ± 17 ^c	* 55 ± 30 ^c	N/A	23 ± 15 ^a

*Significant herbicide by mowing interactions ($\alpha=0.05$). Differences were analyzed between equivalent mowed and non-mowed herbicide treatments at each time of rating within locations.

** DAT= days after treatment.

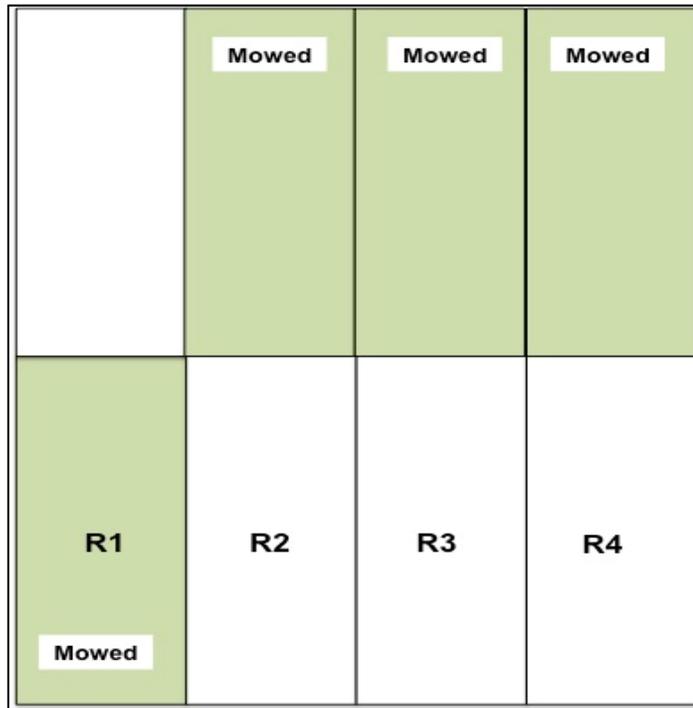


Figure 2.1. Trial design at Reardan, WA. Herbicide treatments were applied parallel to where the main plots were split by mowing. Eighty-eight subplots were included in the trial. Figure not to scale.

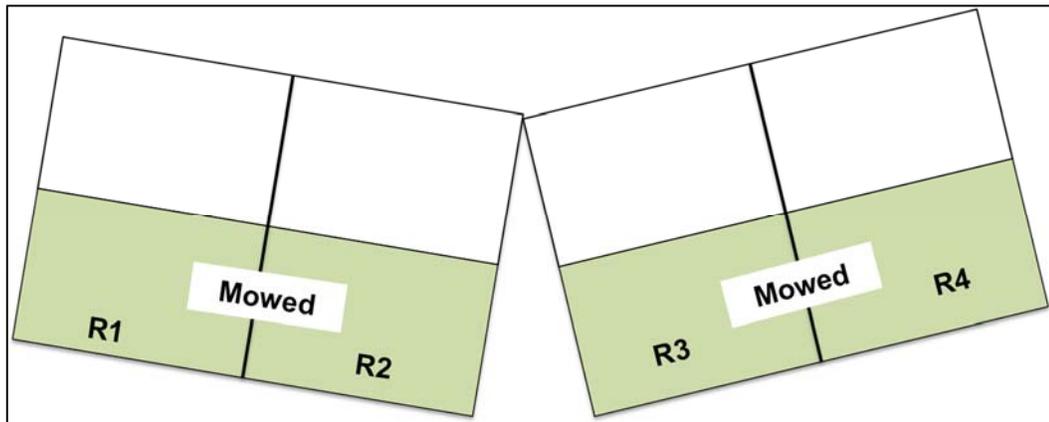


Figure 2.2. Trial design at The Dalles, OR. Herbicide treatments were applied perpendicular to where individual plots were split by mowing. Forty-four plots were included in the trial. Figure not to scale.

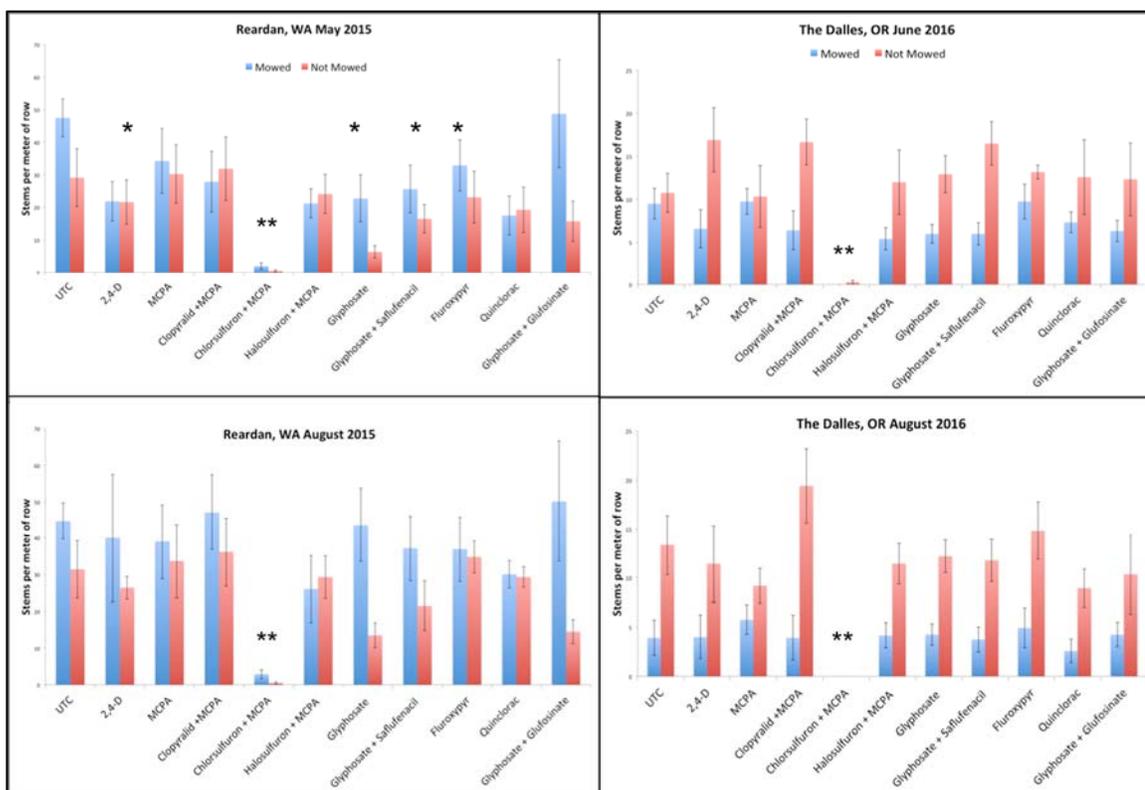


Figure 2.3. Scouringrush stem density per meter of row for at spring and postharvest timings. No mowing effect or mowing by herbicide interaction was observed at Reardan, WA, at the May (Spring) or August (Postharvest) sampling dates ($P > 0.05$). There was a mowing by herbicide interaction at The Dalles, OR at the June (Spring; $P = 0.05$) and August (Postharvest; $P = 0.0124$) sampling dates. Statistical analysis was, therefore, conducted across the combined average of mowed and non-mowed subplots for each herbicide treatment at Reardan, and between mowed and non-mowed subplots at The Dalles. Asterisks signify statistical significance. Chlorsulfuron plus MCPA-ester was the only treatment that was different than untreated controls and all other treatments at both locations ($\alpha = 0.05$). No difference in stems per meter of row was observed between any herbicide treatments within mowed or non-mowed subplots at The Dalles. Error bars show the standard error of the treatment means.

*Different than the untreated control; **Different than all other treatments.

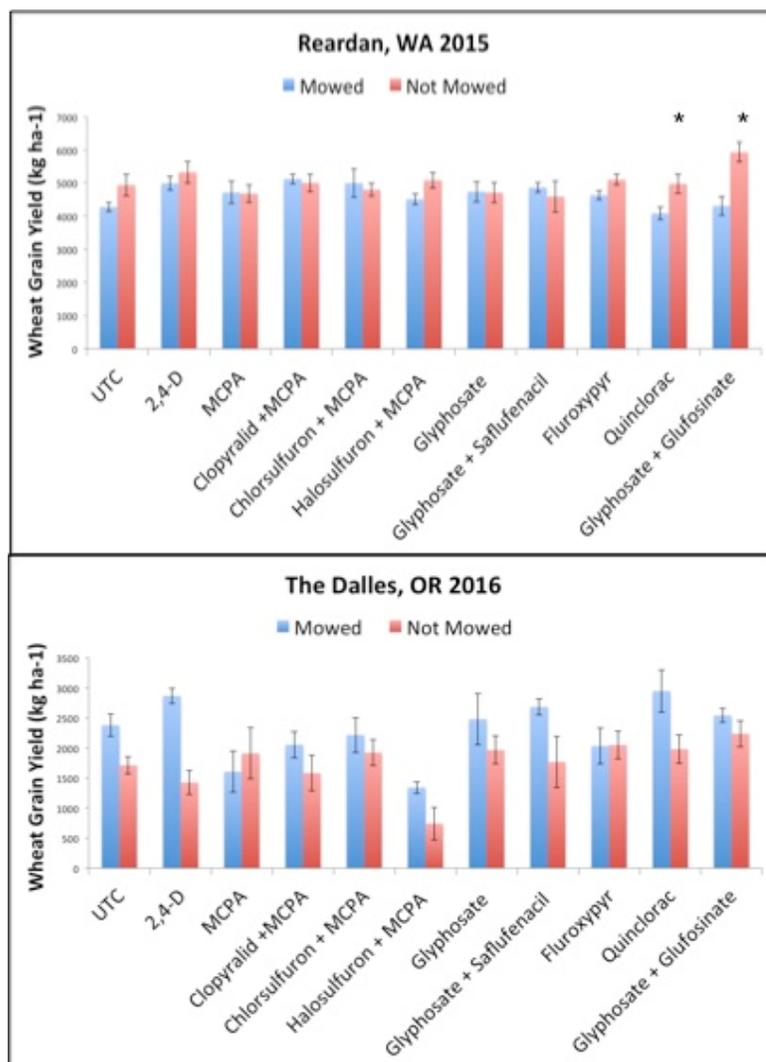


Figure 2.4. Average wheat yield from both trial locations. There was a herbicide treatment by mowing interaction observed at Reardan, WA ($P=0.0027$). A mowing effect was observed at The Dalles, OR ($P=0.01$). Therefore, statistical analysis was conducted between mowed and non-mowed subplots at Reardan and separately across mowed and non-mowed subplots at The Dalles. Plots treated with halosulfuron plus MCPA-ester at The Dalles were excluded from statistical analysis due to injury resulting from an application error. Asterisks signify statistical significance ($\alpha=0.05$). There was no difference in wheat yield between herbicide treatments among mowed or non-mowed plots at The Dalles. Error bars show the standard error of the treatment means. *Difference in yield between mowed and non-mowed plots.

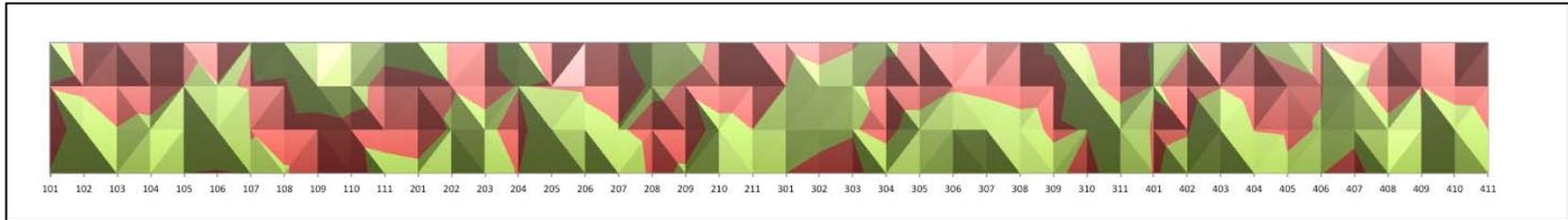


Figure 2.5. Smooth scouringrush density map of the trial area at Reardan, WA, in May 2015. Red and light green areas correspond to low and high smooth scouringrush stem density respectively. Numbers along the x-axis are subplot numbers within each replicate. For subplots 101 to 111, the bottom half of the map represents the mowed plots and for subplots 201 to 411, the top half of the map represents the mowed plots. Density was determined by separately subtracting 1 m smooth scouringrush stem density subsamples from mowed plots from stem density subsamples in the non-mowed plot that received the same herbicide treatment within the same replicate; and then reversing the equation. Positive numbers were considered high density and negative numbers were considered low density. Calculations for this map assume that herbicide treatments other than chlorsulfuron plus MCPA-ester had no activity or reduced scouringrush density by the same proportion in mowed and non-mowed plots within the same replicate. In this scenario “high density” and “low density” are somewhat arbitrary terms, but it does show that the Reardan, WA, site had a more random distribution of smooth scouringrush.

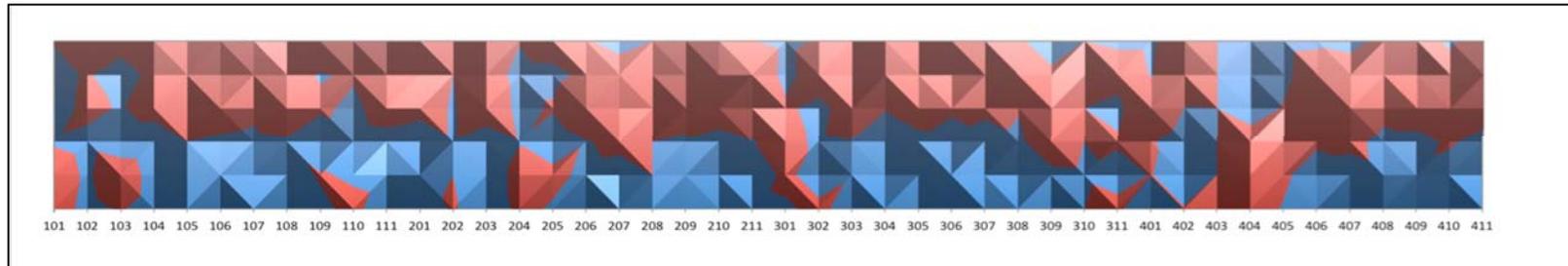


Figure 2.6. Intermediate scouringrush density map of the trial area at The Dalles, OR, in June 2016. Red and blue areas correspond to low and high intermediate scouringrush stem density. The lower half of the map represents the mowed subplots. Numbers along the x-axis are plot numbers within each replicate. Density was determined as described in figure 2.5. High density areas are more concentrated on the top half (non-mowed) of the map, whereas the Reardan, WA map shows high and low density more randomly distributed.

CHAPTER 3: Wheat (*Triticum aestivum* L.) Yield Effects in Response to Scouringrush (*Equisetum* spp. L.) Density

ABSTRACT

Scouringrush (*Equisetum* spp. L.) has invaded direct seeded dryland production fields in the Pacific Northwest. Eastern Oregon wheat growers have reported up to 30% yield reductions in areas of heavy infestation. However, no attempts have been made to quantify what effect scouringrush plant density has on winter wheat grain yield. An additive competition study was used wherein winter wheat was seeded into 17 L pots containing 0, 1, 2, 4, 8, 16, 32, 64 or 100 intermediate scouringrush (*Equisetum x ferrissii* Clute) rhizome fragments. The study was terminated at wheat maturity. Total aboveground biomass, grain yield, height, tiller number, and spike number were regressed against the total number of scouringrush stems per pot. Due to inconsistent establishment by scouringrush, data were reclassified into either a high or low density group for regression analysis. To supplement data from the potted wheat experiment, yield data from trials assessing herbicide efficacy on scouringrush in The Dalles, OR (2015) and Reardan, WA (2014) were analyzed. For field experiments, winter wheat plot yields were regressed against the average number of scouringrush stems m row⁻¹ measured in the plot. Low r^2 values indicated quantifying scouringrush density through stem number was not ideal. However, regression lines from a controlled potted wheat experiment have slopes near zero, which suggested scouringrush is a weak or non-competitor with winter wheat. Regression lines from field studies in Reardan and The Dalles have slopes of ≈ -10 and ≈ -30 respectively, suggesting there was a correlation between low yielding areas and scouringrush density. Although these results are preliminary, there is evidence suggesting negative yield responses currently attributed to scouringrush infestations could be in part the result of nutrient deficiency, as other species within the genus *Equisetum* lack the ability to respond to nitrogen and have been associated with nitrogen deficient field plots in agronomy trials.

INTRODUCTION

Intermediate scouringrush (*Equisetum xferrissii* L.) is a sterile hybrid of smooth scouringrush (*Equisetum laevigatum* L.) and scouringrush (*Equisetum hyemale* L.). In agricultural production, intermediate scouringrush historically has been confined to irrigation ditches and field margins (Rutz and Farrar, 1984). Currently, there is interest in studying scouringrush species in more detail as patches growing in production fields have become more common. Eastern Oregon growers have reported winter wheat (*Triticum aestivum* L.) yield losses of up to 30% in areas of dense intermediate scouringrush infestation. Expanding populations coupled with claims of substantial wheat yield loss have necessitated a need for understanding what effects scouringrush plant density has on winter wheat growth and grain yield.

There are numerous experimental methods utilized to characterize weed-crop interference, with the additive design being the most common (Radosevich et al., 2007; Zimdahl, 2004). In these studies, crop plants are held at a constant density and weed densities are gradually increased. Yield or percent yield loss are then regressed against weed density. Results are often incorporated into competition models used to describe economic and/or biological thresholds of particular weed species. Attempting to quantify the effects of scouringrush plant density on winter wheat yield has a unique set of challenges. Whereas wheat plant density can be determined by seeding rate, intermediate scouringrush can only be propagated using rhizomes making plant density determination difficult when compared to plants grown from seed. Wheat, is an annual grass, and has completely different life cycle characteristics than intermediate scouringrush, being a creeping perennial seedless vascular plant. Plant competition between wheat and species with similar lifecycle characteristics; specifically *Bromus* spp. L., Italian ryegrass (*Lolium perenne* L. spp. *multiflorum* Lam.), blackgrass (*Alopecurus myosuroides* Huds.) and jointed goatgrass (*Aegilops cylindrical* L.) have been extensively studied, but few plant competition studies have been completed with wheat and plants with differing life cycle characteristics (Zimdahl, 2004).

A perennial weed that has been studied in the context of wheat production is Canada thistle (*Cirsium arvense* (L.) Scop.). Mamolos and Kalburtji (2001) found that wheat yield loss increased as field densities of Canada thistle increased from 0 to 64 plants m⁻². Further analysis revealed that competition for nitrogen, as quantified by nitrogen concentration in Canada thistle biomass, was the main factor effecting

wheat yield loss. In contrast, Donald and Kahn (1996) found wheat stand reduction in response to Canada thistle density m^{-2} as the primary factor driving wheat yield loss. Under conditions where a wide range of plant densities m^{-2} are available, a non-linear rectangular hyperbolic model has been shown to produce the most accurate estimates of wheat yield loss to Canada thistle (McLennan et al., 1991). When a narrower range of weed density m^{-2} is used to describe wheat yield loss, rectangular hyperbolic models have been shown to over estimate yield loss (Friesen et al., 1990). Yenish and Durgan (1997) evaluated hard red spring wheat yield reduction in response to common milkweed (*Asclepias syriaca* L.), a perennial forb, density found in Minnesota production fields. In their work, an additive design was used where the percentage wheat yield loss was regressed against common milkweed densities ranging from 0 to 12 plants m^{-2} and total common milkweed dry biomass m^{-2} . Due to low common milkweed density m^{-2} , a simple linear model was shown to most accurately estimate yield loss as a function of weed density when coefficients of determination were compared to linear square root and rectangular hyperbolic models.

The following research was an attempt to quantify what effect, if any, scouringrush stem density has on winter wheat yields. A potted wheat study was completed to evaluate scouringrush-winter wheat competition in a controlled environment where initial weed density was known, because it is extremely difficult to determine exact scouringrush plant number under field conditions. Additionally, winter wheat yield data from two field trials conducted in Reardan, Washington, and The Dalles, Oregon, were regressed against scouringrush stem densities quantified within plots. Results from field trial regression analysis should be viewed with the caveat that herbicide efficacy, and not winter wheat-scouringrush plant interference, was the focus of the trials. However, they do provide an adequate field-scale comparison for data derived from the potted wheat experiment.

MATERIALS AND METHODS

Intermediate Scouringrush Plant Density

Intermediate scouringrush rhizome fragments were collected near The Dalles, OR in October 2015. Rhizomes were washed to remove excess soil, cut into 3-5 cm segments containing a single node and planted into 17 L white plastic pots containing potting mix (Metro-Mix 350 Professional Growing Mix;

Sun Gro Horticulture, Inc., 110th Ave. NE, Suite 490, Bellevue, WA 98004). Fifty 0.3 cm holes were drilled in the bottom of each pot to allow excess moisture to drain. Rhizomes were planted at rates ranging from 0 to 100 fragments per 17 L pot. Beginning with a single rhizome fragment, each subsequent planting rate was doubled (except at the highest density due to pot size limitations) making the final intermediate scouringrush density treatments 0, 1, 2, 4, 8, 16, 32, 64, and 100 fragments per pot.

Plant Material and Growing Conditions

Slow release 14-14-14 fertilizer at 1.3 g l soil⁻¹ (Osmocoat; The Scotts Company LLC, 14111 Scottslawn Rd. Marysville, OH 43040) was incorporated into potting mix prior to planting as recommended by the manufacturer. Intermediate scouringrush growth was initiated in a greenhouse maintained at 20-25° C under ambient light supplemented by lights to achieve 14-hours of 25 mW cm⁻² light per day. After 7 days, pots containing intermediate scouringrush and weed free controls were seeded with a soft white winter wheat ‘Goetze’ at a constant rate of 32 wheat seeds per pot. The seeding rate was adapted from the variety description and management recommendations for this variety (Flowers and Peterson, 2008). Seed was treated with 0.09 ml thiamethoxam, 0.02 ml mfenoxam and 0.21 ml difenoconazole (CruiserMaxx 30.8 kg L⁻¹ thiamethoxam, 5.9 g L⁻¹ mfenoxam and 37.2 g L⁻¹ difenoconazole; Syngenta Crop Protection Inc., 410 Swing Road, Greensboro, NC 27409) per kg seed. Wheat growth was initiated in the greenhouse and pots were placed outside on November 24, 2015, after wheat plants had developed 1 true leaf. Tubs remained outside at the Oregon State University campus in Corvallis, OR, through wheat maturity.

Maintenance

Wheat was treated with 72.9 g ha⁻¹ propiconazole plus 72.9 g ha⁻¹ trifloxystrobin (Stratego, 122.5 g L⁻¹ propiconazole and 122.5 g L⁻¹ trifloxystrobin; Bayer Crop Science, 2T.W. Alexander Drive, Research Triangle Park, NC 27709) on March 7, 2016, as a preventative treatment for septoria blotch (*Septoria tritici* Desm.), and with 107.2 g ha⁻¹ propiconazole plus 123.9 g ha⁻¹ azoxystrobin (Quilt Xcel, 122.2 g L⁻¹ propiconazole and 141.4 g L⁻¹ azoxystrobin; (Syngenta Crop Protection Inc., 410 Swing Road, Greensboro,

NC 27409) on May 2, 2016 for stripe rust (*Puccinia striiformis* Westend.). Wheat was fertilized on March 30, 2016 and April 20, 2016 with 0.7 g 20-20-20 water-soluble fertilizer (All Season Plant Food; Grow More Inc., 15600 New Century Drive Gardena, CA 90248) per pot mixed in 0.2 L water. Tubs were watered as needed, (when potting mix was visibly dry and beginning to crack at the surface) throughout the growing period.

Field Studies

Herbicide efficacy trials were conducted on smooth and intermediate scouringrush in grower fields in Reardan (2014) and The Dalles (2015). Smooth scouringrush was the predominant species present at the Reardan site, while intermediate scouringrush was predominant at The Dalles site. A complete description of the trial sites, maintenance, experimental designs, and general methods for these trials is presented in Chapter 2. Though the purpose of the two field studies was to evaluate herbicide treatments for smooth and intermediate scouringrush control, lack of herbicide efficacy allowed for regression analysis on yield loss in response to smooth or intermediate scouringrush stem density.

Experimental Design and Data Collection

An additive design was used where pots were arranged in randomized complete blocks containing 5 replications for treatments consisting of 8, 16, 32, 64 and 100 rhizome fragments. Seven replications were used for treatments consisting 1 and 2 rhizome fragments and six replications of pots containing 4 rhizome fragments. Additional replications were used for low intermediate scouringrush density treatments to ensure enough plants established to include in the analysis. Grain was hand threshed to measure wheat grain yield. Data collected were wheat grain weight (g), tiller number, total above ground wheat biomass (g), wheat height and spike number. All data were collected on a per pot basis. Intermediate scouringrush stems were counted at tillering and at wheat harvest. Wheat was harvested on July 18, 2016.

Experimental designs differed between field study locations due to terrain and scouringrush patch dynamics. A split-plot arrangement was used in Reardan, while a split-block arrangement was used in The Dalles. The metric used to quantify smooth and intermediate scouringrush density in field studies was

average stems m row⁻¹. Stems were counted in May and August of 2014 at the Reardan site, and June and August of 2015 at The Dalles site (8 months after planting and at harvest) by counting all visible scouringrush stems from row-center to row-center on a 1-meter transect parallel to the wheat rows. Stems along two, 1-meter transects were counted per plot (n=176) at each timing in Reardan, and three, 1-meter transects were counted per subplot (n=240) in The Dalles. Average stems m row⁻¹ was calculated from two samples per plot in Reardan, and 3 samples per subplot in The Dalles. Eight subplots were excluded from The Dalles trial due to wheat stand reductions caused by herbicide injury. An extra subsample in each plot was counted in The Dalles to account for variation introduced by low intermediate scouringrush patch density. Intermediate scouringrush occurs almost exclusively in low density stands (1-50 stems m⁻²), while smooth scouringrush occurs most commonly in medium density stands (50-200 stems m⁻²) (Rutz and Farrar, 1984).

Data Analysis

Not enough intermediate scouringrush was actively growing in pots at the time of wheat tillering for analysis. Intermediate scouringrush stem density at harvest in the potted wheat experiment did not increase proportionally with rhizome planting densities. To maintain balance within the dataset, pots with 1 to 5 intermediate scouringrush stems were considered low density (n=18), and pots with >5 intermediate scouringrush stems were considered high density (n=16). A control group was comprised of all remaining pots lacking intermediate scouringrush stems at harvest (n=16). Levene's analysis of variance (ANOVA) was used to test for homogeneity of variance across smooth or intermediate scouringrush density groupings in the potted wheat experiment and locations in field experiments. Tests for homogeneity of variance were conducted using SAS Proc GLM (SAS version 9.3, 2011). Grain yield, tiller number, spike number, wheat height and aboveground wheat biomass were regressed against scouringrush stem density.

Data from potted wheat and field experiments were fitted to the model:

$$y = \beta_0 + \beta_1 \text{Stems} + \beta_2 \text{Density} + \beta_3 \text{Stems} \times \text{Density},$$

Where y = grain yield/aboveground wheat biomass/tiller number/ wheat height/spike number, β_0 = intercept, β_1 = the regression coefficient for scouringrush stem number, β_2 = the regression coefficient for

smooth or intermediate scouringrush density groupings or location in field experiments and β_3 = the regression coefficient for the interaction between smooth or intermediate scouringrush stem number and density grouping. Smooth and intermediate scouringrush stem number was treated as a continuous variable and density groupings were included as categorical predictors. Wheat yield data from field experiments were regressed against the average number of scouringrush stems $m\ row^{-1}$ at both timings and analyzed using the same method. Stems $m\ row^{-1}$ were treated as a continuous variable and locations were included as categorical predictor values. Multiple linear regression procedures were completed using the Reg procedure of SAS. Outlying data points were included in the analysis, as they did not influence results. All statistical tests were conducted using a 5% significance level.

RESULTS AND DISCUSSION

Potted Wheat Study

Under controlled growing conditions, there was no evidence that intermediate scouringrush stem densities from 1 to 5 stems per pot had a different magnitude of effect on wheat grain yield, aboveground biomass production, tiller number, spike number or height than stem densities ranging from 6 to 74 stems per pot. All regression parameters tested were found to be insignificant ($P>0.05$). The slope of the regression lines for grain yield and all other measurements taken on winter wheat remained near zero for both high and low density treatments (Figure 3.1). However, low coefficients of determination indicate that the total number of intermediate scouringrush stems per pot was an inadequate measure to explain how intermediate scouringrush density affects winter wheat under these conditions. More pots with higher stem counts (i.e. 30-70 stems per pot) would have likely resulted in a better fitting regression model.

Fitting a regression line to the control group was not possible because all pots included in the control group had 0 intermediate scouringrush stems per pot. Control grain weight, aboveground biomass production, number of tillers, number of spikes, and height were averaged and presented as a single point (Figure 3.1-3.5). Though there was no evidence for a violation of homogenous variance between the average grain yield for the control, high and low density groups (Levene's ANOVA; $P>0.05$), no meaningful statistical analysis could be conducted on density grouping means. There was no method to

determine what grain yield effects were a result of intermediate scouringrush-wheat competition, and what was an effect of increasing the overall plant density per pot, which is the fundamental disadvantage of utilizing an additive design to quantify effects plant-plant competition. However, it should be noted that there was seemingly no difference between the average of the control group and the response intercept for either high or low intermediate scouringrush density regression lines for any wheat growth metric or yield component measured (Figures 3.1-3.5). Intermediate scouringrush density group means and standard deviations are presented in Table 3.1.

Through their work with common milkweed-wheat competition, Yenish and Durgan (1997) found that wheat yield loss regressed against common milkweed biomass m^{-2} had higher coefficients of determination than common milkweed stems m^{-2} . While measuring intermediate scouringrush biomass for use in regression analysis may have ultimately resulted in better-fit regression lines, the methodology involved with collecting accurate biomass data presented some complications. Greenhouse grown scouringrush was found to produce 90% of its biomass below ground (Chapter 4). Aboveground intermediate scouringrush biomass could have been measured and used as an explanatory variable in the analysis, but it is unlikely that using only 10% of the total biomass would capture the true effect of the plant-plant association. Furthermore, separating wheat and intermediate scouringrush belowground biomass under potted conditions has the potential to introduce additional error into the data.

Another factor to consider is the general appearance of intermediate scouringrush shoots observed in the potted wheat experiment was different than what is typically observed in natural settings, field experiments, and other greenhouse studies. Stems were generally shorter, thinner and lacked biomechanical strength. All *Equisetum* species have an extremely deep root system that is facilitated by their terraced rhizome architecture. Under field conditions, winter wheat roots can reach depths of up to 2 m (Thorup-Kristensen et al., 2009) while *Equisetum* rhizomes and roots are able to extend downward past 2 m (Husby, 2013; Golub and Whetmore, 1948). By growing wheat and intermediate scouringrush together in pots, a lower bound was created on rooting depth. Greenhouse grown soybeans (*Glycine max* (L.) Merr.) have been shown to partition more resources to root development when multiple plants are grown in a single pot compared to plants grown individually (Gersani et al., 2001). It is a possibility that forcing roots to grow in a more limited space elicited a similar response, but is likely not the sole explanation for the difference in

appearance observed in this study. A longer establishment period in the greenhouse would likely produce intermediate scouringrush that more closely resembles what is observed in agricultural or natural settings.

Field horsetail (*Equisetum arvense* L.), a plant from the same genus as intermediate scouringrush, forms more robust plants growing in denser stands under low nitrogen and high light conditions, making it vulnerable to being outcompeted by faster growing plants that respond to nitrogen (Andersson and Lundgårdh, 1999a,b). Given the short period of time allotted for intermediate scouringrush to establish prior to wheat seeding and supplemental fertilizer inputs, winter wheat could have gained a competitive advantage before intermediate scouringrush began actively growing the spring after planting in the potted experiment.

Field Studies

There was a difference in yield between locations, (Figure 3.6). Average wheat yields were 4821 kg ha⁻¹ ± 590 at Reardan and 2119 kg ha⁻¹ ± 633 at The Dalles. Under field conditions, there was no evidence that smooth or intermediate scouringrush density m row⁻¹ at the May/June timing had an effect on winter wheat yield at either trial location. The regression parameter for stems m row⁻¹ quantified in May and June was found to be insignificant (P>0.05). However, there was evidence that the magnitude of effect of intermediate scouringrush density in August was higher at the Dalles compared to smooth scouringrush at Reardan, as the interaction parameter was significant (P<0.05). Slopes of August regression lines were -9 for Reardan and -33 for The Dalles, which indicated that for every 1 smooth or intermediate scouringrush stem m row⁻¹ increase over a hectare, a 9 kg ha⁻¹ and 33 kg ha⁻¹ reduction would result at the two locations, respectively. Coefficients of determination were greater when wheat yields were regressed against scouringrush stems m row⁻¹ quantified in August than May/June, but overall were low enough to indicate that the number of smooth/intermediate scouringrush stems m row⁻¹ was a poor method of predicting wheat yield. Therefore, model predictions of yield effects at the two locations were not accurate.

Different trends were observed between field and potted wheat experiments. Increasing smooth and intermediate scouringrush density was correlated with a negative yield response at both field locations, while intermediate scouringrush density was found to have no influence on winter wheat in the potted

study. Interpreting results in this fashion should be addressed with caution because it does not account for herbicide treatments applied at field sites and data from potted wheat studies are not transitive to data collected in the field. However, there is enough data to form a preliminary hypothesis that typical field densities of smooth and intermediate scouringrush are correlated with negative yield responses in winter wheat.

Andersson and Milberg (1996) conducted a 26-year running field study examining weed species composition and performance in response to nitrogen rates and cropping systems consisting of fall seeded crops and grass ley, legume-grass ley and spring wheat followed by harrowed fallow rotations. The dominant weed species in unfertilized field plots was always field horsetail regardless of crop rotation. The authors suggest that increasing seeding and/or nitrogen fertilizer rates will control field horsetail, because it is a weak competitor and lacks the ability to respond to increased resource availability. With the understanding that results are being extrapolated between *Equisetum* species, it is possible that a similar scenario is being observed with smooth and intermediate scouringrush in Pacific Northwest directed seeded wheat cropping systems. Winter wheat was direct seeded following a year of chemical fallow at the Reardan and The Dalles site. Few herbicides are known to have activity on scouringrush species (Bernards et al., 2010; Frasure and Bernards, 2011; Finnerty and Glaser, 1980; Yoder et al., 1983), which results in an elimination of all potential competitors with smooth and intermediate scouringrush during the chemical fallow rotation. The spring following winter wheat seeding where noticeable patches of scouringrush remain could be in portions of the field where wheat was slower to establish or established in weaker stands due to nutrient deficiency or other biotic or abiotic stresses. This scenario could partially explain the 30% yield reduction in scouringrush infested wheat fields reported by eastern Oregon growers, and is supported by the potted wheat study results where nutrient and water availability was controlled and intermediate scouringrush was found to be a weak or non-competitor with winter wheat.

CONCLUSIONS

Based on data from field experiments, it does appear that winter wheat yield reductions are correlated with increasing smooth and intermediate scouringrush density. However, no apparent influence

on winter wheat development or grain yield was caused by intermediate scouringrush in a controlled potted wheat experiment. These results lead to a preliminary hypothesis that variable soil fertility or additional biotic or abiotic stresses within winter wheat fields has a larger influence on grain yield than smooth or intermediate scouringrush density. Available literature on field horsetail suggest that it is possible yield reductions currently attributed to smooth and intermediate scouringrush are in reality a symptom of a different agronomic issue (nitrogen deficiency was discussed as a potential factor in this chapter). Additional field studies specifically designed to evaluate how smooth and intermediate scouringrush patch density affects wheat yields, and/or potted wheat studies where more time is allotted for smooth and/or intermediate scouringrush to establish are needed to support this conclusion. Addressing what soil and environmental conditions favor smooth and intermediate scouringrush establishment within production fields would be another direction for future research.

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Table 3.1. Average wheat grain yields and other measurements with standard deviations from potted wheat density groupings and field experiments. Grain yield for potted wheat is expressed as grams per pot, and $t\ ha^{-1}$ for field experiments.

	Grain Yield	Biomass (g)	Spike Number	Tiller Number	Height (cm)
Potted Wheat (Control)	121.8 ± 21.1	282.8 ± 44.9	55 ± 12	116 ± 19	85.8 ± 4.2
Potted Wheat (Low Density)	121.1 ± 20.5	278.8 ± 39.4	53 ± 11	113 ± 16	85.1 ± 11.2
Potted Wheat (High Density)	111.9 ± 16.3	263.3 ± 35.1	47 ± 7	104 ± 11	87.1 ± 4.1
Reardan, WA	4.82 ± 0.4	N/A	N/A	N/A	N/A
The Dalles, OR	2.12 ± 0.3	N/A	N/A	N/A	N/A

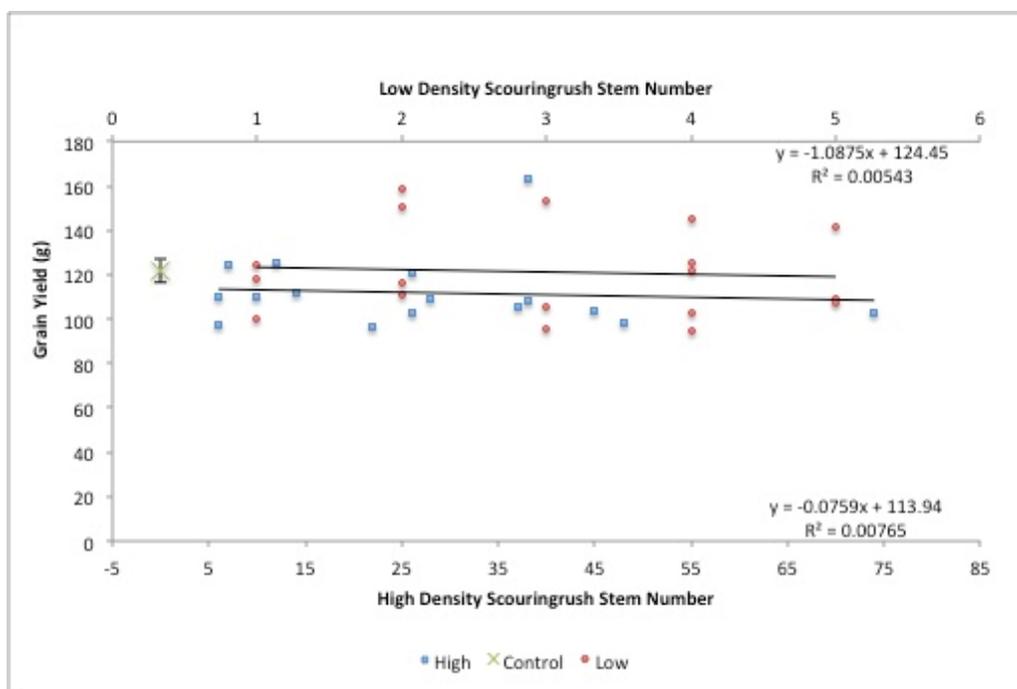


Figure 3.1. Grain yield data with fitted linear regression lines from the potted wheat experiment. The primary x-axis is scaled for high density intermediate scouringrush and the secondary x-axis is scaled for low density intermediate scouringrush. Regression lines, equations and coefficients of determination for the high density group are all on the lower portion of the figure and on the top portion for the low density group. The standard error of the control group mean is shown with the data point.

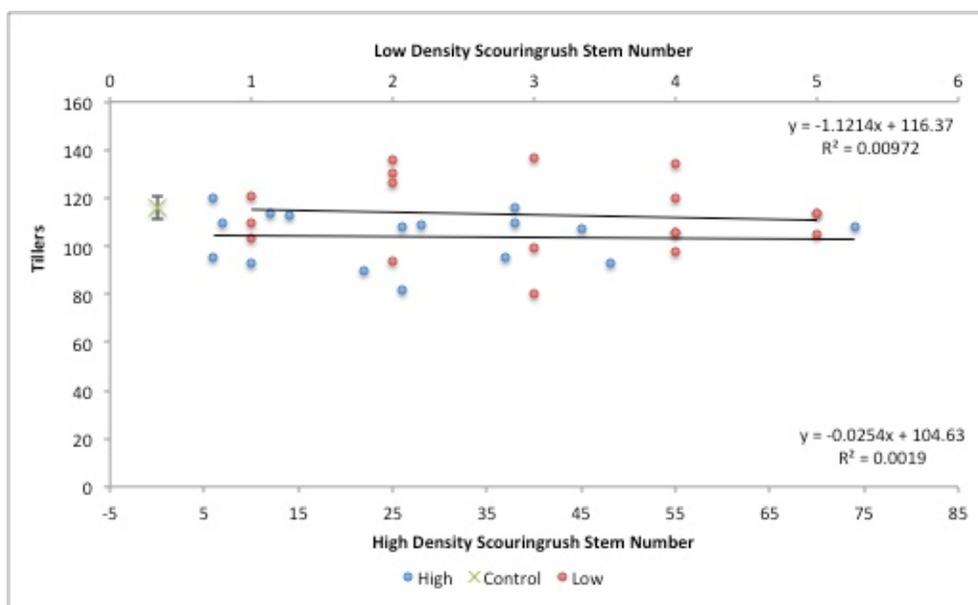


Figure 3.2. Tiller numbers with fitted linear regression lines from the potted wheat experiment. The primary x-axis is scaled for high density intermediate scouringrush and the secondary x-axis is scaled for low density intermediate scouringrush. Regression lines, equations and coefficients of determination for the high density group are all on the lower portion of the figure and on the top portion for the low density group. The standard error of the control group mean is shown with the data point.

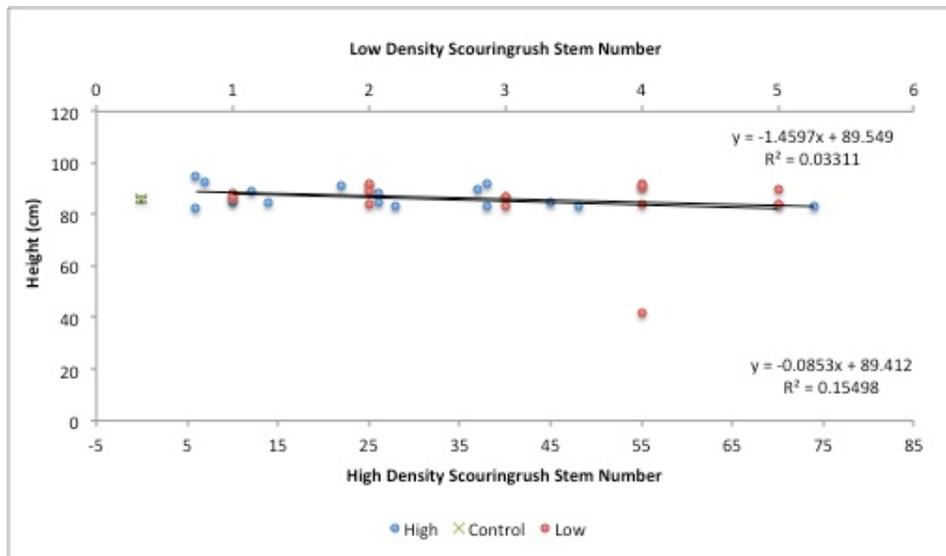


Figure 3.3. Wheat height with fitted linear regression lines from the potted wheat experiment. The primary x-axis is scaled for high density intermediate scouringrush and the secondary x-axis is scaled for low density intermediate scouringrush. Regression equations and coefficients of determination for the high density group are all on the lower portion of the figure and on the top portion for the low density group. The standard error of the control group mean is shown with the data point.

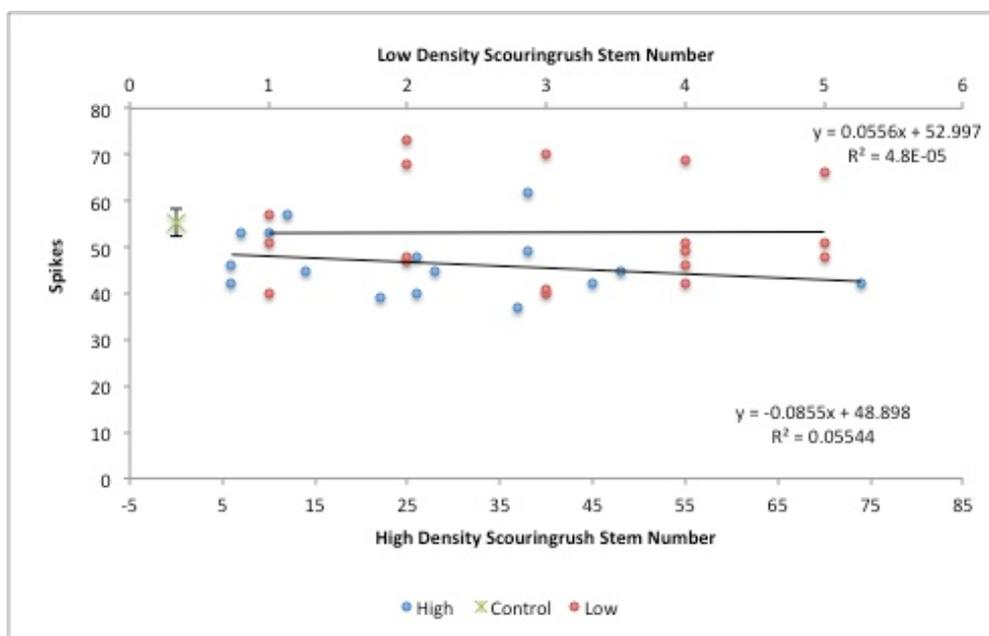


Figure 3.4. Spike numbers with fitted linear regression lines from the potted wheat experiment. The primary x-axis is scaled for high density intermediate scouringrush and the secondary x-axis is scaled for low density intermediate scouringrush. Regression lines, equations and coefficients of determination for the high density group are all on the lower portion of the figure and on the top portion for the low density group. Slopes of regression lines appear to be different, but the density regression parameter was insignificant ($P > 0.05$). The standard error of the control group mean is shown with the data point.

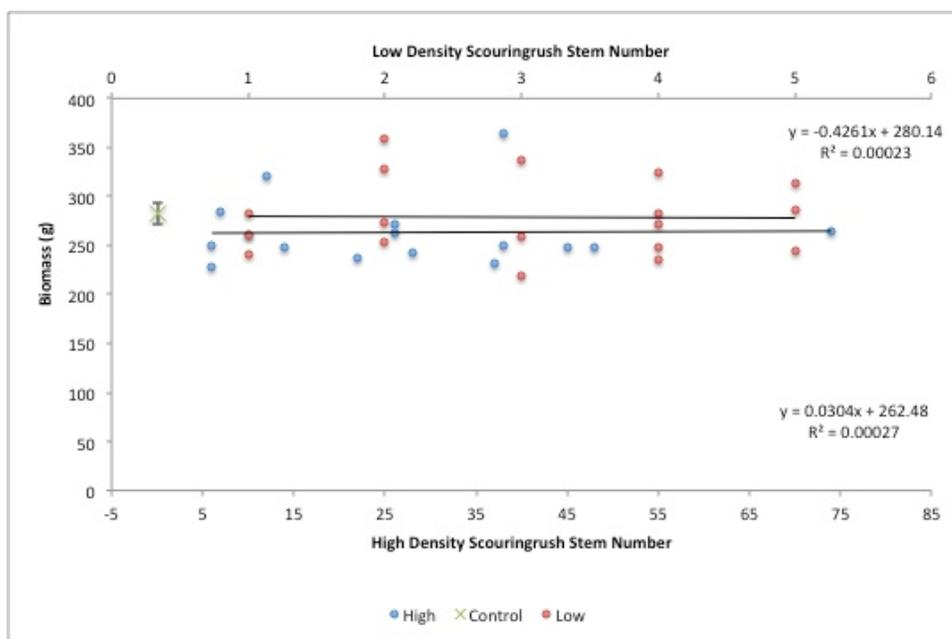


Figure 3.5. Biomass data with fitted linear regression lines from the potted wheat experiment. The primary x-axis is scaled for high density intermediate scouringrush and the secondary x-axis is scaled for low density intermediate scouringrush. Regression lines, equations and coefficients of determination for the high density group are all on the lower portion of the figure and on the top portion for the low density group. The standard error of the control group mean is shown with the data point.

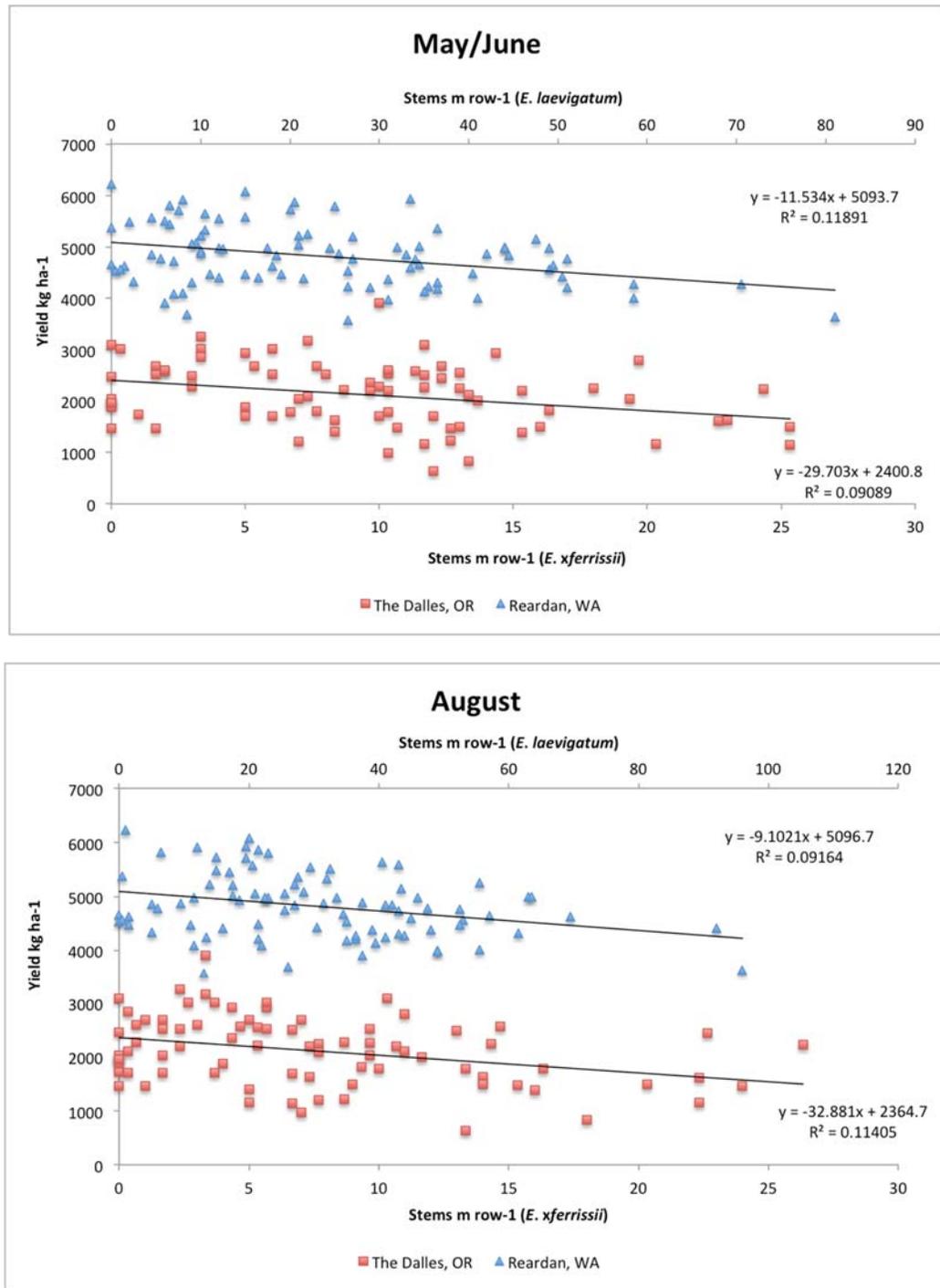


Figure 3.6. Wheat yields with fitted linear regression lines from field experiments. The primary x-axis is scaled for scouringrush stem numbers quantified at The Dalles, OR site and the secondary x-axis is scaled for stem numbers quantified at the Reardan, WA site. Stems were counted in May of 2015 in Reardan, WA, and June of 2016 in The Dalles, OR.

CHAPTER 4: Effects of Soil pH on Growth and Establishment of Scouringrush (*Equisetum* spp. L.)

ABSTRACT

Repeated applications of ammonium based nitrogen fertilizers in agricultural production fields have caused soils to gradually acidify in the inland Pacific Northwest. Scouringrush (*Equisetum* spp. L.) is a species historically associated with wetlands that has begun to expand into agricultural production fields in the same region. Though it is accepted that scouringrush can survive in a wide range of soil conditions, there is little information available on how soil chemistry effects scouringrush growth and establishment. The following research was conducted to more thoroughly examine what effect soil pH has on scouringrush growing in two different soil types. An acidic soil collected near Corvallis, Oregon, was amended with calcium carbonate, and an alkaline soil collected near Madras, Oregon, was amended with sulfuric acid with the goal of creating a matching soil pH range for the two soils. After soils were amended, scouringrush rhizome fragments were planted and grown in a greenhouse for 344 days. Total above and belowground dry biomass was then measured and whole plant tissue samples were analyzed for 13 different nutrient concentrations. Scouringrush had a similar biomass response to soil pH when grown in both soil types. Total biomass production increased as soil pH increased from ≈ 4.6 to ≈ 8.0 . Scouringrush was able to survive in acidic soil conditions (pH ≈ 4.1) with plant tissue concentrations of iron, manganese, and aluminum known to cause toxicity in a majority of crop plants (9916 mg kg⁻¹ iron, 781 mg kg⁻¹ manganese and 5000 mg kg⁻¹ aluminum). Soil acidity was found to negatively impact scouringrush, but the plants were able to establish under conditions that would be unsuitable for winter wheat or other cereal grain crop production.

INTRODUCTION

Repeated applications of ammonium based nitrogen fertilizers in agricultural production fields have caused soils to gradually acidify in the inland Pacific Northwest (Mahler et al., 1985). Decreases in soil pH are of particular concern in direct seeded cereal grain cropping systems. Soil acidification proliferates in these cropping systems due to high nitrogen inputs ($>100 \text{ kg ha}^{-1} \text{ yr}^{-1}$ in high yielding areas) that are not mechanically incorporated or mixed with the subsurface soil as they would be under a conventional tillage regime (Mahler and Harder, 1984; Robbins and Voss, 1989). While the soil pH in the region is typically 6.5 to 7.2 (Brown et al. 2008), Mahler et al. (2015) observed that the percentage of northern Idaho and eastern Washington growers reporting field soil pH ranging from 4.5 to 5.0 increased from 0% in 1981 to 2.3% in 1996 and 5.8% in 2011. In the same years, 19.3%, 35.8% and 44.1% of growers reported soil pH ranges between 5.1 and 5.5. These trends are alarming as they indicate an increasing amount of hectares in cereal grain production are reaching minimum soil pH thresholds for cereal grain production. For example, the minimum soil pH threshold for wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are 5.4 and 5.8, respectively (Hart et al., 2013).

Another issue that has materialized in Pacific Northwest direct seeded cereal grain cropping systems is a weed species shift where scouringrush (*Equisetum* spp. L.) populations have been observed in production fields in eastern Oregon and eastern Washington similar to what Hartzler (2009) identified in no-till cropping systems in the Midwest region. Consistently finding large high-density scouringrush patches in production fields is striking as historically the plant was confined to irrigation ditches, field margins and roadside depressions (Rutz and Farrar, 1984). Under ideal production conditions cereal grain crops will likely out-compete scouringrush as *Equisetum* species have an inability to respond to increased nitrogen availability (Husby, 2013). How cereal grain crops compete with scouringrush in high stress conditions, however, is largely unknown.

Shifts in tillage practices are likely the main contributor to the expanding role scouringrush has as an agricultural weed. However, soil pH could play a role in describing how scouringrush establishes in its expanded range and what effect, if any, it has on cereal grain crops. It is known that scouringrush can survive in an extremely wide range of soil conditions (Juárez-Santillán et al., 2010; Husby, 2013), but the

small amount of information available on how scouringrush responds to soil pH is a sparse collection of anecdotal observations. For example, in a vegetation survey of irrigation canals in New Mexico (Schroeder et al. 2012), *Equisetum* species were found not to be influenced by soil pH and were more likely to be found on fine-textured soils. These results lacked support from scientific literature and their results call for additional studies to define relationships between *Equisetum* abundance and soil texture and chemistry. The following studies were conducted to gain a more specific and quantifiable understanding on how scouringrush responds to soil pH, and to set a foundation to investigate how soil acidification affects the plant-plant interaction between scouringrush and cereal grain crops.

MATERIALS AND METHODS

Soil Collection and Amendments

An acidic Woodburn silt loam soil (pH \approx 4.3, 2.7% organic matter) was collected near Corvallis, Oregon, at the Oregon State University Hyslop Field Research Lab in December 2014. Soil was dried in a greenhouse and sieved to 5mm in preparation for liming. A representative sample was sent to A & L Western Agricultural Laboratories in Portland, OR, to measure the SMP buffer pH.

Acidic soil was amended with solution grade calcium carbonate (CaCO_3) (Microna Ag H_2O ; Columbia River Carbonates, 300 N. Pekin Rd. Woodland, WA 98674) to reach a range of soil pH conditions. Target soil pH levels were 4.6 (untreated), 5.1, 5.6, 6.6 and 7.6. Calcium carbonate rates were developed using the SMP buffer pH, measured at 5.2 for the Corvallis, OR, soil, and the assumption that an oven-dry hectare furrow slice (HFS; the uppermost 15 cm of the soil profile over 1 hectare) weighs 2,240,746 kg. Using recommendations from the Western Oregon Lime Guide (Anderson et al., 2013), SMP buffer pH and HFS it was calculated that 1 metric ton of CaCO_3 would raise the soil pH of an oven-dry HFS by 0.1. Calcium carbonate rates calculated on a tonnes ha^{-1} basis were scaled down to grams CaCO_3 per kilogram oven-dry soil (Tables 2.1 and 2.2). Percent soil water by mass was calculated by drying soil samples for 24 hours in a convection oven at 100° C. After accounting for soil moisture, CaCO_3 treatments were applied to soil then mixed in 19L plastic buckets. In a preliminary experiment (lime-1), treated soil

was incubated for 7 days prior to planting. For the primary CaCO_3 experiment (lime-2), the soil incubation period was increased to 28 days to allow more reaction time for the soil amendments. Further descriptions of the equations and assumptions used to create CaCO_3 amendment rates are included in Appendix C.

A basic Willowdale loam soil ($\text{pH} \approx 8.6$, 5.2% organic matter) was obtained through Central Oregon Seeds Inc. in Madras, OR. Elemental sulfur can be used to amend high pH soils. Sulfur is oxidized to form sulfuric acid (H_2SO_4), which reacts with lime in the soil to form gypsum, carbon dioxide and water resulting in decreased soil pH. The oxidation process is slow and can take multiple sulfur applications over many seasons to cause a reduction in soil pH (Horneck et al., 2006). To expedite the soil acidification process, a titration method derived from Costello and Sullivan (2014) was used to calculate rates of H_2SO_4 needed to create a range of soil pH conditions below that of the CaCO_3 amended soil from Corvallis. From the fizz test described by Horneck et al. (2006) it was assumed that the Madras soil has approximately 5% free lime (CaCO_3). Assuming 1 mol of sulfur is needed to neutralize 3 mol CaCO_3 , it was calculated that 1 ml of 10.4 N H_2SO_4 will neutralize 10 g of soil containing 5% CaCO_3 . To test this estimate, 1 ml of 14.4, 12.4, 10.4, 8.4, 6.4, 4.4, 2.4, 1, and 0.4 N H_2SO_4 was added to 10 g of Madras soil with 19 ml deionized water. One ml of each concentration of H_2SO_4 was added to 19 ml of deionized water to allow for the 1:2 soil to water ratio needed to measure soil pH to be maintained. Soil pH measurements were taken at 24 hours then every 24 hours until stable.

Soil from Madras was dried and sieved as previously described. Target pH and H_2SO_4 rates for each soil treatment were initially going to be based off of the titration curve, but due to soil pH buffering effects (Figure 2.1) and time constraints, rates were chosen based on the initial estimation that 1 ml of 10.4 N H_2SO_4 will neutralize 10 g of soil containing 5% free lime. Rates were then scaled to mmol H^+ per kg dry soil. Sulfuric acid treatments included a neutralizing rate, 1.5 times the neutralizing rate and 2 times the neutralizing rate (Table 2.3). Treatments were applied by subchemigating H_2SO_4 diluted in distilled water through the bottom of 2.6 L pots containing the Madras soil. Dilutions were calculated based on soil subirrigation moisture wicking capacity of soil contained within 5 pots. Entire H_2SO_4 rates were applied in a single subchemigation application for 1x and 1.5x treatments. For 2x treatments half of the H_2SO_4 was subchemigated and after 14 days a second non-diluted (97% H_2SO_4) application was injected directly into soil at the surface of pots. Seven days following H_2SO_4 application soil was removed from pots and mixed

in 39 L #5 plastic tubs to eliminate any soil pH stratification formed by subchemigation or injection treatments. Soil was incubated for 7 days prior planting. Further descriptions of the equations and assumptions used to create H₂SO₄ amendment rates are included in Appendix C.

Soil pH Measurement

All soil pH measurements were taken with a Fisher Scientific Accumet AR-15 pH Meter using the 1:2 soil to water ratio method described by the Oregon State University Soil Testing Laboratory. Soil pH was measured following incubation at planting and immediately following harvest for all experimental runs. For the lime-2 and the H₂SO₄ (acid-1) experiments, additional soil samples were treated to monitor pH through the growing period (Figures 2.2 and 2.3).

Plant Material and Growing Conditions

Scouringrush rhizome fragments from a mixed population of *Equisetum* spp. (*Equisetum hyemale* L. and *Equisetum arvense* L.) were collected near Corvallis in October 2014. Soil pH at the site was measured at 6.1. Two rhizome fragments ranging in length from 7 to 15 cm were planted into 2.6 L pots containing amended soils at a depth of 7 cm. Excess plant material from lime-1 was planted in 52x28x5 cm trays filled with potting mix (Sunshine Mix 1 Potting Mix; Sun Gro Horticulture, Inc., 110th Ave. NE, Suite 490, Bellevue, WA 98004) and kept alive for subsequent experiments. Each fragment was weighed prior to planting. Plants were grown in a greenhouse maintained at 20-25° C under ambient light supplemented by lights to achieve 14-hours of 25 mW cm⁻² light per day. Pots were subirrigated as needed for all experiments. Plants from lime-1 were grown for 45 days, 344 days for lime-2, and 325 days for acid-1.

Experimental Design and Data Analysis

All experiments were arranged using a randomized complete block design. Treatments were replicated 5 times in lime-1 and 4 times in lime-2 and acid-1. An extra replication was utilized in lime-1 because it was unknown how scouringrush would respond to a field soil under greenhouse conditions. For

all experiments, total fresh biomass (g) was measured for each pot. Total fresh biomass increase for each pot was calculated using the rhizome weights recorded at planting. In the lime-2 and acid-1 experiments, soil was washed away from roots and rhizomes, which were then separated from the above ground shoots and dried at 55° C for 48 hours. Total above and below ground dry biomass was then recorded. After dry biomass was recorded, above and below ground plant tissue were combined into a single composite sample from each pot and sent to A & L Western Agricultural Laboratories in Portland, OR for analysis. Samples were analyzed for total nitrogen, phosphorus, potassium, sulfur, magnesium, calcium, sodium, iron, aluminum, manganese, copper, boron and zinc.

Plant tissue analysis data for each nutrient were plotted against the corresponding soil pH values. Total dry biomass plotted against soil pH was included in the same figures (4.9, 4.10 and 4.11). As an initial analysis, plots with total dry biomass and plant tissue nutrient concentration were used to identify possible trends involving scouringrush tissue nutrient concentrations, soil pH and biomass production. Scatter plots used in the initial analysis can be seen in Appendix D.

Analysis of variance (ANOVA) was conducted for soil pH at harvest, total dry biomass, fresh biomass increase, and plant tissue analysis results as responses to soil amendment method using SAS Proc GLM (SAS version 9.3, 2011). Levene's ANOVA and the Shapiro-Wilk test were used to verify that assumptions of homogenous variance and normality were met. T test statistics were used to analyze differences in treatment means using the LSMEANS function of SAS Proc GLM. All ANOVA and T-tests were completed using a 5% significance level.

To further isolate how soil pH affects scouringrush growth independent of soil amendment method, total dry biomass, and plant tissue iron, aluminum and manganese data from scouringrush grown in the Corvallis (lime-2) and Madras (acid-1) soil were regressed against soil pH measured from the individual pot in which they were grown. Regression procedures were completed using SAS Proc Reg (SAS version 9.3, 2011). Soil pH was analyzed as a continuous variable with soil type as a categorical variable. Data were fit to the model:

$$y = \beta_0 + \beta_1pH + \beta_2Soil + \beta_3pH x Soil,$$

where y = dry biomass, β_0 = intercept, β_1 = the regression coefficient for soil pH, β_2 = the regression coefficient for soil type/amending agent and β_3 = the regression coefficient for the interaction between soil pH and soil type/amending agent. All regression tests were completed using a 5% significance level.

RESULTS AND DISCUSSION

Soil pH

There was no evidence for normality violations in the soil pH data for any of the experiments according to the Shapiro-Wilk test ($P > 0.05$). Levene's ANOVA provided no evidence for unequal variance in experiments lime-1 and lime-2 ($P > 0.05$). There was evidence for unequal variance in soil pH for acid-1 according to Levene's ANOVA ($P < 0.05$). However, because the assumption of normality was met and all sample sizes were equal, the T-tests performed were robust to the violation (Ramsey and Shafer, 2013). Further, ANOVA statistics provided no evidence for blocking effects ($P > 0.05$) and significant evidence for H_2SO_4 and $CaCO_3$ treatment effects on soil pH ($P < 0.05$).

There was no difference in soil pH between the 8.98 and 13.46 g $CaCO_3$ kg^{-1} soil treatments in experiment lime-1, but all other differences in soil pH between $CaCO_3$ treatment levels were significant ($P < 0.05$). When soil pH was measured over 370 days in experiment lime-2, increases in pH across all levels continued for approximately 50 days (Figure 2.3). Due to the short soil incubation period, there was likely still unreacted $CaCO_3$ in lime-1 soil, which could describe the lack of separation in soil pH between the 8.98 and 13.46 g $CaCO_3$ kg^{-1} soil treatments. Differences in soil pH between all treatment levels of $CaCO_3$ were significant ($P < 0.05$) in experiment lime-2.

For experiment acid-1, differences in pH between untreated soil, 2x-neutralizing H_2SO_4 treated soil, and all other treatment levels of H_2SO_4 were significant ($P < 0.05$). However, there was no difference in pH between soils treated with a neutralizing and a 1.5x-neutralizing dose of H_2SO_4 . A buffering effect was observed when pH was measured over 380 days in soil from experiment acid-1. Soil pH began to increase 55 days after H_2SO_4 treatment. Soil treated with a neutralizing and a 1.5x-neutralizing H_2SO_4 dose reached a minimum soil pH of 6.2 at 55 days then buffered back to 7.3 throughout the duration of the monitoring period. No buffering effects were observed in surplus soil treated with a 2x-neutralizing dose of

H₂SO₄ (Figure 2.2). It is unknown whether the elevated H₂SO₄ rate or the treatment method reduced pH buffering, as a 2x-neutralizing dose was achieved through a split application where the first dose was diluted and subchemigated into pots containing soil and the second dose was directly injected at the soil surface.

Although there were not equally stratified soil pH responses within and across experiments, and CaCO₃ and H₂SO₄ treatments lacked observations ranging from soil pH 5.0-6.0, variation in pH response within treatment groups was minimal. It is also important to note that a wide enough range of soil pH conditions was achieved to address how scouringrush responds to soil pH in conditions unsuitable for cereal grain production. Average soil pH, standard deviations, and range at harvest for all treatment levels across the three experiments are presented in Table 2.4.

Fresh biomass Increase

There was no evidence for normality or unequal variance violations in the fresh biomass increase data for any of the experiments according to the Shapiro-Wilk test and Levene's ANOVA ($P > 0.05$). Further ANOVA statistics provided no evidence for blocking effects ($P > 0.05$) and significant evidence for H₂SO₄ and CaCO₃ treatment effects on fresh biomass increase.

As a preliminary study, the only biomass measurement taken for experiment lime-1 was total fresh weight increase. Following 45 days of growth, scouringrush biomass production increased as soil pH increased from 4.6 to 7.2. At soil pH 7.3, biomass production decreased (Figure 2.4). There was no difference in total fresh biomass increase between scouringrush grown in soils at pH 4.6 and 7.3 ($P > 0.05$). It is unlikely that a soil at pH 7.3 would elicit that dramatic of a biomass response, so the same measurement was repeated for experiments lime-2 and acid-1. After 370 days, scouringrush fresh biomass production again peaked at soil pH 7.2 then dropped off as soil pH approached 7.4. However, the decrease was not as dramatic as observed in the preliminary experiment and fresh biomass continued to increase as soil pH increased from 4.3 to 8.0 in experiment acid-1, suggests that biomass reductions observed in both lime experiments were likely due to factors other than soil pH (Figures 2.5 and 2.6).

Dry biomass

There was no evidence for normality or unequal variance violations in any dry biomass data for any of the experiments according to the Shapiro-Wilk test and Levene's ANOVA ($P > 0.05$). Further ANOVA statistics provided no evidence for blocking effects and significant evidence for H_2SO_4 and $CaCO_3$ treatment effects on dry biomass ($P < 0.05$).

For experiments lime-2 and acid-1 total, above and belowground dry biomass were measured and analyzed separately. In experiment acid-1, there was no difference in total dry biomass between scouringrush grown in untreated ($pH = 8.0 \pm 0.12$), neutral dosed ($pH = 7.7 \pm 0.1$), and 1.5x neutral dosed ($pH = 7.4 \pm 0.3$) soils. Scouringrush grown in soils with a 2x-neutral dose ($pH = 4.3 \pm 0.3$) produced less biomass than scouringrush grown in soils receiving all other acid treatments ($P < 0.05$). Rhizome biomass accounted for approximately 90% of the total dry biomass across all treatment groups, which made differences in biomass production mirror those of total dry biomass. Less biomass was produced in the rhizomes of scouringrush grown in 2x-neutral dosed soils than any other treatment group. With above ground biomass accounting for approximately 10% of the total biomass across all treatment groups, trends in aboveground biomass production differed from those of total and rhizome biomass. Aboveground biomass produced in untreated soil and 2x-neutral dosed soils were different than other treatment groups ($P < 0.05$). There was no difference in aboveground biomass produced in neutral dosed and 1.5x neutral dosed soil. Dry biomass results from experiment acid-1 are presented in Figure 2.7.

Scouringrush total dry biomass increased as lime rates increased, with the greatest biomass being produced by plants grown in soil treated with $9.37 \text{ g } CaCO_3 \text{ kg}^{-1}$ soil ($pH = 7.2 \pm 0.04$) in experiment lime-2. Total biomass from untreated soils ($pH = 4.6 \pm 0.2$) and soils treated with $9.37 \text{ g } CaCO_3 \text{ kg}^{-1}$ soil were different than all other treatment groups ($P < 0.05$). Again rhizome biomass accounted for approximately 90% of the total biomass across all treatment groups making trends in total rhizome biomass align with the results of total biomass. Patterns in aboveground biomass production differed from total and rhizome biomass production as aboveground biomass again only accounted for approximately 10% of the total biomass across all treatment groups. Aboveground biomass production from untreated soils ($pH = 4.6 \pm 0.2$) was different than all other treatment groups ($P < 0.05$). Total, aboveground and rhizome dry

biomass declined in soils treated with 14.04 g CaCO₃ kg⁻¹ soil (pH= 7.4 +/- 0.07). As discussed previously, this decline in biomass production is likely due to other factors than soil pH.

Results from regression procedures indicated there was no evidence that using two soil types amended with different agents had an effect on how scouringrush biomass production responded to soil pH. Soil type and soil type x soil pH interaction regression parameters were not significant ($P>0.05$). Because soil pH was the only significant regression parameter, data were pooled across experiment lime-2 and acid-1. A linear regression line was fit to describe the relationship of soil pH and scouringrush total dry biomass. Total scouringrush biomass increased as soil pH increased from 4.6 to 8.0 (Figure 2.6). There was a substantial reduction in scouringrush biomass as soil pH dropped below 5.0. Reduced growth in these conditions is not surprising as most plant species have reduced growth at soil pH 4.6 as compared to 6.0 or 7.0. However, it is worth noting that scouringrush was able to establish and grow in acidic soil pH conditions that would be highly problematic when producing cereal grains.

Plant Tissue Analysis

Similar trends in scouringrush plant tissue nitrogen concentration were observed in experiments acid-1 and lime-2. When grown in soils amended with 23, 45, 93 and 140 g kg⁻¹ soil, there was no difference in plant tissue nitrogen ($P>0.05$). Average plant tissue nitrogen across these treatments was 0.6%. Untreated soils in experiment lime-2 produced scouringrush (soil pH=4.6 +/- 0.2) with higher plant tissue nitrogen, which was measured at 0.83% on average. Experiment acid-1 soil treated with a 2x neutralizing rate of H₂SO₄ (Soil pH= 4.3 +/- 0.3) was also found to produce scouringrush with higher plant tissue nitrogen, which was measured at 0.82% on average.

There was no difference in plant tissue nitrogen measured in scouringrush grown in soils treated with a 1.5x neutralizing or neutralizing rate of H₂SO₄. Abiotic stresses can trigger a response in which nitrogen is remobilized similar to how deciduous trees recycle nitrogen from leaves during fall senescence (Masclaux-Daubresse et al., 2010). When exposed to cadmium, tomato (*Lycopersicon esculentum*) seedlings have been shown to remobilize nitrogen in the leaves and increase nitrogen storage in the roots (Chaffei et al., 2004). A similar mechanism could be involved in describing why scouringrush grown in

low pH soils was shown to have high overall plant tissue nitrogen as low soil pH causes aluminum, manganese toxicity in a majority of plants. However, the leaf structure of scouringrush is extremely reduced, which makes a response observed in tomatoes difficult to relate to an *Equisetum* species.

Plant tissue phosphorus concentrations were consistent across all soil pH conditions and treatments between the two experiments (See Appendix D: Phosphorus). Potassium measured in scouringrush plant tissue was consistent across H₂SO₄ and soil pH conditions in experiment acid-1, but potassium measured in plant tissue from experiment lime-2 increased as CaCO₃ rates and soil pH increased. In acid soils, aluminum ions (Al⁺³) in the soil solution have been shown to decrease potassium uptake (Cumming et al., 1985). When CaCO₃ is applied to acid soils, 2Al⁺³ ions exchange with 3Ca⁺² ions, then Al⁺³ hydrolyzes and Al(OH)₃ precipitates, resulting in a net reduction in soluble aluminum (Havlin et al., 2014). Reducing the total soluble aluminum in soil with CaCO₃ is a possible explanation for elevated potassium uptake in experiment lime-2. However, there are likely other factors that contribute to differential potassium uptake by scouringrush between experiments. Trends in plant tissue calcium and sulfur between the two experiments were confounded by the soil amending agents used. Scouringrush grown in soil treated with H₂SO₄ or CaCO₃ had higher plant tissue sulfur or calcium depending on which amendment was used.

Different trends in plant tissue magnesium were observed between the two experiments. Overall, there was a higher percentage of magnesium in scouringrush plant tissue when grown in soil amended with H₂SO₄ than in soil amended with CaCO₃ (P<0.05). Average scouringrush plant tissue magnesium was 0.1 and 0.3% when grown in soils treated with CaCO₃ and H₂SO₄ respectively. Scouringrush plant tissue magnesium was within a normal range of 0.1-0.4% (Havlin et al., 2014). In experiment lime-2, scouringrush plant tissue magnesium decreased as CaCO₃ rates increased (Appendix D: Magnesium). Plants growing in soils treated with the highest rate of CaCO₃ were found to have lower than normal levels of magnesium. When high rates of lime are applied to acid soils, it is possible to reduce the amount of plant available magnesium by an overabundance of calcium in the soil solution. Excess calcium has been shown to cause solution magnesium to move to exchange sites, making it unavailable for plant uptake and potentially causing a deficiency (Carran, 1991; Summer et al., 1978; and Farina et al., 1980). The highest CaCO₃ rate used in experiment lime-2 is equivalent to applying 31 metric tons of lime per hectare, which

likely describes the differential plant tissue magnesium in scouringrush grown in soils treated with H_2SO_4 and CaCO_3 . A calcium-magnesium imbalance is a possible explanation for the decrease in scouringrush biomass production in experiments lime-1 and lime-2. Halvin et al. (2014) suggested that normal plant tissue magnesium is 0.1-0.4%. Scouringrush grown in soils treated with the highest rate of lime were found to have between 0.06 and 0.08% magnesium.

Results from regression procedures indicated there was no evidence that using two soil types amended with different agents had an effect on how scouringrush plants tissue accumulated aluminum, manganese or iron in response to soil pH. Soil type and soil type x soil pH interaction regression parameters were not significant ($P > 0.05$). Because soil pH was the only significant regression parameter, data were pooled across experiment lime-2 and acid-1. Aluminum (Al^{+3}), iron (Fe^{+2} and Fe^{+3}) and manganese (Mn^{+2}) are exchangeable metallic ions that become more soluble as soil pH declines. Though aluminum toxicity is the primary issue in acidic soils, iron and manganese can be toxic to plants at high concentrations (Adams, 1984). Scouringrush was able to survive with plant tissue concentrations of 9916 mg kg^{-1} iron, 781 mg kg^{-1} manganese and 5000 mg kg^{-1} aluminum in acidic soil conditions ($\text{pH}=4.1$). By comparison toxicity generally occurs at plant tissue concentrations greater than 300 mg kg^{-1} iron and 500-1000 mg kg^{-1} manganese. Susceptibility to aluminum toxicity varies widely by species (Havlin et al., 2014; Duncan, 1983). Juárez-Santillán et al. (2010) report that a species of scouringrush (*Equisetum hyemale*) was able to withstand manganese concentrations of 5266.3 mg kg^{-1} in root tissue while keeping stem tissue manganese at 229.4 mg kg^{-1} . They hypothesized that scouringrush has evolved a rizofiltration mechanism where stable manganese complexes are formed in root cells which limit translocation to aboveground stems. Since plant tissue was analyzed on a whole plant basis in these studies it is difficult to compare results to results from Juárez-Santillán et al. (2010). However, all samples analyzed were near 90% belowground biomass making it a reasonable assumption that a majority of the iron, manganese, and aluminum found in scouringrush plant tissue were located in these structures. Results for soil pH effects on tissue aluminum, iron and manganese are presented in Figures 2.8, 2.9 and 2.10.

CONCLUSIONS

Over three experiments scouringrush biomass production increased as soil pH increased from ≈ 4.0 to ≈ 8.1 . Rates of CaCO_3 designed to raise soil pH from 4.6 to 7.6 caused a decrease in scouringrush fresh biomass production that was observed when grown for 45 and 344 days. Average soil pH between treatments was measured as 7.3 and 7.4, respectively, for these two experiments, while in the third experiment where soil was amended with H_2SO_4 , biomass production continued to increase as soil pH surpassed 7.4. This reduction in biomass has been attributed to excess calcium causing an imbalance between calcium and magnesium in the plant rather than an effect of soil pH.

Scouringrush was able to withstand excessively high concentrations of iron, manganese, and aluminum in low soil pH conditions. All of these metallic ions become more soluble at low soil pH causing toxicity in a majority of plants. The plant tissue concentration of these metals, and biomass production response by scouringrush was unaffected by soil type or amending agent. Though other plant tissue nutrient responses were discussed, it should be noted that mechanisms presented were only possibilities and further work is needed to fully understand how soil pH effects scouringrush nutrient accumulation with respect to other plant species.

In the scope of cereal grain production, it should be recognized that scouringrush was able to establish and survive in conditions where grain crops would be under heavy stress. Considering acidified soil in production fields is typically limited to the uppermost 15 cm and scouringrush is an extremely deep-rooted plant compared to a wheat or barley crop, it is a possibility under these conditions that scouringrush could gain a competitive advantage despite having an inability to respond to nitrogen inputs. Investigating how soil pH stratification within the soil profile effects scouringrush, and how soil pH effects the plant-plant interactions between scouringrush and cereal grain crops are both logical progressions to build off of this research.

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Table 4.1. Target soil pH, CaCO₃ rates and soil pH following incubation for lime-1. Soil was incubated for 7 days prior to planting. Harvest soil pH measurements were taken 60 days after liming treatments. Soil pH at planting is presented as an average across all replicates of each treatment.

pH Treatment	Untreated	A	B	C	D
Target pH	4.6	5.1	5.6	6.6	7.6
CaCO₃ (g kg⁻¹ Soil)	-	2.24	4.5	8.98	13.46
Soil pH at Planting	4.9	5.31	5.85	6.35	6.47
Soil pH at Harvest	4.58	5.33	6.21	7.21	7.28

Table 4.2. Target soil pH, CaCO₃ rates and soil pH following incubation for lime-2. Soil was incubated for 28 days prior to planting. Harvest soil pH measurements were taken 372 days after liming treatments. Soil pH at planting is presented as an average across all replicates of each treatment.

pH Treatment	Untreated	A	B	C	D
Target pH	4.6	5.1	5.6	6.6	7.6
*CaCO₃ (g kg⁻¹ Soil)	-	2.33	4.66	9.37	14.04
Soil pH at Planting	4.77	5.42	5.78	6.19	6.29
Soil pH at Harvest	4.68	5.98	6.46	7.24	7.45

*CaCO₃ rates are listed after accounting for soil moisture. Treatments are equivalent per kg oven dry soil for both CaCO₃ experiments.

Table 4.3. Target soil pH, H₂SO₄ rates and soil pH at planting. Pots containing 1.5x neutral and 2x neutral amended soil were planted 55 days after treatment. *Neutral pots were planted 15 days after treatment. Soils were amended with 97% sulfuric acid. Harvest soil pH measurements were taken 385 days after acid treatment for 1.5x and 2x soils, and 340 days after acid treatment for neutral soils. Soil pH at planting is presented as an average across all replicates of each treatment.

pH Treatment	Untreated	Neutral	1.5x Neutral	2x Neutral
Target pH	8.6	7.0	<7.0	< 6.0
H₂SO₄ (mmol H⁺ kg⁻¹ Soil)	-	1044	1440	2088
Soil pH at Planting	7.56	6.33	6.08	4.15
Soil pH at Harvest	7.99	7.67	7.4	4.29

*Additional amended soil was needed to complete the neutral treatment; this resulted in planting closer to H₂SO₄ application.

Table 4.4. Results for soil pH at harvest and plant tissue analysis. Letters signify significance ($\alpha=0.05$) within each column. Statistical tests were conducted separately for experiments lime-2 and acid-1. Included are plant tissue analysis data that could not be pooled across experiments. Data presented are averages across all treatment levels of H_2SO_4 or $CaCO_3$

	Soil pH*	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Sulfur (%)	Magnesium (%)	Calcium (%)	Sodium (%)	Boron (mg kg ⁻¹)	Copper (mg kg ⁻¹)	Zinc (mg kg ⁻¹)
Lime-2											
<i>CaCO₃</i>											
<i>(g kg⁻¹ Soil)</i>											
0 (Control)	4.7 ^a ± 0.17	0.83 ^a	0.13 ^a	0.77 ^a	0.19	0.17 ^a	0.66 ^a	0.075 ^a	10	10.25	231
23	5.9 ^b ± 0.12	0.59 ^b	0.16 ^b	1.13 ^b	0.12	0.10 ^b	0.90 ^b	0.05 ^b	6.75	10.5	237.5
45	6.5 ^c ± 0.03	0.57 ^b	0.15 ^{ab}	1.07 ^b	0.11	0.08 ^b	1.05 ^{bc}	0.04 ^b	4.75	8.25	140.75
93	7.2 ^d ± 0.04	0.56 ^b	0.14 ^{ab}	1.23 ^b	0.14	0.06 ^b	1.10 ^{dc}	0.038 ^b	6.89	7.89	173.02
140	7.4 ^c ± 0.07	0.61 ^b	0.17 ^b	1.28 ^b	0.18	0.06 ^b	1.26 ^d	0.06 ^{ab}	4.75	9.25	201.5
Acid-1											
<i>H₂SO₄</i>											
<i>(mmol H⁺</i>											
<i>kg⁻¹ Soil)</i>											
0 (Control)	8.0 ^a ± 0.25	0.365 ^a	0.13	0.95	0.15 ^a	0.25	0.63 ^a	0.0375	9.5	5.25 ^a	18 ^a
1044	7.7 ^b ± 0.29	0.33 ^a	0.14	0.81	0.24 ^b	0.29	0.58 ^b	0.0275	6.75	9.25 ^a	21.75 ^a
1440	7.74 ^b ± 0.09	0.4 ^a	0.16	0.94	0.35 ^{bc}	0.39	0.69 ^b	0.03	7.0	5.25 ^a	20.75 ^a
2088	4.3 ^c ± 0.12	0.81 ^b	0.12	0.97	0.71 ^d	0.26	1.07 ^b	0.5	7.75	26.42 ^b	53.33 ^b

* Average soil pH is presented with the corresponding standard deviation at each treatment level.

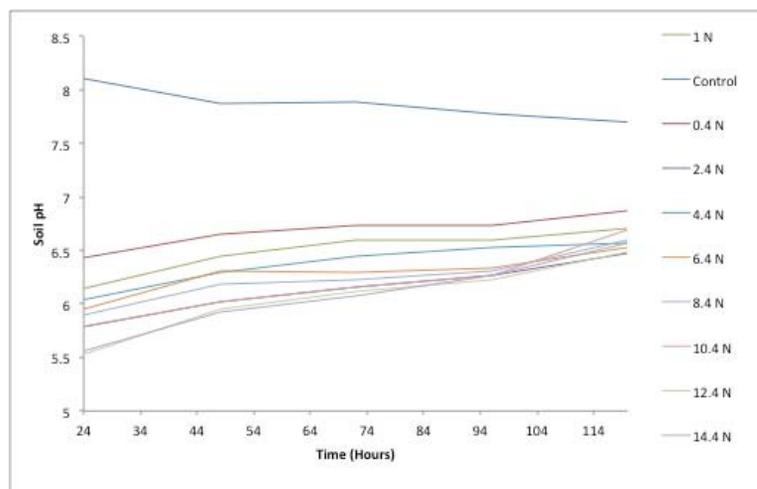


Figure 4.1. Titration curves for soil amended with H_2SO_4 . Each line represents the normality of 1 ml of sulfuric acid diluted in 19 ml of deionized water. Sulfuric acid dilutions were applied to 10 g soil and the pH was measured at intervals labeled on the x-axis.

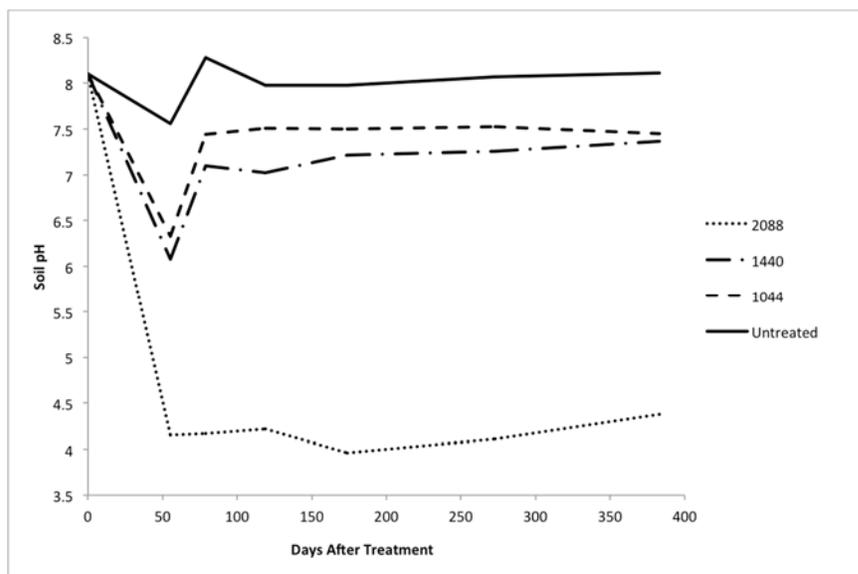


Figure 4.2. pH of soil treated in experiment acid-1. Each line represents a different H_2SO_4 treatment (mmol H^+ kg soil).

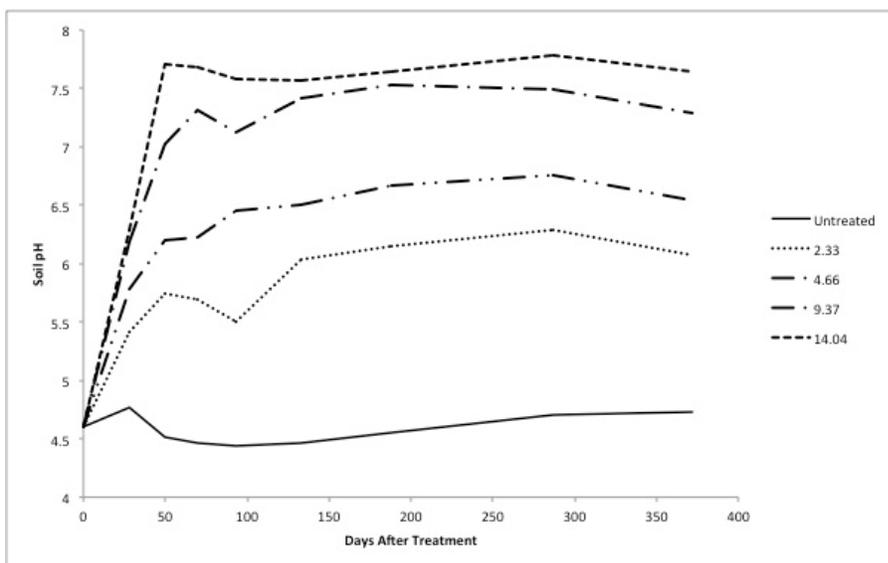


Figure 4.3. pH of soil treated in experiment lime-2. Each line represent a different CaCO_3 treatment. Values presented in the legend are CaCO_3 rates expressed in g kg^{-1} soil.

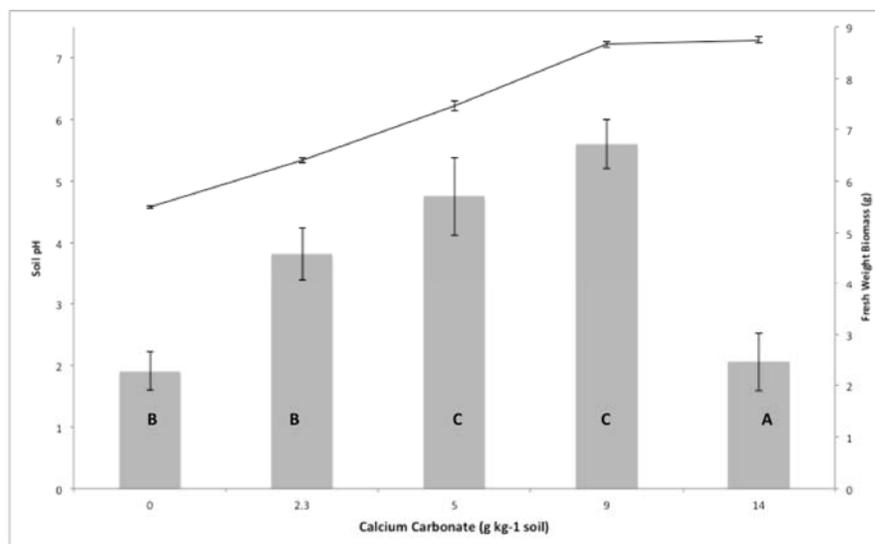


Figure 4.4. Total fresh biomass increase for experiment lime-1. Soil pH is shown with the line. Letters represent statistical significance ($\alpha=0.05$). Error bars express the standard error of the treatment mean.

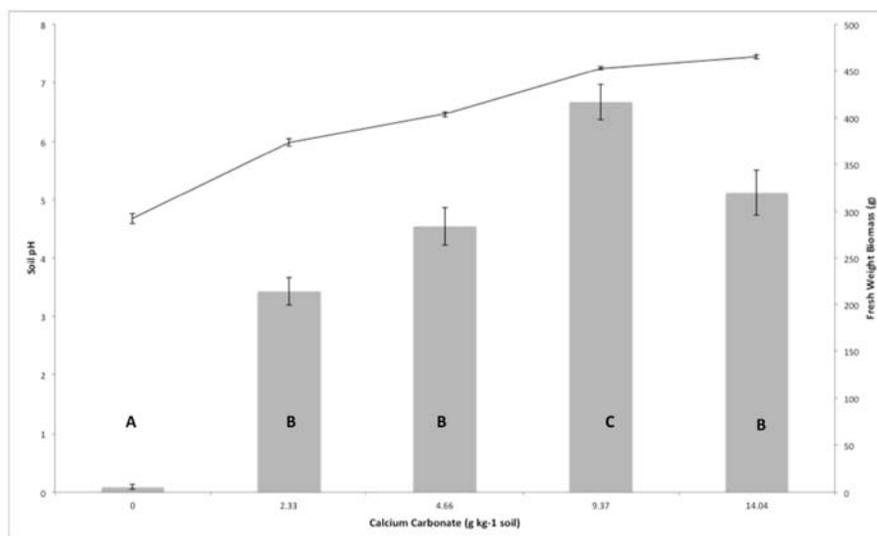


Figure 4.5. Total fresh biomass increase for experiment lime-2. Soil pH is shown with the line. Letters represent statistical significance ($\alpha=0.05$). Error bars express the standard error of the treatment mean.

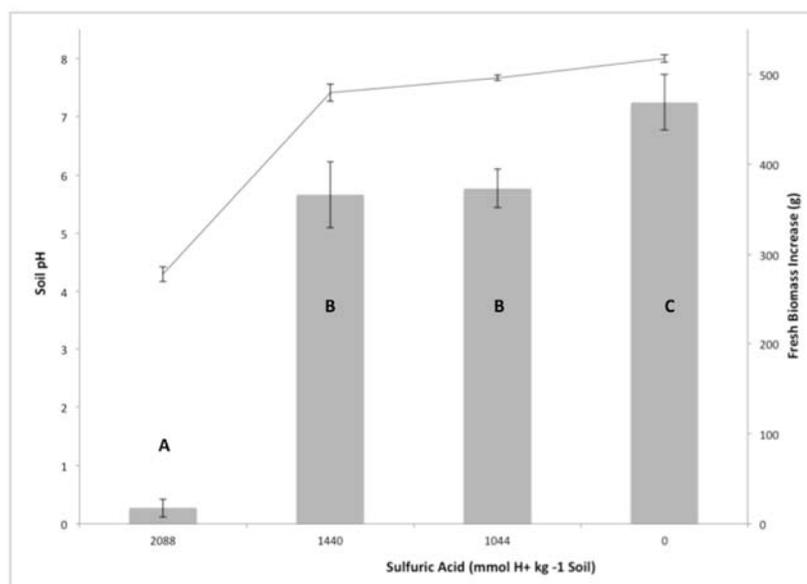


Figure 4.6. Total fresh biomass increase for experiment acid-1. Soil pH for each treatment level is shown with the line. Letters represent statistical significance ($\alpha=0.05$). Error bars express the standard error of the treatment mean.

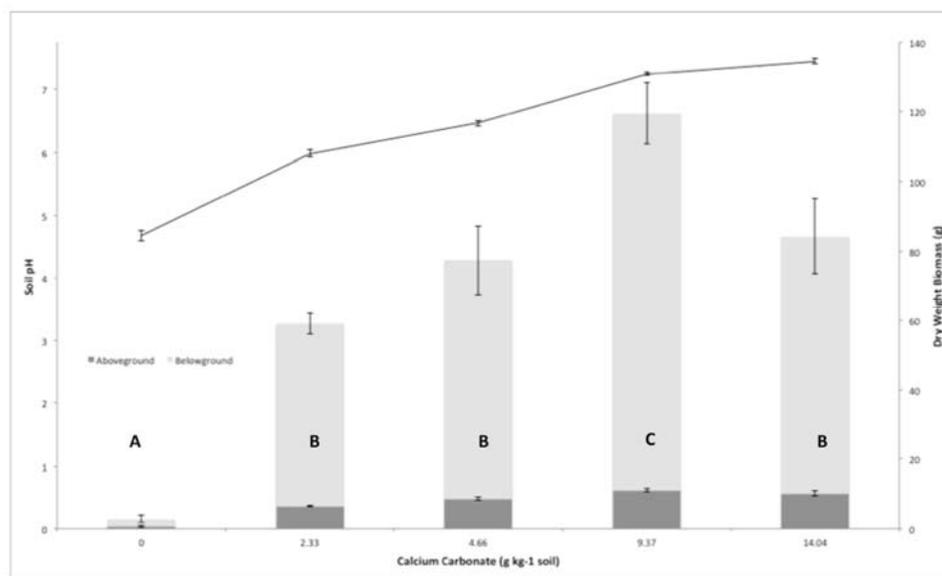


Figure 4.8. Total above and belowground dry biomass from scouringrush grown in experiment acid-1. Soil pH for each treatment level is shown with the line. Letters represent statistical significance for total fresh biomass ($\alpha=0.05$). Error bars express the standard error of the treatment means.

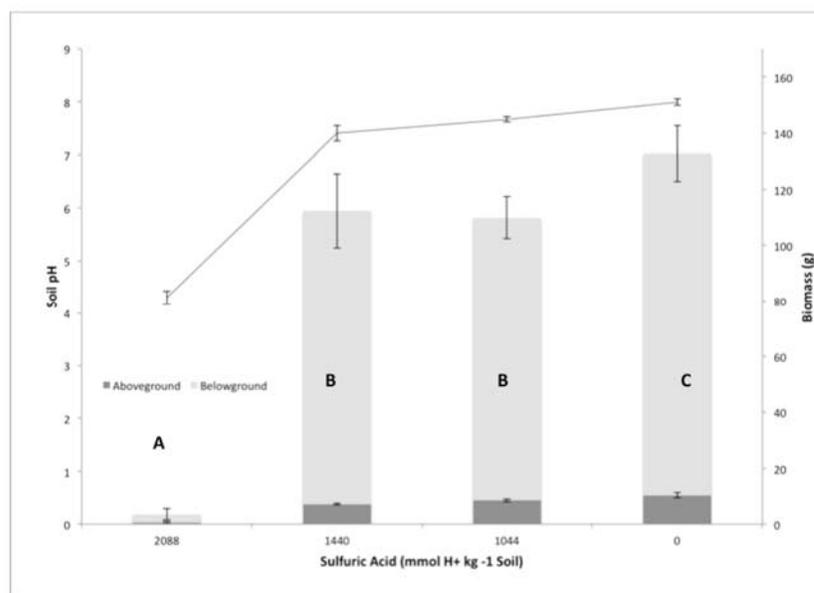


Figure 4.7. Total above and belowground dry biomass from scouringrush grown in experiment lime-2. Soil pH for each treatment level is shown with the line. Letters represent statistical significance for total fresh biomass ($\alpha=0.05$) for total fresh biomass. Error bars express the standard error of the treatment means.

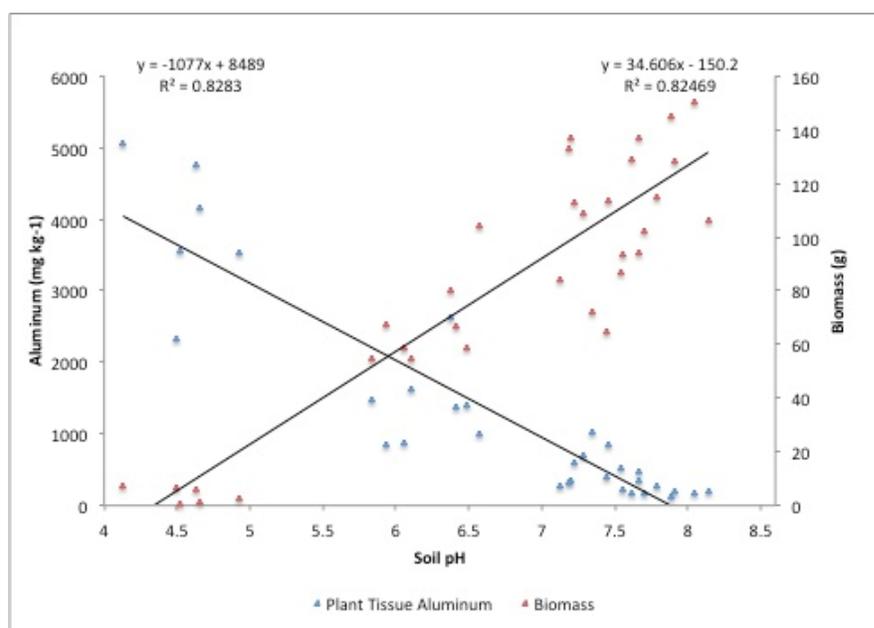


Figure 4.9. Total dry biomass and plant tissue aluminum in response to soil pH. Data were pooled across experiments acid-1 and lime-2.

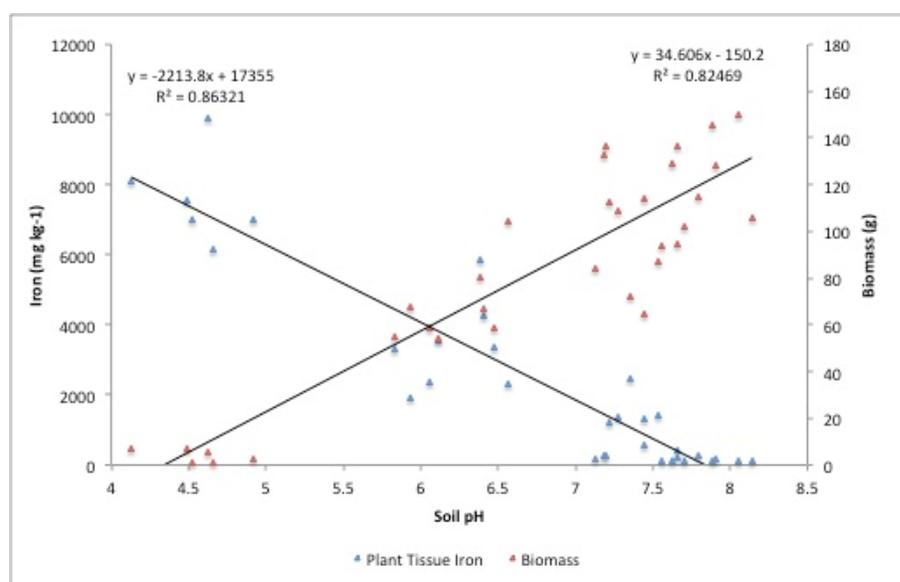


Figure 4.10. Total dry biomass and plant tissue Iron in response to soil pH. Data were pooled across experiments acid-1 and lime-2.

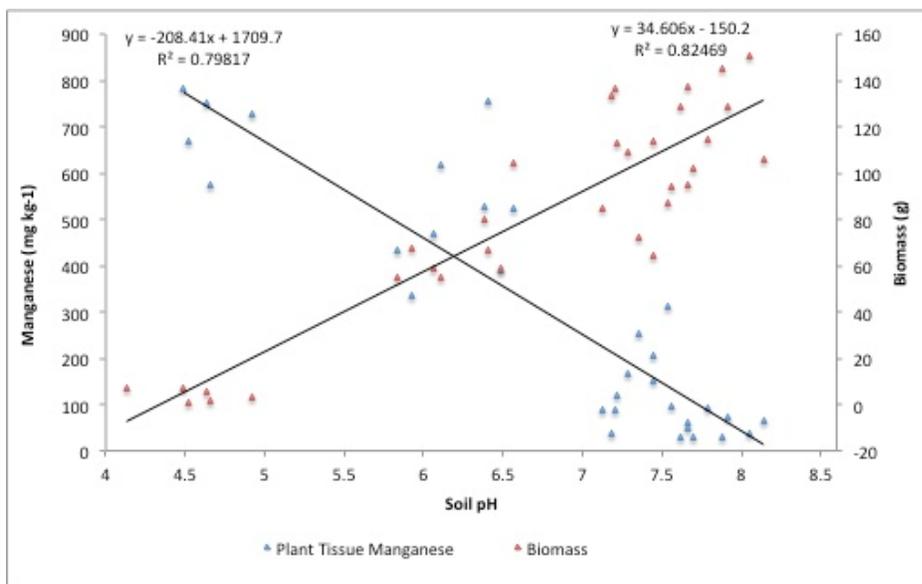


Figure 4.11. Total dry biomass and plant tissue Manganese in response to soil pH. Data were pooled across experiments acid-1 and lime-2.

CHAPTER 5: Control of Intermediate Scouringrush (*Equisetum x ferrissii* Clute) with Herbicides in a Non-Crop Area of Eastern Oregon

ABSTRACT

Due to expanding populations and few effective control options, there is a need for herbicides to be identified that may control intermediate scouringrush (*Equisetum x ferrissii* Clute) in non-crop areas of eastern Oregon. Six herbicide treatments were applied to a patch of intermediate scouringrush located 12 km east of The Dalles, Oregon, on October 8, 2015. Visual control ratings were made 20 days after treatment and live stems were counted using 60 cm² quadrats 7 and 10 months after treatment. Intermediate scouringrush treated with halosulfuron plus 2,4-D and dicamba or triclopyr was controlled 71 and 100%, respectively, 20 days after treatment. However 10 months after treatment, plots where halosulfuron plus 2,4-D and dicamba or triclopyr were applied had 23 and 25 stems 60 cm², respectively. Where chlorsulfuron plus 2,4-D and dicamba and chlorsulfuron plus asulam was applied, visual control of intermediate scouringrush was rated as 60% and 40%, respectively. Ten months after treatment chlorsulfuron plus 2,4-D and dicamba and chlorsulfuron plus asulam were found to have 11 and 0.3 stems 60 cm², respectively. Results suggest the initial response of intermediate scouringrush stems turning dark black 20 days after herbicide application is an inadequate method to predict complete control, and herbicide treatments containing chlorsulfuron and triclopyr were most effective in controlling intermediate scouringrush 10 months after treatment.

INTRODUCTION

Though there has been recent concern over scouringrush expanding into agricultural production fields, the primary habitats for scouringrush from a weed management standpoint are roadside depressions and field margins (Rutz and Farrar, 1984). Controlling scouringrush in these areas before plant have the opportunity to invade production fields is a viable option for growers. Information available on herbicides with activity on scouringrush is dated and tailored towards regions vastly different from eastern Oregon. Additionally, a majority of herbicide evaluations for scouringrush control have been done using *Equisetum hyemale* L., one of the parent species of the hybrid intermediate scouringrush (*Equisetum x ferrissii* Clute) predominantly found in agricultural areas of eastern Oregon. The following study was conducted to evaluate treatments containing triclopyr, chlorsulfuron, asulam and herbicides with similar modes of action for control of intermediate scouringrush on an eastern Oregon roadway bordering a winter wheat (*Triticum aestivum* L.) field.

Richard and Kitchen (1983) evaluated the efficacy of postemergence applications of triclopyr, an auxinic herbicide from the pyridine chemical family, on *Equisetum hyemale* growing in drainage ditches of Louisiana sugarcane production fields. Triclopyr was applied at 3.4 and 4.5 kg ha⁻¹ at a volume of 208 L ha⁻¹. Initial control for both rates was rated at 80%, and substantial reductions in stem number were reported for both rates 21 weeks following application. Chlorsulfuron is an acetolaactate synthase (ALS) inhibiting herbicide from the sulfonyleurea chemical family that has been evaluated for non-crop *E. hyemale* control. Yoder et al. (1983) were able to achieve 87% control of *E. hyemale* with 0.14 kg ha⁻¹ plus a surfactant 180 days after treatment. Research done by Yoder et al. (1983) was conducted on Louisiana sugarcane drainage ditches. Finnerty and Glaser (1980) applied chlorsulfuron at rates of 0.025, 0.05, 0.1 kg ha⁻¹ in the spring and 0.05, 0.1 and 0.2 kg ha⁻¹ in the fall to *E. hyemale* growing on railroad beds in Minnesota. Control per rate five months after application was rated as 80, 90, and 93% for spring applications and 99, 100, and 100% for fall applications, respectively. Asulam is a carbamate herbicide that inhibits the enzyme dihydropteroate (DHP) synthase. Bracken fern (*Pteridium aquilinum* L.) is commonly controlled using asulam (Rowntree and Sheffield, 2005). The plant families containing *E. hyemale* and *P. aquilinum*

(Equisetaceae and Dennstaedtiaceae) are physiologically similar, so it was hypothesized that asulam may have activity on *Equisetum* species.

MATERIALS AND METHODS

Site description

Plots were located 12 km east of The Dalles, Oregon on a fencerow separating a county road and agricultural land. The fencerow contained a patch of intermediate scouringrush with a uniform density of approximately 80-100 stems m². Soil type was a Walla Walla silt loam with a pH of 6.5.

Treatments

Six herbicide treatments were applied on October 8, 2015. A fall application date was chosen because herbicides are known to be more effective on *Equisetum* species once aboveground shoots have matured and rhizome growth accelerates (Bernards et al., 2010). Treatments included were: aminopyralid with metsulfuron (Opensight, 52.5% a.e. by weight aminopyralid and 9.45% by weight metsulfuron; Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268), chlorsulfuron (Telar XP, 75% chlorsulfuron by weight; DuPont, 1007 Market Street, Wilmington, DE 19898) plus 2,4-D and dicamba (Latigo, 0.288 kg L⁻¹ a.e. 2,4-D and 0.216 kg L⁻¹ a.e. dicamba; Helena Chemical Company, 225 Schilling, TN 38017), halosulfuron (Sanda, halosulfuron 75% by weight; Gowan Company LLC., 370 S. Main St. Yuma, AZ 85364) plus 2,4-D and dicamba (Latigo, 0.288 kg L⁻¹ a.e. 2,4-D and 0.216 kg L⁻¹ a.e. dicamba), triclopyr (Garlon 4, 0.479 kg L⁻¹ a.e. triclopyr; Dow AgroSciences), asulam (Asulox, 0.4 kg L⁻¹ asulam; United Phosphorus Inc., 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406) and chlorsulfuron (Telar XP, 75% chlorsulfuron by weight) plus asulam (Asulox, 0.4 kg L⁻¹ asulam). A complete list of herbicide rates and adjuvants is presented Table 5.1

Treatments were applied at a volume of 468 L ha⁻¹ with a CO₂ powered sprayer pressurized to 207 kPa. A boom equipped with 5 TeeJet XR8003 nozzles was used. Environmental conditions at the time of application were 0% cloud cover with an ambient temperature of 24° C and relative humidity of 58%. Winds were east at 3.2 to 9.7 kilometers per hour. Soil temperature was 22° C at a depth of 15 cm.

Trial Maintenance

Two applications of clethodim at 0.6 L ha⁻¹ (Select 2 EC, 0.24 kg L⁻¹ clethodim, Valent U.S.A. Corp., PO Box 8025 Walnut Creek, CA 94596) were applied over the trial to remove downy brome (*Bromus tectorum* L.). The first treatment was applied on February 16, 2016 and the second on April 7, 2016.

Experimental Design/Data Collection and Analysis

A randomized complete block design with 4 replications was used. Plots were 2.4 x 3 m. To maintain scouringrush uniformity, 3 meters was the maximum possible plot length. Visual control ratings of intermediate scouringrush were made 20 days after treatment. On May 10, 2016 the number of emerged stems were counted using two 60 cm² quadrats per plot. A second stem count was taken on August 3, 2016. Analysis of variance was conducted using SAS Proc Mixed (SAS version 9.3, 2012) on visual control ratings and the number of stems m² at both timings individually and together as a split-plot analysis in time. Assumptions of normality and homogeneity of variance were verified using diagnostic and normal probability and residual plots. The COVTEST option of SAS Proc Mixed was used to test significance for variance components introduced by subsampling. T test statistics were used to analyze differences in treatment means using the LSMEANS function of SAS Proc Mixed. All ANOVA and T-tests were completed using a 5% significance level.

RESULTS AND DISCUSSION

Visual Control Ratings

Visual control ratings for all treatments are presented in Figure 5.1. Intermediate scouringrush was 100% controlled in plots treated with triclopyr 20 days after treatment. Asulam controlled 12.5% of intermediate scouringrush on average across four replicates. Aminopyralid plus metsulfuron, asulam plus chlorsulfuron, and halosulfuron plus 2,4-D and dicamba controlled 54%, 40%, and 71% of the intermediate scouringrush, respectively. It should be noted that herbicides from multiple modes of action caused

intermediate scouringrush stems to turn dark black. This black stem response was used for control ratings 20 days after treatment.

Stem Counts May 2016 (7 Months After Treatment)

No significant variance components were detected due to subsampling during May stem counts ($P>0.05$). Seven months after treatment, differences were detected ($P<0.05$) in intermediate scouringrush stem number between plots treated with chlorsulfuron plus 2,4-D and dicamba, triclopyr, and chlorsulfuron plus asulam and all other treatments. On average, there were 9.75, 13.0, and 3.12 stems 60 cm², respectively, for these treatments. Untreated plots averaged 77.25 stems 60 cm². Halosulfuron plus 2,4-D and dicamba did reduce the number of intermediate scouringrush stems compared to the untreated control. However, the average number of stems 60 cm² was 42.4, which would be unacceptable as a commercial treatment for intermediate scouringrush control. Complete results from May 2016 stem counts are presented in Figure 5.2.

Stem Counts August 2016 (10 Months After Treatment)

No significant variance components were detected due to subsampling during August stem counts ($P>0.05$). Ten months after treatment, chlorsulfuron plus asulam was the only treatment different than other treatments ($P<0.05$). Average intermediate scouringrush stem number was 0.4 stems 60 cm² in plots treated with chlorsulfuron plus asulam. Untreated plots averaged 45.25 stems 60 cm². Dalley and Richard (2008) demonstrated that control of rhizome johnsongrass (*Sorghum halepense* L.) improved when treated with a mixture of asulam and trifloxysulfuron compared to either herbicide applied alone. Trifloxysulfuron is a herbicide with the same mode of action from the same chemical family as chlorsulfuron. Therefore, a synergistic relationship between chlorsulfuron and asulam is a possible explanation for increased intermediate scouringrush control with chlorsulfuron plus asulam compared to asulam alone and chlorsulfuron plus 2,4-D and dicamba. However, antagonistic relationships between sulfonylurea herbicides plus MCPA and imidazolinone herbicides plus dicamba have also been reported (Hart and Wax, 1996; Mathiassen and Kudsk, 1993). Without chlorsulfuron alone included in the trial, it is impossible to properly

determine why chlorsulfuron plus asulam achieved better control than chlorsulfuron plus 2,4-D and dicamba. Results from May 2016 stem counts are in Figure 5.2.

Control Differences Between Sampling Dates

No significant variance components were detected due to subsampling while comparing May and August stem counts ($P>0.05$). Results from the split-plot in time ANOVA suggested there was a significant month by herbicide treatment interaction ($P<0.05$). The number of stems decreased from May to August 2016 in the untreated control plots, and plots treated with aminopyralid plus metsulfuron, asulam, and halosulfuron plus 2,4-D and dicamba. There was no significant increase in stem number in plots treated with chlorsulfuron plus 2,4-D and dicamba, triclopyr, or chlorsulfuron plus asulam ($P>0.05$). Decreases in intermediate scouringrush stem number from May to August independent of herbicide treatment is likely due to a combination of the environmental conditions in eastern Oregon and the biology of the plant. The genus *Equisetum* is better adapted to areas with higher soil moisture, and rainfall during July and August in the area surrounding The Dalles typically totals less than 1 cm each month. Another factor to consider is a portion of each intermediate scouringrush stem will die back in late summer and early fall. Plots treated with triclopyr or chlorsulfuron did not show a significant change in scouringrush stem number between May and August. Treatment effects from these herbicides likely outweighed the environmental and/or biological effects observed in plots treated with less effective herbicides.

CONCLUSIONS

Plots treated with triclopyr were rated as 100% control of intermediate scouringrush 20 days after treatment based on the observation that all previously green stems had turned black following application. The same plots were found to have 25 intermediate scouringrush stems 60 cm² on average 10 months after treatment. Alternatively, plots treated with chlorsulfuron plus 2,4-D and dicamba and chlorsulfuron plus asulam were rated as 60% and 40% control, respectively, 20 days after treatment. Ten months after treatment, plots where chlorsulfuron plus 2,4-D and dicamba was applied had 11 intermediate scouringrush stems 60 cm² on average, and plots where chlorsulfuron plus asulam was applied had 0.4 stems 60 cm² on

average. These results indicate that the previously described black stem response is not an adequate indicator of long term herbicide efficacy.

Intermediate scouringrush in plots treated with asulam alone, and aminopyralid plus metsulfuron, were found to have 58 and 48 stems 60 cm², respectively, on average ten months after treatment. For comparison untreated control plots were found to have an average of 45 stems 60 cm², making asulam alone and aminopyralid plus metsulfuron inadequate for commercial intermediate scouringrush control. Plots treated with halosulfuron plus 2,4-D and dicamba were found to have 23 stems 60 cm² on average ten months after treatment, which was nearly the same as plots treated with triclopyr that were found to have 25 stems 60 cm² on average. However, halosulfuron plus 2,4-D and dicamba treated plots were found to have an average of 42 stems 60 cm² seven months after treatment while only 13 stems 60 cm² on average in triclopyr treated plots. Due to untreated control plots following the same trend where less stems were present in the plots in August compared to May, the reduction in stems observed in plots treated with halosulfuron plus 2,4-D and dicamba were likely a result of intermediate scouringrush biology and the surrounding environment rather than a response to the herbicide treatment.

Both treatments containing chlorsulfuron offered adequate control of intermediate scouringrush 10 months after treatment. However, live plants were observed in these plots suggesting a follow-up treatment is needed to achieve complete control. Though not as effective as chlorsulfuron 10 months after treatment, triclopyr was found to offer adequate control up to seven months. Fall application timings were effective for controlling intermediate scouringrush in eastern Oregon, but more work is needed to determine what treatment options are most effective when following a fall herbicide application.

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Table 5.1. Herbicide treatments and adjuvants applied to fencerow plots.

Herbicide Treatments and Adjuvants	Rates
Untreated Control	-
Aminopyralid	0.143 kg ha ⁻¹
Metsulfuron	0.022 kg ha
COC*	1% v/v
Chlorsulfuron	0.14 kg ha ⁻¹
2,4-D	0.672 kg ha ⁻¹ a.e.
Dicamba	0.504 kg ha ⁻¹ a.e.
NIS*	0.25% v/v
Halosulfuron	0.07 kg ha ⁻¹
2,4-D	0.672 kg ha ⁻¹ a.e.
Dicamba	0.504 kg ha ⁻¹ a.e.
NIS	0.25% v/v
Triclopyr	5.6 kg ha ⁻¹
NIS	0.025% v/v
Asulam	3.74 kg ha ⁻¹
COC	1% v/v
Chlorsulfuron	0.14 kg ha ⁻¹
Asulam	3.74 kg ha ⁻¹
COC	1% v/v

*NIS: non-ionic surfactant; COC: crop oil concentrate.

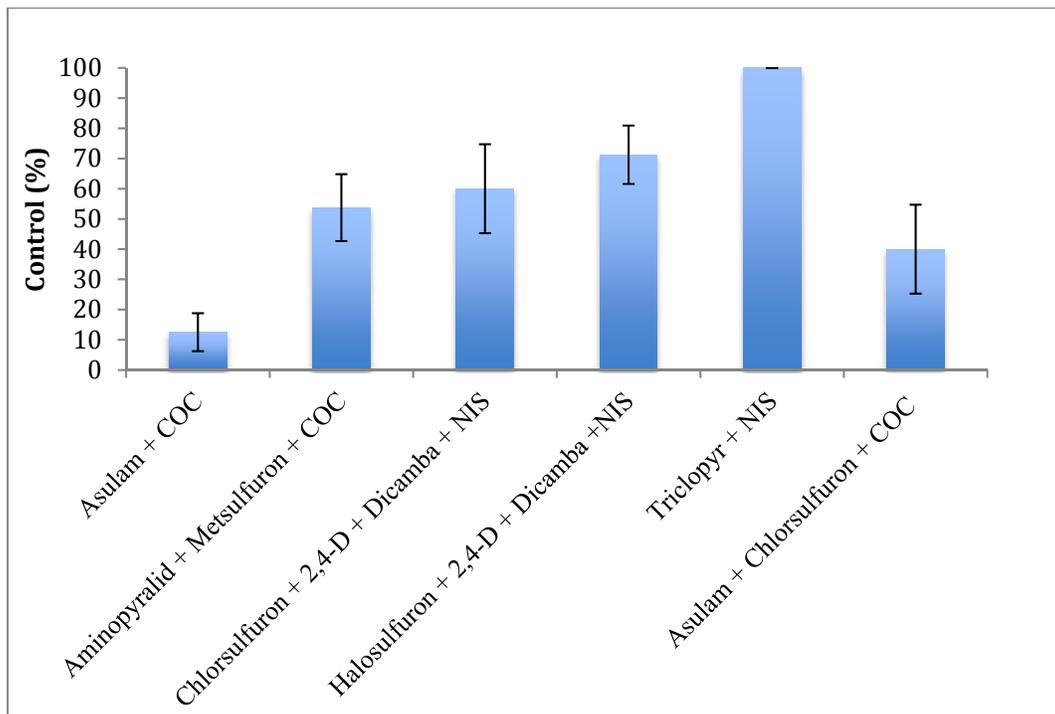


Figure 5.1. Visual control ratings of intermediate scouringrush made 20 days after treatment. Results are presented as the mean percent control across 4 replicates. Error bars show the standard error of the treatment means.

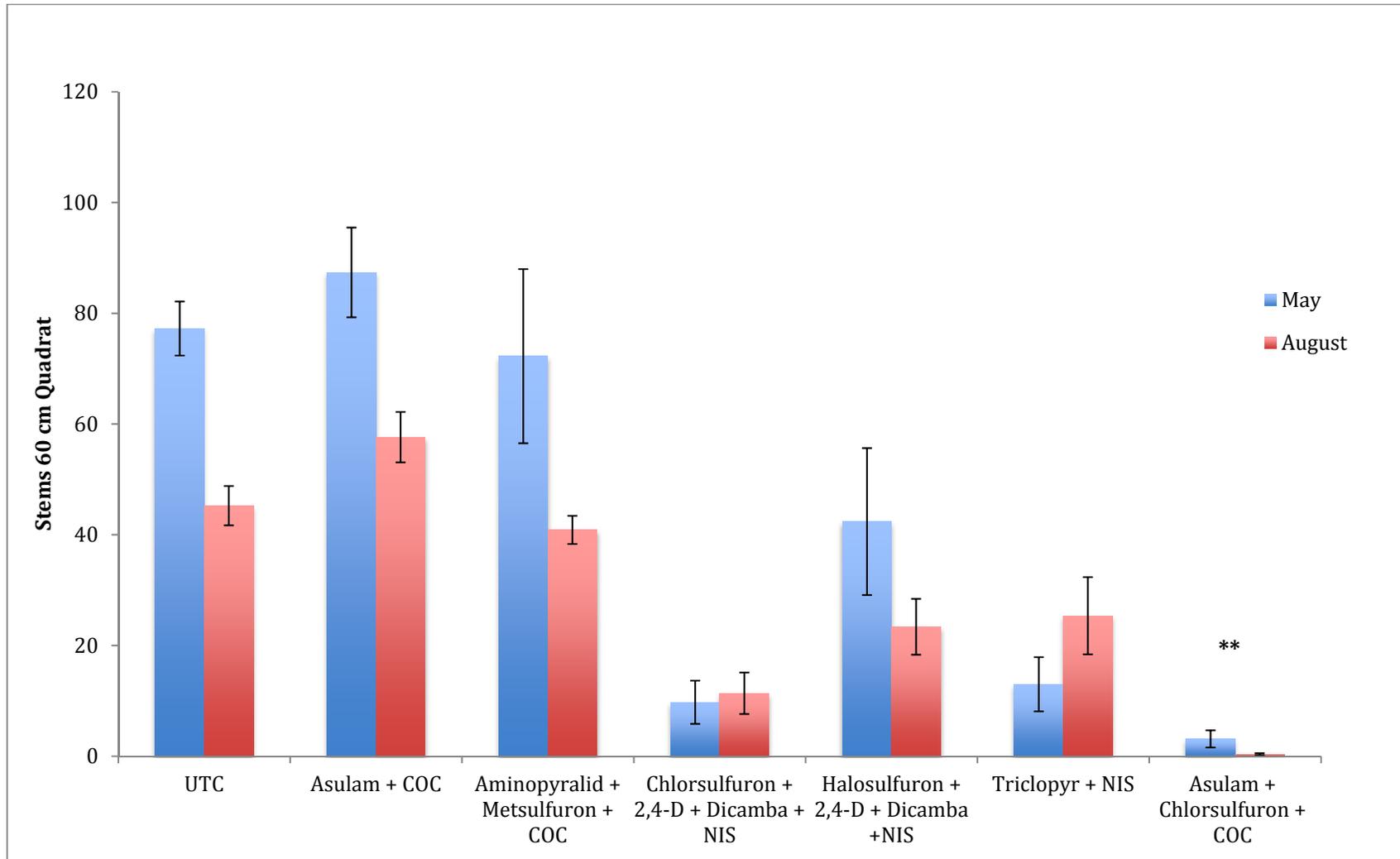


Figure 5.2. Average intermediate scouringrush stem number per 60 cm quadrat. Counts were taken in May (7 months after treatment) and August (10 months after treatment) 2016. Error bars show standard errors of treatment means at both count timings. ** Different than all other treatments at both sampling timings ($\alpha=0.05$).

CHAPTER 6: Conclusions

Both species of scouringrush (*Equisetum laevigatum* L. and *Equisetum xferrii* Clute) were controlled by chemical fallow applications of chlorsulfuron plus MCPA-ester in winter wheat rotations. All other chemical fallow herbicide treatments screened would not be accepted as commercial treatments. Pre-herbicide mowing was found to have no effect on herbicide efficacy when applied to control scouringrush. Although there was a misapplication at The Dalles, OR and a 1.7x rate of the treatment was applied, it does appear that the labeled rate of chlorsulfuron in north central Texas and southern Oklahoma (26 g ha⁻¹) plus 1.12 kg ha⁻¹ MCPA-ester would be an effective treatment for scouringrush control in Pacific Northwest winter wheat-chemical fallow rotations if it were labeled. Although, special considerations should be made to determine if/when scouringrush control is worth increasing selection pressure for ALS resistance by adding another ALS-inhibiting herbicide into a winter wheat-chemical fallow rotation, especially if Clearfield® wheat varieties are common crop rotations.

There was no difference in wheat yield between plots treated with chlorsulfuron plus MCPA-ester and plots treated with any other herbicide treatment within locations. However, there was an apparent yield difference between mowed and non-mowed subplots at The Dalles, but the effect was likely due to factors other than mowing. No differences in yield resulted between herbicide treatments when compared among mowed or non-mowed subplots including where scouringrush was nearly 100% controlled.

Regression analyses from field studies suggest that higher scouringrush stem densities in production fields are correlated with wheat grain yield reduction. Although, low coefficients of determination indicate that stems m row⁻¹ was not an ideal metric when used to quantify scouringrush-winter wheat competition effects. Contrary to field results, regression analysis from a controlled potted wheat-scouringrush competition experiment showed that scouringrush density did not influence winter wheat development or grain yield. The preliminary hypothesis developed from these studies is that abiotic stress could be the true cause of negative yield responses currently attributed to scouringrush infestations in Pacific Northwest winter wheat-chemical fallow cropping systems. Studies completed by Anderson and Millberg (1996) and Anderson and Lundgårdth (1999^{a,b}) are the foundation of the abiotic stress hypothesis.

Under greenhouse conditions scouringrush biomass production increased as soil pH increased from ≈ 4.0 to ≈ 8.1 . However, in two different studies CaCO_3 rates designed to raise an acidic Willamette Valley soil pH from 4.6 to 7.6 caused a decrease in fresh biomass production when plants were grown for 45 and 344 days. Soil pH resulting from these two CaCO_3 treatments was measured as 7.3 and 7.4 respectively. Results from a third experiment where a basic central Oregon soil was acidified with H_2SO_4 showed scouringrush biomass production continued to increase as soil pH surpassed 7.4. This discrepancy was attributed to excess calcium from CaCO_3 treatments causing an imbalance between magnesium and calcium.

Scouringrush was able to withstand excessively high tissue concentrations of iron, manganese and aluminum in low soil pH conditions. Plant tissue concentrations of these metals and subsequent biomass production response by scouringrush was unaffected by soil type or amending agent. Though results from soil pH studies with scouringrush were not necessarily new findings, they did add some quantifiable data that were lacking in what is known about scouringrush biology. In the scope of cereal grain production, what should be recognized that scouringrush was able to establish and survive in soil pH conditions that would cause stress for grain crops.

Chlorsulfuron applied at 0.14 kg ha^{-1} plus 2,4-D and dicamba or asulam was found adequately control intermediate scouringrush 10 months after treatment in a non-crop site in eastern Oregon. Triclopyr applied at 5.6 kg ha^{-1} was found to offer adequate control up to seven months after treatment. However, live plants were observed in all plots treated with chlorsulfuron and triclopyr suggesting a follow-up treatment is needed for complete control. Additionally, every herbicide screening (Reardan chemical fallow; The Dalles chemical fallow and non-crop) evaluated in this thesis contained at least one treatment that induced a color response where nearly all scouringrush stems turned black. These treatments were subsequently rated $>75\%$ control, and did not rank among the most effective treatments the following spring when scouringrush began actively growing. This trend suggests that the color response brought on by herbicides is not completely indicative of long term efficacy.

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APPENDIX A

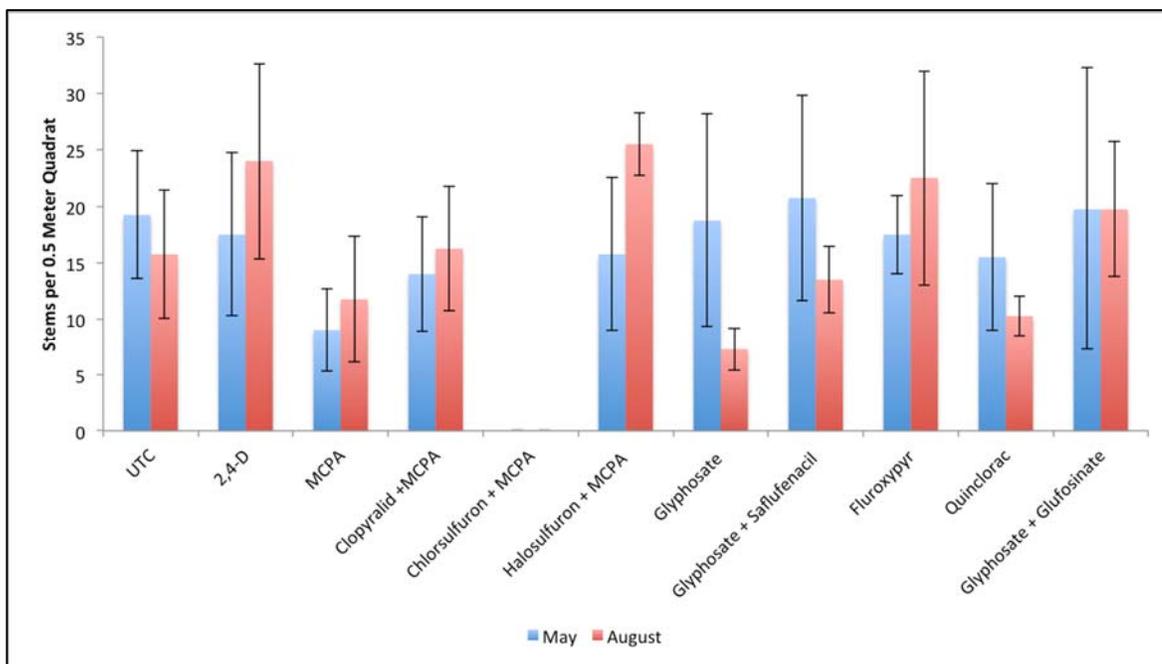


Figure A.1. Scouringrush stems 0.5 m quadrat quantified in May and August 2016. At both sampling timings chlorsulfuron plus MCPA-ester was the only treatment different than untreated controls and all other treatments ($P < 0.05$). Quadrats were counted in portions of winter wheat plots left unseeded by the grower. The same level of control was achieved by chlorsulfuron plus MCPA-ester in the absence of winter wheat-scouringrush plant interference effects. Error bars show the standard error of treatment means.

APPENDIX B

Scouringrush rhizomes collected from the Reardan, WA did not establish consistently in the greenhouse. Therefore, mowed and non-mowed rhizomes from each herbicide treatment were pooled giving each treatment 6 replications. All rhizomes that did not produce new growth were excluded from statistical analysis. All rhizomes from field plots treated with fluroxypyr were excluded from the analysis because only 2 of 6 produced new growth, which was likely not a herbicide treatment effect based on field results. Analysis of variance was conducted on total, stem, and rhizome/root system dry biomass separately using SAS Proc GLM (SAS version 9.3, 2011). T test statistics were used to analyze differences in treatment means using the LSMEANS function of SAS Proc GLM. All analysis of variance and T-tests were completed using a 5% significance level.

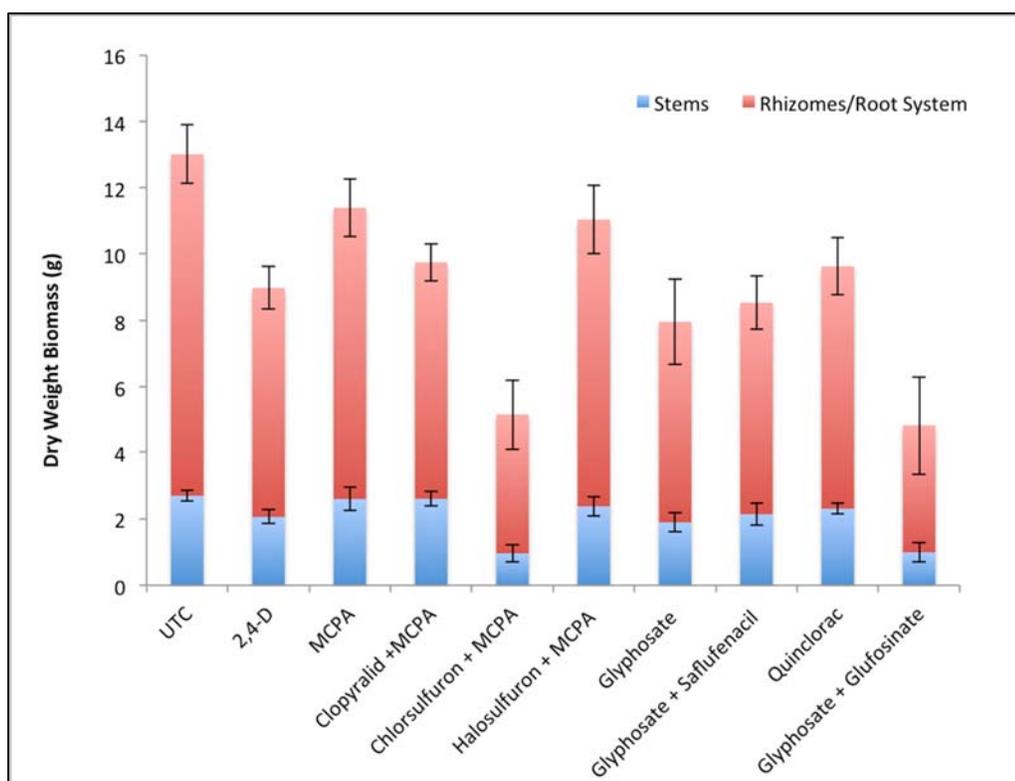


Figure B.1. Average total dry biomass separated by stems and rhizome/root systems produced from rhizomes extracted from plots at Reardan, WA after 200 days. Error bars show the standard error of treatment means. Error bars for total biomass are presented on the rhizome/root system portion of each bar. Rhizome standard error is not presented.

There was a significant treatment effect for total, stem, and rhizome/root system dry biomass ($P < 0.05$). Rhizomes from field plots treated with Chlorsulfuron plus MCPA-ester and glyphosate plus glufosinate produced less stem and total dry biomass than all other treatments ($P < 0.05$). When analyzed separately, rhizomes from plots treated with halosulfuron plus MCPA-ester were the only treatment group that did not produce less new rhizome/root growth than the untreated control group ($P > 0.05$). No treatment group produced new rhizome/root growth that was different than all other treatments at the 5% significance level.

Chlorsulfuron plus MCPA-ester and glyphosate plus glufosinate applied to field plots in Reardan, WA appeared to slow biomass production when scouringrush rhizome fragments were extracted from the field and propagated in a greenhouse. The biomass response from rhizome fragments from plots treated with glyphosate plus glufosinate is surprising as the treatment resulted in poor efficacy in the field. Despite nearly complete control in field plots at Reardan, WA and The Dalles, OR, rhizome fragments extracted from field plots treated with chlorsulfuron plus MCPA-ester were able to produce new growth and establish under greenhouse conditions. These results indicate that there is a possibility viable rhizomes will remain in the field after a single application of chlorsulfuron plus MCPA-ester.

APPENDIX C

Soil Amendment Equations and Assumptions

CaCO₃ Amendments

Moisture Adjusted Soil weight = $\left[\left(\frac{H_2O(\%)_{Mass}}{100} \right) * \text{Oven Dry Soil Weight to be Treated} \right] +$
Oven Dry Soil Weight to be Treated

Greenhouse Scaled Rate = $\frac{\text{Hectare rate CaCO}_3}{\text{HFS kg}}$

H₂SO₄ Amendments

All molar concentrations were calculated using 1.787 g cm⁻³ as the density of H₂SO₄, which assumes temperature is constant at 25° C. The proportion of the H₂SO₄ molar mass from sulfur is 0.327.

H₂SO₄ Needed to Neutralize 1 kg Oven Dry Soil (5% free lime) = $\frac{\frac{1}{3}(0.05 \text{ kg CaCO}_3)}{0.327}$

APPENDIX D

Included figures are plant tissue analysis results from experiments lime-2 and acid-1. Data from both experiments are included in each figure. Results are presented as either the percentage (nitrogen, phosphorous, potassium, sulfur, calcium, magnesium and sodium) or total mg kg⁻¹ (iron, aluminum, manganese, boron, copper and zinc) found in each plant sample.

