AN ABSTRACT OF THE DISSERTATION OF

Bianca Assis Barbosa Martins for the degree of Doctor of Philosophy in Crop Science presented on February 27, 2014.

Title: Hybridization between Imidazolinone-Resistant Wheat (*Triticum aestivum* L.) and Jointed Goatgrass (*Aegilops cylindrical* Host.) and Selection Pressure Impacts on Proportion of Resistance Alleles

Abstract approved:

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Carol A. Mallory-Smith

Imazamox-resistant wheat (Clearfield®) cultivars carry the *Imi1* gene, which confers resistance to the imidazolinone (IMI) herbicide imazamox. Imazamox provides selective control of jointed goatgrass and other weeds in IMI-resistant wheat. *Imi1* gene flow between IMI-resistant wheat and jointed goatgrass may occur via hybridization and backcross events. In 2009 and 2010, surveys were conducted in Eastern Oregon to determine the prevalence of the *Imi1* gene in wheat by jointed goatgrass hybrids in Eastern Oregon. Tissue and spikes from hybrids were collected and *Imi1* presence was detected by PCR assays. We assessed hybrid yield components and explored how these components varied across the sampled sites. The association between the proportion of IMI-resistant hybrids and type of system (agricultural or non-agricultural) or management practice in the commercial fields was determined. A total of 128 sites were surveyed over the two years. Of 1,410 plants sampled, 1,100 were positive for the *Imi1* gene of which 1,087 were heterozygousand 13 samples were homozygous for the gene. The 13 homozygous plants provide evidence that they

are of backcross generations because they no longer carry the wild type allele. This is the first report of natural occurrence of IMI-resistant backcross plants in commercial wheat fields. Non-agricultural sites or fields with IMI-resistant wheat production back-to-back, were two factors associated with a greater proportion of IMI-resistant hybrids. These results indicate that it is important to choose field management practices that reduce the production of IMI-resistant hybrids, and to manage non-agricultural areas with jointed goatgrass infestations to prevent introgression of the *Imi1* gene in these hybridization zones. The most economic and environmental friendly method to selectively control the pathogen *Oculimacula yallundae* in winter wheat is the use of resistant wheat cultivars. These cultivars carry the *Pch1* gene, which provides resistance to foot rot. Once the *Imi1* and *Pch1* genes are introgressed into a jointed goatgrass population, their intraspecific movement and fate in the progeny remains largely unstudied. Therefore, field experiments were conducted using *Imi1* and *Pch1* resistance genes introgressed into a single jointed goatgrass population and selection pressure treatments were applied. The progeny were genotyped to detect the presence of the resistance alleles in order to determine proportion and the level of gene flow. In addition, selection pressure effects on yield components were analyzed. The herbicide-resistance allele proportion in the progeny was greater when parent plants were treated with imazamox. The disease-resistance allele proportion did not differ among the selection pressure treatments in the first year, but was greater with disease in the second year. The herbicide-resistance gene flow was greater with herbicide selection pressure in the first year but did not differ in the second year. Disease resistance gene flow did not differ among the selection pressure treatments. Because the resistance allele proportion increased in the two experiments for herbicide and in experiment one for disease resistance, it is likely that

once introgression takes place, the increase of the resistance alleles in subsequent generations will reach fixation, with selection pressure. In addition, selection pressure treatments reduced yield components in the parental plants compared with the control treatment. This study revealed that there was no fitness cost associated with IMI-resistant or foot rot resistance in jointed goatgrass in the absence of selection pressure. The knowledge of how selection pressure at the field level influences the resistance gene flow and the proportion with which the resistance genes occur in the progeny is important to prevent resistance spread. In addition, it lays the ground work for researchers to continue investigating the impacts of selection pressure on resistance genes in subsequent generations or other genes of ecological significance such as drought, cold or salt tolerance.

Hybridization between Imidazolinone-Resistant Wheat (*Triticum aestivum* L.) and Jointed Goatgrass (*Aegilops cylindrica* Host.) and Selection Pressure Impacts on Proportion of Resistance Alleles

by

Bianca Assis Barbosa Martins

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APPROVED:

Major Professor, representing Crop Science

Head of the Department of Crop and Soil Science

Dean of the Graduate School

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

Bianca Assis Barbosa Martins, Author

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DEDICATION

This dissertation is dedicated to my beloved friend and laboratory colleague Elena Raquel Sánchez Olguín, who I learned so much with in several aspects of work and life and who I am fully thankful for have met in my human experience. Thank you, my dear angel, for everything you taught and made for me. Your energy will always remain strong with me.

HYBRIDIZATION BETWEEN IMIDAZOLINONE-RESISTANT WHEAT (*Triticum Aestivum* L.) AND JOINTED GOATGRASS (*Aegilops Cylindrica* Host.) AND SELECTION PRESSURE IMPACTS ON PROPORTION OF RESISTANCE ALLELES

CHAPTER 1

GENERAL INTRODUCTION

Wheat (*T. aestivum* L.) is among the earliest domesticated crops grown for human consumption, and is believed to have originated in the 'Fertile Crescent' region of southwest Asia (Vavilov, 1951), between 10,000 to 12,000 years ago (Kimber and Sears, 1987). Widespread cultivation of wheat took place as soon as the dough-like properties of wheat grains were recognized (Poehlman and Sleper, 1995). According to FAO (2001), wheat provides about 20% of food energy and protein worldwide, representing one of the most important staple foods. The total world cereal production in 2012 was 2,546,631 tons. Wheat ranked third among cereals,after maize and rice, for grain production, with 216,638.762 million ha harvested in 2012 (FAO, 2013). Wheat can be also cultivated as a forage crop for livestock or as winter pasture for hay and silage (Cash et al., 2007).

Traditionally, wheat cultivation has been of importance in American agriculture, with production in almost every state, it is the main cereal grain grown. The acreage devoted to wheat in the US has shown long-term general downward trend since the early 1980s. American wheat trade faces competition from the Black Sea region, whose wheat exports are expected to rise from 22% in 2013/14 to 30% of global trade over the next decade (USDA Long-term Projections, 2013). Hence, wheat yields should be increased in order to increase American competition and market share worldwide. Approximately 80% of wheat grown in

the Pacific Northwest is winter wheat, primarily because its yield potential is about 14bushels per acre more than spring wheat (WGA, 2009).

Wheat yields are affected by several biotic and abiotic factors. Insects, nematodes, fungi and weeds are included amongst the biotic factors. About 25 to 30% of world wheat production is lost yearly due to stresses during wheat development and in storage (Gill, 2010). Of these losses, about 20% is caused by diseases (Wiese, 1991). There are more than 30 diseases that impact wheat production in the US Pacific Northwest. The most economically important wheat diseases are caused by fungal pathogens. The cool, wet winters of the Pacific Northwest favor the development of fungal diseases, including strawbreaker foot rot or eyespot, caused by the fungi *Oculimacula acuformis* and *Oculimacula yallundae*. Amongst the tactics available for controlling foot rot, the most economically and environmentally friendly is the employment of foot rot-resistant wheat cultivars that carry the resistance gene *Pch1*, introgressed from the wheat wild relative *Aegilops ventricosa*.

Losses from weed contamination in harvested wheat can be the most significant cost during crop production (Bowran, 2000). Amongst the weeds that impact wheat production, jointed goatgrass is recognized as a major weed of winter wheat in the Western US (Zemetra et al., 1998). Jointed goatgrass causes yield losses because it competes with wheat for light, nutrients and moisture (Johnston, 1931). Average wheat yield loss with moderate to dense jointed goatgrass infestation has been estimated to be 25% (Donald and Ogg, 1991). In 2004, more than 2.5 million hectares of winter wheat in the Pacific Northwest, Intermountain West and the Central Great Plains of the United States were reported to be as infested with jointed goatgrass, costing producers $145 million yearly (Hanavan et al., 2004). In addition, jointed

goatgrass reduces winter wheat by reducing harvested grain quality. Discounts from $0.04 to $0.18 per bushel (27.3 kg), depending on the percent of contaminants, have been reported (Hanavan et al., 2004).

Wheat and jointed goatgrass co-exist in commercial winter wheat fields sharing several similar growth and development habits, including temperature optimums, vernalization requirements of several weeks at 0° to 5°C, maximum photosynthetic rates and growth and flowering periods that overlap, which allow low levels of cross-pollination (Hegde and Waines, 2004). These similarities make jointed goatgrass a difficult-to-control weed species in winter wheat. Several mechanical and cultural control methods for jointed goatgrass have been studied in winter wheat, including tillage, one-time moldboard plowing, mowing infested areas, crop rotation, chemical fallow, wheat seeding time, nitrogen fertilization, seeding density manipulation, use of tall wheat varieties, and field burning (Wicks et al., 2003; Young et al., 1990; Anderson, 1993; Anderson, 1997; Anderson, 2004).

The Genus *Aegilops* and the Species *Aegilops cylindrica* Host. (jointed goatgrass)

Aegilops is a Mediterranean–Western Asiatic genus having species that occur in the Mediterranean and Irano-Turanian regions (Hedge et al., 2002). *Aegilops* has an extensive geographical growth range, occuring in Mediterranean Europe and southern Ukraine, the Crimea, Cis- and Transcaucasia, North of the Sahara in Africa, in Western and Central Asia, in the region bordered by the deserts of the Arabian Peninsula in the South and by the Tian Shan Mountains in the East (Kilian et al., 2011). *Aegilops* species have been introduced in many states of the US, of which *Ae. cylindrica* (Bayer code AEGY) has become widespread and reported in 32 states since the end of the 19 century (USDA-NRCS, 2006). In 1995,

jointed goatgrass was reported in Chihuahua (Northern Mexico) and near Port Colborne in the Niagara Region at Southern Ontario (Canada). One year later, another population of jointed goatgrass was found 4 km Southeast from the first one in Canada (Oldham and Brinker, 2009). In the United States, jointed goatgrass is a noxious weed in seven states (Arizona, California, Colorado, Idaho, New Mexico, Oregon, and Washington). In Canada, jointed goatgrass is listed as a Class 1 Prohibited Noxious Weed Seed. Australia, United States and Mexico also restrict its movement due to its noxious weed status.

Aegilops species show adaptation to ruderal and disturbed sites, including dry hilland mountain slopes, pastures and roadsides, and close by or within cultivation (Kilian et al., 2011). *Aegilops* species grow intermingled with other grasses (including other *Triticum* species) and with shrubs. Jointed goatgrassmay grow in large stands after recent disturbances. In the United States, jointed goatgrass is well adapted to reduced tillage farming systems, especially where there is continuous wheat cultivation or winter wheat fallow rotation (Donald, 1991). Jointed goatgrass also infests rangelands surrounding wheat fields in Conservation Reserve Program (CRP) areas throughout the Western United States (Donald and Ogg, 1991; NAPPO, 2003). In terms of climate, jointed goatgrass is generally adapted to temperate climates with hot summers and cold winters. In agro-ecosystems in the United States, jointed goatgrass behaves as a winter annual grass.

Recently harvested or shattered seeds are dormant and require a post-harvest ripening period that usually occurs in the field during the summer. Exposure to warm and dry conditions breaks seed dormancy. Jointed gostgrass seed dormancy break occurred after 16 weeks of after-ripening at 22 ºC (Fandrich and Mallory-Smith, 2006).

In addition, flowering requires a vernalization period of several weeks (Donald, 1984). In the spikelet, there are 3-5 florets of which the lower 1-2 usually are fertile (Johnston and Parker, 1929), but there can be up to five fertile florets per spikelet. According to Donald and Zimdahl (1987), approximately 20% of spikelets had one seed, 80% had two seeds, and less than 1% had three seeds, based on a study conducted with two jointed goatgrass populations.

One jointed goatgrass plant can produce more than 100 spikes, 1,500 spikelets, and 3,000 seeds (Gealy, 1988). However, around 130 seeds per plant are produced when growing in a wheat crop with adequate moisture (Morishita, 1996). Seeds from jointed goatgrass can remain viable for several years; however, studies have shown that, after three years at a burial depth of 5 cm, few seeds remained intact (Donald and Zimdahl, 1987).

Molecular studies using nuclear and chloroplastic markers have shown that *Aegilops cylindrica* has low levels of genetic diversity (Pester et al., 2003; Gandhi et al., 2005). In addition, phenotypic variation among jointed goatgrass populations is minimal.

Some species from the *Aegilops* genus participated in wheat evolution and played an important role in wheat domestication. Thus, the largest part of the secondary gene pool of wheat is represented by the genus *Aegilops*, and several species have been used in wheat breeding programs.

Aegilops species are a source of valuable traits for wheat, including long ears (Millet et al., 1988), a high content of protein, lysine, and iron and zinc in the kernels (Rawat et al., 2009), resistance to leaf rust (Spetsov et al., 2006), powdery mildew (Miranda et al., 2007), tan spot (Tadesse et al., 2006), and to strawbreaker foot rot (Doussinault et al., 1983; Thiele

et al., 2002). In addition, *Aegilops* species are a source of tolerance for soil salinity, drought (Farooq et al., 1989) and soil acidification (Berzonsky and Kimber, 1986).

A translocation of genes *Pch1* and *Pch2* from the species *Ae. ventricosa* to the winter variety VPM1 conferred resistance to foot rot, caused by *Oculimacula* spp. (Doussinault et al., 1983). The first evidence that another species, *Ae. longissima*, contained resistance to foot rot was published recently (Sheng, 2011). The high frost tolerance levels found in jointed goatgrassmake it promising for cold tolerance improvement in bread wheat (Limin and Fowler, 1981). The species also has been described as a gene source for salt and drought tolerance (Farooq and Azam, 2001).

Biological and ecological features make jointed goatgrass an aggressive colonizer: short reproductive cycle, large tiller production, spike shattering into dispersal units (spikelets), where seeds remain protected within the though structure of the spikelet protection during dispersal and long-term burial, movement of spikelets beyond the mother plant by cattle, wild animals, humans, and equipment and ability to germinate and establish on the soil surface.

Hybridization and Gene Flow between Wheat and Jointed Goatgrass

A stepwise interspecific hybridization is believed to have formed common wheat (West et al., 1988). Such events were initiated with a cross between the species *Triticum urartu* Tumanian ex Gandilyan (2n = 14, genome AA) and a B-genome donor species related to *Ae. speltoides* Tausch (2n = 14, SS genome), which formed a sterile hybrid. Meiotic error and self-fertilization of such hybrid produced the allotetraploid *Triticum turgidum* (2n = 28, genome AABB) (Gandhi et al., 2006), which migrated through domestication to the habitat

of the wild diploid *Ae. tauschii* (2n = 14, genome D) (Weissman et al, 2005). Hybridization between those species gave rise to the current domestic wheat. Thus, wheat is an allohexaploid species with 2n = 6x = 42 chromosomes that usually forms 21 pairs of chromosomes during meiosis (Kimber and Sears, 1987). Three homoeologous genomes, A, B, and D, each compose seven pairs of homologous chromosomes (AABBDD). A cross between *Ae. tauschii* (2n = 14, genome DD) and *Ae. markgrafii* (Greuter) Hammer (2n = 14; genome CC) produced a hybrid, which through amphiploidization doubled its chromosome number, originating the species jointed goatgrass (*Ae. cylindrica*) (2n = 28, genome CCDD) (Gandhi et al, 2006). Thus, wheat and jointed goatgrass have *Ae. tauschii* the donor of the D genome as a common ancestor (Kimber and Sears, 1987).

Wheat pollen is shed before the flower opens (Frankl and Galun, 1977); therefore, wheat is a primarily self-pollinating species with low rates of out-crossing. Out-crossing occurs primarily by wind dispersal. Wheat flowers lack nectaries to attract insects (Eastham and Sweet, 2002), so cross-pollination by insects is considered to be minimal (Glover, 2002). Jointed goatgrass is a primarily self-pollinated species, with outcrossing rates varying from 0 to 2% (Cannon, 2006). Cross-pollinationin jointed goatgrass occurs primarily by wind.

Jointed goatgrass by wheat hybrids have been reported by several authors within the native regions of jointed goatgrass distribution in Eastern Europe and Asia (van Slageren, 1994). In the United States, jointed goatgrass was first identified at the taxonomic level in 1917 in Kansas (Johnston, 1931), and in Oregon in 1926. Hybrids between wheat and jointed goagrass were first reported in Kansas (Johnston and Parker, 1929; Mayfield, 1927). The extent of hybridization varies between growing seasons, indicating that the amount of hybridization may depend upon environmental conditions (Johnston and Parker 1929).

Hybrids are consistently found in commercial wheat fields of the Pacific Northwest (Watanabe and Kawahara, 1999; Morrison et al., 2002).

Hybrids that produced low numbers of seeds were reported in Europe (Rajhaty, 1960); however, hybrids were described as sterile for several decades. In addition, products of controlled crosses between wheat and jointed goatgrass were traditionally considered sterile (Johnston and Parker, 1929; Priadcencu et al., 1967). No seed was found on hybrid spikes from 10 surveyed sites in Kansas (McGregor, 1987). Hybrids in commercial wheat fields from Oregon were incorrectly reported to be sterile (Watanabe and Kawahara, 1999) and thought to be of little consequence (Donald and Ogg, 1991). During the late 1990's, natural seed-producing hybrids were documented in Idaho, Washington, and Oregon (Mallory-Smith et al., 1996; Seefeldt et al., 1998). A one percent (1%) seed production rate was found in hybrids collected from a three-year survey (Morrison et al., 2002).

When jointed goatgrass and wheat cross-pollinate, the different genomes (A, B and C) are brought together from the two different parents, and exchange genetic material to form a new mixed genome (hybrid). Therefore, when wheat and jointed goatgrass come into contact, spontaneous hybridization can occur (Morrison et al., 2002; Zaharieva and Monneveux, 2006; Loureiro et al., 2008). However, fertility in the hybrid is greatly reduced compared to wheat or jointed goatgrass and it should not be totally dismissed.

Hybrids between winter wheat and jointed goatgrass have 35 chromosomes (21 from wheat and 14 from jointed goatgrass), and ABDDC as their genomic constitution. One cause of sterility in the hybrids is the lack of chromosome pairing during meiosis, except for the D genomes, leading to unbalanced distribution of chromosomes in the gametes and non-viable gametes (Seefeldt et al., 1998; Zemetra et al., 1993). However, hybrids possess low female

fertility, which allows fertilization byother wheat or jointed goatgrass plants. Gametocidal genes, transferred to wheat from wild related *Aegilops* species, are known to induce chromosome breakage in wheat gametophytes lacking them, leading to preferential transmission of the gametocidal-carrier chromosome (Friebe and Gill, 1996). A gametocidal gene responsible for hybrid sterility was identified in wheat by *Aegilops* crosses (Endo, 1988, 1996). For example, backcrosses that did not carry a specific C chromosome had chromosome deletions and rearrangements. This sterility system does not occur in the first generation of hybrids, but causes sterility in the first backcross generation if the recurrent parent is wheat.

Several studies in the Pacific Northwest have shown that backcrosses are male sterile but do have low female fertility (Zemetra et al., 1998; Wang et al., 2001; Cremieux et al., 2001; Cannon, 2006; Gandhi et al., 2006).

When the hybrid backcrosses to jointed goatgrass, thesterility system does not take place, which makes backcrosses to jointed goatgrass have higher percent of female fertility. Therefore, backcrosses to jointed goatgrass can produce more seed each generation than if the backcrosses were to wheat. In partially female-fertile hybrids, there is an instability caused by the three unmatched genomes (ABC) due to a disproportionate number of univalents migrating to one pole in female gametogenesis (unreduced gametes).

Hybrids can be detected by initial morphological observations. Hybrids have wider spikes 6-18 cm long, and have more awns than jointed goatgrass (Morrison, et al, 2002). At maturity, the spikes disarticulate at the base and fall to the ground as a whole dispersal unit (Spetsov et al, 2006), and height was recorded as varying from 46-114 cm (Stone and Peeper, 2004). Hybrids may be more competitive than either of the parents, although the low seed

production indicates reduced fitness of the hybrid population as a whole. According to Zaharieva and Monneveux (2006), case-by-case and region-by-region assessments are needed to evaluate the risk associated with production and competitiveness of hybrids and their progeny. Hybrid spike color at harvest is usually darker than mature wheat spikes, however hybrids with light brown spikes, like wheat, can also be found (Martins, personal observation). Natural hybrids have also been reported with 4 other *Aegilops* species: *Ae. crassa*, *Ae. columnaris*, *Ae. triuncialis*, *Ae. biuncualis* (van Slageren, 1994). First backross generation plants possess spikes that vary in morphology (Morrison et al., 2002). If hybrids backcross mostly with jointed goatgrass, progenies will increasingly look like jointed goatgrass. Similarly, if hybrids backcross mostly with wheat, resulting progenies will increasingly resemble wheat.

Heterosis (or hybrid vigor) occurs when hybrid plants show phenotypic performance that is superior to that of their parents. Hybrid vigor results from several features, including biomass increase, a typically improved characteristic in heterosis (Birchler et al., 2010). Greater hybrid vigor is commonly observed in interspecific crosses. Indeed, wheat by jointed goatgrass hybrids do display hybrid vigor, being usually taller and more vigorous than either of the parents, indicating that hybrids may be more competitive for resources (Johnston and Parker, 1929).

If a selective advantage occurred in hybrids and their offspring, there could be a counterbalance for low female fertility, male-sterility and low seed set. Even in absence of selection pressure, a single introgressed herbicide-resistant *Brassica rapa* plant was discovered in a canola field in Canada (Warwick et al., 2008), showing evidence that introgression occurred and allowed a herbicide-resistance gene to persist in the fields at low

frequency. Under herbicide-selection pressure, this frequency would increase due to the selective advantage provided by the resistance gene.

Hybrids that backcross with wheat and have a subsequent selfing tend to recover the chromosome number of 42, whereas backcrosses with jointed goatgrass tend to recover the chromosome number of 28. Because there are two possibilities of pollen donor parents and pollination may vary from one generation to another, the population structure in the field can become complex (Cremieux, 2000). In experimental plots for production offirst backcross (BC_1) plants, both wheat and jointed goatgrass had an equal chance toserve as the paternal parent of BC_1plants, although the pollination success ratio appeared to be infavor of wheat (Cremieux, 2000).

In experimental plots where hybrids had an equal chance of pollination by wheat or jointed goatgrass (Snyder et al., 2000). However, wheat was the prevalent pollen donor compared to jointed goatgrass (Cremieux, 2000; Perez-Jones et al., 2010). Hybridization rates of 0.048 to 7% have been reported between wheat and jointed goatgrass (Guadagnuolo et al., 2001; Hanson et al., 2005; Gaines et al., 2008).

The sexual transfer of a crop gene to a wild relative weed species takes place via crop-weed hybridization is also called introgressive hybridization (Ellstrand and Hoffman, 1990). Hybridization and the potential for gene flow from cultivated crops to weed relatives by introgressive hybridization have been demonstrated in many crops. All crops, except the clonally reproduced, have the potential for gene flow via pollen movement, even those that are predominately self-pollinated, because some level of outcrossing will occur. With each successive backcross to jointed goatgrass there is a greater number of potential C- and D-genome bivalent chromosome pairings that can occur during meiosis, resulting in increased

seed set on first generation backcrosses compared to wheat and jointed goatgrass hybrids. Based on manual pollinations with jointed goatgrass pollen in controlled conditions, rates of female fertility of first backcross generations have been estimated between 4.4% and 7.5% (Mallory-Smith et al., 1996; Zemetra et al., 1998). Male fertility was estimated at 1.8% when the first backcross plants were pollinated with jointed goatgrass pollen, which indicates the possibility that first backcross plants may self-pollinate (Wang et al., 2001). More recently, field trials having jointed goatgrass as the only pollen source were conducted, and female fertility rate of first backcross plants from was 0.03% (Beil, 2013). By bagging the spikes separately and bagging the whole plant, Beil found a self-fertility rate of 0.0% and 0.004%, respectively, in first backcross generation plants. Although these studies showed the possibility of producing second backcross generations after two generations of backcrossing jointed goatgrass to the hybrids, these rates are based on emasculation and manual pollination in controlled conditions and thus are not be representative of field conditions. However, in both field and controlled crosses, partial and complete self-fertility was found in the second backcross generation and in first backcross selfings, with either jonted goatgrass or wheat as the pollen parent, indicating that stable, self-fertile individuals can develop in one to two backcross generations (Zemetra et al., 1998; Wang, 2001; Cremieux et al., 2001). The increasing pollen viability over the backcross generations is due to reduction in the chromosome number, which leads to a more normal chromosome distribution during meiosis (Zemetra et al., 1998).

Other research indicates that advanced backcross generations and their self-pollinated backcross lines can reach relatively high levels of seed production: up to 73% fertility for third backcross generation to jointed goatgrass (Zemetra et al., 1998), and up to 93% for

second backcross to jointed goatgrass that went through two cycles of self-pollination (BC_2S_2) (Wang et al., 2000). Beil (2013) transplanted 65 first backcross plants into field plots and planted jointed goatgrass seeds within and between rows of the first backcross plants over the entire plot, so that jointed goatgrass served as the dominant pollen source. The average backcrossing rate for two growing seasons was 0.404%. Econopouly (2010) suggested that backcrossing rates vary by environment and that multiple field studies are necessary to estimate the range of backcrossing rates across the western United States. Cannon (2006) conducted a field study over two years at four locations and determined outcrossing rates of four jointed goatgrass populations varied from 0.38% to 2.24%.

Seed-mediated gene flow may also occur through loss of seed or natural seed dispersal. The term ‘admixture’ or ‘commingling’ refers to when herbicide-resistant seed is mixed with non-herbicide-resistant seed, most frequently due to human error and the plant biology *per se*. Volunteer herbicide-resistant crop plants from a previous season, field machinery activities or storage can also lead to admixture. Reducing seed-mediated gene flow includes practices such as control of herbicide-resistant crop volunteer plants, clear labeling and cultivar identification, cleaning of field machinery and adequate handling during processing and storage (Mallory-Smith and Zapiola, 2008).

In the wheat production scenario, before the mid-1990’s, gene flow was mostly a concern for the seed industry because genetic purity had to be assured to the wheat buyers. Today, the concern still exists; however, it has been extended to the possibility of genes that confer selective advantages could move from wheat to jointed goatgrass.

Movement of a single wheat chromosomal segment, containing a novel gene, into a jointed goatgrass genome could lead to the expression of the novel trait. Although there are a

number of documented cases for gene flow from crops to wild relatives (Ellstrand et al., 1999), little information is available about the long-term persistence of crop genes in wild populations or the impact of fitness-related genes on weedy species population dynamics. Three mechanisms are known that allow the sexual transfer of genetic material from wheat into jointed goatgrass to occur. Although the D genomes of wheat and jointed goatgrass originated from different *Aegilops tauschii* biotypes, the first and most frequent mechanism is recombination of the homologous chromosomes of their D genomes due to successful pairing at meiosis and formation of 14 bivalents (Zemetra et al., 1998; Badaeva et al., 2002). Intergenomic translocations or chromosome rearrangement is the second method. As the number of backcross generations increases, the chromosomes not found in the recurrent parent are eliminated (Zemetra et al., 1998). However, the herbicide-resistance gene may be retained through chromosome translocation from the A or B genome to the C or D genomes of jointed goatgrass. For example, when jointed goatgrass was the male recurrent backcross parent, a second backcross generation was self-pollinated and yielded a chromosome number close to that of jointed goatgrass, with plants having 28 chromosomes and a high level of pollen viability / self-fertility (Wang et al., 2001). Therefore, the gene flow from wheat to jointed goatgrass via backcrossing may be a function of the genome location of the gene. Genes located in the D genome may be retained at expected Mendelian frequencies (Kroiss, 2001), while genes located in different genomes can be transferred via disomic chromosome retention (addition or substitution). In this process, either one chromosome from A or B genome is present in a homologous pair or three different chromosomes from A or B genome occur as monosomes, which indicates a wheat chromosome substitution in the first backcross generation, describing the third method of sexual transfer of genetic material from wheat into

jointed goatgrass. Second backcross plants that were self-pollinated had chromosome numbers ranging from 28 to 40, indicating that extra chromosomes are present (Zemetra et al., 1998). Thus, the potential of gene transfer from other wheat genomes (A or B) to jointed goatgrass is possible. Fragments from A and B wheat genomes were introgressed into jointed goatgrass (Schoenenberger et al., 2005), suggesting that a gene occurring in the A or B wheat genomes cannot completely prevent introgression (Wang et al., 2001).

Introgression is defined by the infiltration of germplasm from one species into another via repeated backcrossing of the hybrids to one of the parental species (Arnold, 1997). Successive backcross generations progressively accumulate the traits of the backcross parent. The recovery of fertility in backcross individuals is necessary for a successful introgression between any two taxa (Hedge and Waines, 2004). Thus, recurrent backcrosses to jointed goatgrass in natural field conditions are imperative for gene introgression into jointed goatgrass to occur (Econopouly et al., 2011).

Crop genes are expected to be introgressed into wild relativesunder selection pressure effects, linkage to other traits, and heterosis or outbreeding depression. Genes for traits like seed color may not promote any selective advantage to the wild populations, while a drought tolerance or an insect or herbicide resistance gene has, under selection pressure, the potential to enhance the fitness of a wild population (Hedge and Waines, 2004). Transgenes conferring glyphosate and glufosinate resistance were found in volunteer and wild populations of canola (*Brassica napus* L.), via pollen- and seed-mediated gene flow (Hall et al., 2000). In another study, after four years of glyphosate-resistant canola hybridized with susceptible *Brassica rapa* in two fields in Canada, glyphosate-resistant *B. rapa* was detected (Warwick et al., 2008). Both pollen and seed escape led to unadvertent gene flow from glyphosate-resistant

creeping bentgrass (*Agrostis stolonifera*) to susceptible bentgrass and wild relatives in Oregon (Watrud et al., 2004; Zapiola et al., 2008). Hence, if the crop and its wild relative co-exist, share flowering periods, and hybridize, it seems likely that neutral or beneficial crop alleles could persist in free-living populations.

Imazamox (IMI)- and Foot Rot-Resistant Wheat Varieties in the Pacific Northwest of the US

A mutation was induced in a conventional wheat cultivar, resulting in resistance to the herbicide imazamox and later, to the development of imazamox-resistant wheat varieties known as Clearfield. The IMI-resistant commercial winter wheat cultivars carry the S653N mutation, which is a substitution of the amino acid asparagine to serotonine in the acetolactate synthase (ALS) enzyme (Newhouse et al., 1992; Tan et al., 2005). This mutation occurs in a gene known as *Imi1*: a single, incompletely dominant nuclear gene located in chromosome 6DL (Anderson et al., 2004; Pozniak et al., 2004). The Clearfield production system for wheat combines the use of imazamox with a winter wheat cultivar containing the *Imi1* gene, conferring minimal risk of injury to the crop. Winter wheat cultivars that do not contain the resistance gene are injured or killed when treated with imazamox. Other Clearfield crops that have also been developed, which include corn, canola, and sunflower.

The first Clearfield wheat varieties released in the US occurred in 2001. The varieties were ‘Above’ and ‘AP502 CL’, grown in the central Great Plains. In 2003, General Mills marketed the first Clearfield wheat variety for the Pacific Northwest. The first Clearfield wheat variety developed by Oregon State University and BASF Corporation in a joint project with USDA-ARS was the soft white winter wheat (ORCF-101), released in 2003. The variety

‘ORCF-101’ had resistance to imidazolinone herbicides but no disease resistance. In 2004, variety ‘ORCF-102’ was released with the *Imi1* and the *Pch1* genes, which provided resistance to the herbicide imazamox and strawbreaker foot rot pathogen (*Oculimacula* spp.), respectively. The variety ‘ORCF-103’ was released in 2008 and has the same *Imi1* gene. ORCF-103 has resistance to some races of stripe rust (*Puccinia striiformis*) and moderate resistance to pink snow mold (*Microdochium* (*Fusarium*) *nivale*), Fusarium crown rot or dryland foot rot (*Bipolaris sorokiniana*), and Cephalosporium stripe (*Cephalosporium gramineum*), but is susceptible to foot rot (Flowers et al., 2010).

In the United States, IMI-resistant wheat is currently cultivated in Oregon, Idaho, Washington, Montana, Norht and South Dakota, Wyoming, Nebraska, Colorado, Kansas, Oklahoma and Texas (BASF, 2013). Because of the hexaploid nature of wheat, there are 3 copies of the ALS enzyme on genomes A, B and D. Thus, there are wheat varieties carrying only the *Imi1* gene and varieties carrying the *Imi1* plus the *Imi2* genes. No varieties with the *Imi3* have been released. The mutant ALS genes are on the long arms of chromosomes 6D, 6B and 6A (Anderson et al., 2004) and are referred to as *Imi1*, *Imi2* and *Imi3*, respectively (Pozniak and Hucl, 2004). Pozniak et al. (2004) reported that despite *Imi2* having the identical base pair mutation as *Imi1*, it codes for less resistant ALS activity, possibly due to a lower level of resistance expressed by *Imi2*, compared to *Imi1*, whereas the *Imi3* gene did not appear to code for the S653N substitution, leading to an inconclusive result about resistance provided by the *Imi3* gene. Pozniak and Hucl (2012) reported wheat plants with multiple resistant alleles and increased IMI resistance compared to wheat plants possessing only one resistant allele. The wheat plants from the study were generated by combining *Imi1+Imi2* or *Imi1+Imi2+Imi3* each conferring resistance to IMI herbicides. One issue of concern to

growers about the 1-gene wheat varieties available was that these varieties could sometimes be injured, which possibly lead to yield decrease. Crop injury risk increased with cold weather conditions (Ball and Peterson, 2007). Therefore, the 2-gene IMI-wheat varieties, which have a mutation on a second genome so that 2 of the 3 genomes produce resistant ALS enzyme were developed. The other single genome lacks a mutation and produces sensitive ALS. Two-gene IMI cultivars have greater levels of resistance and are not injured even in cold weather. The Washington State University wheat breeding program transferred the S653N mutation from two genomes to isolines of soft white winter wheat varieties Eltan and Madsen (Kumar et al., 2010).

Selection Pressure

When interspecific hybridization occurs, the hybrids typically have reduced fitness and maladapted phenotypes (Snow et al., 2008). With only a small proportion of the interspecific hybrid offspring being viable, selection pressure (e.g., frequent herbicide applications in fields where herbicide-resistant hybrids occur) could favor the persistence of the offspring that carry escaped genes (Snow et al., 1999). Crop-wild hybridization may produce several generations towards the wild phenotype carrying beneficial crop-derived traits, such as resistance to certain diseases or herbicides (Warwick et al., 2008). Ellstrand and Schierenbeck (2000) provide several examples of invasive taxa that evolved after intertaxon hybridization, including *Bromus hordeaceus* (*B. arvensis* x *B. scoparius*), *Spartina anglica* (*S. alterniflora* x *S. maritmia*) and *Sorghum almum* (*S. propinquum* x *S. bicolor*). Several species became successful only after an unusually long lag time after initial arrival, and/or after multiple introductions. In the case of hybridization between a crop that carry a

beneficial trait and its wild relative, the trait may be passed to the wild relative. This indicates that introductions of the beneficial trait into new sites can take place via seed.

Introgressed genes from domesticated plantsthat confer resistance to herbicides, disease or insects may affect the survival and fecundity of non-cultivated species, potentially making existing weeds more difficult to control or increasing the weediness of species that are not currently an issue (Snow et al., 2003).

The long-term persistence of genes that provide fitness depends on the balance between the cost of the trait expression, if any, and the strength of the selection pressure favoring the trait (Snow and Moran Palma, 1997). The fate of weedy populations that acquired transgenes through gene flow is largely different, depending on the fitness effect of the introgressed transgenes under given environmental conditions (Lu and Yang, 2009). Hybridization between wheat varieties that are foot rot- and IMI-resistant is a process that is currently occurring in the wheat production area in Eastern Oregon. There is no published information about foot rot and imazamox resistance cost in resistant jointed goatgrass. However, even if these resistance traits incurred a cost, this could still be favored because its benefits under disease and herbicide pressure in the field are great enough. Consequently, it is helpful to understand the potential for jointed goatgrass, carrying the IMI- and foot rot resistance alleles, and its subsequent generations, to survive and reproduce in different selection pressure conditions of the herbicide imazamox and foot rot disease.

The herbicide imazamox (Beyond®)

Imazamox ($C_{15}H_{19}N_3O_4$) (Figure 1) was first registered under the commercial formulation Raptor® in 1997 by the American Cyanamid Company, which now belongs to BASF Corporation. The chemical structure of imazamox is shown in Figure 1.

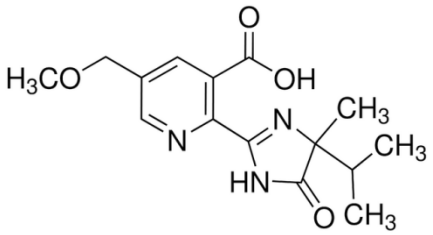

Figure 1. Chemical structure of the imidazolinone herbicide imazamox.

Imazamox belongs to the imidazolinone chemical group of herbicides (Group 2) (Mallory-Smith and Retzinger, 2003), and was developed for weed control in several crops, including IMI-resistant wheat and various legume crops. When this herbicide is used in the IMI-resistant system for wheat, imazamox is sold under the name ‘Beyond’, or as ‘Raptor’ when used in legume crops. Imazamox binds to the enzyme ALS (acetolactate synthase), which prevents the production of three essential branched chain amino acids (valine, leucine and isoleucine), leading to plant death. The substrate (pyruvate or 2-ketobutyrate) binds to the ALS enzyme, producing those three amino acids. When imazamox is applied and absorbed into the plant cells, it blocks the substrate, inhibits the enzyme, prevents the production of amino acids, and consequently, the production of proteins, which leads to plant death (Duke, 1990). Absorption and translocation of imazamox in plants varies depending on the species and environmental conditions, and the differential response of jointed goatgrass

and feral rye to imazamox was related to differences in translocation and metabolism rather than absorption (Pester et al., 2001).

The advent of imazamox-resistant wheat led to control of several difficult-to-control weed species in conventional winter wheat production. Imazamox is a broad-spectrum herbicide that provides post-emergence and some in-season residual weed control in many grass and broadleaf weeds, including jointed goatgrass, downy brome (*Bromus tectorum* L.), California brome (*Bromus carinatus* L.), rattail fescue (*Vulpia myuros*), wild oats (*Avena fatua* L.), Italian ryegrass (*Lolium multiflorum* Lam.), feral rye (*Secale cereale* L.) and volunteer cereals (Ball and Peterson, 2007). Imazamox also controls several winter annual broadleaf weeds, including flixweed, henbit, chickweed, shepherd's purse, field pennycress, and other mustard species. Summer annual broadleaf weeds including common lambsquarters, pigweed, and wild buckwheat are controlled with spring applications of imazamox (Ball and Peterson, 2007).

Strawbreaker foot rot disease

Strawbreaker foot rot or eyespot is a stem base disease of wheat caused by the pathogen *Oculimacula* spp. The disease is considered to be the most important stem base disease of cereals in temperate countries. The characteristic symptom is an eyeshaped elliptical lesion on the stem base or basal leaf sheaths. Initial symptoms are pin-point lesions and water-soaked areas in the wheat stem base (Sprague and Fellows, 1934). The stem base is weakened, leading to its breakage and lodging, a consequence that originated the disease popular name “strawbreaker foot rot”. Other cereals, such as triticale, rye, oats, and other

related grasses can be affected by foot rot, but wheat is the most susceptible. Winter wheat and fall-sown spring wheat are more frequently damaged.

Foot rot was first reported in France in 1912 (Sprague, 1936) and now it occurs wherever wheat is grown and weather conditions are favorable to the causal agent (Sheng, 2011). Wheat of northwestern Europe and the Pacific Northwest of the United States is prone to the occurrence of foot rot. In the US, foot rot occurred occasionally in the Great Plains, Midwest, and Northeast, and the Pacific Northwest by 1919 (Sprague, 1936).

The detrimental effects of foot rot include reduced tiller number per plant, kernel number per head, and 1,000 kernel weight (Murray and Bruehl, 1986). Under severe foot rot occurrence in commercial wheat fields, yield reductions of up to 50% have been documented (Murray, 2010). Wheat ear weight reduction from 3 to 7% and yield reduction from 6 to 11% were documented by Ray et al. (2006) when foot rot was severe. When logding is present, yield losses are greater (Sheng, 2011). It was thought that the foot rot pathogen had only the anamorph stage in the life cycle, reproducing only asexually, by means of conidia. However, apothecia belonging to the genus *Tapesia* were discovered in Australia in 1987 on wheat residue, and single ascospore isolates derived from these apothecia yielded pathotype isolates of the species (Wallwork, 1987). Later, apothecia were found in England, indicating that sexual reproduction could occur within both existing pathotypes, establishing the anamorph-teleomorph connection of the fungus. Two species of the genus *Oculimacula* (known previously as "*Tapesia*"), *O. yallundae* and *O. acuformis*, were defined as the teleomorph states. A heterothallic mating system has since been described for both species, based on the failure of isolates from the two groups to intercross, despite having apparently morphologically identical apothecia. The accepted classification now places the wheat foot

rot pathogen in the *Oculimacula* genus, phylum Ascomycota, subphylum Pezizomycotina, class Leotiomycetes, order Helotiales, family Dermateaceae (Dyer et al., 2001).

There are four pathotypes of the foot rot pathogen: wheat (W), rye (R), couch (C), and squarrosa (S), and all are virulent to wheat (Scott and Hollins, 1980), with different degrees of virulence to the other hosts. Formerly, the W- and R-type isolates were the accepted major groups of *Pseudocercosporella herpotrichoides*, which are now placed into two species: *O. yallundae* and *O. acuformis*, respectively (Lucas et al., 2000). Because there were no pairings between strains of *O. yallundae* and *O. acuformis in vitro*, they were considered sexually incompatible species (Moreau and Maraite, 1995). The predominant species of the foot rot pathogen in the Pacific Northwest since 1919 has been *O. yallundae* (Douhan et al., 2002). *Oculimacula yallundae* is commonly referred to as 'W type', while and *O. acuformis* as 'R type' foot rot, which refers back to a previous name change when they thought to be two pathotypes within the *Pseudocercosporella herpotrichoides* species. These nomenclatures refer to their relative pathogenicity. W type is highly pathogenic on wheat, but less so on rye and on barley, whereas the R type is equally pathogenic on wheat, barley and rye (Scott et al., 1975).

The two *Oculimacula* species have similar life cycles, and foot rot is considered to be a monocyclic disease because secondary infections originating from conidia produced on plant lesions are considered to play little role during the season epidemics (Lucas et al., 2000). Ascospores may be dispersed further distances by wind, but they probably do not constitute a major source of inoculum. Fungal mycelium survives on infested stubble, volunteer wheat and barley plants (Kelly et al., 2008), and during summer, the pathogen occurs in the dormant or least active period. When fall, winter and spring temperatures vary between 0ºC and 20ºC,

with an optimum of 10ºC, sporulation of *Oculimacula* spp. takes place (Murray, 2010). Infection of plants occurs via conidia dispersal from infested debris a short distance by rain splash with an optimum temperature between 6 and 15ºC (Fitt and Bainbridge, 1983).

Germination of *Oculimacula* spp. conidia and infection require moisture for many hours. During this process, conidia penetrate coleoptiles and outer leaf sheaths of lower stems directly through epidermal cells (Bateman and Taylor, 1976), or stomatal openings (Sprague and Fellows, 1934). In the Pacific Northwest, most infections occur in November and December (Bruehl et al., 1982).

Symptoms have been reported to develop between 2 to 8 weeks after infection, or longer, depending on weather conditions (Murray, 2010). During the first weeks after infection, there are no macroscopically visible symptoms of the disease, but pathogen growth on the coleoptiles and the first leaf sheath can be observed microscopically (Daniels et al., 1991). *Oculimacula* spp. invades successive leaf sheaths while the disease develops, spreading from the innermost leaf sheaths to the true stem after stem elongation begins. Both relative humidity and temperature affect the foot rot development.

Control of foot rot usually requires a combination of cultural practices, fungicides and host resistance (Sheng, 2011). Cultural methods of foot rot control include tillage, delayed seedling and crop rotation. Fungicides are used worldwide to control foot rot. According to Russel (2005), there are three main fungicide groups used to control foot rot: methyl benzimidazole carbamates (MBC), sterol demethylation inhibitors (DMI), and anilinopyrimidines (AP). However, selection of resistant pathotypes has been documented worldwide to several active ingredients (Sheng, 2011).

Kimber developed the first foot rot-resistant wheat cultivar in 1967, based on the discoveries by Sprague (1936) that the wheat wild relatives *Aegilops ventricosa* and *Haynaldia villosa* were highly resistant to eyespot. The development of the first foot rot-resistant wheat cultivar through introgression of the foot rot-resistance gene *Pch1* was achieved by using cytogenetic manipulations (Kimber, 1967). Thus far, the most popular breeding line, VPM-1 (VPM = Ventricosa x Persicum x Marne), has been the source of *Pch1* in wheat breeding programs (Doussinault et al., 1983). Madsen, released in 1988, was the first cultivar with resistance to foot rot (Allan et al., 1989). It was extensively grown in Oregon due to its good yield potential and broad-spectrum disease resistance.

The *Pch1* was reported to be a single dominant gene (Strausbaugh and Murray, 1989), which was confirmed by Allan and Roberts (1991). Worland et al. (1988), by using the endopeptidase isozyme allele *EP-D1b* as a marker for *Aegilops ventricosa*-derived foot rot resistance, found that the *Pch1* was mapped to the distal end of the long arm of chromosome 7D as a single dominant gene. Several PCR-based molecular markers linked to the *Pch1* gene have been developed to select for the *Pch1* gene (Chao et al. 1989; Groenewald et al., 2003; Santra et al., 2006; Leonard et al., 2008; Chapman et al., 2008). Burt et al. (2010) found that *Pch1* conferred resistance to both *O. acuformis* and *O. yallundae* at the seedling stage. A second foot rot resistance gene, *Pch2*, was identified on chromosome 7A in the French wheat cultivar Cappelle Desprez (CD) (Law et al., 1976; de la Peña et al., 1996). Klos et al. (2013) confirmed that *Pch2* conferred some degree of resistance against both *O. yallundae* and *O. acuformis*.

Foot rot management can include several tactics; however, resistant wheat cultivars are economically the best option, reducing costs. In addition, some of the fungicides available

are no longer effective due to selection of resistant *O. acuformis* and *O. yallundae* pathotypes. Crop rotation is an effective cultural control tactic of foot rot in wheat.

RATIONALE

It is well known that wheat and jointed goatgrass outcross and produce hybrids and backcross generations, and that gene flow between the two species takes places where they co-exist. In addition, the genetics of hybrids and backcrosses have been extensively studied over the past 16 years. However, with the advent of IMI-resistant wheat carrying the *Imi1* gene, knowledge about the extent of the hybridization between IMI-resistant wheat and jointed goatgrass, as well as the *Imi1* gene flow from IMI-resistant wheat into hybrids and backcross generations in commercial wheat fields in Eastern Oregon has not been analyzed. The movement of the imazamox-resistant gene from wheat to jointed goatgrass could reduce the benefits of the IMI-resistant wheat technology, limiting weed management options and making jointed goatgrass more challenging to control in rotational crops or in fallow where herbicides with the same site of action are used. Thus, the knowledge of IMI-resistance spread in hybrids and backcrosses will allow growers, agronomists, extension educators and regulators to recognize the serious implications of introgression of the IMI-resistance gene into jointed goatgrass populations.

Evolutionary processes are influenced by levels of gene flow in populations with selection pressure. While low levels of gene flow may allow local adaptation, high levels may prevent this process (Star et al., 2007). If the magnitude of selection pressure is greater

than the arrival of individuals from other populations, selection pressure will cause allele frequency divergence among populations. Gene flow may homogenize this divergence, particularly in small, isolated populations where migration is limited (Storfer, 1999). Fitness costs caused by herbicide-resistance in the absence of herbicide may prevent fixation of the herbicide-resistance allele in some situations. In similar circumstances, there is a consensus that gene flow slows down the development of resistance in these circumstances.

Resistance to xenobiotics is a local adaptation process, while a migration-selection pressure balance is established between areas differing in the fitness cost associated with the different alleles. Selection-migration equilibrium is not reached if there is no fitness cost associated with the resistant alleles (Comins, 1977). In addition, if the resistance trait promotes selective advantage to the resistant individuals, an increase in frequency of resistance genes is likely to occur (Lenormand and Raymond, 1998). According to Wolfe et al. (2001), increase in frequency of resistance genes might cause potential ecological consequences, such as the ecological fitness enhancement of the wild relative in regions where it is already abundant. While several studies have focused on gene flow between wheat and jointed goatgrass, the fate of genes that provide resistance in jointed goatgrass remains largely unstudied (Willenbourg, 2011).

Because the resistance gene flow to jointed goatgrass seems unavoidable, the spread of the IMI- and foot rot resistance genes from IMI- and foot rot-resistant wheat cultivars to jointed goatgrass populations via gene flow creates a major concern for the maintenance of resistant cultivar production. Therefore, characterization of potential fitness effect brought by these resistance genes is essential to assess environmental consequences caused by wheat-jointed goatgrass gene flow. The study of potential ecological consequences due to gene flow

will provide data for environmental risk assessment and will facilitate the development of models based on gene flow.

Finally, if the introgressed herbicide and foot rot resistant genes increase fitness, the genes will enhance the competitiveness and invasiveness of jointed goatgrass populations, leading to the spread of the resistance genes in the jointed goatgrass populations. Thus, estimating fitness effect of the herbicide and foot rot genes on a jointed goatgrass population is essential.

HYPOTHESES

Therefore, questions to be addressed are: are resistance genes being introgressed into jointed goatgrass? How would the resistance allele proportion and gene flow from the resistant individuals respond to selection pressure of herbicide and disease? Would the resistant individuals have a selective advantage? These answers will be valuable to recognize the implications introgression can have on the resistance allele frequencies in a jointed goatgrass population.

The two hypotheses for this research were:

1) Imazamox-resistance hybrids and backcrosses are naturally occurring in the wheat production areas of Eastern Oregon, and

2) Herbicide and disease resistance will provide fitness advantage with selection pressure

OBJECTIVES

In order to address the hypotheses, the research was divided in two parts. Each part had three objectives:

Part 1: Conduct field surveys in the wheat production area from Eastern Oregon,

1) To determine the prevalence of the *Imi1* gene in wheat by jointed goatgrass hybrids by screening hybrids collected from commercial fields and adjacent areas where IMI-resistant wheat had been grown;

2) To assess hybrid yield components, and explore how those hybrid yield components vary across the sampled sites and

3) To determine whether there was an association between proportion of imazamox-resistant hybrids with area and / or field management practices.

Part 2: Conduct field experiments to study allele frequency with selection pressure,

1) To determine herbicide- and disease-resistant allele proportion in the first progeny with and without herbicide and disease selection pressure;

2) To determine gene flow from resistant to susceptible individuals with and without herbicide and disease selection pressure and

3) To evaluate yield components in herbicide- and disease-resistant jointed goatgrass with and without selection pressure.

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CHAPTER 2

HYBRIDIZATION BETWEEN CLEARFIELD® WHEAT AND JOINTED GOATGRASS (*Aegilops cylindrica* Host.) AND IMAZAMOX RESISTANCE IN HYBRIDS AND BACKCROSSES ON WHEAT FIELDS FROM EASTERN OREGON

ABSTRACT

Clearfield® wheat varieties carry the *Imi1* gene, which is responsible for conferring resistance to the imidazolinone (IMI) herbicide imazamox. This trait allows the selective control of jointed goatgrass, a difficult to control annual grass weed in winter wheat. Gene flow between Clearfield (IMI-resistant) wheat and jointed goatgrass may occur via hybridization and backcross events. Hybrids (F_1) of IMI-resistant wheat and jointed goatgrass were identified in 2008 in a commercial wheat field in Eastern Oregon. In 2009 and 2010, surveys were conducted in Eastern Oregon to determine the prevalence of the *Imi1* gene in wheat by jointed goatgrass hybrids. Polymerase chain reactions (PCR) assays were performed to detect the presence of the *Imi1* gene. We assessed hybrid plant yield components and principal component analyses explored how these components varied across the sampled sites. The association between the proportion of IMI-resistant hybrids and the area or management practice in the commercial fields was determined. A total of 128 sites were surveyed over the two years. Of 1,410 plants sampled, 1,100 were positive for the *Imi1* gene; 1,087 were heterozygous, with a copy of both wild type and mutant alleles, and 13 plants were homozygous for the mutant allele, respectively. The 13 homozygous plants provide evidence that they are of backcross

generations because they no longer carry the wild type allele. This is the first report of natural occurrence of IMI-resistant backcross plants in commercial wheat fields.Seed number per spike was positively correlated with seed number per plant, and there was no correlation between spike number per plant and seed number per plant. Non-agricultural sites or production of IMI-resistant wheat in consecutive years were two factors associated with a greater proportion of IMI-resistant hybrids. Our results demonstrate that the *Imi1* gene is moving from IMI-resistant wheat to jointed goatgrass, hybrids and backcross generations. Therefore, it is important to implement field management practices that reduce the potential of IMI-resistant hybrid production, and to effectivelymanage non-agricultural areas with jointed goatgrass infestations to prevent introgression of the IMI-resistance gene in these areas where hybridization occurs.

INTRODUCTION

Many crops have weed relatives, and hybridization is known to occur (Hieser 1973; Small 1984; Ellstrand & Hoffman 1990; Klinger *et al*. 1992). The transfer of genes that enhance survival, such as genes for biotic or abiotic stress tolerance/resistance, could improve the competitiveness of a weed species, making it more invasive than the wild type. It is also possible to obtain a more invasive weed by selection of natural variants in commercial production fields where the weedy species grows in close proximity to a related crop species (Hancock, 2005). One such species is jointed goatgrass (*Aegilops cylindrica*Host.) growing in association with wheat (*Triticum aestivum*).

Jointed goatgrass is a winter annual grass weed that infests 5 million hectares, including winter wheat and fallow land in the Pacific Northwest (PNW) and Great Plains of the United States (U.S.). Jointed goatgrass has been declared a noxious weed in Arizona, California, Colorado, Idaho, New Mexico, Oregon and Washington and exists throughout much of the mainland U.S., infesting areas across 14 Western and Midwestern states (USDA, NRCS, 2010). The average yield loss on fields moderately to densely infested with jointed goatgrass was estimated to be 25% (Donald & Ogg, 1991). Reduced grain yields were estimated at $45 million and indirect losses, including control costs and dockage at more than $90 million annually. In 1998, Westbrooks reported that total losses from this species in the Western U.S. exceeded $145 million annually (Westbrooks, 1998). Estimated annual wheat yield loss due to jointed goatgrass infestations for the Intermountain Region, including Utah, southern Idaho, and parts of

Nevada, was approximately 139,000 bushels (Quinn et al., 2003). The rate of jointed goatgrass spread has been estimated to be 20,000 to 50,000 hectares per year through seed movement facilitated by combines, grain trucks and contaminated wheat seed (White et al., 2004; Washington State University, 2009; USDA, 2013). Jointed goatgrass establishes easily in disturbed sites (van Slageren, 1994), such as agricultural fields, roadside ditches, fencerows, farm access roads, scablands and pastures.

Wheat and jointed goatgrass belong to the Triticeae tribe (Poaceae family), and there is a close genetic relationship between the two species. Wheat is an allohexaploid (2n = 6x = 42) with three genomes (AA, BB and DD). The A and B genomes of wheat originated from *Triticum turgidum*, and the D genome from *Ae. tauschii* Coss (Kimber & Sears, 1987). Jointed goatgrass is an allotetraploid (2n = 4x = 28), with two genomes (CC and DD). The C genome was contributed by *Ae. markgrafii* (Greuter) Hammer (2n = 2x = 14), and the D genome was contributed by *Ae. tauschii* Coss. (2n = 2x = 14) (Linc *et al*., 1999). Therefore, wheat and jointed goatgrass have the D genome in common, which allows them to cross and produce a hybrid with fivesets of seven chromosomes, ABCDD. The lack of the two chromosome sets forthe A, B, and C genomes produce male-sterile hybrids. However,the presence of two sets of D chromosomesallows chromosome pairing of the D genomeand partial female fertility of the hybrid and viable seed formation when backcrossed with wheat or jointed goatgrass (Zemetra et al., 1998). Kroiss *et al*. (2004) reported that normal genetic recombination between homologous D genome chromosomes of winter wheat and jointed goatgrass in backcross progenies can occur.

The D genome acts as a buffer, ensuring some female fertility in the F_1 progeny (Zemetra *et al*., 1998a; Zaharieva & Monneveux, 2006).

Introgression of the *Imi1* gene into jointed goatgrass was reported by Perez-Jones *et al*. (2006a). In that study, two backcross populations were artificially produced and self-fertilized three times, and the chromosome number of jointed goatgrass was restored. These jointed goatgrass plants were resistant to imidazolinone herbicides with restored fertility.

Although jointed goatgrass and wheat are primarily self-pollinated species, outcrossing has been reported for both jointed goatgrass and wheat. An outcrossing rate of 1.3% was quantified for jointed goatgrass (Cannon, 2006).Outcrossing rates of 5.6% to 10% have been reported for wheat (Martin, 1990; Hucl, 1996; Enjalbert et al, 1998). Spontaneous hybridization has been reported between wheat and other related *Aegilops* species (Hegde and Waines 2004; Loureiro et al. 2008b; Zaharieva and Monneveux 2006).

Hybridization rates between wheat and jointed goatgrass ranging from 0.1 to 8% have been reported (Guadagnuolo et al. 2001; Morrison et al. 2002a; Stone and Peeper, 2004).Because of the evolutionary genetic relationship and the similarities in appearance and life cycles between wheat and jointed goatgrass, chemical, cultural and mechanical control of jointed goatgrass in wheat has been challenging. Under conditions of adequate precipitation, wheat is more competitive than jointed goatgrass (Donald & Ogg, 1991; Dewey, 1996). When precipitation is limited, this relationship reverses (Quinn et al.,

2003) and this competition becomes important in the Intermountain Region of the US because of limited moisture in many dryland cropping systems.

One of the most successful methods for control of jointed goatgrass in winter wheat has been the use of non-transgenic Clearfield® wheat cultivars, which are resistant to the herbicide imazamox in the imidazolinone chemical family. The molecular basis of imazamox-resistance in most IMI-resistant wheat varieties, including those from Oregon State University breeding program, is the mutation S653N (Newhouse et al., 1992) in the gene that encodes the enzyme acetohydroxyacid synthase (AHAS), also called acetolactate synthase (ALS). Herbicides that inhibit the acetolactate synthase (ALS) enzyme are known as ALS inhibitors. Imazamox, an ALS-inhibitor herbicide, provides control of jointed goatgrass and some other grass and broadleaf weeds. However, the movement of the resistance gene from imazamox-resistant wheat to jointed goatgrass could limit the long-term utility of the Clearfield technology, limiting weed management options and making jointed goatgrass more challenging to control in rotational crops where herbicides with the same site of action are used. It is important to note that the production of hybrids and even early backcross generations does not ensure the introgression of the herbicide-resistant gene into a population of susceptible jointed goatgrass.

The *Imi1* is a single semi-dominant genethat can betransferred via pollen from wheat to jointed goatgrass (Anderson et al., 2004; Perez-Jones et al., 2006a). Wheat by jointed goatgrass hybrids carrying this resistance gene were identified in a research study

under natural field conditions (Seefeldt et al., 1998) and in a commercial wheat field (Perez-Jones et al., 2010).

During the 2010-2011 season in Oregon, 107,475 ha (32% of the planted wheat area) were planted with IMI-wheat (NASS, 2013), and the variety ORCF-101 was ranked as the most planted wheat variety in Oregon at 20.1% (67,514 ha). The variety ORCF-102 contributed 11.9% (39,971 ha) of the total area planted to IMI-wheat (NASS, 2013). Thus, IMI-resistant hybrid occurrence has serious implications because a large portion of the winter wheat in Oregon is IMI-wheat, where management of jointed goatgrass relies heavily on the use of imazamox.

The objectives of this research were: 1) to determine the prevalence of the *Imi1*gene in wheat by jointed goatgrass hybrids by screening hybrids collected from fields in Eastern Oregon where wheat, carrying the *Imi1* gene, had been grown; 2) to assess hybrid yield components fertility (seed set), seed per spike, seed per plant and spike per plant, and explore how these hybrid yield components varied across the sampled sites and 3) to determine whether there was an association between proportion of imazamox-resistant hybrids with area type and / or management practice.

MATERIALS AND METHODS

Hybrid Sampling

A list of wheat growers from Eastern Oregon, who had been participated in a previous sampling survey (Morrison et al., 2002b), was compiled. Field consultants from Morrow County Grain Growers in Lexington and Wasco Counties, as well as weed scientists from the Oregon State University (OSU) Columbia Basin Agricultural Research Center, Pendleton, and the Union County OSU Extension Office, La Grande, provided grower contact information. Additional growers were identified by referral. Growers were contacted to determine their willingness to participate in the study. From the growers whoagreed to participate, only those having fields that had been seeded previously with Clearfield wheat were chosen. Non-cropping areas such as roadsides, pastures and fencerows close to these fields, as well as Conservation Reserve Program (CRP) sites close to these fields, were sampled.

The survey was conducted across Oregon from The Dalles to La Grande, during the summers of 2009 and 2010(Figure 1). The sampled fields differed between 2009 and 2010. Most of the sampled fields in 2010 were in summer fallow during 2009. In 2010, new growers and fields were added to the survey.

The sampling technique in this study was non-probability purposive sampling; thus not all individuals in a population have an equal chanceof being selected (Doherty, 1994). Within this sampling technique, subjects were chosen to be part of the sample

because of a specific criterion. We purposely chose to sample fields that previously had Clearfield wheat production. With this non-probability sampling technique,neither the counties nor the wheat growers were randomly selected, so the representativeness of the sample is unknown. Therefore, care must be takennot to generalize the results of this sampling study to the entire area of winter wheat fields from Eastern Oregon or other regions.

To sample the sites, the first step was to look for jointed goatgrass patches, because hybrids usually occur close to where the parents occur. If there were not known jointed goatgrass patches, random walking within field and field edges would be done. If hybrids were not detected after one hour of walking, then that particular site would be considered as having no hybrids. Thirty collected hybrids was the number established for the sites that had hybrids. If a certain site was heavily infested with hybrids, then more than thirty hybrids would be collected due to the high amount of hybrids and shorter time of collection.

Sampled sites and plants were geo-referenced using a hand-held eTrex Legend global positioning system (GPS) (Garmin, Olathe, KS, USA). Spatial maps were prepared byusing ARCMAP v8.3 and the ARCGIS 8.3 (Environmental Systems Research Institute, Redlands, CA).

In 2009, sampling was conducted from July through the end of August. At this time, wheat and most of the hybrid plants were already dry and the spikes mature, so leaves and spikes from each plant were collected separately and placed in paper bags at one sampling time. In 2010, sampling was conducted in two steps. First, hybrids were

marked and geo-referenced, fresh green tissue was collected, placed in a plastic bag and kept in a cooler containing ice. Later, mature spikes of the marked hybrid plants were collected.

Imi1 Gene Identification

Genomic DNA was extracted from the field collected tissue samples. For the dry leaves, DNA was extracted using a DNA isolation kit (QIamp® Micro Kit, Qiagen, Valencia, CA, USA), which included a proteinase K pre-treatment for 12 h at 56°C in the automated shaker. For the fresh green leaves, a DNA isolation kit (DNeasy® 96 Plant Maxi Kit, Qiagen, Valencia, CA, USA) was used. The manufacturer's recommendations were followed in both cases.

The presence of the *Imi1* gene was detected through a PCR allele specific assay (PASA) (Zhao et al, 2005). This PCR reaction was composed of two polymerase chain reaction rounds. In the first round, primers (Table 1) amplified one fragment of the three ALS genes located on chromosomes 6D, 6B and 6A, respectively. In the second round, primers (Table 1) were used in two different reactions to detect both the wild type and the mutant allele (*Imi1*). A second gene-specific primer set was used in the second round upstream of the allele-specific primer, which added a further refinement in that each reaction has an internal control band for DNA quality/quantity. The "diagnostic" band was the product of the allele-specific and gene specific primer pair. The higher molecular weight "control" band was the product of both gene specific primers (BASF, 2010). Therefore, the presence of the control band and absence of the diagnostic band indicated

that the PCR reaction worked and the allele in question was not present in the sample (Figure 1). The heterozygosity or homozygosity for either the wild type or the mutant alleles was determined using this PCR method.

Table 1. Primer sets used for ALS amplification and detection of the specific alleles.

	Primer	Sequence	Fragment size
First round	CM-F	5'-CCGCCGCAATATGCTATCCAG-3'	852bp
	CM-R	5'-GTAGGACAAGAAACTTGCATG-3'	852bp
Second round	1AD-F	5'-GGGAGGCGATCATTGCCACT-3'	775bp
	WT-F	5'-GTGCTGCCTATGATCCGAAG-3'	267bp
	MU-F	5'-CGTGCTGCCTATGATCCGAAC-3'	268bp
	1D-R	5'-GCACATCCCTACAAAAGAGAAGAT-3'	775bp

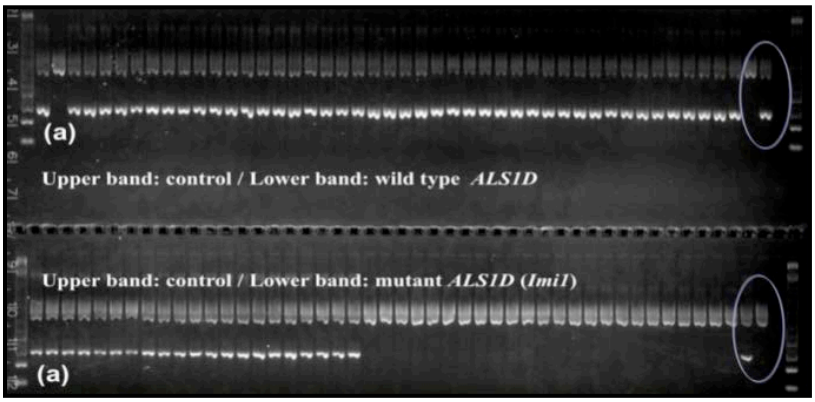

Figure 1. A diagnostic gel showing presence/absence of the*Imi1* gene. Letter “a” indicates asample homozygous for the mutant allele. In the gel, there is absence of the wild type allele band in the *ALS1D* lane. In the *ALS1D (Imi1)* lane, the mutant allele band was amplified. Circles indicate control samples (IMI-resistant wheat, susceptible JGG and water, respectively).

Yield Components and Female Fertility

Hybrid female fertility was determined using the formula:

$$Fertility = \frac{\#\,seeds}{\#\,florets} * 100$$

The yield components, number of spikes produced per hybrid plant, seed number per spike, and seed number per hybrid plant, were determined.

Data Analysis

Hybrid yield components

For both years, the Pearson product-moment correlation coefficient wasused to assessthe linear relationshipsamong number of sampled hybrids, number of IMI-resistant hybrids, seed number per plant, seed number per spike and spike number per plant. We explored the data from the 2 years separately by using principal components analysis (PCA), to visually verify how the sampled sites grouped based on the hybrid yield components measured.

Principal component analysis (PCA) was based on the correlation matrix of the survey variables. There was some redundancy in those variables, i.e., some of thevariables were correlated. Thus, PCA was used to reduce theobserved variables into a smaller number of principal components (artificial variables) that would account for most of the variance.Each variable wasstandardized so that it had a mean of zero and a variance of one. The “total variance” in this data set was, therefore, the sum of the variances of the observed variables. Because the variableswere standardized to have a variance of one, each observed variable contributed one unit of variance to the “total variance” in the data set.The number of principal components retained in a PCA analysis depends on the relative sizesof the eigenvalues of the variancematrix,which depend on

the magnitude of the variances and covariances of the original variables(Hatcher and Stepanski, 1994). Eigenvalues are the variances of the linearcombinations (principal components).

In order to select the appropriate number of principal components to be retained for further analysis, three methods were applied. The eigenvalue-one criterion, also known as the Kaiser criterion (Kaiser, 1960), is an approach in which only components with an eigenvalue greater than 1.00 are retained. The scree test (Cattell, 1960) is a method in which the eigenvalues associated with each component are plotted. A “break” between the components with relatively large eigenvalues and those with small eigenvalues is identified. The components that appear before the break are assumed to be meaningful and are retained. The third method, “component retention”, was used to analyze the proportion of variance accounted for. A component should be retained if it accounted for enough percentage of variance in the data set. Biplots for each year were generated as graphical alternatives to conventional scatter plots using the PROC PRINCOMP procedure in SAS (SAS v.9.3; SAS Institute; Cary, NC).Sites that occurred out of the main clusters were further analyzed in relation to the management practices information available to detect putative associations between measured variables and management practices in a field.

Field factors associated with proportion of IMI-resistant hybrids across counties

Data from the 2 years of sampling were analyzedto determine whether there was an association between the proportion of imazamox-resistant hybrids present in a field and management practices. Data from the 2 years were pooled and analyzed in terms of Counties. There were only 2 sites sampled at The Dalles, and these occurred relatively close to Wasco County; thus, data from those sites were combined with data from Wasco County. Logistic regression was used to assess the importance of the five field associated factors counties (location), sampling year, tillage system in the last 4 years (conventional vs. no-tillage), type of system (agricultural ‘yes’ vs. agricultural ‘no’) and consecutive Clearfield wheat production at least once (consecutive ‘yes’ vs. consecutive ‘no’) with the proportion of IMI-resistant hybrids present.

The number of resistant hybrids of the total number of sampled hybrids was treated as a binomial proportion for each field. Each observation (proportion) was a count of resistant hybrid (“successes”) divided bythe total number of hybrids (“trials”). In this sampling study, the data were discrete, and we detected overdispersion, i.e., the observed variance of the proportions was larger than the expected variance based on the binomial model. Therefore, we considered the quasi-likelihood approach to accommodate the overdispersion. Quasi-likelihood models were fitted using the PROC GENMOD procedure in SAS (SAS v.9.3; SAS Institute; Cary, NC).

Field or site was considered to be the basic sampling unit. Pooling data from the 2 years, the proportion of resistant hybrids as a function of location and the other field-associated predictors was modeled. Because of the non-probability sampling, the variable “location” was treated as a fixed effect in multiple-variable models. The probability of a

sampled hybrid being an IMI-resistant hybrid was modeled as a logistic regression with the field-associated factors of interest via the PROC LOGISTIC procedure in SAS. Interaction and odds ratio plots were generated to evaluate the relationships between the factors and the probability of IMI-resistance for a sampled hybrid.

RESULTS

Objective 1

Hybridization and imazamox resistance in sampled plants

Over the two survey years, 128 sites were sampled. Seventy-seven sites (57.4%) had at least one hybrid plant (Figures 2 and 4). The number of hybrids collected per site varied, as well as the site characteristics (Tables 2 and 3). A total of 1,410 hybrids were analyzed.

In 2009, 62 sites were surveyed, of which 35 had at least one hybrid (Figure 2). From those 35 sites, 34 had at least one hybrid with one copy of the *Imi1* gene(Table 2, Figure 3). Difficulties were encountered running the allele specific PCR for the *Imi1* gene in the dry tissue samples, because of the low DNA quantity and quality. Thus, of 512 hybrids collected, 497 were analyzed to the molecular level. Of the 497 hybrids analyzed, 76.7% were identified as carrying the *Imi1* gene,288 were heterozygous, with a copy of both the wild and the mutant alleles, and93 hybrids (19%) were homozygous for the wild type allele.

In 2010, 66 sites were surveyed, of which 40 had at least one hybrid (Figure 3). The number of hybrids collected per site varied (Table 3). Of the40 sites, 37had at least one hybrid with one copy of the *Imi1* gene (Table 3, Figure 5). Of the 923 hybrids analyzed, 75% had the *Imi1* gene of which 693 were heterozygous, with a copy of both the wild and the mutant alleles, 230 were homozygous for the wild type allele and 13 were homozygous for the mutant allele.

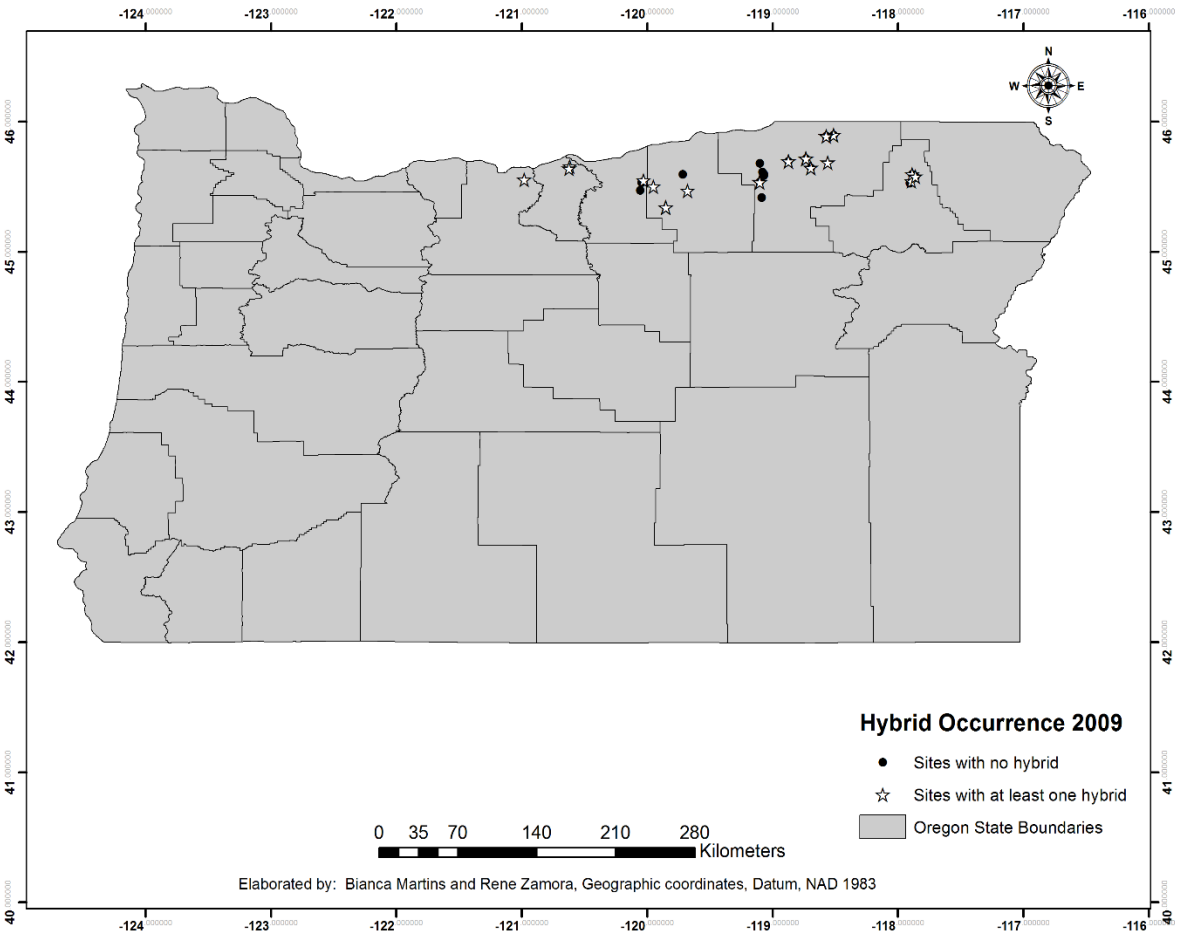

Figure 2. Survey area conducted during 2009, OR. Survey sites were located from The Dalles to La Grande, OR.

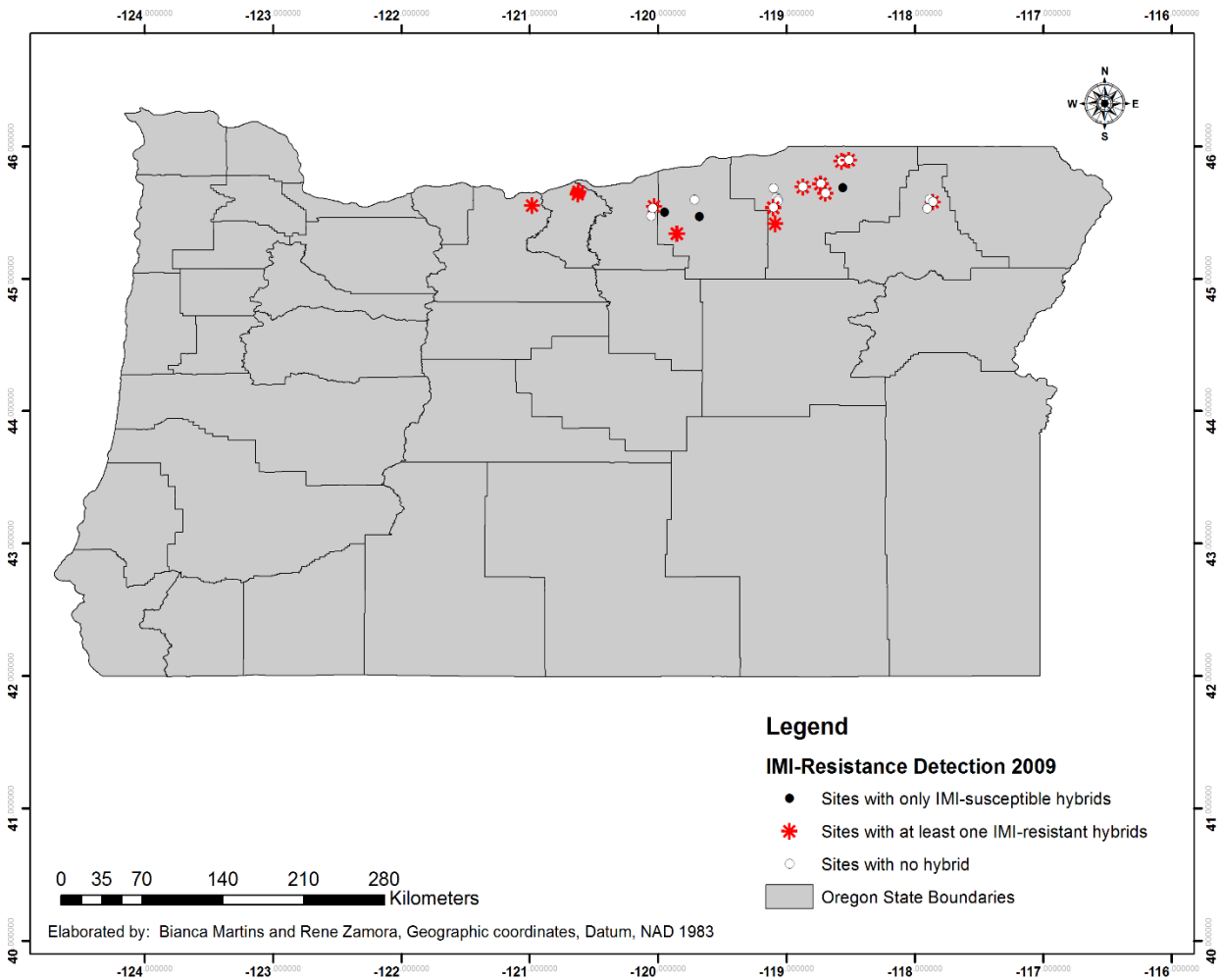

Figure 3. IMI-resistant hybrid occurrence throughout Eastern Oregon, in 2009.

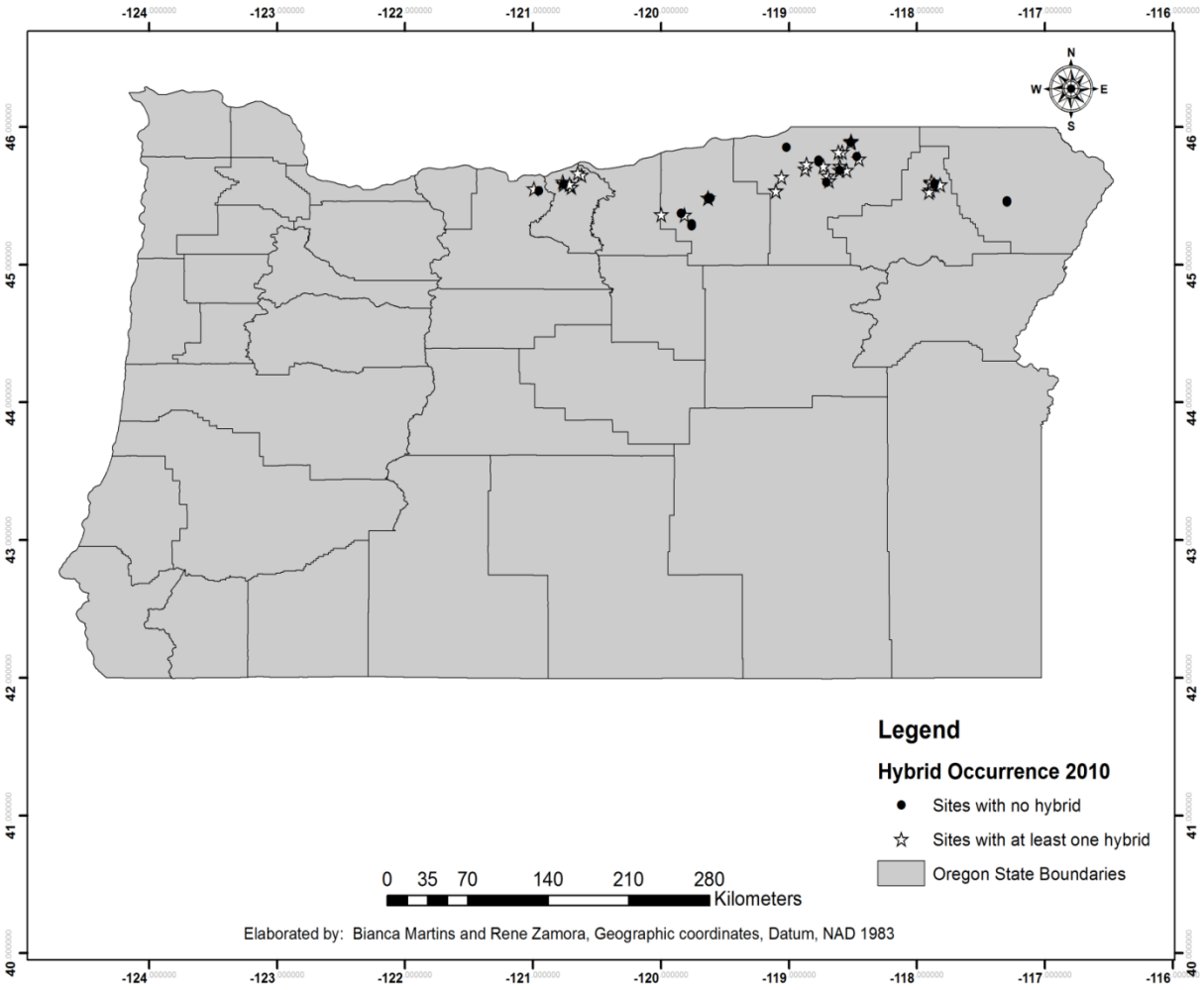

Figure 4. Survey area conducted during 2010, OR. Survey sites were located from The Dalles to Wallowa, OR.

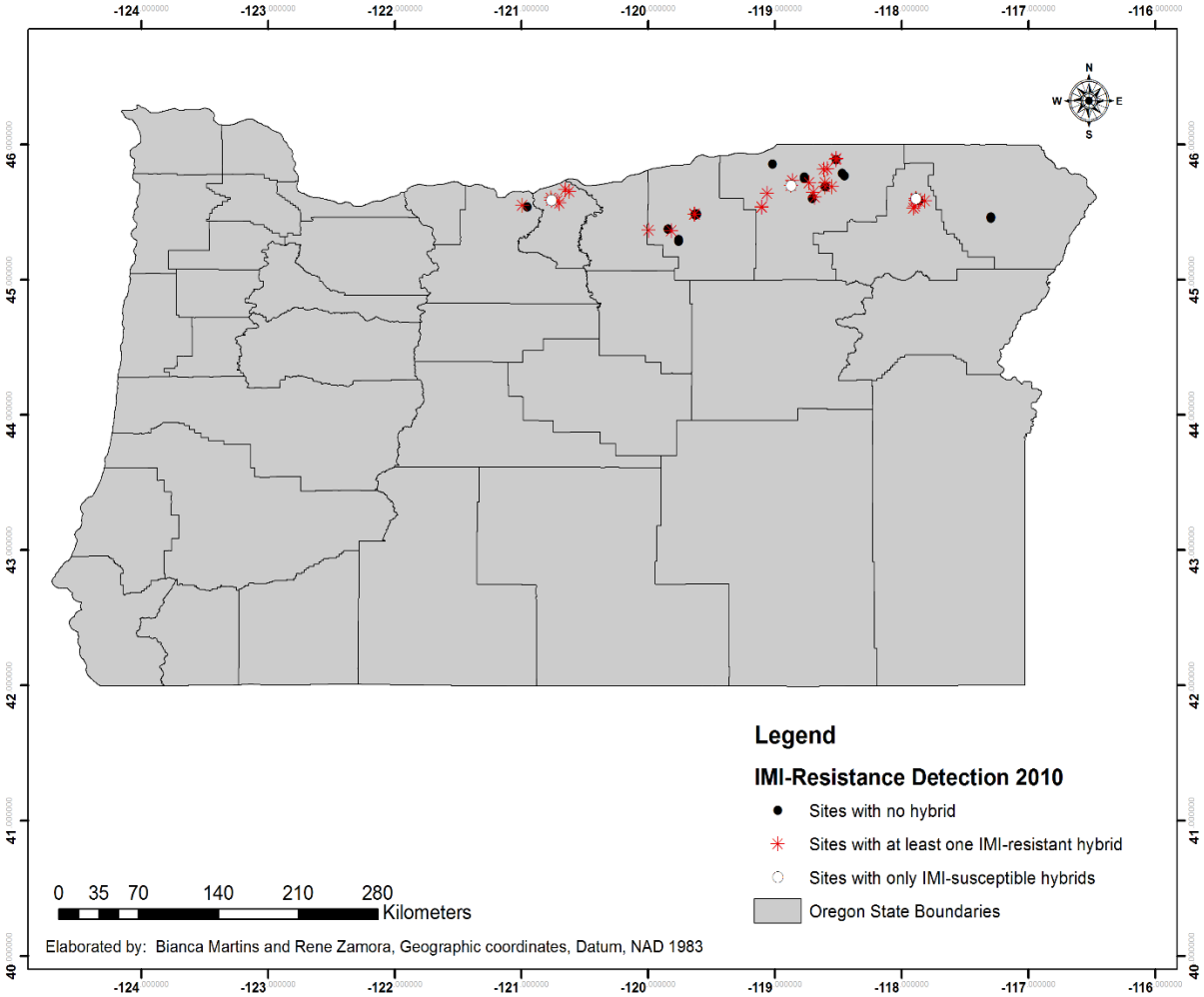

Figure 5. IMI-resistant hybrid occurrence throughout Eastern Oregon, in 2010.

Table 2. Site ID, type and total number of tested and imazamox-resistant hybrids in 2009.

Site ID	Location	Site Type*	No. of Tested Hybrids	No. of IMI-Resistant Hybrids	% IMI-Resistant Hybrids
TD1	The Dalles	winter wheat field	9	6	66.70
W1	Wasco	winter wheat field	27	24	89.00
W2	Wasco	winter wheat field	31	31	100.0
W3	Wasco	winter wheat field	42	38	90.50
W4	Wasco	winter wheat field	10	7	70.00
W5	Wasco	winter wheat field	22	20	91.00
W6	Wasco	winter wheat field	20	20	100.0
W7	Wasco	winter wheat field	8	5	63.00
W8	Wasco	winter wheat field	9	1	11.11
L1	Lexington	winter wheat field	1	0	0.000
L2	Lexington	winter wheat field	2	2	100.0
I1	Ione	winter wheat field	1	1	100.0
I2	Ione	winter wheat field	1	0	0.000
P0	Pendleton	CRP	97	94	97.00
P1	Pendleton	winter wheat field	20	1	5.000
P2	Pendleton	road construction area	41	40	98.00
P3	Pendleton	winter wheat field	16	8	50.00
P4	Pendleton	winter wheat field	1	1	100.0
P5	Pendleton	winter wheat field	2	2	100.0
P6	Pendleton	CRP	2	1	50.00
P7	Pendleton	winter wheat field	1	1	100.0
P8	Pendleton	winter wheat field	30	24	80.00
LG1	La Grande	winter wheat field	5	2	40.00
LG2	La Grande	winter wheat field	4	2	50.00
LG3	La Grande	winter wheat field	30	17	57.00
LG4	La Grande	winter wheat field	1	0	0.000
LG5	La Grande	winter wheat field	2	2	100.0
LG6	La Grande	winter wheat field	32	27	84.40
LG7	La Grande	winter wheat field	8	3	37.50
LG8	La Grande	winter wheat field	1	0	0.000
LG9	La Grande	winter barley field	1	0	0.000
LG10	La Grande	winter barley field	20	1	5.000
Total			497	381	76.70**

*CRP = Conservation Reserve Program; **Average percentage of IMI-resistant hybrids

Table 3. Site ID, type and total number of tested and imazamox-resistant hybrids in 2010.

Site ID	Location	Site Type[1]	No. of Tested Hybrids	No. of IMI-Resistant Hybrids	% IMI-Resistant Hybrids
TD1	The Dalles	winter wheat field	29	29	100.0
W1	Wasco	winter wheat field	33	33	100.0
W2	Wasco	winter wheat field	29	25	86.20
W3	Wasco	winter wheat field	27	3	11.11
W4	Wasco	winter wheat field	33	27	82.00
W5	Wasco	winter wheat field	25	25	100.0
W6	Wasco	winter wheat field	35	35	100.0
I1	Ione	winter wheat field	36	36	100.0
I2	Ione	winter wheat field	45	35	78.00
I3	Ione	winter wheat field	25	15	60.00
L1	Lexington	winter wheat field	21	21	100.0
L2	Lexington	winter wheat field	29	19	65.51
P1	Pendleton	road construction area	30	28	93.33
P2	Pendleton	winter wheat field	9	6	67.00
P3	Pendleton	winter wheat field	11	10	91.00
P4	Pendleton	winter wheat field	21	19	90.50
P5	Pendleton	winter wheat field	32	32	100.0
P6	Pendleton	winter wheat field	28	8	28.60
P7	Pendleton	CRP*	84	82	98.00
P8	Pendleton	winter wheat field	28	27	96.42
P9	Pendleton	roadside	3	3	100.0
P10	Pendleton	winter wheat field**	31	4	13.00
P11	Pendleton	roadside	1	0	0.000
P12	Pendleton	winter wheat field	5	2	40.00
P13	Pendleton	winter wheat field	7	3	43.00
P14	Pendleton	winter wheat field	14	1	7.140
P15	Pendleton	winter wheat field	8	2	25.00
P16	Pendleton	winter wheat field	9	0	0.000
P17	Pendleton	winter wheat field	1	1	100.0
P18	Pendleton	winter wheat field	29	28	96.55
LG1	La Grande	winter wheat field	30	1	3.333
LG2	La Grande	spring wheat field	1	1	100.0
LG3	La Grande	fall pea field	5	5	100.0
LG4	La Grande	fall pea field	7	4	57.14
LG5	La Grande	winter wheat field	39	36	92.30
LG6	La Grande	winter wheat field	9	6	67.00
LG7	La Grande	winter wheat field	34	34	100.0

LG8	La Grande	winter wheat field	15	15	100.0

Table 3. Continuation

LG9	La Grande	winter wheat field	33	0	0.000
LG10	La Grande	winter wheat field	32	32	100.0
Total			923	693	69.8***

[1]*Conservation Reserve Program; **Field without history of IMI-wheat in previous seasons; ***Average percentage of IMI-resistant hybrids

Objective 2

Hybrid fertility and IMI-resistance

The biplots for both years displayed the hybrid yield components and thesampled sites in a single plot by projecting them onto the plane of two principal components (Figures 8 and 10). In Figures 8 and 10, sampled sites are indicated according to their ID displayed in Tables 2 and 3. In addition, sites that were tightly clustered in the biplot representedsites that had hybrids with similar patterns across the variables.

2009 Sampling

In 2009, of the 1,000 spikes analyzed, variation was observed in fertility among spikes, ranging from 0 to 29% (Figure 6). The average fertility was 1.82% (Table 4). The average seed number and number of spikesproduced per hybrid plant were1 and 4, respectively.

The Pearson’s correlation procedure indicated that number of sampled hybrids and number of imazamox-resistant hybrids were positively correlated. The more hybrids sampled and tested, the greater the number of imazamox-resistant hybrids identified

(Table 5). Seed number per spike and seed number per plant were positively correlated, whereas there was no correlation between seed number per spike and spike number per plant or for spike number per plant vs. seed number per plant.

Seventy-nine and 17 percent of the variability could be explained with the first two principal components. Therefore, only the first 2components were extracted and retained (Tables 5 and 8). Components with an eigenvalue of less than 1 accounted for less variance than did the original variable (which had a variance of 1), so were excluded. The principal components analysis redistributed the variance in the correlation matrix (using the method of eigenvalue decomposition – Figures 7 and 9) to the first components extracted.

Component 1 accounted for 42% of the variance within the data. Component 2 accounted for 38% of the variability (Table 6, Figure 7). Because the first two components accounted for a large percent of the variance in the dataset, only these components were retained, interpreted, and used in subsequentanalyses.A biplotof component 2 by component 1, was generated (Figure 8) and some sites were identified as further from the main site clusters. Site P0 had the greatest number of hybrids sampled and IMI-resistant compared with the rest of the sites. Visually, this site was the furthest site from the site clusters in Figure 8. Sites W2 and W3, P2 and LG6 (Figure 8) were also further from the main clusters for number of sampled and IMI-resistant hybrids. For both seed number per spike and per plant, site LG2 had the greatest numbers of seed per spike and per plant. Site I2 was an further from the main clusters for spike number per plant and had hybrids with the greatest spike number per plant compared to the rest of the sites.

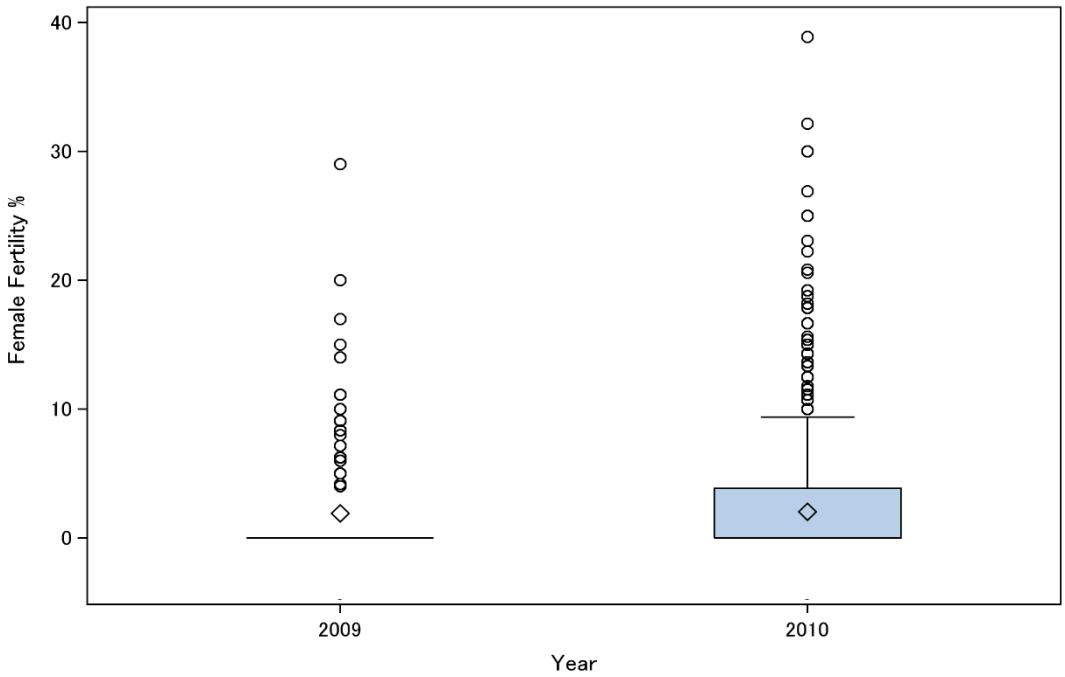

Figure 6. Percent averages of hybrid female fertility in 2009 and 2010. Diamond symbol indicates the median of female fertility (%). Straight horizontal line and blue box indicates 50% of the most likely values for female fertility in 2009 and 2010, respectively. T-bar indicates the maximum observed value for female fertility and circles indicate outliers.

Table 4. Summary of hybrid female fertility averages over the 2-year survey.

Year	2009	2010
Spike number analyzed	1,100	2,936
Average fertility (%)	1.8% (0-29%)[a]	1.9% (0-38%)
Average spike number per plant	4	8
Average seed number per plant	1.4	1.5

[a] Numbers in parentheses are the range of % female fertility.

Table 5. Pearson's correlation coefficients for explanatory variables seed number per plant, seed number per spike and spike number per plant for 2009.

2009	No. of sampled hybrids	No. of IMI-resistant hybrids	Seed no. per plant	Spike no. per plant	Seed no. per spike
No. of sampled hybrids	1.0000	0.92630***	0.01177^{ns}	-0.16736^{ns}	-0.02655^{ns}
No. of IMI-resistant hybrids	0.92630***	1.0000	0.06247^{ns}	-0.1381^{ns}	0.00863^{ns}
Seed no. per plant	0.01177^{ns}	0.06247^{ns}	1.0000	-0.09037^{ns}	0.86419***
Spike no. per plant	-0.16736^{ns}	-0.1381^{ns}	-0.09037^{ns}	1.0000	-0.2008^{ns}
Seed no. per spike	-0.02655^{ns}	0.00863^{ns}	0.86419***	-0.2008^{ns}	1.0000

*** Significant correlation (p<0.0001); ^{ns} non-significant correlation (p>0.05)

Table 6. Eigenvalues of the correlation matrix of variables from the 2009 survey.

Component	Eigenvalue	Difference	Proportion	Cumulative
1	2.0763	0.1943	0.4153	0.4153
2	1.8820	0.9940	0.3764	0.7917
3	0.8879	0.7655	0.1776	0.9693
4	0.1223	0.0911	0.0245	0.9938
5	0.0312	-	0.0062	1.0000

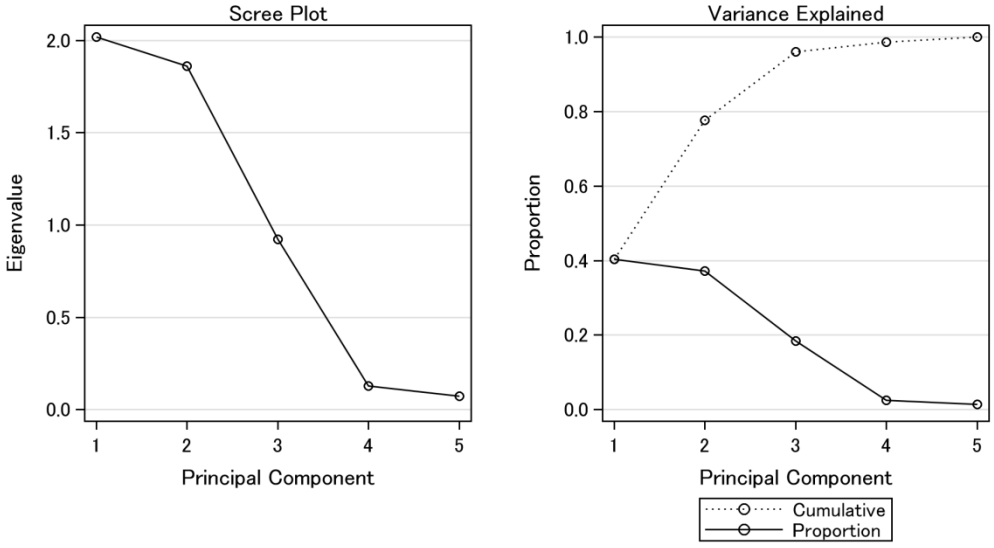

Figure 7. Summary of variability and eigenvalues generated for the 2009 survey variables from principal components analysis.

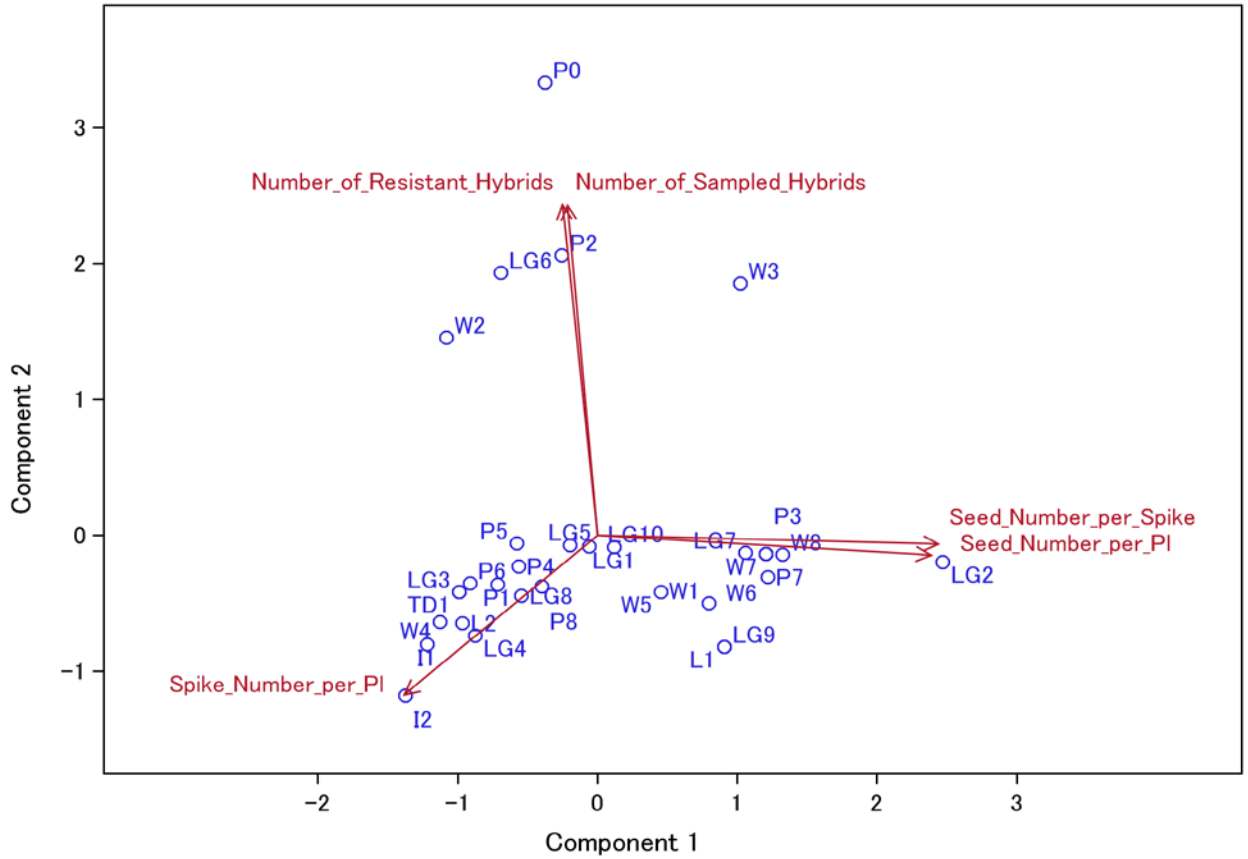

Figure 8. Biplot of the first and second components for the hybrid variables measured and sampled areas from 2009.

2010 Sampling

Approximately 3,000 spikes were analyzedin 2010. Variation was observed in fertility among spikes, ranging from 0 to 38% (Figure 6). The average fertility was 1.9% (Table 4). The average seed number and number of spikesproduced per hybrid plant were1.5 and 8, respectively.

The correlation procedures for 2010 were the same as those used for 2009. Number of sampled hybrids and number of IMI-resistant hybrids were positively correlated. The more hybrids sampled and tested, the greater the number of IMI-resistant hybrids (Table 8). Seed number per spike and seed number per plant were highly correlated.

The principal component analysis for 2010 data was conducted with all pairs of variables. Because the first 2 components accounted for meaningful amounts ofvariance (78%), only these first were retained, interpreted and used in subsequentanalyses. The scree test showed the first 2 components generated eigenvalues greater than 1 (Table 7, Figure 9). Thus, only these components were retained for analysis. Components 1 and 2 accounted for 54% and 24% of the total variance, respectively (Figure 9).

Four sites were detected as further from the main site clusters (Figure 10). With exception of P7, which was the same CRP site sampled in 2009 (P0), sites I1 and LG5, which were wheat fields, had the greatest number of sample and IMI-resistant hybrids. These sites had the greatest spike number and seed number per plant as well. The average spike number produced per hybrid collected in these sites was 23, and average seed produced per spike and plant were 3 and 1, respectively. At site P17, a wheat field edge,

one hybrid was collected which produced 24 spikes, thus being further from the main site clusters in the biplot. Sites P1, P4, P5, L1, L2, and P15 had high average seed number per plant and spike, which were 3.5 and 0.96, respectively (Figure 10). A cluster including sites P9 and P11, and LG 2, LG3 and LG4 differentiated from the rest, with hybrids producing the least seed number per spike and plant.

Table 7. Eigenvalues of the correlation matrix of variables from the 2010 survey.

Component	Eigenvalue	Difference	Proportion	Cumulative
1	2.7110	1.5050	0.5422	0.5422
2	1.2059	0.4973	0.2412	0.7834
3	0.7086	0.4663	0.1417	0.9251
4	0.2422	0.1102	0.0485	0.9736
5	0.1320	-	0.0264	1.0000

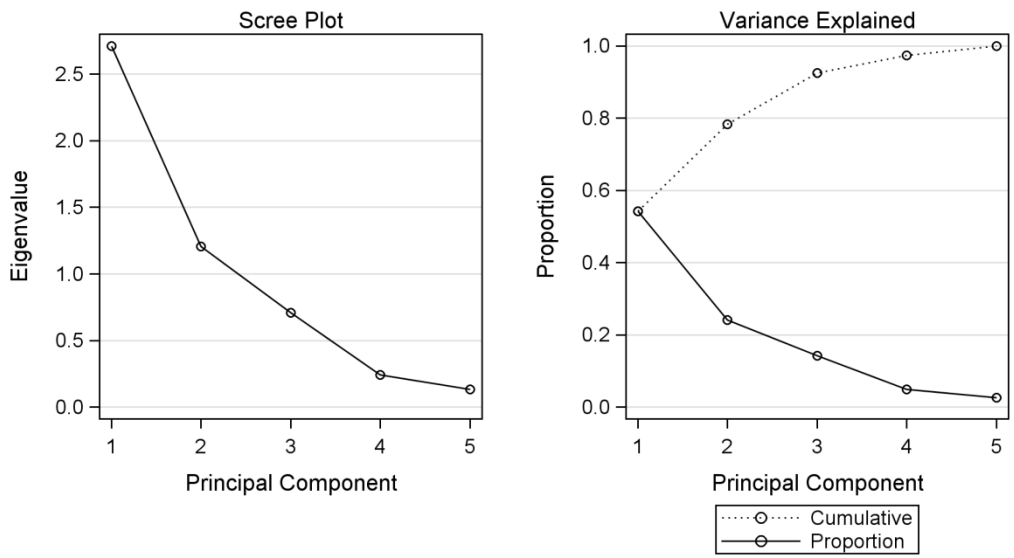

Figure 9. Summary of variability and eigenvalues generated for the survey variables from principal components analysis for 2010.

Table 8. Pearson's correlation coefficients for explanatory variables seed number per plant, seed number per spike and spike number per plant for 2010.

2010	No. of sampled hybrids	No. of IMI-resistant hybrids	Seed no. per plant	Spike no. per plant	Seed no. per spike
No. of sampled hybrids	1.0000	0.85719***	0.32468*	0.22141^{ns}	0.29722^{ns}
No. of IMI-resistant hybrids	0.85719***	1.0000	0.33051*	0.33925*	0.31835*
Seed no. per plant	0.32468*	0.33051*	1.0000	0.32302*	0.72337***
Spike no. per plant	0.2214^{ns}	0.33925*	0.32302*	1.0000	0.51023***
Seed no. per spike	0.29722^{ns}	0.31835*	0.72337***	0.51023***	1.0000

*** Significant correlation (p<0.0001); * Significant correlation (p<0.05); ^{ns} non-significant correlation (p>0.05)

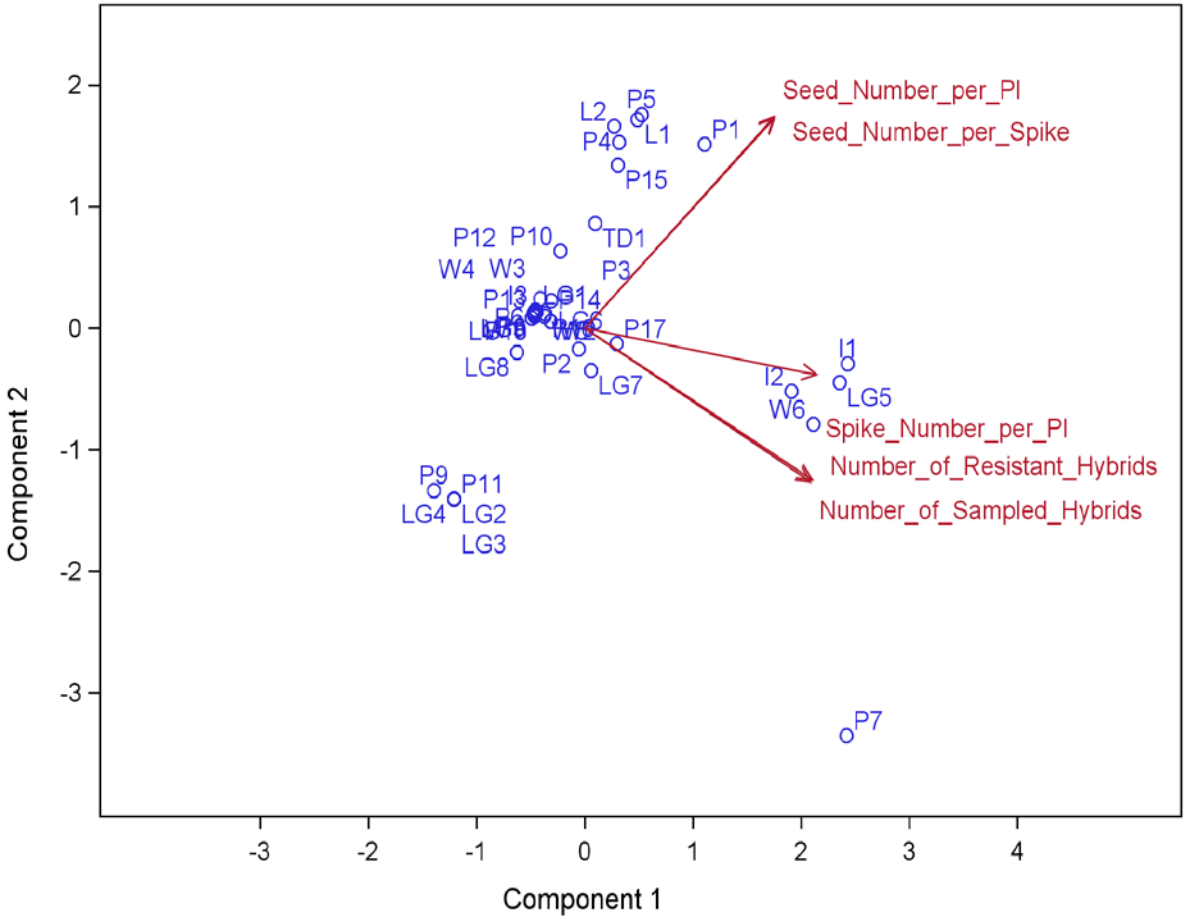

Figure 10. Biplot of the first and second components for the hybrids variables measured and sampled areas from 2010.

Hybrid yield components analyzed over the 2-year survey

2009

Components 1 and 2 explained 79% of variation in the data. The biplot illustrates the site groupings after taking into account the variables: proportion of resistant hybrids per site, seed number per spike, seed number per plant and spike number per plant (Figure 8).

Pendleton 0, the furthest site from the main site clusters in the biplot, had the greatest number of sampled and IMI-resistant hybrids compared to all the other sites. This site was a CRP area that was heavily infested with volunteer IMI-wheat, jointed goatgrass and hybrids. Ninety-seven hybrids were sampled in this site.

La Grande 2 and I2 were further from the site clusters (Figure 8). La Grande 2 had the greatest seed number per spike and seed number per plant averages compared to all other sites (Figure 8). In this site, more than half of the samples had 3 or more seeds per spike. Seventeen of the samples collected in this site were heterozygous and 13 were homozygous for the wild type allele and produced up to 4 seeds. These data suggest that: 1) the homozygous samples with greater seed number than the average may not be F_1 hybrids but advanced backcross plants, or 2) the homozygous samples may be hybrids between conventional wheat and jointed goatgrass and are therefore homozygous for the wild type allele. Site La Grande 2 had IMI-wheat planted only in 2006 and had been under no-till for at least 3 years. The imazamox application rate was 35 g a.i./ha. However, there was large infestation of jointed goatgrass plants, which enabled cross-pollination and seed production.

Ione 2 had hybrids with the greatest spike number per plant, more than 16 spikes per plant on average. This site was under conventional tillage.

Sites P2, LG 6, W2 and W3 had the greatest number of sampled and IMI-resistant hybrids (Figure 8). Site P2 was an IMI-wheat field in 2008, which had history of jointed goatgrass infestation. The grower of site P2 stated that imazamox was sprayed at this site during spring of 2008; however, jointed goatgrass control was unsatisfactory, and plants survived and set seeds. Site P2 was taken out of production for road expansion and this site was located between a summer fallow field and a wheat field that did not have history of IMI-wheat. The summer fallow field had IMI-wheat in 2008. Because wheat pollen pressure in the field is greater than jointed goatgrass pollen pressure, wheat pollinating jointed goatgrass is the prevailing cross-pollination direction (Morrison et al., 2002b; Perez-Jones et al., 2010). The soil in this road construction area was worked with a levelling disc harrow in 2008 and by the summer of 2009, the area was infested with hybrid plants, due to the soil seedbank, seed input by other means, or a combination of the two. The majority of the hybrids collected were IMI-resistant.

La Grande 6 had IMI-wheat in 2007 and 2009, with spring wheat in 2008. Tillage was used in this field. There was large infestation of jointed goatgrass plants with mature spikes, indicating that either imazamox was not applied in this field during the spring of 2009 or it was applied but jointed goatgrass plants were not killed.

There was little information available for W2 and W3. These sites had IMI-wheat in 2008 and conventional wheat in 2009. Wasco 2 had been under conventional tillage and W3 under no-till for several years. Although not distanciated from the main site

clusters, site W1 was under wheat-fallow-wheat rotation and had a high number of sampled hybrids (27) and IMI-resistant hybrids (24) with an average of 3 seeds per plant. Conventional tillage had been used for soil preparation and the field was planted with IMI-resistant wheat only in 2007. Interestingly, 24 out of 27 hybrids collected at this site were IMI-resistant. If the seeds that produced the IMI-resistant hybrids were not brought in by machinery or in seed, they likely were produced during the year IMI-resistant wheat was grown and were in the soil seedbank through the summer fallow year.

For the sites that distanciated from the main site clusters, there was no association of specific management practices with any of the yield components measured in 2009, but the greatest incidence of hybrids was found in non-agricultural areas. Because the number of sampled hybrids was positively correlated with number of resistant hybrids, the greatest incidence of IMI-resistant hybrids also occurred in those areas.

2010

For the 2010 survey, components 1 and 2 explained 78% of variation in the data. The biplot generated for this year illustrates the site clusters after taking into account all variables measured in the survey (Figure 10).

Visually, Pendleton 7 was the furthest site from the main site clusters in the biplot. This site had the greatest number of sampled (84) and IMI-resistant hybrids (82). This site was the same large CRP area sampled in 2009 (site ‘P0’ in 2009). This CRP area was mowed every summer, with no additional management. This site was heavily infested with volunteer IMI-wheat, jointed goatgrass and hybrids, as in 2009.

Four sites that distaciated from the main site clusters were detected, which were associated with hybrids that had greatest spike number per plant. Those sites were I1, I2, LG5 and W6 (Figure 10), which had hybrids with more than 20 spikes. Pendleton 1 was the same road construction area sampled in 2009 (site ‘P2’ in 2009). The average seed number per plant and spike for this site in 2009 was 0.3 and 0.14, respectively. One year later, in 2010, these averages were 3.9 and 0.9, for approximately the same number of plants analyzed. The increase in seed number per plant in samples from the same site may indicate that more backcross plants were produced in this site from 2009 to 2010.

Pendleton 4 and P5 belong to one grower, and L1 and L2 belong to another grower. These sites were infested with hybrids, with more than 20 hybrids sampled from each site, most were close to field edges and produced an average of 3.6 seeds produced per plant. No-till had been adopted in these sites, and imazamox sprayed when IMI-wheat was grown.

Pendleton 9 had no IMI-wheat history, but was next to a field with history of IMI-wheat production. Three hybrids were collected on the edge of this field and all were IMI-resistant. These IMI-resistant hybrids were most likely the result of either seed or pollen movement from the field next to it; either IMI-resistant hybrid seed was produced in the field and brought to the field edge, or IMI-wheat pollinated jointed goatgrass plants that were on the edges of the field that produced hybrid seed.

La Grande 2 was a spring wheat field and had only one hybrid collected, which was IMI-resistant. La Grande 3 and 4 werefall pea fields, and both sites had IMI-wheat in

the past. Five and 7 hybrids were collected in the fall pea fields in LG3 and LG4, respectively, of which 5 and 4 were IMI-resistant.

La Grande 9 was an interesting field because over 30 hybrids were collected but none were IMI-resistant. This field has been under no-till and wheat-fallow rotation since 2006. This field was planted to IMI-wheat in 2008 and 2010, with summer fallow between crops. Hybridization is occurring, and it appears that the rotation between conventional wheat, fallow and IMI-wheat, slowed down production of IMI-resistant hybrids. However, the more hybrids produced, the greater the production of backcross plants and the greater the chance of cross-pollination between these plants and IMI-wheat.

We also sampled a field site that was infested with hybrids, but without a history of IMI-wheat. Of 31 hybrids collected in this site, 4 were IMI-resistant. This field is surrounded by three other fields with a history of IMI-wheat production. Possible explanations for the IMI-resistant hybrid occurrence in this field are either a seed contaminant or a more likely explanation is pollen-mediated gene flow from IMI-wheat from the surrounding fields.

Objective 3

Field parameters associated with proportion of IMI-resistant hybrids across counties

Data were fitted to a generalized linear model (logistic regression) with extra-binomial variation using the quasi-likelihood approach. The model accounted for the factors associated with the site management information and allowed overdispersion

(scale parameter = 3.116), which allows the response variable proportion of IMI-resistant hybrids be treated as having a binomial distribution (Table 9).

Table 9. Analysis of Maximum Likelihood Parameter Estimates in a single, multiple-variable model*

Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr>ChiSq
Intercept	1	5.2569	1.1981	2.9086	7.6052	19.25	<0.0001
Year 1	1	-0.4755	0.5093	-1.4736	0.5226	0.87	0.3505
Year 2	0	0.0000	0.0000	0.0000	0.0000	.	.
Morrow	1	0.5191	0.894	-1.2332	2.2714	0.34	0.5615
Umatilla	1	-0.4473	0.7234	-1.8651	0.9706	0.38	0.5364
Union	1	-1.2053	0.6191	-2.4187	0.0081	3.79	0.0516
Wasco	0	0.0000	0.0000	0.0000	0.0000	.	.
Tillage no	1	-0.6651	0.5005	-1.646	0.3158	1.77	0.1839
Tillage yes	0	0.0000	0.0000	0.0000	0.0000	.	.
Agricultural no	1	-2.3722	0.8446	-4.0276	-0.7167	7.89	0.005
Agricultural yes	0	0.0000	0.0000	0.0000	0.0000	.	.
Consecutive no	1	-1.4644	0.6424	-2.7236	-0.2052	5.2	0.0226
Consecutive yes	0	0.0000	0.0000	0.0000	0.0000	.	.
Scale	0	3.1158	0.0000	3.1158	3.1158		

*The scale parameter was estimated by the square root of Deviance/Degrees of Freedom. The zero rows indicate the reference levels of the different parameters.

Table 10. Likelihood Ratio Type 3 Statistics

Source	Num DF	Den DF	F Value	Pr>F
Year	1	65	0.87	0.3534
Location	3	65	2.38	0.078
Tillage	1	65	1.84	0.1799
Agricultural	1	1	10.67	0.0017
Consecutive	1	65	5.36	0.0238

The Maximum Likelihood analysis estimated the parameters of the field associated factors (Table 9), and the Likelihood Ratio test indicated the significance of the factors in the model. Two factors (agricultural ‘yes’ vs. agricultural ‘no’ and consecutive ‘yes’ vs. consecutive ‘no’) were identified for analysis (Table 10).

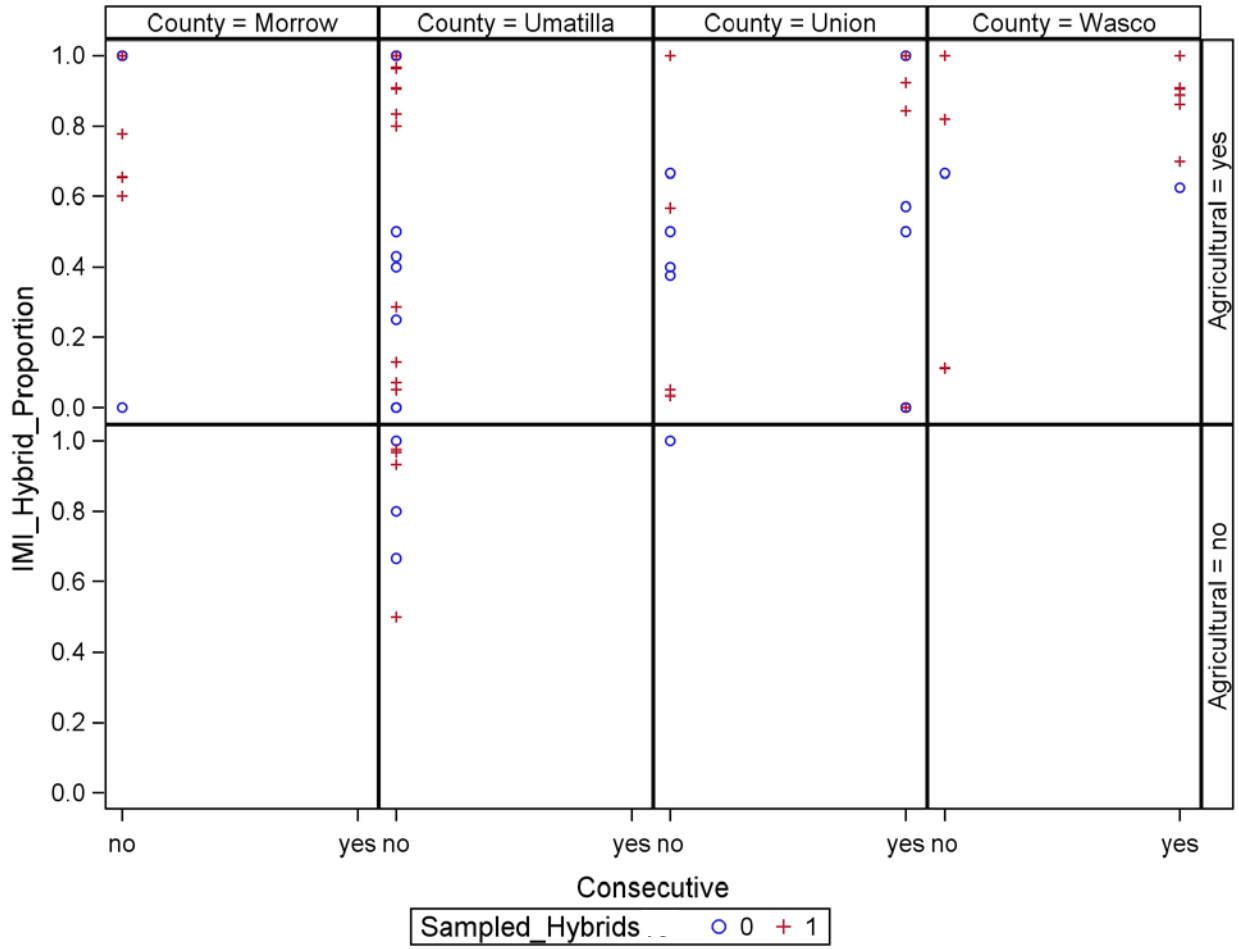

Figure 11. Scatter plot of IMI-resistant hybrid proportion (RHP) as a function of field-associated factors Agricultural and Consecutive across counties.

A scatter plot was generated to visualize the data based on their resistant hybrid proportion (IMI_Hybrid_Proportion) within each of the 2 factors. In Figure 11, the sampled hybrids (Sampled_Hybrids) symbol was '+1' if the total number of sampled hybrids was greater or equal to 10, and zero, otherwise. Resistant Hybrid Proportion is the number of resistant hybridsofthe total number of sampled hybrids. The most information for the type of system factor occurs for Umatilla County and that only Wasco and Union Counties had information for the factor consecutive (IMI-wheat back-to-back production). There was only one non-agricultural site in Union County; so, more non-agricultural sites would be needed to draw conclusions about the proportion of IMI-hybrids in non-agricultural sites. It was evident that, in the model that included all counties, Umatilla County produced a significant effect for the type of system factor, while Wasco and Union Counties produced significant effects for the factor "consecutive".

The model indicated that sites with non-agricultural type of system (Agricultural 'no') or sites that had IMI-wheat back-to-back production (Consecutive 'yes') had a greater proportion of resistant hybrids ($p<0.0008$), in Umatilla County and Wasco and Union Counties, respectively (Figure 11). The probability of IMI-resistance in a sampled hybrid is greater within non-agricultural sites close to wheat fields or fields with history of IMI-wheat production back-to-back (Figure 12).

In addition, this model indicated that the odds of IMI-resistance in a sampled hybrid in a non-agricultural situation are 7.5 times the odds of IMI-resistance in a sampled hybrid in an agricultural situation, i.e., in a wheat field. In terms of percent change, from an agricultural to a non-agricultural situation, there is 750% increase in the

odds of a sampled hybrid being IMI-resistant. This result corroborates the results for number of sampled and IMI-resistant hybrids in the surveyed sites, where the non-agricultural sites, i.e., CRP and road construction areas, had the greatest numbers of sampled and IMI-resistant hybrids. One potential reason for this is that these sites are not managed to reduce jointed goatgrass, hybrids and backcross plants survival and seed production. In addition, practices such as mowing or disc leveling during the summer only favor the spread of jointed goatgrass, hybrids and backcrosses seeds in the area. Thus, the occurrence of IMI-resistance in these areas likely has been increasing over years.

In the same manner, the odds of IMI-resistance in a sampled hybrid in a wheat field without history of IMI-wheat back-to-back production are 0.3 times the odds of IMI-resistance in a sampled hybrid in a wheat field that had have IMI-wheat back-to-back production (Figure 12). For a change from Consecutive ‘no’ to Consecutive ‘yes’, a 233% increase in the odds of a sampled hybrid being IMI-resistant is expected.

The IMI-wheat stewardship program does not encourage the use the IMI-wheat more than twice every six years, i.e., IMI-wheat production in a three-year rotation containing a late-spring seeded crop and summer fallow. In the case of winter wheat-fallow rotation, the program recommends the avoidance of the use of IMI-wheat in more than two consecutive wheat crops (BASF, 2013). It is important to emphasize the benefit of crop rotation and / or fallow, whose key management practice is to control volunteer IMI-wheat, jointed goatgrass, hybrids and backcrosses, with non-ALS herbicides. Non-ALS herbicides have different mode of action than imazamox, which is an ALS herbicide (see section ‘The herbicide imazamox’ in General Introduction).

In the absence of crop rotation or fallow, wheat is cultivated back-to-back. In this case, it is imperative that IMI-resistant wheat and conventional wheat be rotated. Even though this system allows hybridization between wheat and jointed goatgrass to occur, rotating IMI-resistant wheat and conventional wheat can at least reduce the production of IMI-resistant hybrids and IMI-resistant backcross plants. Results for the “Consecutive” factor support these stewardship recommendations because when there is an interruption of IMI-resistant wheat production in the same field, the chances of hybridization and gene flow are reduced given the fact that a different crop is grown in the field. Consequently, herbicides with different modes of action can be used in the rotation crop, reducing the chances of ALS resistance development. In addition, management strategies should prioritize preventing contamination of agricultural machinery, transport vehicles, and wheat seed to minimize the spread of IMI genotypes.

The results of this research confirm pollen-mediated gene flow of the *Imi1* gene from IMI-wheat to F_1 hybrid plants and to backcross generations in commercial wheat production fields in Eastern Oregon. These results are consistent with previous data that confirmed the existence of the *Imi1* in hybrid plants from a commercial wheat field with a history of IMI-wheat production.

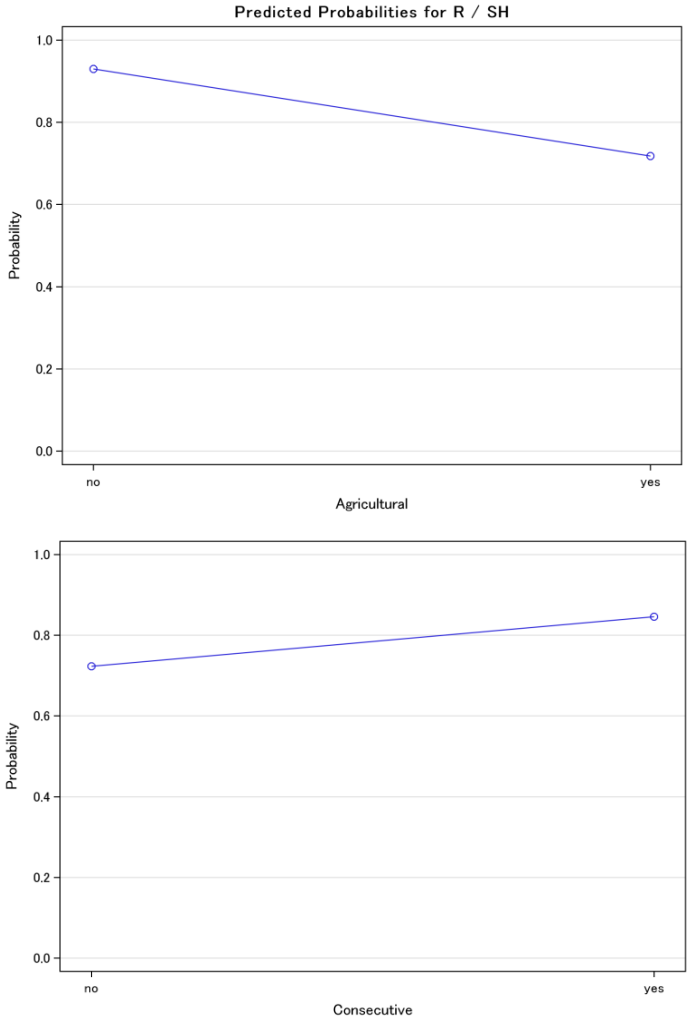

Figure 12. Predicted probability plots for IMI-resistant hybrid proportion (R/SH) within each field-associated factors: type of system (Agricultural ‘no’ vs. ‘yes’) and IMI-wheat production history (Consecutive ‘no’ vs. ‘yes’).

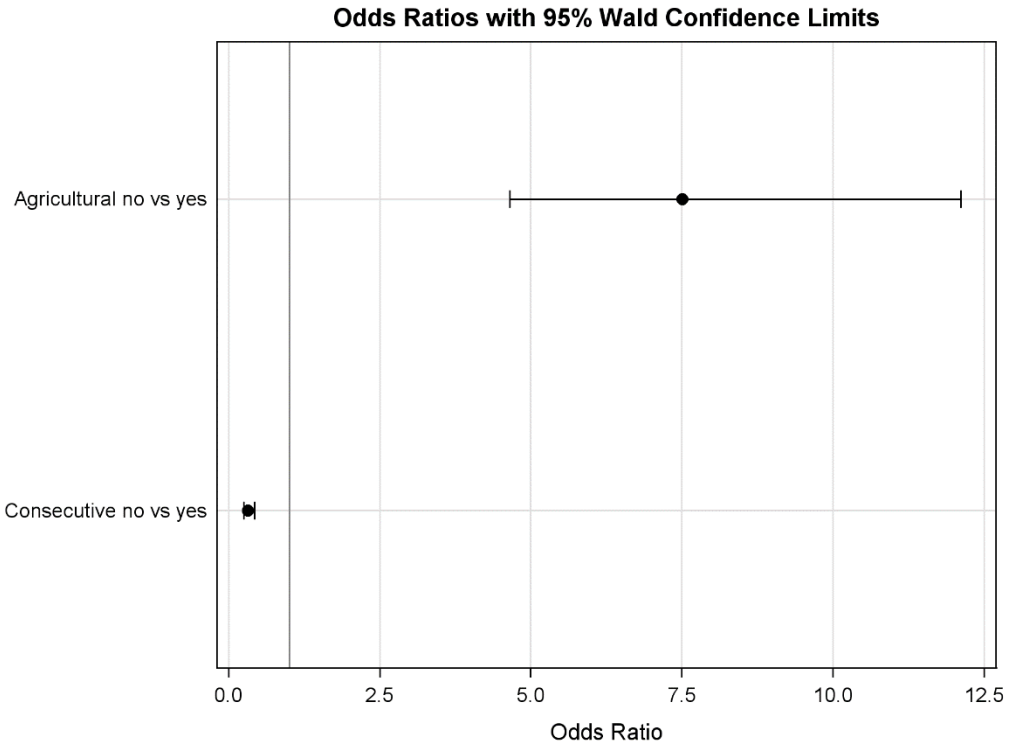

Figure 13. Odds ratios with 95% confidence intervals for type of system (Agricultural 'no'/ Agricultural 'yes') and IMI-wheat production history (Consecutive 'no'/ Consecutive 'yes;').

DISCUSSION

Extent of hybridization in the surveyed area

Data from this survey were considered to explore three issues: 1) the magnitude of hybrid presence (hybridization) in the surveyed sites; 2) the relationship between seed number per spike, spike number per plant and seed number per plant; and 3) the relationship between number of analyzed hybrids and imazamox-resistant hybrids, associated that with site management practices. We did this fully aware of some limitations of the data. One issuewas lumping some sites into a 'non-agricultural' category, i.e. roadsides, road construction or CRP areas. There was additional variation because this survey was conducted over a large geographical area, different environmentalconditions and cropping systems. Some important variables that were not included in the analysis, such as use of the herbicide imazamox, application timing, jointed goatgrass control, and crop rotation could directly or indirectly cause some of the variation. Despite this, these data are useful because a data set on hybridization, production of IMI-resistant hybrids and rate of backcross seed production on hybrid plants under natural field conditions, with a large number of hybrid plants for statistical power and over a large area in Eastern Oregon does not exist. Morrison et al. (2002b) conducted a survey in wheat fields from the same area of this present survey; however, only 93 sites were visited and hybrids were collected from only 45 sites. Moreover, in that survey, the maximum numbers of hybrids collected from winter wheat fields were 5, 10 or 15. Variation in the data can obscure patterns but, conversely, detectable patterns must be relatively strong and consistent over a large geographical area. One reason for

variation in the data was that some sites had relatively few or only one hybrid while others had many hybrids.Furthermore, we looked at groups of sites from a field management perspective.

Of the 128 sites surveyed, 77 had at least one hybrid sample, which indicates that hybridization between wheat and jointed goatgrass is occurring in commercial wheat fields from Eastern Oregon. The average percent of spike fertility of the two years was 1.88%, which aligns with previous research, in which the average seed set of a jointed goatgrass by wheat hybrid spike was reported to be approximately 2% (Zemetra et al., 1998).

Morphological identification of hybrid plants in the field is relatively easy, but differentiation of backcrosses is challenging at the landscape level. Samples homozygous for the mutant allele are certainly backcrosses; however, due to the several outcrossing possibilities between two of these plants (IMI-resistant wheat, conventional wheat, jointed goatgrass, hybrids and backcrosses), the backcross plants can have all the possible genotypes (heterozygous, homozygous for the wild type or the mutant alleles). A plant sample with hybrid type morphology that is homozygous for the wild type allele can be a non-IMI-resistant hybrid from the cross between conventional wheat and jointed goatgrass or a backcross plant. However, a plant that is homozygous for the mutant allele is certainly a backcross plant. Therefore, there may be backcrosses among the heterozygous and homozygous for the wild type allele plants that were considered as hybrids. In addition, it is possible that some samples with relatively high seed number per plant and per spike (Figures 7 and 9) and / or that are homozygous for the mutant allele are advanced backcross generations. The prerequisite for the unidirectional movement of

the *Imi1* gene from wheat to jointed goatgrass is that backcross plants continue to backcross with jointed goatgrass. This represents a scenario that can lead to the *Imi1* introgression into jointed goatgrass.

The greater spike number in wheat by jointed goatgrass hybrids may be the result of hybrid vigor. Hybrids typically have hybrid vigor but very low seed set (Zemetra et al., 1998; Morrison et al., 2002b). Samples collected in Ione 2 and Lexington 2 had high spike number per plant, averaging 16, and set very low number of seeds, averaging 0.25. These samples were heterozygous, with a copy of both the wild type and mutant alleles. Thus, it is more likely that these samples are F_1 hybrids between wheat and jointed goatgrass than backcross generations. These hybrids were mostly collected in the field borders, where the major jointed goatgrass infestation occurred, indicating the need to manage this weed not only within fields but also on the field edges, where the plants can take advantage of the lower competition for resources.

Further backcross generations, in turn, have higher seed number per spike and per plant compared to hybrids (Wang et al., 2001), which may be the reason why some spikes had higher seed set (Figure 4 A and B). Hybrids have low seed set (Wang et al., 2001), so the low seed production and the increased biomass produced by more spikes per plant may be a reason that spike number per plant and seed number per plant were not correlated in 2009 (Table 5) and had low correlation in 2010 (Table 8).

Association of field management information and hybrid IMI-resistance

Non-production areas, such as the CRP and road construction sites sampled, were the areas with the greatest number of hybrids and IMI-resistant hybrids produced.

Volunteer IMI-wheat and jointed goatgrass plants occurred with high incidence in these areas as a consequence of the lack of management. Another concern regarding these non-agricultural areas is the build-up of a diverse seedbank, containing both hybrid and backcross seed generations.

The statistical analyses indicated that the proportion of IMI-resistant hybrids responded in concert to the type of system and IMI-wheat back-to-back production history factors. Therefore, an association between the two field-associated factors and the proportion of IMI-resistant hybrids could be determined with satisfactory confidence in the sampled sites. However, it is important to state thatthe fixed observational factors are not well balanced, and the modeling of the data can only be applied to the sampled sites, which means that we should be careful about generalizing the pattern seen in these Counties. We cannot assume that these results are representative of the whole population of wheat fields with Clearfield production history from Eastern Oregon. The scope of inference from this sampling study is therefore, narrow. In order to generalize these results, a more comprehensive sampling should be conducted, to determine if the patterns seen here occur in the entire wheat production area of Eastern Oregon. These data are, however, important because they can provide interesting exploratory evidence for hypothesis generation and further research.

CONCLUSIONS

We identified plants in the field with the *Imi1* gene. We assessed hybrid heterozygosity and homozygosity for both the wild and mutant alleles. A plant with only the mutant allele is homozygous, and the plant is considered to be a backcross. Similarly, a plant without the mutant allele is homozygous for the wild allele. However, homozygous plants for the wild type allele might be either a non-resistant hybrid or a putative backcross. A heterozygous plant has a 50% probability of being a F_1hybrid or a backcross.

Hybrids and backcross plants can cross with either wheat, jointed goatgrass or other backcross plants. However, a study that analyzed the parentage of hybrid and BC_1plants determined that all F_1 plants tested had jointed goatgrass as the female parent and wheat as the male parent, and all BC_1 plants tested had wheat as the male backcross parent (Gandhi et al., 2006; Perez-Jones et al., 2010). The authors associated the prevalence of wheat pollen over jointed goatgrass pollen in the wheat fields as the reason wheat was the male parent.

Wheat pollen movement depends on several biotic and abiotic factors, including variety, wind speed, air temperature and humidity, and size of the pollen source. It has been reported that wheat pollen can travel up to 1,000 m (Virmani and Edwards, 1983), although most studies have reported shorter distances, ranging from 3 m to 100 m (De Vries, 1971; Suneson and Cox, 1964; Khan et al., 1973; Hucl & Matus-Cadiz, 2001; Matus-Cadiz et al. 2004; Hanson et al., 2005; Loureiro et al., 2011; Beckie et al., 2012). Clearfield wheat seed production guidelines state that a 30-meter isolation buffer should

be maintained among adjacent wheat fields or that the isolation buffer distance should meet or exceed state certification recommendations (Bond, 2010). In Oregon, the isolation distance among wheat fields is 27 meters for foundation seed production and 3 meters for registered or certified seed production (Oregon Seed Certification Service, 2013). Thus, it is evident that gene flow cannot be prevented with the current isolation distances, because wheat pollen is capable of reaching further distances.

In non-agricultural sites, wheat and jointed goatgrass are geographically sympatric, allowing interspecific hybridization and gene flow to occur, even with male-sterility and low female fertility of the hybrid plants. Acreage enrolled in the Conservation Reserve Program (CRP) is projected to rise to approximately13,000,000hectares by the end of 2020 (USDA Long-term Projections, 2013). Therefore, careful attention should be given to CRP areas where jointed goatgrass occurs. These non-agricultural areas can act as hybridization zones, both facilitating and speeding up the introgression process of the herbicide-resistance gene into jointed goatgrass.

Successful hybridization produces viable F_1hybrids,whose survival and reproduction are fundamental for geneflow. The fate of the resulting plants depends on the segregationof parental traits and chromosome transmission, which are usually independent of the gene in question. Introgression is the final result of the sum of these processes that incorporate cropgenes in the gene pool of the related wild species (Liu et al., 2013).Thus, the backcross plants contribute to the *Imi1* gene flow serving as both advanced backcross seed producers and / or pollen donors.

Moreover, because fertility increases with each generation, backcross plants in the field produce more seed, resulting in increased backcross populations. With more backcross plants in the field, there is a potential that those plants can be pollinated by wheat or jointed goatgrass.

Seed-mediated gene flow is influenced by production practices in seed production and commercial fields, and can be reduced by preventing hybrid/backcross seed production during the growing season and cleaning of planters and combines. Likewise, jointed goatgrass should be removed from IMI-wheat fields before spike production, to reduce the chance of hybridization and subsequent transfer of imazamox resistance into weed populations (Wang et al., 2002). In Oregon, certified IMI-wheat seed must be planted. Avoiding IMI-wheat planting back-to-back, using recommended rates of the herbicide imazamox, managing jointed goatgrass in wheat-fallow-wheat rotations and in non-cropped areas, including fence rows, as well as ensuring adequate jointed goatgrass control within IMI-wheat fields are important to prevent pollen-mediated gene flow between wheat and jointed goatgrass.

Although the model from this study did not include some other important factors, including number of imazamox applications per year, imazamox dose and crop rotation, we conclude that there was evidence that IMI-wheat back-to-back production history was a significant field-associated factor parameter in the model applied for the sampled sites. The same result holds for the type of system field-associated parameter. These data may indicate that the patterns seen are valid for the whole population of wheat fields from Eastern Oregon; however, these patterns cannot be extrapolated from our statistical analyses because there was no probability sampling.

Results generated in this research are important to alert growers that it is possible that the *Imi1* gene will be introgressed into jointed goatgrass and jointed goatgrass populations will no longer be controlled by imazamox.

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CHAPTER 3

SELECTION PRESSURE EFFECTS ON INTROGRESSED HERBICIDE AND DISEASE RESISTANCE ALLELE PROPORTION, GENE FLOW AND YIELD COMPONENTS

ABSTRACT

Gene flow facilitates the production of hybrid plants, introgression of novel alleles into a plant populations, and evolution of new genotypes. Gene flow can occur on both spatial and temporal scales. Given a population large enough to avoid genetic drift, introgressed alleles with fitness cost will persist in the population with a constant allele frequency. However, alleles that confer advantages will be selected under selection pressure. This rate of selection is faster in self-pollinated species compared to outcrossing species. Jointed goatgrass (*Aegilops cylindrica* Host.) is one of the most troublesome weed species in winter wheat, and the pathogen *Oculimacula* spp. causes strawbreaker foot rot disease, capable of reducing wheat yields by 50%. The most effective method to selectively control jointed goatgrass in winter wheat (*Triticum aestivum* L.) is to use imazamox (IMI) resistant wheat cultivars coupled with imazamox application. IMI-resistant and foot rot resistant wheat cultivars are widely grown in the Pacific Northwest, representing effective tools to control both the weed and the pathogen. However, gene flow from wheat to jointed goatgrass, hybrid or backcross plants could move the resistance genes to jointed goatgrass populations. Once the resistance genes are introgressed into a jointed goatgrass population, their intraspecific movement and fate in the progeny remains largely unstudied. Therefore, field experiments were conducted using herbicide and foot rot resistance genes introgressed into a single jointed goatgrass

line to study allele frequency and gene flow with herbicide and disease selection pressure. The progeny were genotyped to detect the presence of the resistance alleles to determine their proportion and the level of gene flow from resistant to susceptible plants. In addition, selection pressure effects on jointed goatgrass yield components were analyzed. The herbicide-resistance allele proportion in the progeny was greater when parent plants were treated with imazamox in both years. The disease-resistance allele proportion did not differ among the selection pressure treatments in the first year, but was greater with disease occurrence in the second year. The herbicide-resistance gene flow from resistant to susceptible plants was greater with herbicide selection pressure than without it only in the first year. Disease resistance gene flow did not differ in either year. The results from these field experiments showed that if a jointed goatgrass population acquires the herbicide resistance alleles, there will be increases in their proportions eachgeneration, ultimately reaching stabilization in the population.It is likely that, under selection pressure, more resistant jointed goatgrass plants will prevail compared to the susceptible ones. Selection pressure of herbicide plus disease reduced the yield components in the susceptible parent plants, including total spikelet weight per plant, 1,000 spikelet weight, spikelet number per spike and number of emerged seedlings per spikelet, when compared to the control treatment. The proportional increase in the yield components of the resistant parent plants may favor seed mediated spread of the resistant alleles.

INTRODUCTION

Jointed goatgrass (*Aegilops cylindrica* Host.) competes with winter wheat for nutrients, moisture, and sunlight, reducing wheat (*Triticum aestivum* L.) yields if not controlled (Fleming et al., 1998).The most effective method to manage a winter wheat field infested with jointed goatgrass is to use imazamox (IMI) resistant wheat coupled with the herbicide imazamox (Seefeldt et al., 1998), which defines the IMI-resistant wheat production system. This system is a tactic that has been widely adopted by growers throughout the Pacific Northwest and Great Plains of the United States (US)to control jointed goatgrass and some other annual winter weeds, i.e., downy brome (*Bromus tectorum* L.).

The disease strawbreaker foot rot, caused by the pathogen *Oculimacula* spp., is one of the most important stem base-diseases of cereals in temperate countries (Ray et al., 2006). Among the cultivated cereals, wheat is the most susceptible, especially in the Pacific Northwest, where foot rot occurrence is a management concern in north Idaho, north central Oregon, and some parts of Washington. Foot rot management can include several tactics. In addition, some fungicides are no longer effective due to selection of resistant *Oculimacula yallundae* and *Oculimacula acuformis* pathotyopes, so foot rot control in wheat is now focused on cultural practices, such as crop rotation and variety selection (Sheng, 2011).

Wheat by jointed goatgrass hybrids in field conditions were reported to produce seed (Mallory-Smith et al., 1996) andSeefeldt et al. (1998) found IMI-resistant hybrids in research plots, raising the concern about the negative impacts herbicide-resistance gene

flow and its introgression could have in this production system. In addition, concerns of loss of species diversity due to increased fitness of IMI-resistant hybrids were raised.Several crops in the United States have sexually compatible wild relatives, including oats, sorghum, sugarcane, sunflower and wheat, and hybridization and gene flow from these cultivated crops to their wild relatives is known to occur (Gealy et al., 2007).

Gene flow among individuals of a species is important to promote genetic diversity (Slatkin, 1973; Endler, 1973). Gene flow is an inherent process; however, how much gene flow occurs within and among populations of organisms depends on several factors. Gene flow can take place via seed or pollen dispersal. Pollen mediated gene flow provides an opportunity for crop genes to be introgressed into wild relatives (Hancock et al., 1996), and depends on many factors including inter- and intrageneric fertility, pollen biology (production, viability, dispersal and longevity), size of the pollen donorand recipient populations,flowering duration and synchrony or isolation distances of certified seed production fields (Willenborg, 2009). Seed mediated gene flow is independent of pollination characteristics and is dependent on factors including germination and establishment characteristics of seeds, seed shattering, seed contamination levels in certified and farm-saved seed lots, seed dormancy, planting, harvest and post-harvest operations (Mallory-Smith and Zapiola, 2008).

The incorporation of DNA from one species into the gene pool of a different species is defined as introgression. It has been an important genetic process in the in the evolution of several plant species (Andersonand Hubricht, 1938; Allard, 1999). Gene introgression from crops into their wild relativesmay increase the wild species adaptation

to agricultural environments and allow them to compete with the crops or displace other species in native habitats (Ellstrand, 2003).

Hybridization, gene flow and introgression were reported in several species, including *Brassica* spp., wheat and *Agrostis stolonifera*L. (Watrud et al., 2004; Kwit et al., 2011;Zapiola and Mallory-Smith, 2012). In the case of wheat, introgression into the wild tetraploid relative *Aegilops peregrina* (syn. *Aegilops variabilis*) and the stabilization of a DNA sequence in wild populations occurred, despite not having homologous chromosomes (Weissmann et al., 2005). This result raises questions about the fate of the introgressed DNA sequence within the wild species population. In the IMI-resistant or foot rot resistant wheat cultivars, the fate of the introgressed herbicide-resistance allele within a jointed goatgrass population under herbicide selection pressure is unknown.

Therefore, this study was conducted to determine the proportion of the introgressed herbicide- and disease-resistance alleles within a jointed goatgrass population with and without herbicide and disease selection pressures and the impact of selection pressure on gene flow and yield components.

MATERIALS AND METHODS

Production of Jointed Goatgrass Lines Resistant to Imazamox and Strawbreaker Foot Rot

A foot rot resistant jointed goatgrass line was developed by Perez-Jones et al. (2006a) using Wanget al. (2001) lines. Wang et al. (2001) developed a backcross progeny through manual crossing in the greenhouse of the wheat cultivar 'Madsen' (foot rot resistant) as the female parent and a jointed goatgrass accession (ID). The resulting F_1 hybrids were backcrossed twice using the same jointed goatgrass accessionas the male recurrent parent to restore self-fertility. Asecond backcross-secondself (BC_2S_2) that was foot rot resistant was developed by Perez-Jones et al. (2006a).

An imazamox-resistant $BC_2S_3$was developed by Perez-Jones et al. (2006b) through controlledcrosses in the greenhouse, where imazamox-resistant winter wheat (cv. FS-4) was used as thefemale parent and manually crossed with a jointed goatgrass collection from Idaho. The resulting F_1hybridswere backcrossed twice using the same jointed goatgrass collection as the male recurrent parent to restoreself-fertility. The second backcross generation (BC_2) was treated with the herbicide imazamox (0.044 kg a.i. ha^{-1}) to select resistant plants, whose spikes wereisolated and allowed to self-pollinate. This process was repeated to produce BC_2S_2 plants. After application of imazamox rate in this progeny, the resistant plants were selfed to produce an imazamox-resistant BC_2S_3 generation.

A jointed goatgrass line that was foot rot- and imazamox-resistant was developed at the Univeristy of Idaho, Moscow, ID, via the approach cross method (Kroiss, 2002).

The imazamox-resistant line was used as the female parent and the foot rot-resistant line was used as the male parent. A jointed goatgrass head that was to be usedfor crossing was emasculated just before it completely emerged from the flag leaf.To completely expose the head, the flag leaf was pulled back. By using a forceps, the middle floretwas plucked from each flower, leaving the two outside florets. Theflowers were trimmed with scissors, making the anthers more accessible. The head was covered with a glycine bag after theanthers had been removed with forceps, and the bag attached to a stake. Two days after emasculation, a 5-ml water vial was attached to the stake just below the head. A spikewith exposed anthers was cut, andpositioned in the vial so that it was directly overthe male jointed goatgrassline head. The heads were bagged together in 8 cm of 45-mm dialysis tubing (VWR Scientific), to prevent foreign pollen contamination. The dialysis tubing allowed gas exchange and increased visibility. Bags were made by wetting the portion of the dialysis tubingto be sealed, pressing that portion of the tubing together. Heads of the foot rot-resistant line were replaced as their anthers whitened, for as long as the imazamox-resistant line stigmas appearedreceptive.

The progeny of this cross (F_1) was germinated in the greenhouse. The plants were allowed to self-pollinate to produce a F_2 generation. To ensure self-pollination, the heads were covered, priorto anthesis, with dialysis tubing bags. Seeds were germinated in the greenhouse to produce a F_3 generation. The progeny seeds (F_4) of the F_3 plants served as the germplasm for the field experiments of 2010 and 2011.

Field Experiment Design and Treatments

Spikelets of jointed goatgrass were sown in 267-mL plastic pots containing

commercial potting mix(Sunshine Mix #1, Sun Gro Horticulture Inc,Bellevue, WA, USA). Six thousand seeds were sown, of which, approximately, 4,300 susceptible and 1,700imazamox- and foot rot-resistant. Plants weregrown in the greenhouse under 25/20ºC day/night temperature and a 16-h photoperiod, with daily irrigation.

In September, the field experimental area was treated with glyphosate (0.91 kg a.i. ha^{-1}) to eliminate emerged vegetation. Winter barley (*Hordeum vulgaris*L.) was drilled in 18-cm rows at a rate of 56 kg ha^{-1}in the borders of 3 m by 3 m field plots, on October 13 and 16 of 2010 and 2011, respectively, so that the plots were surrounded on all sides with 2 meters of winter barley to reducecross-pollination among plants in different plots (Figure 2). The greenhouse grown plants were set in the field, to allow the seedlings to acclimatize for 2 days before transplanting. On October 15 and 18 of 2010 and 2011, respectively, the plots were planted with the jointed goatgrass seedlings, at the 2 to 3-leaf growth stage. The plots were arranged in a completely randomized design (Figure 1), with four treatments (Table 1) and four replications. The experiment was repeated.One hundred jointed goatgrass plants were transplanted into each plot, of which 90 plants were susceptible and 10 plants were herbicide- and disease-resistant (Table 1, Figure 1). These plants are referred to as *parent* plants (Figure 2).

Forty-five milligrams (45 mg) of fresh tissue was sampled from each resistant parent plant to confirm the genotype of the resistance traits by polymerase chain reaction (PCR) for imazamox resistance and by Kompetitive Allele Specific Platform (KASP) genotyping for foot rot resistance. The KASP genotyping methodology is described in page 113. For imazamox resistance, PCR assays were conducted as described in Chapter 2.

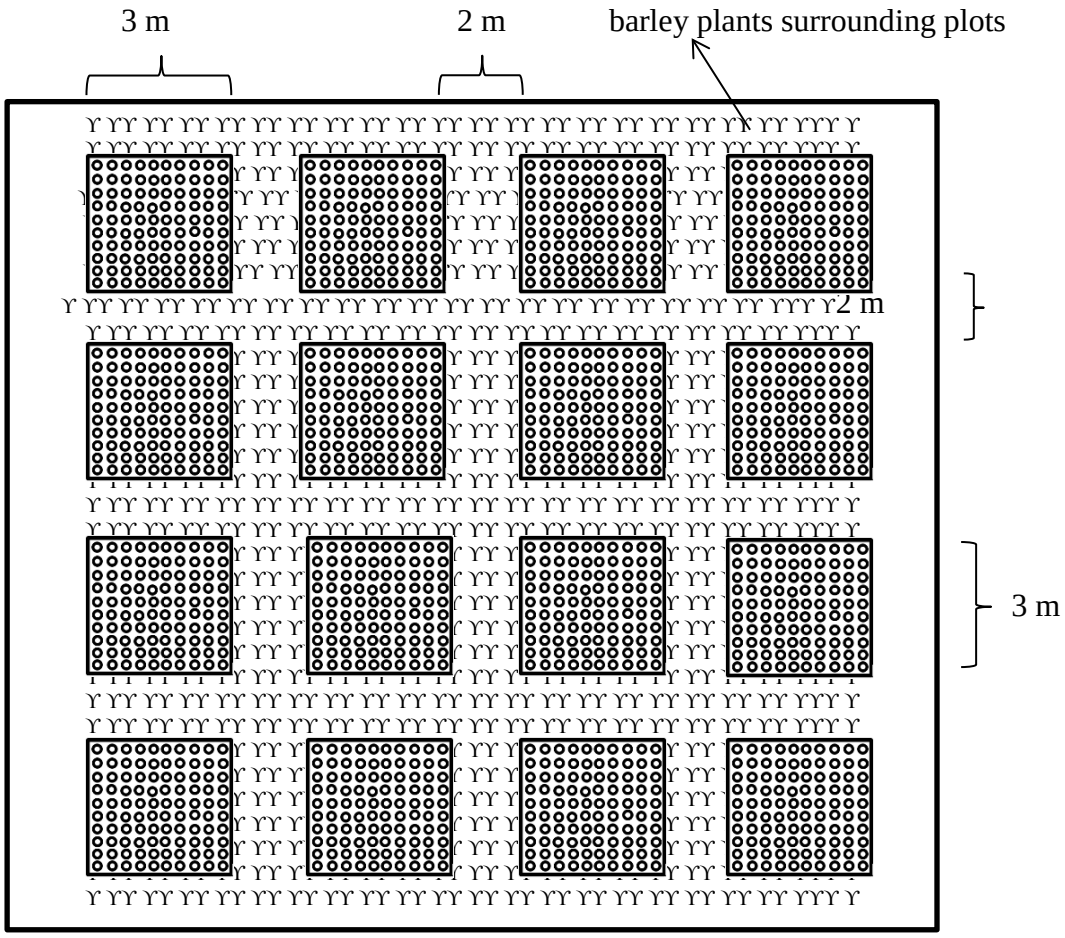

Figure 1. Layout of the field experiments under a Completely Randomized Design.

Results from the PCR and KASP genotyping indicated that not all resistant parent plants were homozygous for the traits. Thus, the number of resistant alleles was not the same for all plots.

The first experiment was referred to as ‘2010 experiment’ (year 1) with the plants grown in the field during the 2010-2011 season. The second experiment was referred to as ‘2011 experiment’ (year 2) with the plants grown during the 2011-2012 season.

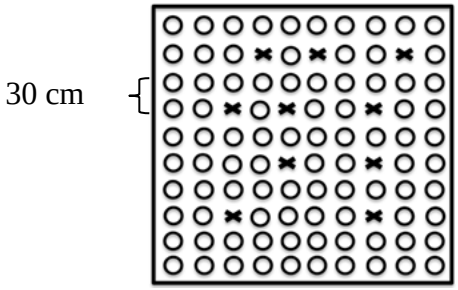

Figure 2. Example of a single experimental unit. The symbols ‘x’ and ‘o’ represent resistant and susceptible jointed goatgrass plants, respectively.

Table 1. Treatments used in the field experiments.

	Treatments
1	Non-Inoculated / No Herbicide
2	Inoculated / No Herbicide
3	Non-Inoculated / Herbicide
4	Inoculated / Herbicide

On October 22, 2010,and December 2, 2011,slug bait (metaldehyde at 1.35 kg ha^{-1}, Deadline®, 13.6 kg ha^{-1}) was applied to the plots. On December 2, 2010, and January 28, 2011, a selective herbicide (72 g a.i. ha^{-1}pyrasulfotoleand 15.4 g a.i. ha^{-1}bromoxynil; Huskie®, 0.073 L ha^{-1}), was sprayed in the plots to kill broadleaf weeds. Herbicidetreatments were applied within the plots with a CO_2-pressurized bicycle sprayer delivering the spray mix at a rate of 9.3 mL/m^2, at 2.11 kgf $cm^{-2}$with TeeJet 11002DG nozzles. From late February until June in both years, plots were then hand-weeded once or twice per month.On April 19, 2010, and April 21, 2011, the herbicide imazamox (0.044 kg a.i. ha^{-1}; Beyond®, 0.438 l ha^{-1}) was sprayed within the assigned plots. Jointed goatgrass plants had approximately 5 tillers. This phenological stage was chosen to

prevent complete control of the imazamox-susceptible plants and allow cross-pollination to take place among plants within each plot.

Oculimacula yallundae Inoculum Preparation

All steps described below were conducted in both 2010 and 2011 to inoculate the jointed goatgrass plants in the plots. To isolate the foot rot pathogen *O.yallundae* for inoculum preparation, wheat stems infected with *O.yallundae* were cut at the base. During September and October, stems were soaked in 10% bleach for approximately 5 min and dried in a laminar flow hood. *O.yallundae* mycelia from the inside of the infected stems were extracted with a dissection instrument and placed at the center of Corn Meal Agar (CMA) Petri dishes (3.4 g corn meal agar, 6 g agar and 10 mg gentimicin antibiotic/L to prevent bacterial growth). In late October, the Petri dishes were water flooded. Mycelia containing water was transferred to 15% water agar (15g agar/L water) plates, which were stored on a bench in the laboratory for about 3 weeks to promote enough colonization for inoculating oat (*Avena sativa*) kernels to increase inoculum amount.

One-liter glass jars were filled with approximately 200 g of oats and 100 ml of water, two days before inoculation with the mycelia. The oats were wet by stirring. Jars were covered with a foam plug and aluminum foil and autoclaved for 60 min. After cooling, jars were shaken to distribute the water evenly. Twenty-four hours later, the jars were autoclaved again for 60 min, and were shaken after cooling to distribute the moisture.

Each 3-week-old plate was used to inoculate 3 jars of oats. Each plate was flooded with 9 ml of sterile water and the agar surface was scraped with a sterilized glass microscope slide. Three milliliters of the suspension were pipetted into each jar. Jars were swirled to distribute the inoculum and were stored in a dark incubator, at room temperature. After inoculation, jars were shaken once every two days.

Two weeks after inoculation, the oats in the jars were infiltrated with *O.yallundae* mycelium. The oats were transferred to 0.5 mm x 0.5 mm window-screen bags that were then stapled shut and placed in an outdoor cold frame, open to the air, but sheltered from the rain. The cold frame were lined with about 5 cm of wet sand to aid in keeping the humidity high, while allowing drainage. The oats were watered with distilled water to maintain moisture.

To prepare liquid inoculum for the field application, the window screen bag was submerged in distilled water in a large cooler, which was agitated to form a spore suspension. A sample was collected of the resulting spore suspension and strained through 4 layers of cheesecloth to prevent contamination by mycelial fragments. Spores were counted with a hemacytometer and the suspension was diluted to a concentration of 2.8×10^5 spores/ml. The suspension also contained 8µl of Tween 20 as a wetting agent. The inoculation at the field plots was conducted by using a backpack sprayer to deliver 15 ml/s of the spore suspension directly at the crown of each jointed goatgrass plant in sufficient amounts to completely cover the plant, which was approximately 25 ml/plant. The jointed goatgrass plants were at the tillering stage at the time of inoculation. For the 2010 field experiment, inoculation was done on January 6, 2011. For the 2011 field

experiment, inoculation was done twice, January 12 and February 7, 2012, due to the high precipitation levels between inoculations (Figure 3).

The control of foot rot in the check plots was achieved by spraying the plot twice (on March 4 and March 25, 2011, and March 2 and March 25, 2012) with the dry flowable formulation of the triazole fungicide triadimefon applied at 284 g a.i./ha (Bayleton®50%, 568 g ha^{-1}). Stripe rust control in all plots was achieved by spraying the experimental area on April 2 and 20, 2010, and March 31 and April 19, 2011, with a broad-spectrum fungicide formulation with two active ingredients, azoxystrobin and propiconazole applied at 75 and 125 g a.i. ha^{-1}, respectively (Quilt®, 1 L ha^{-1}).

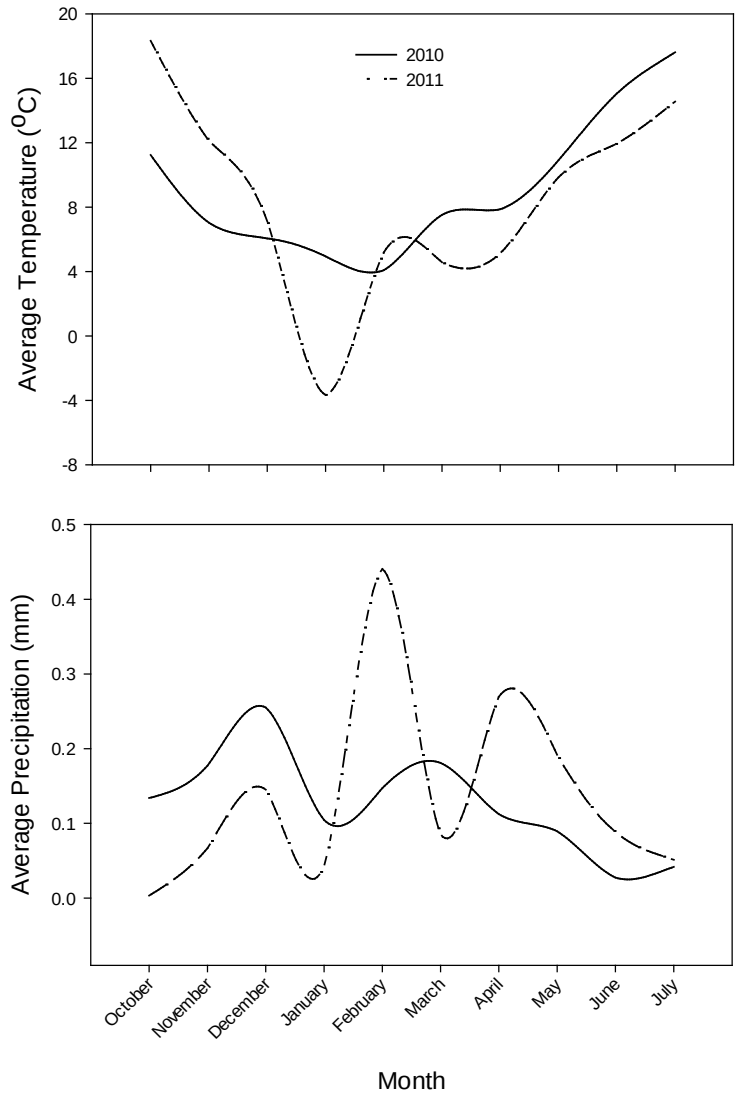

Figure 3. Average monthly air temperatures and precipitation recorded in the 2010-2011 and 2011-2012 seasons at the Corvallis East weather station, at the Botany and Plant Pathology Farm, approximately 1 km from the experiments.

Harvest

Plantswere harvested individually and spikes were placed in paper bags identified with plant number, treatment and replication. The samples were brought to the laboratory for yield component analysis and progeny seed planting in the greenhouse. Vacuuming of the plots post-harvest was conducted to recover any remnant seed that shattered pre-harvest and destroyed by autoclaving at 125°C for 2 hours.

Progeny Germination in the Greenhouse and DNA Extraction

Ten seeds from each parent plant were sown in one row in plastic trays 25 by 50 by 6 cm containing commercial potting mix in the first year. Six rows were sown per tray. Twelve seeds from each parent plant were planted per row in the second year. Plants weregrown in the greenhouse under 25/20ºC day/night temperature and a 16-h photoperiod, with daily irrigation. Fifteen days after sowing, the number of emerged seedlings per parent plant was recorded.

Fresh tissue(25-50 mg) was collected from each seedling and placedin 1 ml-microtubes marked with identification numbers. DNA was extracted from the tissue as described by Riera-Lizarazu et al. (2000).

For both years, a volume of 10 µl of DNA (~ 60 ng/µl) of each sample was transferred to 96-well PCR plates. Each plate was labeled and matched to the ID in the ‘Plate Map’ file of LGC Genomics. PCR plates were stacked with a cardboard spacer between plates to provide protection among plates. Plates were bubble wrapped and put in foam boxes surrounded by peanut pins inside a cardboard box. The boxes were shipped overnight to LCG Genomics laboratory in Boston, Massachussets (Suite 420H,

100 Cummings Center Beverly, MA 01915), where Kompetitive Allele Specific Platform (KASP) genotyping was conducted.

Kompetitive Allele Specific (KASP) Genotyping Platform

KASP is a high-throughput technology that relies on allele-oligo extension and fluorescence resonance energy transfer (FRET) for signal generation (Kumpatla et al., 2012). To identify the herbicide and disease resistance SNPs, the LGC Genomics laboratory (http://www.lgcgenomics.com/) designed assays for the 2 SNPs and validated the efficacy of the assays by using known susceptible and resistant jointed goatgrass control samples for the two traits.

There were two reagent components to the KASP system: the KASP primer mix, and the KASP master mix. The KASP primer mix contained a set of three allele-specific primers for the SNP locus assayed (two allele-specific forward primers and one common reverse primer) (Tables 2 and 3). The KASP master mix contained the universal assay components, including a Taq DNA polymerase, free nucleotides (dNTP's), and two proprietary FRET (Fluorescence Resonance Energy Transfer) cassettes that were part of the detection scheme. A total of 8,297 DNA samples were evaluated for both herbicide and disease resistance, totaling 16,594 PCR reactions. KASP used a set of thermal cycling conditions comprised of two temperature steps, rather than the traditional three step process. The KASP thermal cycling protocol was as follows: 94°C for 15 minutes (for Hot-start activation), 94°C for 20 s for 10 cycles, 65-57°C for 60 s (dropping 0.8°C per cycle), 94°C for 20 s and 57°C for 60 s for 26 cycles. The assay set-up and analysis

were carried out in 384 well PCR plates. Samples from only three replications were genotyped due to the high number of samples.

Table 2. Allele-specific forward primers labeled with FAM and HEX and the common reverse primer to detect the SNPs for imazamox (IMI) and foot rot (FR) resistance.

Trait	Primer_AlleleFAM	Primer_AlleleHEX	Primer_Common
IMI resistance	ATGTCCTTGAAAGCACCACCGC	CATGTCCTTGAAAGCACCACCGT	CATCAGGAGCACGTGCTGCCTA
FR resistance	GGATAGTTGGGTCAAGCATAGTC	GGGATAGTTGGGTCAAGCATAGTT	CTGGGGTSCCTTTCGTCGATGTT

Table 3. Resistant and susceptible-labeled alleles, FAM/HEX-labeled primers, common primers and their respective melting temperatures and cytosine and guanine (CG) contents for the two traits, herbicide and disease resistance.

Trait	AlleleFAM	AlleleHEX	Tm_FAM (°C)	CG%_FAM	Tm_HEX (°C)	CG%_HEX	Tm_Common	CG%_Common
IMI resistance	G	A	64	54.5	65.2	50	65.9	59.1
FR resistance	DV	D	62.9	47.8	63.5	45.8	66.4	56.5

Yield Components and Number of Emerged Seedlings per Spikelet

Total spikelet weight per plant, 1,000 spikelet weight per plant, number of spikelets per spike and number of emerged seedlings per spikelets were measured. For analyzing these variables, the four replications were considered. Analysis of variance (ANOVA) was conducted to determine if selection pressure treatments had any effect on the yield components. After checking the data for homogeneity of variance, the procedure PROC GML in SAS was used (SAS v.9.3; SAS Institute; Cary, NC).

Resistant Allele frequency in the Progeny under Herbicide and Disease Selection Pressure Treatments

There were homozygous and heterozygous parent plants for the herbicide and disease-resistant alleles, so the initial resistant allele frequency (INRAF) varied among the field plots.The frequency of the resistance alleles in the progeny for herbicide and disease resistance was analyzed separately because the alleles are independently inherited.

Pearson’s correlation analysis was conducted to verify the correlation between INRAF and final resistant allele frequency (FRAF). Analysis of variance (ANOVA) with INRAF as response and herbicide and inoculation as predictors was conducted to verify whether or not INRAF was confounded with treatments.

For both herbicide and inoculation data, generalized linear models were fit to investigate the impact of those explanatory variables and their interactions on FRAF. There were only two alleles involved for each trait (herbicide and disease resistance); therefore, both the covariate, INRAF and the response variable (FRAF) were treated as

binomial distributed random variables (A/(A+a)).

Because FRAFwasof primary interest, a general linear model with FRAF as the response variable was used. On the linear predictor level, the covariate INRAF was transformed to the logit scale: log(odds $_{\text{INRAF}}$), where:

$$Odds_{INRAF} = \frac{(p_{INRAF}) \times N}{(1 - p_{INRAF}) \times N}$$

with N as the total number of alleles in the field plot level. The 'p_{INRAF}' was considered as the probability of an allele being dominant ('A') and 1-p_{INRAF} the probability of an allele being recessive ('a'). The log(odds $_{\text{INRAF}}$) was in the appropriate scale because when p_{INRAF} varies from 0 to 1, the possible range of the log(odds $_{\text{INRAF}}$) can vary from minus infinity to plus infinity (- ∞, + ∞). INRAF was used as a covariate in the generalized linear model.

Binomial logistic regression models with the logit link function were fit to the models including the independent exploratory variables and all the possible interactions to test whether there was extra-binomial variation (over-dispersion). The chi-square deviance goodness-of-fit test was used to check whether the theoretical variance in binomial distribution was sufficient to model the variation in the data.

Analysis of deviance was based on a chi-squared test because there was no over-dispersion in the data for herbicide selection pressure in 2010 or 2011 and disease selection pressure in 2010. This analysis tested the significance of the model terms and interactions among them.For model checking, PROC GENMOD procedure in SAS was

used (SAS v.9.3; SAS Institute; Cary, NC). The dependent response variable was the proportion of the resistant allele to the total alleles within the progeny. The model used the log(odds $_{INRAF}$), the herbicide and inoculation terms and their interactions as independent variables.

For the disease selection pressure in 2011, over-dispersion was detected; therefore, the quasi-likelihood approach was considered to accommodate the over-dispersion. In this approach, the overdispersion parameter is set to one, and the variance-mean relationship of the distribution is recovered. By adopting the quasi-likelihood approach, a quasi-likelihood model was fitted using the GENMOD procedure in SAS.

Gene Flow from Resistant to Susceptible Plants with Herbicide and Disease Selection Pressure Treatments

The response variable for gene flow from was the resistance allele frequency within the offspring originating from the susceptible parent plants. To analyze the effect of selection pressure on the response variable, the NPAR1WAY procedure in SAS was used to perform nonparametric tests and compare treatments effects. Standard parametric procedures (two-sample t test and one-way ANOVA) were not used because the normal distribution assumption was not achieved with the study sample size. The PROC NPAR1WAY provides a standard analysis of variance on the raw data and tests based on the empirical distribution function of the raw data (SAS Institute Inc., 2008). This procedure also performs tests for differences based on several scores of a response variable including Wilcoxon. The Wilcoxon rank sum test was chosen to compare levels of each selection pressure factor. The Wilcoxon rank sum test is the analog of the two-

sample t test in nonparametric frameworks. Exact test results were used because the study sample size was small.

RESULTS

Resistant Allele Proportion in the Progeny under Herbicide and Disease Selection Pressure Treatments

Pearson's correlation analysis between INRAF and FRAF revealed that those variables were positively correlated (Pearson's correlation coefficient r_p=0.724, $p<0.05$). Based on one-way analysis of variance (one-way ANOVA), the initial resistant allele frequencies for each trait were evenly distributed among treatments, so INRAF was not a confounding variable (Tables 4 and 5). Consequently, differences in FRAF among treatments were due to treatment effects and not to INRAF.

Table 4. Analysis of variance for the effects of treatments on initial herbicide-resistance allele frequency in herbicide-resistant parent jointed goatgrass plants among field plots from 2010 and 2011.

Type 3 Tests of Fixed Effects					
Year	Effect	Num DF	Den DF	F Value	Pr > F
2010	Treatments	3	8	1.84	0.2183
2011	Treatments	3	8	0.06	0.9783

Table 5. Analysis of variance for the effects of treatmentson initial disease-resistance allele frequency in disease-resistant parent jointed goatgrass plants among field plots from 2010 and 2011.

Type 3 Tests of Fixed Effects					
Year	Effect	Num DF	Den DF	F Value	Pr > F
2010	Treatments	3	8	0.89	0.4869
2011	Treatments	3	8	0.46	0.7175

Selection Pressure Effects on the Herbicide Resistance Allele Proportion in the Progeny

After submitting INRAF to the logit transformation (log($odds_{INRAF}$))analysis indicated no interactions among the explanatory variables year, log($odds_{INRAF}$), herbicide and inoculation. In addition, variances between the two years were homogeneous for the herbicide trait, so the data were pooled, increasing statistical power.

The p-value for the chi-square goodness-of-fit test was 0.471,indicating the binomial logistic regression model was adequate to describe the proportion of the herbicide-resistant alleles in the progeny.

Year and its interactions with herbicide, inoculation and herbicide plus inoculation were not associated with the proportion of the herbicide-resistant alleles (Table 6). Based on these results, year and its interactions were dropped from the model (Table 7). A deviance that is approximately equal to its degrees of freedom is an indication of a good model fit (SAS Institute Inc., 2008.). The binomial model was sufficient to account for the variation of the data because the deviance/df ratio was close to one, indicating that the null hypothesis could not be rejected (Table 8). Scaled Deviance and Scaled Pearson X^2 are Deviance and Pearson Chi-Square, respectively, divided by the dispersion parameter.

Their values are the same because for the binomial distribution the dispersion parameter is 1 (Table 8). The fitted values were not different from the observed values; therefore, the binomial model was chosen.

Table 6. Logistic regression statistics for the effects of year, herbicide, disease and their interactions on the proportion of herbicide-resistant allele in the progeny in herbicide-resistant parent jointed goatgrass plants.

LR Statistics for Type 3 Analysis			
Source	DF	Chi-Square	Pr > ChiSq
$\log(\text{odds}_{\text{INRAF}})$	1	88.87	< 0.0001
Year	1	1.30	0.2549
Herbicide	1	8.41	0.0037
Year*Herbicide	1	0.00	0.9631
Inoculation	1	0.21	0.6506
Year*Inoculation	1	0.50	0.4794
Herbicide*Inoculation	1	2.71	0.0995
Year*Herbicide*Inoculation	1	0.34	0.5582

Table 7. Main effects of herbicide, disease and their interactions on the proportion of herbicide-resistant alleles in the progeny from herbicide-resistant parent jointed goatgrass plants.

LR Statistics for Type 3 Analysis			
Source	DF	Chi-Square	Pr > ChiSq
$\log(\text{odds}_{\text{INRAF}})$	1	115.98	< 0.0001
Herbicide	1	8.31	0.0040
Inoculation	1	0.09	0.7605
Herbicide*Inoculation	1	3.11	0.0776

Table 8. Chi-square goodness-of-fit test for the binomial model.

Criteria For Assessing Goodness Of Fit			
Criterion	DF	Value	Value/DF
Deviance	19	17.242	0.9075
Scaled Deviance	19	17.242	0.9075
Pearson Chi-Square	19	17.4971	0.9209
Scaled Pearson X^2	19	17.4971	0.9209

Because the response variable was assumed to be binomial distributed and the logit link function was used, the proportion of the herbicide-resistant alleles of the total alleles within the jointed goatgrass progeny could be interpreted as the following: for every generation of jointed goatgrass, the odds of an allele being herbicide-resistant without herbicide selection pressure was 0.868 (exp(-0.1414)) times the odds of an allele being herbicide-resistant with herbicide selection pressure (Table 9). For every generation of jointed goatgrass, the odds of an allele being herbicide-resistant with herbicide selection pressure is 1.16 times the odds of the same allele being herbicide-resistant without herbicide (exp(-2.8019) divided by exp(-2.9461)) (Table 9, Figure 4). This indicates that the odds of for an allele being herbicide-resistant with herbicide selection pressure are 16% the odds without herbicide. In other words, there is a 16% increase in the odds of an allele being herbicide-resistant with herbicide compared without herbicide selection pressure. Over generations with herbicide selection pressure, fixation of the herbicide-resistant allele in the jointed goatgrass population would take place and the herbicide-resistant allele would be incorporated in the gene pool of the jointed goatgrass population. The line in Figure 4 does not cross the 45-degree reference line, confirming the difference between the presence and absence of herbicide on the proportion of herbicide-resistant alleles in the progeny.

Because the model included the interaction term (Herbicide*Inoculation), the mean differences between combinations of the levels of Herbicide and Inoculation were analyzed Table 10, Figure 5). Analyses of these mean differences indicated that the odds of an allele being herbicide-resistant for every generation of jointed goatgrass with herbicide plus disease selection pressure was estimated to be 1.17 times the odds without any selection pressure present or 117% the odds without any selection pressure. Therefore, there is an increase in 17% in the odds of an allele being herbicide-resistant in the progeny with herbicide plus disease selection than without any selection pressure.

When herbicide plus disease occurred, the odds of an allele being herbicide-resistant in the progeny was 1.26 times of that when only the disease selection pressure occurred. Therefore, there is an increase in 26% in the odds of an allele being herbicide-resistant for every jointed goatgrass generation with herbicide plus disease selection pressure compared to the odds of an allele being herbicide-resistant when only disease occurs but no herbicide was applied. These results indicate that, when disease occurred, the odds of an allele being herbicide-resistant were the same whether the herbicide was applied or not, with a p-value of 0.07 (Table 10). Thus, disease did not have an effect in the response variable.

Occurrence of the disease did not affect the herbicide-resistance allele proportion in the progeny because the initial resistant allele frequency (INRAF) was computed out of the herbicide-resistant parent plants, and not the disease-resistant parent plants; not all the resistant parent plants were both herbicide plus disease resistant, so the resistant parent plants in each plot were composed of some herbicide-resistant plants, some disease-resistant plants and some herbicide- *and* disease-resistant plants. Thus, only the

herbicide-resistant or the herbicide- and disease-resistant parent plants were considered when analyzing the effect of herbicide selection pressure on the herbicide-resistant allele proportion in the progeny.

Table 9. Estimated difference in means for the proportion of the herbicide-resistance allele in the jointed goatgrass progeny between levels of each selection pressure factor, with 95% confidence limits (CL).

Differences of Herbicide Selection Pressure Least Squares Means										
Herbicide	Estimate	Standard Error	z Value	Pr > |z|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Lower Confidence Limit for Odds Ratio
No vs. Yes	-0.141	0.0491	-2.88	0.004	0.05	-0.238	-0.0452	0.868	0.788	0.956
Disease										
No vs. Yes	-0.0148	0.04910	-0.30	0.7605	0.05	-0.111	0.08127	0.985	0.895	1.085

Table 10. Least squares means differences between the levels of Herbicide and Inoculationselection pressure on the herbicide-resistance allele proportion in the progeny.

Differences of Herbicide*Inoculation Least Squares Means													
Herb.	Inoc.	Herb.	Inoc.	Estimate	Standard Error	z Value	Pr > |z|	Alpha	Lower	Upper	Odds Ratio	Lower CL for Odds Ratio	Lower CL for Odds Ratio
No	No	No	Yes	0.07292	0.07214	1.01	0.3121	0.05	-0.06848	0.2143	1.076	0.934	1.239
No	No	Yes	No	-0.0535	0.06963	-0.77	0.4422	0.05	-0.19	0.08296	0.948	0.827	1.086
No	No	Yes	Yes	-0.1564	0.07088	-2.21	0.0274	0.05	-0.2953	-0.01744	0.855	0.744	0.983
No	Yes	Yes	No	-0.1264	0.06797	-1.86	0.0629	0.05	-0.2596	0.00679	0.881	0.771	1.007
No	Yes	Yes	Yes	-0.2293	0.07021	-3.27	0.0011	0.05	-0.3669	-0.09168	0.795	0.693	0.912
Yes	No	Yes	Yes	-0.1029	0.06763	-1.52	0.1283	0.05	-0.2354	0.02969	0.902	0.79	1.03

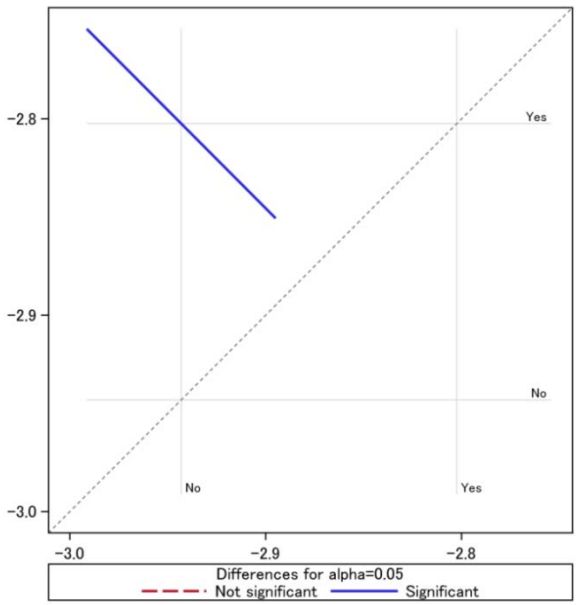

Figure 4. Least squares means of the herbicide-resistance allele proportion in the progeny for the treatments with and without herbicide selection pressure.

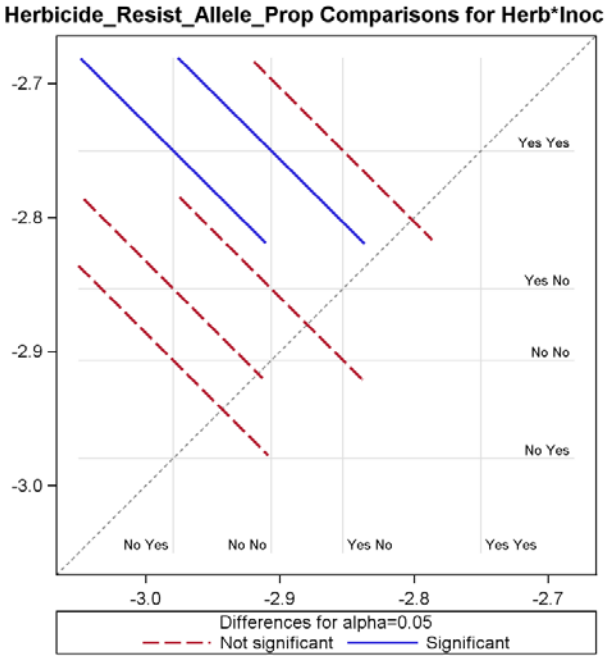

Figure 5. Least squares means difference between the levels of Herbicide and Inoculationselection pressure on the herbicide-resistance allele proportion in the progeny (‘Yes Yes’ = Herbicide ‘yes’ and Inoculation ‘yes’; ‘Yes No’ = Herbicide ‘yes’ and Inoculation ‘no’; ‘No Yes’ = Herbicide ‘no’ and Inoculation ‘yes’).

Selection Pressure Effects on the Disease-Resistance Allele Proportion in the Progeny

The initial disease-resistance allele frequency was submitted to the logit transformation as for herbicide-resistance allele frequency. For the disease resistance trait, there was a significant interaction between the variables inoculation and year (Table 11). The herbicide plus disease treatment differed between 2010 and 2011 (p=0.01), which likely made the interaction between year and inoculation significant. Thus, data from the two years were analyzed separately.

Table 11. Logistic regression statistics for the effects of year, herbicide, disease and their interactions on the proportion of disease-resistant allele in the progeny in disease-resistant parent jointed goatgrass plants, 2010.

LR Statistics for Type 3 Analysis			
Source	DF	Chi-Square	Pr > ChiSq
$\log(\text{odds}_{\text{INRAF}})$	1	260.24	< 0.0001
Year	1	0.80	0.3705
Herbicide	1	0.03	0.8606
Year*Herbicide	1	2.75	0.0972
Inoculation	1	0.27	0.6034
Year*Inoculation	1	6.58	0.0103
Herbicide*Inoculation	1	0.48	0.4891
Year*Herbicide*Inoculation	1	0.46	0.4957

2010 Field Experiment

Using the terms herbicide, inoculation and their interactions in the model, the p-value for chi-square goodness-of-fit test was 0.44, which indicated the binomial logistic regression model was adequate to fit the data.

Herbicide, inoculation and their interactions were not associated with the proportion of the disease-resistant alleles to the total alleles within the progeny (Table 12). The deviance/df ratio was close to 1, indicating that the null hypothesis could not be rejected. The fitted values were not different from the observed values. Therefore, the binomial model was chosen to fit the data for disease selection pressure in 2010 (Table 13).

Analyses of the proportion of the disease-resistant alleles of the total alleles within the progeny revealed that, for every jointed goatgrass generation with disease selection pressure, the odds of an allele being disease-resistant is (exp(-2.9781) - exp(-3.0793)), which is 0.004 times the odds of an allele being disease-resistant without disease selection pressure. This result indicates that there was no difference on the proportion of the disease-resistant allele in the progeny with or without disease selection pressure (Figure 6).

Table 12. Main effects of herbicide, disease and their interactions on the proportion of disease-resistant allele in the progeny from disease-resistant parent jointed goatgrass plants, 2010.

LR Statistics for Type 3 Analysis			
Source	DF	Chi-Square	Pr > ChiSq
$\log(\text{odds}_{\text{INRAF}})$	1	124.73	< 0.0001
Herbicide	1	1.93	0.1647
Inoculation	1	1.59	0.2070
Herbicide*Inoculation	1	0.00	0.9642

Table 13. Chi-square goodness-of-fit test for the binomial model, 2010.

Criteria For Assessing Goodness Of Fit			
Criterion	DF	Value	Value/DF
Deviance	7	4.9235	0.7034
Scaled Deviance	7	4.9235	0.7034
Pearson Chi-Square	7	4.9626	0.7089
Scaled Pearson X^2	7	4.9626	0.7089

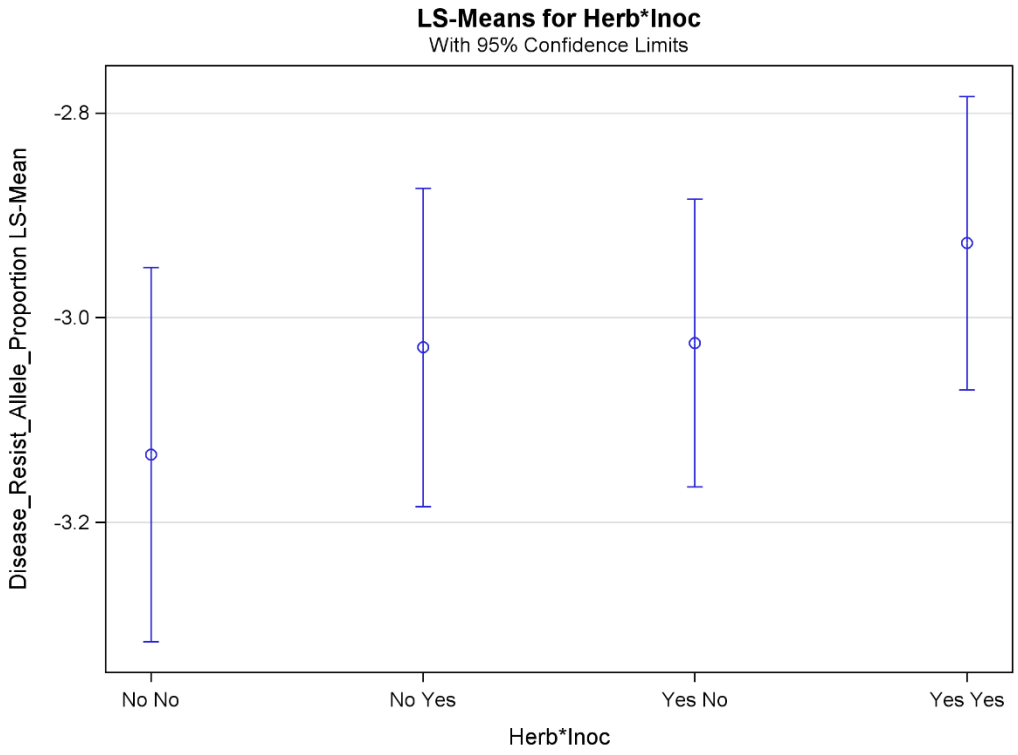

Figure 6. Least squares means between the levels of Herbicide and Inoculationselection pressure on the disease-resistance allele proportion in the progeny, 2010.

2011 Field Experiment

Data were fitted to a generalized linear model (logistic regression) with extra-binomial variation using the quasi-likelihood approach. The model accounted for herbicide, disease and their interaction and allowed over-dispersion (scale parameter = 2.0974), indicating that the response variable proportion of disease-resistant alleles to the total alleles in the progeny could be described adequately by the quasi-binomial model (Table 15). The proportion of disease-resistance alleles of the total alleles in the progeny increased with disease selection pressure (p = 0.0383) (Table 14).

Table 14. Main effects of herbicide, disease and their interactions on the proportion of disease-resistant allele in the progeny from disease-resistant parent jointed goatgrass plants, 2011.

LR Statistics for Type 3 Analysis				
Source	Num DF	Den DF	F Value	Pr > F
$\log(\text{odds}_{\text{INRAF}})$	1	7	13.19	0.0084
Herbicide	1	7	0.02	0.8827
Inoculation	1	7	6.48	0.0383
Herbicide*Inoculation	1	7	3.92	0.0883

Analyses of the proportion of the disease-resistant alleles of the total alleles within the progeny revealed that, for every jointed goatgrass generation, the odds of an allele being disease-resistant with disease selection pressure is (exp(-2.4477)/exp(-2.7807), which is 1.4 times the odds of an allele being disease-resistant without disease. This result indicates that, with disease selection pressure, for every jointed goatgrass

generation, there is a 40% increase in the odds of an allele being disease-resistant (Table 16, Figure 7). The line in Figure 7 does not cross the 45-degree reference line, confirming the difference between the presence and absence of disease on the proportion of disease-resistant alleles in the progeny.

Table 15. Chi-square goodness-of-fit test for the binomial model, 2011.

Criteria For Assessing Goodness Of Fit			
Criterion	DF	Value	Value/DF
Deviance	7	30.7935	4.3991
Scaled Deviance	7	7.0000	1.0000
Pearson Chi-Square	7	31.8772	4.5539
Scaled Pearson X^2	7	7.2464	1.0352

Because the model included the interaction term (Herbicide*Inoculation), the mean differences between combinations of the levels of herbicide and inoculation were analyzed (Table 17). The odds of an allele being disease-resistant in the progeny for every generation with disease and no herbicide were 1.83 times the odds in the control treatment. This result indicates that disease selection pressure alone promoted an increase of 83% in the odds of an allele being disease-resistant with disease occurrence but no herbicide selection pressure (p=0.0026) (Figure 8). Otherleast squares means differences between treatment combinations are shown in Table 17 and Figure 6.

Table 16. Estimated difference in means for the proportion of the disease-resistant allele in the jointed goatgrass progeny between levels of each selection pressure factor, with 95% confidence limits (CL), 2011.

Differences of Herbicide Selection Pressure Least Squares Means										
Herbicide	Estimate	Standard Error	z Value	Pr > |z|	Alpha	Lower	Upper	Odds Ratio	Lower Confidence Limit for Odds Ratio	Lower Confidence Limit for Odds Ratio
No vs. Yes	-0.0206	0.1351	-0.15	0.8784	0.05	-0.285	0.2441	0.980	0.752	1.277
Disease										
No vs. Yes	-0.3330	0.1317	-2.53	0.0115	0.05	-0.591	-0.07478	0.717	0.554	0.928

Table 17. Least squares means differences between the levels of Herbicide and Inoculationselection pressure on the disease-resistance allele proportion in the progeny, 2011.

Differences of Herbicide*Inoculation Least Squares Means													
Herb.	Inoc.	Herb.	Inoc.	Estimate	Standard Error	z Value	Pr > |z|	Alpha	Lower	Upper	Odds Ratio	Lower CL for Odds Ratio	Lower CL for Odds Ratio
No	No	No	Yes	-0.6053	0.2012	-3.01	0.0026	0.05	-0.9997	-0.2110	0.546	0.368	0.810
No	No	Yes	No	-0.2930	0.2147	-1.36	0.1724	0.05	-0.7139	0.1279	0.746	0.490	1.136
No	No	Yes	Yes	-0.3537	0.2047	-1.73	0.0840	0.05	-0.7549	0.04755	0.702	0.470	1.049
No	Yes	Yes	No	0.3123	0.1712	1.82	0.0681	0.05	-0.02326	0.6479	1.367	0.977	1.912
No	Yes	Yes	Yes	0.2517	0.1690	1.49	0.1364	0.05	-0.07957	0.5829	1.286	0.924	1.791
Yes	No	Yes	Yes	-0.0606	0.1800	-0.34	0.7361	0.05	-0.4134	0.2921	0.941	0.661	1.339

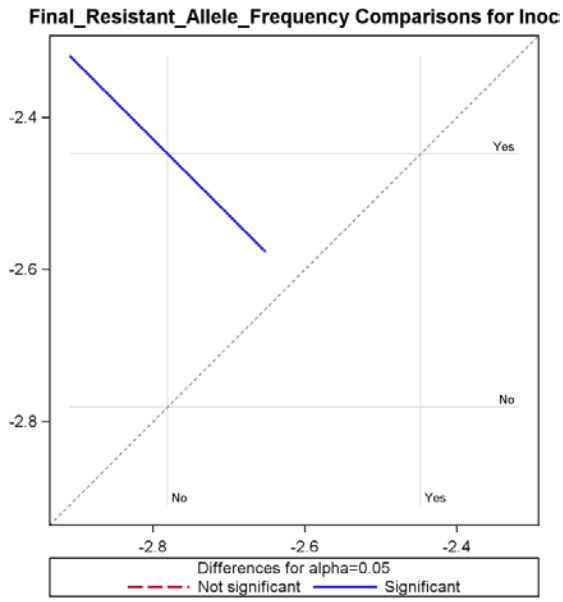

Figure 7. Least squares mean difference between the treatments with and without diseaseselection pressure, 2011.

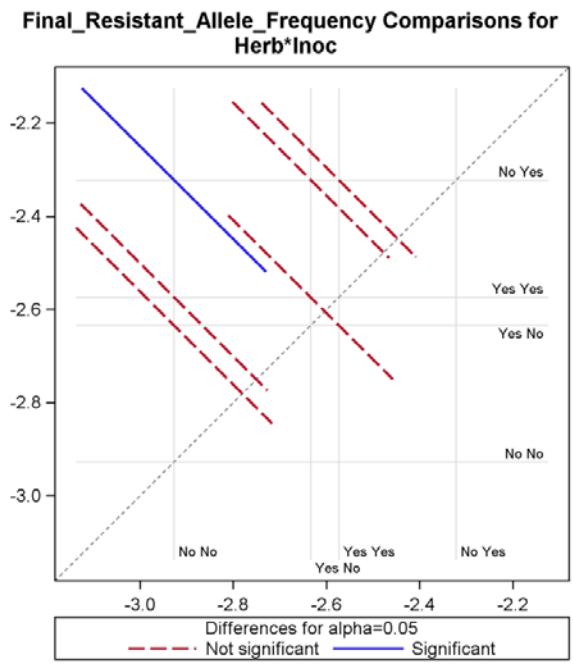

Figure 8. Least squares means difference between the levels of Herbicide and Inoculation selection pressure on the disease-resistance allele proportion in the progeny (‘Yes Yes’ = Herbicide ‘yes’ and Inoculation ‘yes’; ‘Yes No’ = Herbicide ‘yes’ and Inoculation ‘no’).

Gene Flow from Resistant to Susceptible Parents with Selection Pressure

Gene flow from resistant to susceptibleplantswithherbicide selection pressure

In 2010, the Wilcoxon test score plus the two-sided p-value (0.0152) indicated that the gene flow was greater with herbicide selection pressure (Tables 18 and 19). The herbicide-resistant plants were visually more vigorous than the susceptible ones and were taller, greener and had more tillers and spikes (based on visual observations). The greater height of the herbicide-resistant plants increased the probability of pollination of a susceptible plant, especially the ones nearest the herbicide-resistant plants. In fact, all the heterozygous progeny originated from the susceptible plants surrounded by herbicide-resistant plants.

In 2011, the Wilcoxon test score plus the one-sided p-value (0.252) indicated that the gene flow did not differ among the selection pressure treatments (Tables 18 and 19). There were fewer heterozygote plants in the progeny originating from susceptible parent plants in 2011 than in 2010.

Table 18. Wilcoxon scores for herbicide-resistance allele frequency in progeny from herbicide-susceptible parent plants classified by the herbicide selection pressure.

Herbicide	Year	N	Sum of Scores	Expected under H0	Std Dev under H0	Mean Score
Yes	2010	6	54.00	39.0	5.600325	9.00
No		6	24.00	39.0	5.600325	4.00
Yes	2011	6	34.50	39.0	6.223124	5.75
No		6	43.50	39.0	6.223124	7.25

Table 19. Wilcoxon two-sample test between presence and absence of herbicide for 2010 and 2011 experiments.

Year	2010	2011
Statistic (S)	54.00	34.50
Exact Test		
One-Sided Pr >= S	0.0125	0.252
Two-Sided Pr >= |S - Mean|	0.0249	0.504

Gene flow from resistant to susceptible plants with disease selection pressure

In 2010, the Wilcoxon rank sum test score for treatments without disease was greater than the expected score under the null hypothesis of no difference between treatments (Table 20). One-sided and two-sided p-values were 0.2273 and 0.4545, respectively, indicating no difference in the gene flow with or without the pressure of disease (Table 21).

In 2011, the Wilcoxon rank sum test score with disease was greater than the expected score under the null hypothesis of no difference between treatments with and without disease; however, the two-sided p-value was 0.1667, indicating that the gene flow did not differ with and without disease selection pressure (Tables 20 and 21).

Although foot rot symptoms were more evident in 2011 than in 2010, gene flow did not differ among the treatments for either year.

Table 20. Wilcoxon scores for disease-resistance allele frequency in progeny from disease-susceptible parent plants classified by the disease selection pressure.

Herbicide	Year	N	Sum of Scores	Expected under H0	Std Dev under H0	Mean Score
Yes	2010	6	45.00	39.0	4.062019	7.50
No		6	33.00	39.0	4.062019	5.50
Yes	2011	6	30.00	39.0	6.190168	5.00
No		6	48.00	39.0	6.190168	8.00

Table 21. Wilcoxon two-sample test between presence and absence of disease for 2010 and 2011 experiments.

Year	2010	2011
Statistic (S)	45.00	45.00
Exact Test		
One-Sided Pr >= S	0.2273	0.0833
Two-Sided Pr >= |S - Mean|	0.4545	0.1667

Selection Pressure Effect on Yield Components of Parent Plants in the Field

Total spikelet weight

In 2010, for the resistant parent plants, total spikelet weight did not differ across treatments. For the susceptible parent plants, herbicide reduced total spikelet weight per plant regardless of presence or absence of disease (Tables 22 and 23). In 2011, total spikelet weight did not differ across treatments for the resistant parent plants. For the susceptible parent plants, both herbicide and disease decreased total spikelet weight per plant compared with the control (Table 23). With no herbicide, disease decreased total

spikelet weight, while with herbicide, total spikelet weight did not differ between the presence and absence of disease (Tables 22 and 23).

Table 22. Selection pressure treatments effect on total spikelet weight (g) per resistant parent plant and 95% confidence intervals (CI) for 2010 and 2011.

Treatment	2010	95% CI	2011	95% CI
Control	20.382 a*	(13.183 27.581)	25.202 a*	(18.53431.869)
NoHerb/Inoc	21.165 a	(13.356 28.973)	25.888 a	(20.68331.093)
Herb/Inoc	25.286 a	(18.261 32.312)	30.244 a	(26.12934.358)
Herb/NoInoc	27.225 a	(20.653 33.796)	29.338 a	(25.22333.452)

*Means in columns, followed by the same letter are not significantly different according to Fisher's protected LSD values (p = 0.05)

Table 23. Selection pressure treatments effect on total spikelet weight per susceptible parent plant and 95% confidence intervals (CI) for 2010 and 2011.

Treatment	2010	95% CI	2011	95% CI
Control	13.009 a*	(12.414 13.604)	15.874 a*	(15.45316.296)
NoHerb/Inoc	10.773 b	(10.184 11.361)	13.847 b	(13.43014.264)
Herb/NoInoc	6.311 c	(5.715 6.907)	12.903 c	(12.48013.326)
Herb/Inoc	5.678 c	(5.084 6.272)	13.240 c	(12.82013.661)

*Means in columns, followed by the same letter are notsignificantly different according to Fisher'sprotected LSD values (p = 0.05)

One thousand spikelet weight

In 2010, for the resistant parent plants, 1,000 spikelet weight did not differ across treatments. For the susceptible parent plants, herbicide, and herbicide plus disease reduced 1,000 spikelet weight per plant. For disease alone, 1,000 spikelet weight was not reduced (Tables 24 and 25).

In 2011, the 1,000 spikelet weight did not differ across treatments for the resistant plants. For the susceptible plants, herbicide, disease, and herbicide plus disease reduced 1,000 spikelet weight per plant (Tables 24 and 25).

Table 24. Selection pressure treatments effect on 1,000 spikelet weight per resistant parent plant and 95% confidence intervals (CI) for 2010 and 2011.

Treatment	2010	95% CI	2011	95% CI
Control	28.217 a*	(24.946 31.488)	32.625 a*	(30.967 34.283)
NoHerb/Inoc	33.400 a	(30.826 35.974)	32.647 a	(31.037 34.257)
Herb/Inoc	30.044 a	(27.224 32.863)	31.477 a	(29.819 33.135)
Herb/NoInoc	29.697 a	(27.338 32.056)	30.951 a	(29.197 32.705)

*Means in columns, followed by the same letter are not significantly different according to Fisher's protected LSD values (p = 0.05)

Table 25. Selection pressure treatments effect on 1,000 spikelet weight per resistant parent plant and 95% confidence intervals (CI) for 2010 and 2011.

Treatment	2010	95% CI	2011	95% CI
Control	21.384 a*	(21.039 21.729)	16.515 a*	(16.262 16.769)
NoHerb/Inoc	19.919b	(19.567 20.272)	15.637 b	(15.382 15.829)
Herb/NoInoc	14.031c	(13.673 14.389)	12.187 c	(11.930 12.443)
Herb/Inoc	13.839c	(13.498 14.180)	11.485 d	(11.245 11.726)

*Means in columns, followed by the same letter are not significantly different according to Fisher's protected LSD values (p = 0.05)

Number of emerged seedlings per spikelet

For the resistant parent plants, the number of emerged seedlings did not differ across treatments in either year (Table 26).

In 2010, for the susceptible plants, herbicide plus disease reduced the number of emerged seedlings per spike. Number of emerged seedlings per spikelet did not differ between the control and the treatments with only one selection pressure.

In 2011, for the susceptible plants, herbicide, disease, and herbicide plus disease reduced the number of emerged seedlings per spikelet compared to the control (Table 27). Without herbicide selection pressure, susceptible plants produced more emerged seedlings per spikelet compared to treatments with herbicide, regardless of presence or absence of disease. However without herbicide, disease reduced the number of emerged seedlings per spikelet when compared to the control.

Table 26. Selection pressure treatments effect on number of emerged seedlings per spikelet per resistant parent plant and 95% confidence intervals (CI) for 2010 and 2011.

Treatment	2010	95% CI		2011	95% CI	
Control	11.071 a*	(8.787	13.355)	13.775 a	(12.250	15.299)
NoHerb/Inoc	11.578 a	(9.618	13.539)	12.000 a	(10.475	13.524)
Herb/Inoc	10.769a	(8.399	13.139)	12.200 a	(10.675	13.724)
Herb/NoInoc	10.470 a	(8.397	12.543)	12.750 a	(11.225	14.274)

*Means in columns, followed by the same letter are not significantly different according to Fisher's protected LSD values (p = 0.05)

Table 27. Selection pressure treatments effect on number of emerged seedlings per spikelet persusceptible parent plant and 95% confidence intervals (CI) for 2010 and 2011.

Treatment	2010	95% CI		2011	95% CI	
Control	9.933 a*	(8.787	13.355)	12.038 a*	(11.825	12.191)
NoHerb/Inoc	10.039 a	(9.618	13.539)	10.177 b	(9.5211	11.123)

Herb/NoInoc	9.684	b	(8.397	12.543)	7.244 c	(6.2110	8.2450)
Herb/Inoc	8.6154	b	(8.399	13.139)	9.497 c	(7.681110.123)	

*Means in columns, followed by the same letter are not significantly different according to Fisher's protected LSD values (p = 0.05)

In the 2010 experiment, foot rot symptoms were observed in the susceptible parental jointed goatgrass plants. Susceptible parental plants produced seed, and the inoculation did not affect the susceptible parent plants as much as the herbicide did on a visual basis, although all susceptible parent plants survived in the plots where herbicide was sprayed.

In 2011, inoculation promoted more noticeable symptoms, causing more lesions on the stem base or basal leaf sheaths of the susceptible plants than in 2010. In addition, jointed goatgrass plants lodged more later in the season in 2011, but survived. There was an increase in the proportion of the disease-resistance allele in the progeny under the disease selection pressure in 2011, whereas in 2010, the disease occurrence did not lead to an increase of the disease-resistance allele in the progeny, but did reduce yield components.

DISCUSSION

Resistance Allele Proportion in the Progeny under Herbicide and Disease Selection Pressure Treatments

Results of this research support the initial hypothesis that selection pressure would increase resistance alleles in the jointed goatgrass progeny. With herbicide selection pressure, there was an increase of 16% in the odds of the herbicide-resistance allele occurrence compared to no herbicide selection pressure. With disease selection pressure, there was an increase of 40% in the odds of the disease-resistance allele occurrence compared to no disease selection pressure in 2011.

Experiments conducted in Colorado from 2007 to 2009 determined hybrid backcrossing rates from 0.03 to 0.6%, when jointed goatgrass was the only pollen source (Econopouly et al., 2011). In a commercial winter wheat field, where wheat and jointed goatgrass were the pollen sources, Perez-Jones et al. (2010) determined that 14.3% of the first backcross plants analyzed had jointed goatgrass as the male backcross parent. As the premise for resistance allele introgression into jointed goatgrass is a hybrid backcrossing to jointed goatgrass and not to wheat, these results indicate the real possibility of resistance allele introgression.

Many studies have investigatedwhether there was an associated decrease in plant fitness without herbicide selection pressure (Jasieniuk et al., 1996), because in the absence of herbicide selection pressure, a fitness cost associated with herbicide resistance will decrease the likelihoodor rate of weed populations developing resistance (Tranel and Wright, 2002). For example, Christoffoleti et al. (1997) studied a kochia (*Kochia*

scoparia L.) population that had resistance-conferring mutations in the acetolactate synthase (ALS) enzyme and did not find significantdifferences in biomass production, number of seeds produced, or competitiveness.Mutations in the ALS enzyme conferring herbicide resistance in weed species has, in many cases, negligible fitness costs of theresistance gene in the absence of herbicide selection (Tranel and Wright, 2002). In addition, there is no published information whether there is fitness cost associated with foot rot resistance in wheat without the disease selection pressure.

There was no fitness cost from the IMI- and foot rot-resistance traits, with respect to the yield components in this study. It is likely that by using imazamox and with foot rot occurrence, the jointed goatgrass gene pool that is initially mixed, will be increasingly comprised of imazamox- and foot rot-resistance genes, until the resistance alleles reach fixation. Resistance management strategies only delay this fixation (Comins, 1977). Resistance to xenobiotics is a local adaptation process, while a migration-selection pressure balance is established between areas differing in the fitness cost associated with the different alleles. Selection-migration equilibrium is not reached if there is no fitness cost associated with the resistant alleles.Thus, if the resistance trait promotes selective advantage, an increase in frequency of resistance genes is likely to occur (Lenormand and Raymond, 1998), which would be the case for IMI resistance.

The greater seed production by the resistant individuals will favor the spread of the resistant alleles to further distances via seed movement. Thus, resistance management strategies to reduce resistance allele proportions over generations and resistance gene flow are important to delay this process.

Selection Pressure Effect on Yield Components of Jointed Goatgrass Parent Plants

The herbicide imazamox was sprayed in the field plots when jointed goatgrass parental plants had approximately 5 tillers; therefore, the herbicide did not kill the susceptible parent plants, which survived and produced seeds in both years. However, selection pressure affected the yield components analyzed. All yield components were reduced in susceptible jointed goatgrass parent plants by the selection pressure treatments compared to the control. Although disease did not affect the disease-resistance allele proportion in the progeny in 2010 or gene flow from resistant-to-susceptible plants in the two years, it did promote visual lesions and lodging in the plants, with lodging being more evident in 2011. However, diseasereduced susceptible plant yield components in both years, probably by interfering with the metabolism of the plants and obstructing the normal transport of water and nutrients via the stem. In general, foot-rot does reduce grain yield and the components of yield, with or without plant lodging (Mungarro, 1991).

Because genotyping for identifying foot-rot resistance in the progeny was conducted in the same number of seedlings originated from each parent plant, the reduction in seed production in the susceptible plants with selection pressure did not influence the results of disease-resistance allele proportion in the progeny and gene flow. For example, if all seeds produced of every parent plant from each plot were mixed, and a random sample of 1,000 seeds were chosen from each plot, then the reduced seed production by the susceptible plants in the selection pressure treatments might influence the results of resistance allele proportion and gene flow.

Yield components were not reduced in the resistant jointed goatgrass plants compared to the susceptible ones with selection pressure, exemplifying a negative consequence in the environment by the introgression of a trait that alters the fitness of the plants. Thus, resistance alleles that have positive impacts on fitness in the presence of a selection pressure will increase, promoting shifts in allele frequencies.

Usually, plant pollen represents the major reproductive method for gene flow across areas, while seed and vegetative propagules tend to promote the movement of genes across time and space (Mallory-Smith and Sanchez-Olguin, 2011). However, seeds tend to be more persistent than pollen and can be moved further distances through seed dispersal, which in turn, has essentially limitless dispersal capability due to human activities during commerce (Mallory-Smith and Zapiola, 2008). Thus, once there is greater seed production by the resistant jointed goatgrass plants under selection pressure compared to the susceptible plants, the potential for the resistance genes to move increases.

Gene Flow from Resistant to Susceptible Plants with Herbicide and Disease Selection Pressure in Jointed Goatgrass Progeny

Movement of the IMI- and foot rot-resistance allele among jointed goatgrass plants would occur bidirectionally because these are nuclear traits (Newhouse et al., 1992), transmitted via pollen and seed.

There were differences in the herbicide-resistance allele movement among different selection pressure treatments for 2010. However, no difference in gene flow was found in 2011. The disease-resistance allele movement was the same among the selection pressure

treatments in both years.

Our results showed that, as expected, the *introgressed* resistance allele movement within the jointed goatgrass population was low. This movement differed among selection pressure treatments only in one year and for one trait, herbicide resistance. It is likely that the increase in the proportion of the resistance allele in the jointed goatgrass progeny did not have gene flow as a cause, but the significant reduction in spikelet emergence of the susceptible parent plants, thus reducing the number of individuals from susceptible parents tested. In addition, the selection pressure treatments promoted selective advantage for the more adapted (resistant) alleles.

The gene flow required to swamp localadaptation depends on the magnitude and direction ofselection pressure in the interacting populations (Shafer, 1990). For example, gene flow from resistant-to-susceptible plants was not affected by the selection pressure in this study probably because sample size was not large enough to detect the effects. However, the level of gene flow required is also a function of the population size. If the size is large enough, strong selection pressure of more adapted individuals will likely occur. The allele-frequency distribution patterns varyas a function oflevels of gene flow, underlining the importance of correctly sampling spatial structureif these patterns are to be used in estimation of population-genetic processes (Star et al., 2007). The sample size was large enough for detecting differences in the proportion of resistance alleles in the progeny in this study (but a larger sample size may be necessary to analyze gene flow more realistically).

There were heterozygotes originating from heterozygous resistant plants whose

resistance alleles could be from that same plant or from pollination by a different resistant plant. Similarly, there is a possibility that the gene flow from susceptible to resistant plants could have been affected by selection pressure, i.e., greater gene flow from susceptible to resistant plants in the control plots as opposed to plots with selection pressure. Thus, the overall transfer of the resistant alleles via gene flow is likely under-estimated in this study. Further studies with a larger sample size, or more importantly, analyzing progeny over generations from bulk seed samples should be conducted to draw conclusions more carefully. Despite the limitations of our study, knowledge about the dynamics of gene flow in jointed goatgrass increased and can be used to develop further hypotheses and research.

Gene flow from resistant wheat towards jointed goatgrass is more prevalent than from jointed goatgrass in a wheat field condition, because wheat is more abundant than jointed goatgrass, and wheat pollen amounts are greater than jointed goatgrass pollen amounts.

An empirical model developed by Currat et al. (2008) predicts that the asymmetric demography of two species makes the expandingspecies more likely to incorporate genes from the resident species than vice versa. This is probably the case of wheat and jointed goatgrass occurring in sympatry, where wheat is the resident species and jointed goatgrass is the expanding species.The model also predicts that once genes from the resident species have introgressed in the genome of the invading species, they will persistif intraspecific gene flow is limited.

Although in the field experiments of this research, jointed goatgrass was not in

sympatry with wheat, the results agree with the first and second predictions of the model by Currat et al. (2008). Low intraspecific gene flow is the case for both species because outcrossing rates are low. Thus, although theoccasional backcross of F_1 hybrids with jointed goatgrass is verylow, it is of far more significance than intraspecific gene flow in jointed goatgrass because the occasional backcross can lead to the eventual introgression of the resistance allele into jointed goatgrass.

In addition, the persistence and increase of the resistance allele proportion in the progeny took place under selection pressure conditions. Therefore, our results suggest that, for a model to better explain actual patterns of introgression, information on both the presence of selection pressure on more adapted alleles and the differences in relative species abundance should be added.

Imazamox-resistance (*Imi1*) gene flow from IMI-resistant wheat into hybrids and backcrosses is the current scenario encountered in commercial wheat production fields from Eastern Oregon (Perez-Jones et al., 2010). This data also can be applied to *Pch1* gene flow from foot rot-resistant wheat varieties to hybrids and backcrosses. The frequency of alleles conferring disease resistance may increase in the population in generations where the pathogen is prevalent, while alleles conferring herbicide resistance will neither increase nor decrease in the population where the herbicide is not used (Brule-Babel, 2006). Once the resistance gene is introgressed, it will likely persist in the population.

Data show that the process of herbicide-resistant allele movement from IMI-resistant wheat to hybrids and backcross generations and introgression has features that

help to reduce its speed (Zemetra et al. 1998; Gandhi et al., 2006, Perez-Jones et al., 2010; Econopouly et al., 2013). Those factors include low outcrossing rates of both species and reduced hybrid fitness, such as hybrid male sterility and low female fertility.

Several strategies can be adopted to prevent gene flow. For example, herbicide- and disease-resistant hybrids and/or backcross generations are not produced during rotation with non-IMI wheat. Production of hybrids and/or backcrosses is prevented during the season if a rotational crop is grown and jointed goatgrass is controlled. Imazamox should be used when growing IMI-resistant wheat to control jointed goatgrass and to prevent or reduce hybrids/backcrosses seed production. It is important to control jointed goatgrass to prevent cross-pollination with the IMI-resistant hybrids/backcrosses that are no longer controlled by imazamox and to prevent cross-pollination with IMI-resistant wheat.

CONCLUSIONS

The spread of the IMI-resistance allele in the progeny represents a threat to profitable winter wheat production in areas where jointed goatgrass is a weed management concern. Wheat and jointed goatgrass cross-pollinate and produce hybrids carrying the herbicide-resistance allele. In field conditions, the herbicide-resistance allele has not been yet identified in jointed goatgrass due to gene flow from IMI-wheat or from selection of resistant individuals, but has been identified in hybrids and, for the first time, in backcross plants.

Under herbicide selection pressure, once the herbicide-resistance allele is

introgressed into jointed goatgrass, it will be selected and transmitted to the next generation, and will increase in frequency within the progeny. It is likely that the herbicide-resistance allele frequency will increase even more in subsequent generations because seed production increases with subsequent backcross generations (Wang et al., 2001; Gandhi et al., 2006).

The results of this research have important implications for agricultural practices that have been adopted by some wheat growers in Eastern Oregon. Some growers do not follow stewardship guidelines in order to avoid gene flow from IMI-resistant wheat to hybrids, backcrosses and eventually the *Imi1* introgression into jointed goatgrass. For foot rot resistance, cultural practices can be a very effective control tool. Crop rotation is the best preventive strategy for managing foot rot, because the level of consecutive inoculum production is reduced. Thus, avoiding planting wheat back-to-back reduces the overall frequency of the foot rot pathogen host and IMI-resistant hybrids. Short periods between successive wheat crops tend to favor foot rot development, whereas long rotations (2 or more years between wheat crops) appear to limit inoculum and disease development due to non-host species planted between wheat crops. The drawback for wheat growers is that rotational cropsdo not provide as much income as wheat does. Although wheat as the previous crop has long been known to favor foot rot, the effect of tillage, which is highly controversial, can only be considered beneficial or not if its interaction with the crop species previously grown is considered. Therefore, crop rotation still remains the best management tactic to control foot rot in wheat. In addition, proper jointed goatgrasscontrol continues to the best preventive way to avoid hybridization with

wheat or backcross with hybrids/backcrosses. Our results represent a situation where theresistance alleles were introgressed in a jointed goatgrass population but were not yet fixed (stabilized) in the population. For the herbicide and disease traits, the gene pool of the parent plants from the field experiments was comprised of two alleles, which occurred in different frequencies. Under selection pressure, the first jointed goatgrass progeny had a significant increase of the proportion of resistance alleles.

Favorable genotypes for a certain trait usually become stabilized faster in self-pollinated than in outcrossing species (Allard, 1999). Thus, even though the resistance allele proportion increased in the two experiments for herbicide and in only one for disease resistance, it is likely that once introgression takes place, the increase of the resistance alleles in subsequent generations will move towards stabilization, under selection pressure.

Although no single jointed goatgrass population having individuals carrying the resistance alleles may be able to guarantee the persistence and possibly stabilization of the resistance alleles, the combined effect of many populations having few resistant individuals within the meta-population of jointed goatgrass from the wheat fields in Eastern Oregon may be able to do this. The outcome from the selection pressure in this study for the resistance alleles proportions in the jointed goatgrass first progeny could be used for certain population-genetic evaluations, especially because the spatial structure was known.

Gene flow patterns may vary with different levels of selection pressure; however, for this study situation, the relative importance of selection pressure on gene flow was not

significant. Thus, although resistance allele gene flow did not differ among the selection pressure treatments, occasional gene flow events including hybridization and backcrossing are of more importance than intraspecific gene flow because the events can lead to the eventual introgression of the resistance alleles into jointed goatgrass.It is likely that gene flow from resistant to susceptible plants would be an additional factor to favor the spread of the resistance alleles and consequently, favor local adaptation of resistant jointed goatgrass in a situation where selection pressure is present.

In addition, resistant wheat will likely continue to pose problems of resistance gene flow to other wheat cultivars, hybrids, backcrosses or jointed goatgrass. Thus, understanding how selection pressure at the field level influences the resistance gene flow and the proportion with which the resistance genes occur in the progeny will inform growers about the importance of controlling this weed species in their wheat fields and prevent further spread of resistance genes. Thus, it lays the ground work for researchers to continue investigating the impacts of selection pressure on resistance genes in subsequent generations.

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GENERAL CONCLUSIONS

CHAPTER 4

Wheat by jointed goatgrass hybrids and backcrosses carrying the *Imi1* gene were identified in commercial wheat production fields. Hybrid heterozygosity and homozygosity for both the wild and mutant alleles were assessed and the natural occurrence of IMI-resistant backcrosses was confirmed for the first time in wheat fields from Eastern Oregon. Non-agricultural sites were heavily infested with jointed goatgrass, volunteer IMI-resistant wheat, hybrids and / or backcross plants and the probability of IMI-resistance in hybrids was greater in these sites compared to agricultural sites. In non-agricultural areas, wheat and jointed goatgrass are geographically sympatric, allowing interspecific hybridization and gene flow to occur, even with male-sterility and low female fertility of the hybrid plants. These areas can act as hybridization zones, both facilitating and speeding up the introgression process of the herbicide-resistance gene into jointed goatgrass. In addition, the consecutive IMI-resistant wheat production also increases the probability of IMI-resistance in hybrid plants. Given the current scenario of IMI-resistance prevalence in hybrids and backcrosses in Eastern Oregon, it is evident that gene flow cannot be prevented in commercial wheat fields. Therefore, results of this research will help in understanding the resistance gene flow in field conditions and introgressed resistance gene among jointed goatgrass plants, as well as making predictions about the increase of resistance in subsequent generations. These results also

will aid in the establishment of tactics to delay the introgression of the resistance genes into jointed goatgrass and for those involved in wheat production to recognize that the gene flow process has already started.

The results from the field experiments showed that if a jointed goatgrass population acquires the herbicide and foot rot resistance alleles, there will be predictable increases in their proportions every generation, ultimately reaching fixation in the population. However, for resistance alleles to be added to the gene pool (fixed) of jointed goatgrass, selection pressure must be present and more than one generation is needed, providing time to adopt management tactics to delay the gene flow in jointed goatgrass.

Wheat production back-to-back increases the foot rot pathogen inoculum and IMI-resistant wheat production back-to-back increases the proportion of IMI-resistant hybrids in the field. Wheat fields infested with jointed goatgrass may justify the production of IMI-resistant wheat back-to-back, assuming careful practices are adopted over the season, which includes imazamox application in proper weather conditions and jointed goatgrass phenological stage, monitoring for non-controlled hybrids or jointed goatgrass within the field and in field edges, waste areas and Conservation Reserve Program (CRP) areas.

For foot rot, selection of resistant *Oculimacula* spp. strains to the fungicides used for its control make cultural practices the best management tactic employed against foot rot, with foot rot resistant cultivars, crop rotation and tillage as the most important and effective tools in a management strategy. However, whether to grow IMI-wheat back-to-

back or to rotate crops should be a decision based on which factor is of more concern, jointed goatgrass and hybrid infestation or foot rot.

Future research should be done to better associate IMI-resistance in hybrids, backcrosses and jointed goatgrass with management practices adopted in winter wheat fields. Information on whether imazamox was used in IMI-wheat, imazamox dose, number of imazamox applications per wheat season and frequency of summer fallow and/or crop rotation will strengthen the knowledge generated in this research.

The results from the field experiments could be strengthened by conducting further field experiments using bulk samples of the jointed goatgrass progeny seeds and subject them to the same selection pressure treatments. These field experiments also could be improved by utilizing a larger sample size. In addition, conducting field experiments over multiple jointed goatgrass generations will generate information on the number of generations necessary to the stabilization of the resistance genes within the jointed goatgrass population under selection pressure of herbicide and disease.

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APPENDIX A

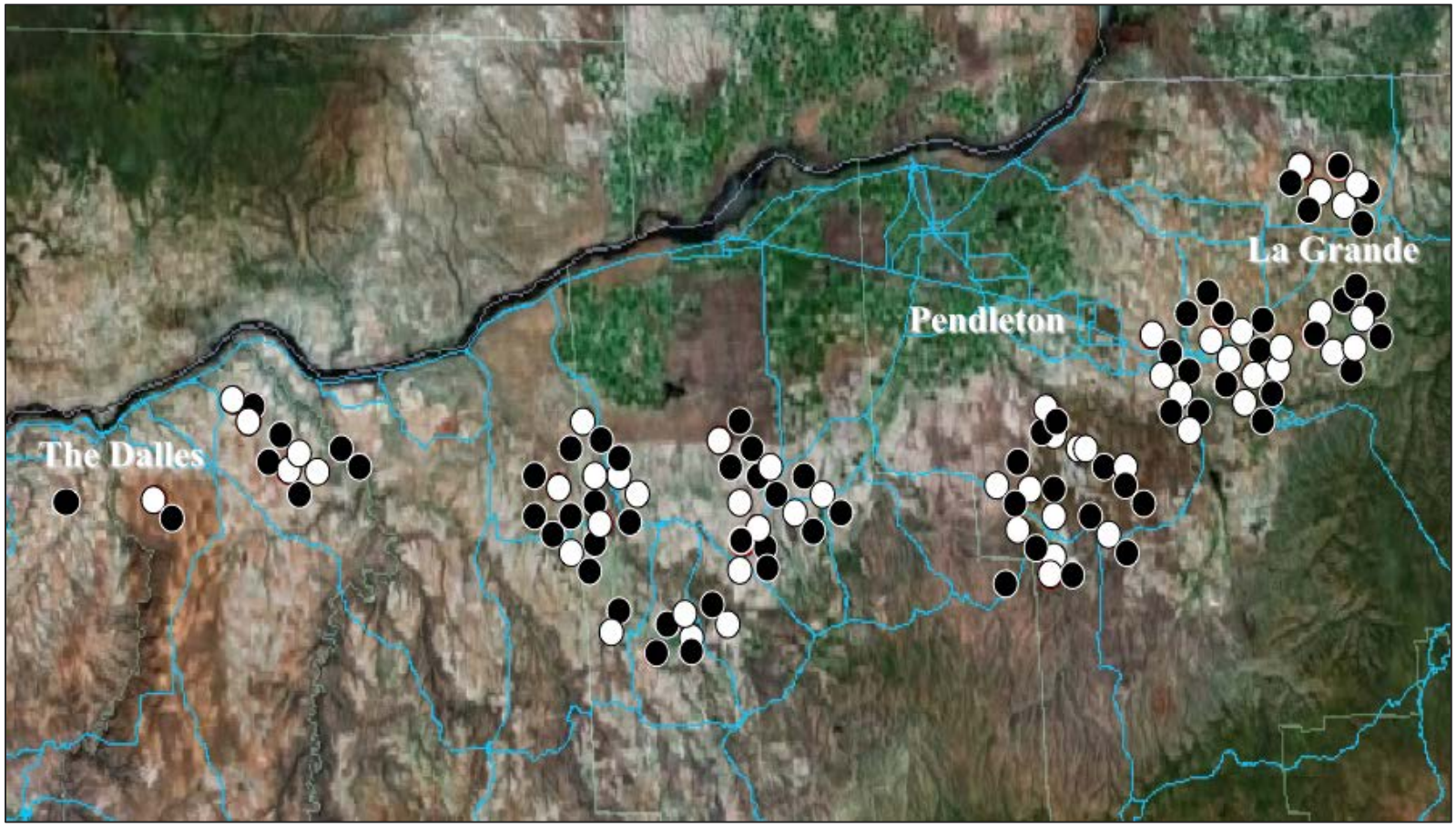

Figure 1. Survey sites located from The Dalles to La Grande, OR. Black circles indicate sites with at least one IMI-resistant plant sample collected. White circles indicate sites with at least one hybrid sampled, but with none IMI-resistant hybrid.

APPENDIX B

Table 1. Estimated mean difference of total spikelet weight per resistant parent plant among the selection pressure treatments and confidence intervals (CI) for 2010.

Treatment Comparison	Mean Difference	Simultaneous 95% CI	Significance
Control vs No Herb/Inoc	1.782676	(-8.838 12.403)	NS
Control vs Herb/No Inoc	7.842500	(-1.905 17.590)	NS
Control vs Herb/Inoc	5.904262	(-4.154 15.963)	NS
No Herb/Inoc vs Herb/No Inoc	6.059824	(-4.146 16.265)	NS
No Herb/Inoc vs Herb/Inoc	4.121585	(-6.382 14.625)	NS
Herb/No Inoc vs Herb/Inoc	-1.938238	(-11.558 7.682)	NS

Table 2. Estimated mean difference of total spikelet weight per susceptible parent plant among the selection pressure treatments and confidence intervals (CI) for 2010.

Treatment Comparison	Mean Difference	Simultaneous 95% CI	Significance
Control vs No Herb/Inoc	-2.236233	(-3.073 -1.399)	NS
Control vs Herb/No Inoc	-6.697936	(-7.540 -5.855)	***
Control vs Herb/Inoc	-7.330580	(-8.171 -6.489)	***
No Herb/Inoc vs Herb/No Inoc	-4.461703	(-5.299 -3.624)	***
No Herb/Inoc vs Herb/Inoc	-5.094347	(-5.930 -4.257)	***
Herb/No Inoc vs Herb/Inoc	-0.632643	(-1.474 0.208)	NS

† Control corresponds to No Herb/No Inoc. *** and NS correspond to significant and non-significant treatment comparisons at the 0.05 level, respectively

Table 3. Estimated mean difference of total spikelet weight per susceptible parent plant among the selection pressure treatments and confidence intervals (CI) for 2011.

Treatment Comparison	Mean Difference	Simultaneous 95% CI	Significance
Control vs No Herb/Inoc	1.0462	(0.271 1.820)	***
Control vs Herb/No Inoc	1.8792	(1.136 2.621)	***
Control vs Herb/Inoc	1.8840	(1.156 2.611)	***
No Herb/Inoc vs Herb/No Inoc	0.8330	(0.185 1.480)	***
No Herb/Inoc vs Herb/Inoc	0.8378	(0.207 1.468)	***
Herb/No Inoc vs Herb/Inoc	0.0049	(-0.585 0.595)	NS

† Control corresponds to No Herb/No Inoc. *** and NS correspond to significant and non-significant treatment comparisons at the 0.05 level, respectively

Table 4. Estimated mean difference of total spikelet weight per resistant parent plant among the selection pressure treatments and confidence intervals (CI) for 2011.

Treatment Comparison	Mean Difference	Simultaneous 95% CI	Significance
Control vs No Herb/Inoc	-0.379487	(-9.834 9.075)	NS
Control vs Herb/No Inoc	0.379487	(-9.075 9.834)	NS
Control vs Herb/Inoc	-0.589744	(-10.104 8.925)	NS
No Herb/Inoc vs Herb/No Inoc	0	(-9.395 9.395)	NS
No Herb/Inoc vs Herb/Inoc	-0.969231	(-10.424 8.485)	NS
Herb/No Inoc vs Herb/Inoc	0.969231	(-8.485 10.424)	NS

† Control corresponds to No Herb/No Inoc. NS: non-significant treatment comparisons at 0.05 level

Table 5. Estimated mean difference of 1,000 spikelet weight per susceptible parent plant among the selection pressure treatments and confidence intervals (CI) for 2010.

Treatment Comparison	Mean Difference	Simultaneous 95% CI	Significance
Control vs No Herb/Inoc	1.6808	(0.894 2.466)	***
Control vs Herb/No Inoc	7.4919	(6.842 8.141)	***
Control vs Herb/Inoc	7.5643	(6.917 8.211)	***
No Herb/Inoc vs Herb/No Inoc	5.8111	(5.191 6.431)	***
No Herb/Inoc vs Herb/Inoc	5.8836	(5.265 6.501)	***
Herb/No Inoc vs Herb/Inoc	0.0724	(-0.358 0.503)	NS

† Control corresponds to No Herb/No Inoc. *** and NS correspond to significant and non-significant treatment comparisons at the 0.05 level, respectively

Table 6. Estimated mean difference of 1,000 spikelet weight per resistant parent plant among the selection pressure treatments and confidence intervals (CI) for 2010.

Treatment Comparison	Mean Difference	Simultaneous 95% CI	Significance
Control vs No Herb/Inoc	-5.183	(-10.816 0.449)	NS
Control vs Herb/No Inoc	-1.480	(-6.938 3.977)	NS
Control vs Herb/Inoc	-1.827	(-7.671 4.017)	NS
No Herb/Inoc vs Herb/No Inoc	3.703	(-1.021 8.427)	NS
No Herb/Inoc vs Herb/Inoc	3.356	(-1.809 8.522)	NS
Herb/No Inoc vs Herb/Inoc	-0.347	(-5.321 4.628)	NS

† Control corresponds to No Herb/No Inoc. NS: non-significant comparisons at the 0.05 level

Table 7. Estimated mean difference of spikelet number per 2 spikes for thesusceptible parent plants among the selection pressure treatments and 95% confidence intervals (CI) for 2010.

Treatment Comparison	Mean Difference	Simultaneous 95% CI		Significance
Control vs No Herb/Inoc	0.910861	(-0.395	0.123)	***
Control vs Herb/No Inoc	-5.674953	(-5.933	-5.416)	***
Control vs Herb/Inoc	-5.538857	(-5.798	-5.279)	***
No Herb/Inoc vs Herb/No Inoc	-4.764092	(-5.023	-4.504)	***
No Herb/Inoc vs Herb/Inoc	-4.627996	(-4.888	-4.367)	***
Herb/No Inoc vs Herb/Inoc	-0.136096	(-5.321	4.628)	NS

† Control corresponds to No Herb/No Inoc. *** and NS correspond to significant and non-significant treatment comparisons at the 0.05 level, respectively

Table 8. Estimated mean difference of spikelet number per 2 spikes for theresistant parent plants among the selection pressure treatments and 95% confidence intervals (CI) for 2010.

Treatment Comparison	Mean Difference	Simultaneous 95% CI		Significance
Control vs No Herb/Inoc	-0.914551	(-2.574	0.745)	NS
Control vs Herb/No Inoc	-1.480	(-6.938	3.977)	NS
Control vs Herb/Inoc	-1.827	(-7.671	4.017)	NS
No Herb/Inoc vs Herb/No Inoc	3.703	(-1.021	8.427)	NS
No Herb/Inoc vs Herb/Inoc	3.356	(-1.809	8.522)	NS
Herb/No Inoc vs Herb/Inoc	-0.347	(-5.321	4.628)	NS

† Control corresponds to No Herb/No Inoc. NS: non-significant comparisons at the 0.05 level

Table 9. Selection pressure treatments effect on spikelet number per 2 spikes for resistant and susceptible parent plants and 95% confidence intervals (CI) for the first (2010) year.

Treatment	Resistant Parent Plants			Susceptible Parent Plants		
	2010	95% CI		2010	95% CI	
Control	16.046 a*	(15.103	16.990)	17.683 a	(17.500	17.865)
NoHerb/Inoc	16.961 a	(16.128	17.794)	16.772 b	(16.588	16.956)
Herb/Inoc	17.116 a	(16.167	18.066)	12.144 c	(11.960	12.328)
Herb/NoInoc	16.794 a	(15.961	17.627)	12.008 c	(11.825	12.191)

† Control corresponds to No Herb/No Inoc. *Means in columns, followed by the same letter are not significantly different by Fisher's protected LSD values (p = 0.05)

Table 10. Estimated mean difference of number of emerged seedlings per spikelet per resistant parent plants among the selection pressure treatments and 95% confidence intervals (CI) for 2010.

Treatment Comparison	Mean Difference	Simultaneous 95% CI		Significance
Control vs No Herb/Inoc	0.507519	(-2.849	3.447)	NS
Control vs Herb/No Inoc	-0.600840	(-3.685	2.483)	NS
Control vs Herb/Inoc	-0.302198	(-3.593	2.989)	NS
No Herb/Inoc vs Herb/No Inoc	-1.108359	(-3.961	1.744)	NS
No Herb/Inoc vs Herb/Inoc	-0.809717	(-3.885	2.266)	NS
Herb/No Inoc vs Herb/Inoc	0.298643	(-5.321	4.628)	NS

† Control corresponds to No Herb/No Inoc. NS: non-significant comparisons at the 0.05 level

Table 11. Estimated mean difference of number of emerged seedlings per spikelet per susceptible parent plants among the selection pressure treatments and 95% confidence intervals (CI) for 2010.

Treatment Comparison	Mean Difference	Simultaneous 95% CI		Significance
Control vs No Herb/Inoc	-0.1062	(-1.068	0.856)	NS
Control vs Herb/No Inoc	0.2487	(-0.698	1.196)	NS
Control vs Herb/Inoc	1.3177	(0.307	2.328)	***
No Herb/Inoc vs Herb/No Inoc	0.3549	(-0.607	1.317)	NS
No Herb/Inoc vs Herb/Inoc	1.4239	(0.399	2.448)	***
Herb/No Inoc vs Herb/Inoc	1.0690	(0.058	2.079)	***

† Control corresponds to No Herb/No Inoc. *Means in columns, followed by the same letter are not significantly different by Fisher's protected LSD values (p = 0.05)

Table 12. Estimated mean difference of number of emerged seedlings per spikelet per resistant parent plants among the selection pressure treatments and 95% confidence intervals (CI) for 2011.

Treatment Comparison	Mean Difference	Simultaneous 95% CI		Significance
Control vs No Herb/Inoc	1.775	(-0.380	3.930)	NS
Control vs Herb/No Inoc	-1.025	(-3.180	1.130)	NS
Control vs Herb/Inoc	-1.575	(-3.730	0.580)	NS
No Herb/Inoc vs Herb/No Inoc	0.75	(-1.405	2.905)	NS
No Herb/Inoc vs Herb/Inoc	0.20	(-1.955	2.355)	NS
Herb/No Inoc vs Herb/Inoc	0.55	(-1.605	2.705)	NS

† Control corresponds to No Herb/No Inoc. NS: non-significant comparisons at the 0.05 level

Table 13. Estimated mean difference of number of emerged seedlings per spikelet per susceptible parent plants among the selection pressure treatments and 95% confidence intervals (CI) for 2011.

Treatment Comparison	Mean Difference	Simultaneous 95% CI		Significance
Control vs No Herb/Inoc	0.910861	(-0.395	0.123)	***
Control vs Herb/No Inoc	-5.674953	(-5.933	-5.416)	***
Control vs Herb/Inoc	-5.538857	(-5.798	-5.279)	***
No Herb/Inoc vs Herb/No Inoc	-4.764092	(-5.023	-4.504)	***
No Herb/Inoc vs Herb/Inoc	-4.627996	(-4.888	-4.367)	***
Herb/No Inoc vs Herb/Inoc	-0.136096	(-5.321	4.628)	NS

† Control corresponds to No Herb/No Inoc. *Means in columns, followed by the same letter are not significantly different by Fisher's protected LSD values (p = 0.05)