BREEDING FINE FESCUES FOR LOW MAINTENANCE; UNDERSTANDING DOLLAR SPOT RESISTANCE AND INCREASING TOLERANCE TO MESOTRIONE.

By

TRENT MATTHEW TATE

A dissertation submitted to the

School of Graduate Studies

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

Graduate Program in Plant Biology

Written under the direction of

Dr. William A. Meyer

And approved by

____

____

____

____

New Brunswick, New Jersey

May 2019

ABSTRACT OF THE DISSERTATION

Breeding fine fescues for low maintenance; understanding dollar spot resistance and increasing tolerance to mesotrione.

by TRENT MATTHEW TATE

Dissertation Director:

Dr. William A. Meyer

Fine fescues are group of cool-season grasses that are utilized as low maintenance grasses. The three main species most commonly utilized are Chewings fescue (*Festuca rubra* L. subsp. *commutata* Markgr.-Dann.), hard fescue (*Festuca trachyphylla* (Hack.) Hack.), and strong creeping red fescue (*Festuca rubra* L. subsp. *rubra*). The objectives of this research were to (i) determine the maternal and reciprocals effects of dollar spot resistance in hard fescue by performing a diallel cross between three resistant endophyte containing and three susceptible endophyte free parents, (ii) determine the physiological behavior of mesotrione associated with differential tolerance levels of three fine fescue species and (iii) to utilize a recurrent selection method to breed for increased tolerance to mesotrione in fine fescues and test the selections in field trials.

Maternal and reciprocal effects were significant in the diallel cross of three E+ resistant parents and three E- susceptible parents. All progeny from E+ resistant mothers that got more than 40% dollar spot averaged over the 2 year study did not contain the endophyte. The high maternal inheritance of dollar spot resistance and the maternal inheritance of the *Epichlöe festucae* fungal endophyte along with demonstration of susceptibility in progeny from an E+ resistant maternal parent that did not get the endophyte suggest the endophyte presence is a major factor in the resistance to dollar spot in hard fescue.

In the dose response study, mesotrione tolerance from highest to lowest was: hard > Chewings > strong creeping red fescue. For the absorption study foliar uptake from highest to lowest was: Chewings > strong creeping red > hard fescue, while root absorption was comparable among species. Overall, less foliar uptake and acropetal translocation may be associated with enhanced tolerance of hard fescue to broadcast mesotrione applications compared to Chewings and strong creeping red fescues.

A total of 29 fine fescue selections were developed with mesotrione tolerance and evaluated over three generations. The hard fescues were consistently the most tolerant in each generation. The Chewings fescue were the second most tolerant group in the first and second generations and the strong creeping red fescues were the least tolerant in the first and second generations. In the third generation one strong creeping red fescue selection was ranked 5th overall for mesotrione injury behind two third generation hard fescue selections.

ACKNOWLEDGEMENTS

I would like to thank my family for all of their support and encouragement while I worked on my graduate degree. I would not be in the position I am in without them. Specifically, I want to thank my wife, Ruying, for always being there for me and keeping me motivated and helping me get to where I am now. Her support has been vital to my success. To my PhD advisor Dr. Meyer, thank you for the opportunity to come to Rutgers and pursue my degree working in such an amazing turfgrass breeding program. This experience has prepared me well for my career. To all of my committee members, thank you for all of your support and guidance throughout my time working on my degree. I could not have done it all without your help and guidance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Literature Review

Fine fescues

The fine-leaved fescues, also commonly known as the fine fescues, are a group of cool-season turfgrass species in the *Festuca* genus. Although *Festuca* is an old Latin name for weedy grass (Hitchcock and Chase, 1951), extensive breeding work conducted on many species in this genus transformed them into a widely accepted turf of high quality (Meyer and Funk, 1989; Ruemmele et al., 1995). The fine fescues have been documented as being used as early as the 16th century for golf turfs (Beard, 1973b). They are characterized by their fine bristle-like leaf texture. The very narrow leaf blades of the fine fescues are usually less than one mm in width (Beard, 1973a). This group of species is adapted to cool-humid regions of the world and is generally tolerant of shade and drought. They are adapted for growth on infertile and acidic soils with a pH ranging from 5.5–6.5 (Beard, 1973a; Hanson et al., 1969; Ruemmele et al., 1995; Turgeon, 1996). This group of species is found in a wide array of habitats from beaches, sand dunes, coastal rock, cliffs, salt marshes, riverine gravel, moist meadows, boreal grasslands, and disturbed roadsides (Pavlick, 1985). They generally do not tolerate saturated or wet soils or high nitrogen fertilization (Beard, 1973a; Meyer and Funk, 1989).

The base chromosome number for *Festuca* is x = 7. There have been reports of ploidy level ranging from diploid to decaploid (Ruemmele et al., 1995). The ploidy of strong creeping red fescue (*Festuca rubra* L. subsp. *rubra*) is 2n =

56, while both Chewings (*Festuca rubra* L. subsp. *commutata* Markgr.-Dann.) and hard fescue [*Festuca trachyphylla* (Hack.) Hack.] are 2n = 42 (Huff and Palazzo, 1998; Schmit et al., 1974). Many of the species in the Festuca genus are able to produce viable hybrids but outcrossing frequency between the different species of fine fescue is limited by anthesis date, hour of pollen shed, and/or differences in chromosome number (Schmit et al., 1974). The different fine fescues species flower at different times in the season as well at different times of the day. Sheep fescues (*Festuca ovina* L.) flower earliest in the season followed by the hard, Chewings, and strong creeping red fescues. Pollen is shed prior to 6 am for the Chewings fescues, prior to 8 am for the hards and between 3 and 5 pm strong creeping red fescues according to a study by Schmit et al., 1974.

The species of fine fescues are divided into two major groups, the red fescue (*Festuca rubra*) complex and the sheep fescue (*Festuca ovina*) complex (Ruemmele et al., 2003). Within the red fescue complex there are rhizomatous or creeping growth habits as well as non-rhizomatous or bunch-type growth habits. The sheeps fescue complex contains only non-rhizomatous growth habits. The three species that are most commonly utilized for turfgrass are hard fescue, Chewings fescue, and strong creeping red fescue. Hard fescue is in the sheep fescue complex and both Chewings and strong creeping red fescue are in the red fescue complex (Ruemmele et al., 2003). The research in this dissertation will involve these three species which are most commonly utilized for turfgrass.

Hard fescue

Hard fescue is a perennial C3 turfgrass that has a bunch-type growth habit and spreads by tillers. It is known for high shoot density and a somewhat tufted growth habit (Meyer and Funk, 1989). This species has a dark-green to grey-green color, a reduced vertical growth, and extensive root system which make them a very good choice for low-maintenance sites (Beard, 1973a; Meyer and Funk, 1989; Turgeon, 1996).

In general, the hard fescues are the most disease resistant species of fine fescues. Diseases such as red thread [*Laetisaria fusiformes* (McAlp.) Burdsall], net blotch (*Drechslera dictyoides* F. sp. *Dreschs*), and dollar spot (*Clarireedia jacksonii* C. Salgado, L.A. Beirn, B.B. Clarke, & J.A. Crouch sp. nov.) are not as devastating in hard fescue as in the other fine fescues (Bonos et al., 2006; Meyer and Funk, 1989). However, summer patch is a weakness for hard fescue and can be very destructive. There are two pathogens in the *Magnaporthiopsis* genus which cause summer patch disease in hard fescue, *M. poae* and a recently discovered pathogen, *M. meyeri-festucae* (Luo et al., 2017).

Chewings fescue

Chewings fescue is a perennial C3 turfgrass with a bunch-type growth habit that spreads by tillers. It is tolerant of both drought and traffic. It has an semi-prostrate growth habit and can be very dense due to extensive tillering (Ruemmele et al., 1995). It is tolerant of close mowing and has been used on golf course greens and fairways on links style courses since before 1850 (Beard, 1973a; Beard, 1998). With the recent emphasis on more environmentally friendly turfgrass, research has shown that the use of Chewings fescues alone and in blends with *Agrostis* sp. can produce an acceptable playing surface for golf courses (Bonos et al., 2001; Christians, 2000; Horgan et al., 2007; Watkins et al., 2010).

Chewings fescue is named after George Chewings who was a farmer in New Zealand in 1800's (Ruemmele et al., 2003). Chewings fescue is susceptible to red thread, net blotch, leaf spot [*Bipolaris sorokiniana* (Sacc.) Shoemaker] and dollar spot (Meyer, 1982). Other weaknesses of Chewings fescue include it accumulating excess thatch and having poor low temperature color retention (Ruemmele et al., 2003).

Strong creeping red fescue

Strong creeping red fescue is a perennial C3 turfgrass usually loosely tufted with long rhizomes. Strong creeping red fescue has the coarsest texture and least dense canopy of the fine fescues (Meyer and Funk, 1989)., and a medium- to dark-green color (Ruemmele et al., 1995). Strong creeping red fescue has good tolerance to shade. It is also the most compatible fine fescue in seed mixtures with perennial ryegrass (*Lolium perenne* L.), and Kentucky bluegrass (*Poa pratensis* L.), due to comparable texture and density (Meyer and Funk, 1989). Strong creeping red fescue is susceptible to red thread, net blotch, dollar spot and leaf spot, but it has good recuperative ability following periods of biotic and abiotic stresses due to its long rhizomes (Ruemmele et al., 2003). The rhizomes give strong creeping red fescue good recuperative ability from disease and summer stress. The presence of rhizomes also make it a good species to utlilize in mixtures for sod because it provides greater sod strength.

The *Epichlöe festucae* fungal endophyte

Epichlöe and *Neotyphodium* species (Ascomycota) are mutualistic symbionts (endophytes) of temperate grasses. Endophytes are estimated to occur in 20-30% of all grass species (Leuchtmann, 1993). This group of fungal endophytes was brought to prominence when Bacon et al. (1977) associated their presence in forage grasses with toxicity to grazing animals. Many more studies were conducted on the effects of the *Epichlöe* endophytes and it was discovered that these fungi play a role in host plant defense and ecology (Clay and Schardl, 2002). *Epichlöe festucae* grows in the intercellular spaces of above ground vegetative tissue (leaf sheaths, culms, and inflorescences), ovules, and seeds (Schardl, 2001). Endophytes are transmitted vertically from the mother plant to the progeny. In some cases, *Epichlöe festucae* can become pathogenic where the fungus enters the sexual stage and arrests the development of an inflorescence by forming a stroma that emerges from the culm; a condition called choke disease. This arrests the complete formation of the inflorescence and prevents seed from developing on the infected culm (Kirby, 1961; Sampson, 1933). The cause of this change from asexual mutualistic symbiosis to a pathogen is not well understood. Sexual reproduction occurs when the anthomyiid fly transfers the opposite mating type spermatia to fertilize stroma of nearby culms exhibiting choke disease. This results in the formation of ascospores that can then infect neighboring plants (Chung and Schardl, 1997). Aside from the pathogenic phase of *Epichlöe*, there are many benefits that have been documented by the presence of the endophyte in fine fescues. Endophytes have been reported to deter insects from feeding on turf (Funk et al., 1985; Saha et al., 1987; and Yue et al., 2000). Resistance to fungal pathogens has also been reported and is specific to the fine fescue –*Epichlöe festucae* symbiotic relationship (Rodriguez et al., 2009). Resistance to red thread has been demonstrated in strong creeping red fescue and Chewings fescue (Bonos et al., 2005). Resistance to dollar spot has also reported to occur from the presence of *Epichlöe festucae* in Chewings (*F. rubra* subsp. *commutata*), hard (*F. trachyphylla*), blue (*F. ovina*), and strong creeping red fescue (*F. rubra* subsp. *rubra*) (Clarke et al., 2006). Endophyte-mediated suppression disease and damage from feeding insects along with the additional benefits of enhanced stress tolerance is a key focus of turfgrass breeders in developing low maintenance cultivars. The least expensive, most effective and safest way of controlling a plant disease is the use of resistant cultivars (Agrios, 2005). This can be done by developing cultivars with high levels of *E. festucae* so those cultivars can be more resistant to diseases and insects while being more stress tolerant as well. This will make them more adapted to low maintenance areas.

Dollar spot disease

Dollar spot disease caused by the fungus *Clarireedia jacksonii* sp. nov. (Salgado-Salazar et al., 2018) is a devastating foliar turfgrass disease in both warm- and cool-season turfgrass (Smiley et al., 2005). It is the most common turfgrass disease on cool-season turfgrasses throughout the world (Couch, 1995). According to Goodman and Burpee (1991), more money is spent to control dollar spot than any other turfgrass disease.

The symptoms of dollar spot initially appear as white- to straw-colored leaf lesions with dark borders which can occur at leaf tips or laterally across the leaf blade. On higher cut turf, older lesions may appear hour-glassed shaped with the center being narrower than the edges (Couch, 1995). Infected patches appear as numerous, small, bleached out spots the size of a quarter to a dollar (2-6 cm), which during severe infection can coalesce into larger areas blighted turf (Agrios, 2005). Signs of dollar spot disease include a greyish-white cottony mycelium that forms in the turf canopy when environmental conditions are conducive to infection. Ariale mycelium is produced from the symptomatic tissue. Mycelium of *C. jacksonii* is distinct having acute angle branching with a hyphal septa. The fungus produces a stroma which is a matrix of vegetative hyphae that can survive for long periods in soil, grass clippings and thatch (Couch, 1995; Smiley et al., 2005). When the environment is conducive for disease, the mycelium grows from the stroma and infect nearby plants. Infection from the mycelium can occur through the openings of the stomata, the cut leaf tips or it can penetrate the leaves directly. The hyphae then colonize the epidermal and mesophyll cells secreting toxins and enzymes that cause tissue necrosis (Couch, 1995). Spores are not produced by *C. jacksonii*, it is disseminated by infected plants or stroma in leaf debris and dead plant tissue by people, animals, water or wind. Dollar spot occurs at temperatures ranging from 15° to 32° C when extended leaf wetness periods occur (Couch, 1995). Nitrogen deficiency in turfgrass can increase the incidence and severity of dollar spot (Walsh et al., 1999).

There are numerous classes of fungicides that are labelled for dollar spot control. These include the benzimidazoles, demethylation inhibitors (DMIs), carboximides, dicarboximides, dithiocarbamates, strobilurins (QoIs), succinate dehydrogenase inhibitors (SDHIs), nitriles, and dinitro-anilines (Smiley et al., 2005). Care should be taken when using these fungicides to reduce the risk of developing resistance by rotating chemical classes because resistances have been reported for the benzimidazoles, dicarboximides, and demethylation inhibitors (Detweiler et al., 1983; Golembiewski et al., 1995; Vargas Jr et al., 1992) and most recently for the SDHIs (Popko et al., 2018).

4-Hydroxyphyenyl Pyruvate Dioxygenase Inhibiting herbicides

The 4-Hydroxyphyenyl Pyruvate Dioxygenase Inhibitor (HPPD) class of herbicides are used in controlling many important broadleaf and grassy weeds in agriculture and turfgrass. The HPPD enzyme acts as a catalyst in the conversion of p-hydroxyphenyl pyruvate (4-HPP) to homogentisate (HGA), which is an important precursor to α-tocopherol and plastoquinone (Crouch et al., 1997; Pascal Jr et al., 1985; Que Jr and Ho, 1996). Inhibition of the enzyme disrupts the downstream biosynthesis of carotenoids and results in the bleaching of the foliage due to loss of chlorophyll (Meazza et al., 2002). Herbicides in the HPPD inhibitor class include : amides, anildidex, furanones, phenoxybutan-amides, pyridiazinones, pyridines, callistemones, isozaxoles, pyrazoles, and trikeones

(Senseman, 2007). Scientists from Zeneca discovered the HPPD inhibitor activity of benzoylcyclohexanedione in 1982 while working on a functional mimic of sethoxydim, an acetyl-CoA carboxylase (ACCase) inhibiting herbicide. That molecule acts as a competitive inhibitor of HPPD by interrupting the synthesis of plastoquinone and α-tocopherol (Lee et al., 1997; Mitchell et al., 2001). The development of mesotrione started when scientist observed allelopathic effects from the bottlebrush plant (*Callistemon citrinus* Stapf.). They isolated leptospermone and demonstrated bleaching symptoms in broadleaf and grassy weeds at rates of 1000 g/ha (Beaudegnies et al., 2009; Lee et al., 1997).

Mesotrione

Mesotrione (2-[4-mesyl-2-nitrobenzoyl]cyclohexane-1,3-dione) is a member of the benzoylcyclohexanedione chemical family. Mesotrione was registered with the U.S. Environmental Protection Agency (EPA) in 2001. It is considered a 'reduced risk pesticide' by the EPA. It was initially labeled for use in corn production for controlling broadleaf and grassy weeds (Mitchell et al., 2001). In 2007, mesotrione was registered for use in turfgrass. Applications of mesotrione can be made anytime seeding occurs, i.e. bare ground seeding or renovations and overseeding established turf whether in the fall or spring. It controls both monocot and dicot weeds and once applied it is absorbed in the leaves and roots and translocated to the xylem and phloem. Mesotrione should not be used on new fine fescue seedings and on seed mixtures containing more than 20 percent by weight of hard or fine fescue because it may reduce the density of fine fescue seedings (Anonymous, 2008).

Currently there are very few options available for selective weed control at establishment for fine fescues. Development of tolerance to mesotrione in fine fescues would give turf managers the ability to control problematic weeds such as *Poa annua* at establishment. Controlling weeds during establishment is critical for the seedlings to develop a dense monostand of turfgrass. Herbicide tolerance development in hard fescue has been demonstrated before with the non-selective herbicide glyphosate. ‘Aurora Gold’ is an advanced-generation synthetic cultivar derived from ‘Aurora’ hard fescue after using five cycles of phenotypic recurrent selection over a 10-yr period following direct applications of glyphosate at 0.8 to 1.6 kg/ha (Hart et al., 2005). A study conducted by McCullough et al. in 2015 determined the mechanism of resistance to glyphosate in ‘Aurora Gold’ hard fescue was due to less target site inhibition. An aminotriazole tolerant Chewings fescue cultivar ‘Countess’ was developed using recurrent selection (Johnston and Faulkner 1986). This provided a selective control of *P. annua* in but unfortunately aminotriazole became a restricted use chemical not long after its release.

Heritability

Heritability is defined as the inherited portion of variation that is observed in a progeny (Poehlman et al., 1995). Estimates of heritability are used by plant breeders to predict the expected improvements that can be made after selection (Holland et al., 2003; Nyquist and Baker, 1991). There are two ways that heritability can be expressed; broad-sense heritability and narrow-sense heritability (Fehr, 1991). To calculate heritability ratios, observations are made of various genotypes over multiple environments and years (Gordon et al., 1972). Traits that have a high heritability value are less influenced by environmental effects. These traits can be improved more rapidly than traits with lower heritability (Holland et al., 2003; Nyquist and Baker, 1991). Total genetic variance is the portion of the phenotypic variance that is due to the genotypic differences among the phenotypes. The additive, the dominance, and the epistatic genetic variance are what makes up the total genetic variance (Dudley and Moll, 1969). Heritability estimates are used by plant breeders to predict gain from selection. Gain from selection can be calculated using the formula $Gs = i\sigma_p h^2$, where Gs is the genetic gain from selection, i is a constant based on selection intensity, σ_p is the standard deviation of the phenotypic variance, and h^2 is the narrow- sense heritability (Poehlman et al., 1995). Determining the portion of the total observed variation that is from additive genetic effects is the narrow-sense heritability estimate (Holland et al., 2003; Nyquist and Baker, 1991; Poehlman et al., 1995). Determining narrow-sense heritability can be accomplished by conducting diallel mating design and testing the progeny along with the parents in multiple locations. Understanding that ratio of additive to the total phenotypic variance is very important in cross-pollinated grasses because the recurrent selection breeding technique maximizes the use of additive genetic variation (Vogel and Pedersen, 1993). Data obtained from a diallel mating design can also be used to determine general combining abilities (GCA) and specific combining abilities of the parents used in the cross. This is accomplished by using the statistical methods outlined by Griffing (1956).

Combining abilities are also useful for studying aspects of quantitative traits (Sprague and Tatum, 1942). General combining ability is calculated by comparing the mean performance from an individual parent to the mean of all other crosses. These estimates of GCA provide an expected value for a specific cross which is equal to the sum of the GCA from both parents. Specific combining ability is the deviation from the expected value of a cross. These estimates of GCA and SCA can be used to evaluate additive vs. non-additive gene effects that are contributing to a phenotype and also identify parents to use or not use in breeding for a particular trait (Cisar et al., 1982; de Araujo and Coulman, 2004; Falconer and Mackay, 1996; Van Becelaere and Miller, 2004).

Another informative component that can be estimated from a diallel mating design is heterosis. Heterosis gives plant breeders an indication of dominance from a cross and is calculated by comparing the mid-parent means of a specific cross to the progeny means. A lack of heterosis is indicated by progeny means that are similar to the mid-parent mean. This can mean that gene effects are additive in nature or that the parents from that cross have groups of loci that oppose responses for the trait of interest (Falconer and Mackay, 1996). Maternal effects are calculated by comparing the progeny means of reciprocal crosses. This measure informs the breeder if the trait of interest is transferred to the progeny unequally among the egg and pollen donor plants. Heterosis and maternal effects are measured by implementing a two sample t test using data from a diallel cross (Kitchens, 1998). Many turfgrass pathosystems have been studied to determine heritability estimates both in the broad-sense and narrow-sense. The broad-sense heritability for dollar spot in *Poa trivialis* ranged between 0.57 to 0.90 on a three plant mean basis (Hurley and Funk, 1985). Broad-sense heritability estimates for rust caused by *Puccinia graminis* subsp. *graminicola* in perennial ryegrass (*Lolium perenne*) ranged from 0.13 to 0.70 (Rose-Fricker et al., 1986). Broad-sense heritability for dollar spot (*Clairireedia jacksonii*) in creeping bentgrass (*Agrostis stolonifera*) were estimated at 0.56 on a single plant basis and 0.90 on an 11-plant clonal mean basis (Bonos et al., 2003) and narrow-sense heritability for dollar spot in creeping bentgrass was estimated at 0.79 (Bonos, 2006). Narrow-sense heritability for gray leaf spot (*Pyricularia oryzae* Cavara) in perennial ryegrass was estimated from 0.57 to 0.76 (Han et al., 2006). Narrow-sense heritability for brown patch (*Rhizoctonia solani*) in tall fescue (*Festuca arundinacea*) was estimated at 0.62 and 0.57 over a two year study (Bokmeyer et al., 2009). A broad-sense heritability for *Bipolaris* leaf spot in fine textured germplasm of zoysiagrass (*Zoysia* spp.) was 0.40 (Schwartz et al., 2009). A narrow-sense heritability estimate was estimated at 0.23 for dollar spot in seashore paspalum (*Paspalum vaginatum*) (Flor et al., 2013).

Goal of this dissertation

The objectives of this dissertation are to:

a) Determine the maternal and reciprocals effects, presence of the *Epichlöe festucae* fungal endophyte association with dollar spot disease resistance in hard fescue by performing a diallel cross between three resistant and three susceptible parents and testing in a field trial.

b) Determine mesotrione tolerance levels and ^{14}C-mesotrione absorption and translocation in three fine fescue species .

c) Utilize a recurrent selection method to breed for increased tolerance to mesotrione, subject that material to field trials treated with applications of mesotrione, and compare injury and quality with non mesotrione-selected experimental selections and commercially available cultivars.

REFERENCES

Agrios GN (2005) Plant pathology. 5th edn: Elsevier Acad. Press.

Anonymous (2008) Tenacity herbicide label. Pages 8. Greensboro, NC: Syngenta Corp Protection

Bacon CW, Porter JK, Robbins JD, Luttrell ES (1977) *Epichlöe typhina* from toxic tall fescue grasses. Applied and environmental microbiology 34:576–81

Beard JB (1973a) Cool season turfgrasses. Pages 54–131 Turfgrass: Science and Culture. Englewood Cliffs, NJ: Prentice-Hall

Beard JB (1973b) Turfgrass: science and culture. Englewood Cliffs, NJ: Prentice-Hall, Inc.

Beard JB (1998) Traditional fine-leaf fescue putting greens. Turfax 6:3

Beaudegnies R, Edmunds AJ, Fraser TE, Hall RG, Hawkes TR, Mitchell G, Schaetzer J, Wendeborn S, Wibley J (2009) Herbicidal 4-hydroxyphenylpyruvate dioxygenase inhibitors—a review of the triketone chemistry story from a Syngenta perspective. Bioorganic & medicinal chemistry 17:4134–4152

Bokmeyer JM, Bonos SA, Meyer WA (2009) Inheritance characteristics of brown patch resistance in tall fescue. Crop sci. 49:2302-2308.

Bonos SA (2006) Heritability of dollar spot resistance in creeping bentgrass. Phytopathology 96:808–812

Bonos SA, Casler MD, Meyer WA (2003) Inheritance of dollar spot resistance in creeping bentgrass. Crop science 43:2189–2196

Bonos SA, Clarke BB, Meyer WA (2006) Breeding for disease resistance in the major cool-season turfgrasses. Annual review of phytopathology 213–234

Bonos SA, Watkins E, Honig JA, Sosa M, Molnar T, Murphy JA, Meyer WA (2001) Breeding cool-season turfgrasses for wear tolerance using a wear simulator. Int. turfgrass soc. res. J 9:137–145

Bonos SA, Wilson MM, Meyer WA, Funk CR (2005) Suppression of red thread in fine fescues through endophyte-mediated resistance. Applied turfgrass science

Christians N (2000) Fairway grasses for Midwest golf courses. Golf course manage 68:49–56

Chung K-R, Schardl C (1997) Sexual cycle and horizontal transmission of the grass symbiont, Epichloë typhina. Mycological research 101:295–301

Cisar G, Brown C, Jedlinski H (1982) Diallel analyses for tolerance in winter wheat to the barley yellow dwarf virus. Crop Science 22:328–333

Clarke BB, White JF, Hurley RH, Torres MS, Sun S, Huff DR (2006) Endophyte-mediated suppression of dollar spot disease in fine fescues. Plant disease 90:994–998

Clay K (1988) Fungal endophytes of grasses- a defensive mutualism between plants and fungi. Ecological society of aAmerica 69:10-16

Clay K, Schardl C (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. the american naturalist 160:S99–S127

Couch HB (1995) Diseases of turfgrasses. 3rd edn. Malabar, FL: Krieger Publishing Company. Pp. 421

Crouch NP, Adlington RM, Baldwin JE, Lee M-H, MacKinnon CHJT (1997) A mechanistic rationalisation for the substrate specificity of recombinant mammalian 4-hydroxyphenylpyruvate dioxygenase (4-HPPD). 53:6993–7010

de Araujo MR, Coulman BE (2004) Parent-offspring regression in meadow bromegrass (Bromus riparius Rehm.): Evaluation of two methodologies on heritability estimates. Canadian journal of plant science 84:125–127

Detweiler AR, Vargas Jr JM, Danneberger TK (1983) Resistance of Sclerotinia homoeocarpa to iprodione and benomyl. Plant disease 67:627–630

Dudley JW, Moll RH (1969) Interpretation and use of estimates of heritability and genetic variances in plant breeding. Crop science 9:257–262

Falconer DS, Mackay TFC (1996) Heritability. Pages 160–183 Introduction to quantitative genetics: Pearson

Fehr WR (1991) Heritability. Pages 95-105 Principles of cultivar development: theory and technique: Macmillian Publishing Company

Flor N, Munoz P, Harmon P, Kenworthy K (2013) Response of seashore paspalum genotypes to dollar spot disease. Int. turfgrass soc. res. j. 12:119–126

Funk CR, Halisky PM, Ahmad S, Hurley RH (1985) How endophytes modify turfgrass performance and response to insect pests in turfgrass breeding and evaluation trials [Acremonium spp.; Crambus spp., Sphenophorus parvulus, Blissus leucopterus hirtus; Lolium perenne, Festuca arundinacea, Festuca longifolia, cool-season grass. Proceedings of the fifth international turfgrass research conference Avignon, France: American Society of Agronomy, Crop Science Society of America International turfgrass research conference.

Golembiewski RC, Vargas JM, Jones AL, Detweiler AR (1995) Detection of demethylation inhibitor (DMI) resistance in Sclerotinia homoeocarpa populations. Plant disease 79:491–493

Goodman DM, Burpee LL (1991) Biological control of dollar spot disease of creeping bentgrass. Phytopathology 81:1438–1446

Gordon IL, Byth DE, Balam LN (1972) Variance of heritability ratios estimated from phenotypic variance components. Biometrics:401–415

Griffing B (1956) Concept of general and specific combining ability in relation to diallel crossing systems. Australian journal of biological sciences 9:463–493

Han Y, Bonos SA, Clarke BB, Meyer WA (2006) Inheritance of resistance to gray leaf spot disease in perennial ryegrass. Crop science 46:1143–1148

Hanson AA, Juska FV, Burton GW (1969) Species and varieties. Pages 715 Turfgrass science. Madison, Wisconsin: American Society of Agronomy

Hart SE, Derr JF, Lycan DW, Rose-Fricker C, Meyer WA (2005) Increased glyphosate tolerance in ‘Aurora Gold’ hard fescue (Festuca longifolia). Weed technology 19:640–646

Hitchcock AS, Chase A (1951) Manual of the grasses of the United States: US Department of Agriculture

Holland JB, Nyquist WE, Cervantes-Martínez CT (2003) Estimating and interpreting heritability for plant breeding: an update. Plant breeding reviews 22:9–112

Horgan B, Hollman A, Koeritz E, Stier J (2007) Fine fescues and colonial bentgrasses for fairways. Golf course mgt 75:112–117

Huff DR, Palazzo AJ (1998) Fine fescue species determination by laser flow cytometry. Crop science 38:445–450

Hurley R, Funk C (1985) Genetic variability in disease reaction, turf quality, leaf color, leaf texture, plant density and seed shattering of selected genotypes of Poa trivialis Sclerotinia homeocarpa, Erysiphe graminis, Ustilago striiformis. Proceedings of the fifth international turfgrass research conference Avignon, France: American Society of Agronomy, Crop Science Society of America International turfgrass research conference.

Johnston DT, Faulkner JS (1986) Countess and Duchess-Aminotriazole-tolerant cultivars of Chewings fescue and browntop bent. Journal sports turf research institute 62:217

Kirby EJM (1961) Host-parasite relations in the choke disease of grasses. Transactions of the British mycological society 44:493–503

Kitchens LJ (1998) Exploring statistics: A modern introduction to data analysis and inference: Duxbury Press Pacific Grove, CA:

Lee DL, Prisbylla MP, Cromartie TH, Dagarin DP, Howard SW, Provan WM, Ellis MK, Fraser T, Mutter LC (1997) The discovery and structural requirements of inhibitors of p-hydroxyphenylpyruvate dioxygenase. Weed science:601–609

Leuchtmann A (1993) Systematics, distribution, and host specificity of grass endophytes. Natural toxins 1:150–162

Luo J, Vines PL, Grimshaw A, Hoffman L, Walsh E, Bonos SA, Clarke BB, Murphy JA, Meyer WA, Zhang N (2017) Magnaporthiopsis meyeri-festucae, sp. nov., associated with a summer patch-like disease of fine fescue turfgrasses. Mycologia 109:780–789

McCullough PE, Yu J, Shilling DG, Czarnota MA (2015) Physiological basis for glyphosate tolerance in hard fescue and perennial ryegrass cultivars. Crop sci. 55:2352-2358

Meazza G, Scheffler BE, Tellez MR, Rimando AM, Romagni JG, Duke SO, Nanayakkara D, Khan IA, Abourashed EA, Dayan FE (2002) The inhibitory activity of natural products on plant p-hydroxyphenylpyruvate dioxygenase. Phytochemistry 60:281–288

Meyer WA (1982) Breeding disease-resistant cool-season turfgrass cultivars for the United States. Plant disease 66:341–344

Meyer WA, Funk CR (1989) Progress and benefits to humanity from breeding cool-season grasses for turf. Pages 31–48 *in* Sleper DA, Asay KH, Pedersen JF, eds. Contributions from breeding forage and turf grasses. Madison, WI: Crop Science Society of America

Mitchell G, Bartlett DW, Fraser TEM, Hawkes TR, Holt DC, Townson JK, Wichert RA (2001) Mesotrione: a new selective herbicide for use in maize. Pest management science 57:120–128

Nyquist WE, Baker RJ (1991) Estimation of heritability and prediction of selection response in plant populations. Critical reviews in plant sciences 10:235–322

Pascal Jr RA, Oliver MA, Chen YJ (1985) Alternate substrates and inhibitors of bacterial 4-hydroxyphenylpyruvate dioxygenase. Biochemistry 24:3158–3165

Pavlick LE (1985) A new taxonomic survey of the Festuca rubra complex in northwestern North America, with emphasis on British Columbia. Phytologia 57:1–17

Poehlman JM, Sleper DA, Rudd J (1995) Breeding field crops: Springer

Popko JT, Sang H, Lee J, Yamada T, Hoshino Y, Jung G (2018) Resistance of Sclerotinia homoeocarpa Field Isolates to Succinate Dehydrogenase Inhibitor Fungicides. Plant disease 102:2625–2631

Que Jr L, Ho RY (1996) Dioxygen activation by enzymes with mononuclear non-heme iron active sites. Chemical reviews 96:2607–2624

Rodriguez RJ, White Jr JF, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New phytologist 182:314–330

Rose-Fricker CA, Meyer WA, Kronstad WE (1986) Inheritance of resistance to stem rust (Puccinia graminis subsp. graminicola) in six perennial ryegrass (Lolium perenne) crosses. Plant disease 70:678–681

Ruemmele BA, Brilman LA, Huff DR (1995) Fine fescue germplasm diversity and vulnerability. Crop science 35:313–316

Ruemmele BA, Wipff JK, Brilman L, Hignight KW (2003) Fine-leaved Festuca species. Pages 129–174 Turfgrass biology, genetics, and breeding. Hoboken, NJ, USA: John Wiley & Sons, Inc.

Saha DC, Johnsoncicalese JM, Halisky PM, Vanheemstra MI, Funk CR (1987) Occurrence and significance of endophytic fungi in the fine fescues. Plant disease 71:1021–1024

Salgado-Salazar C, Beirn LA, Ismaiel A, Boehm MJ, Carbone I, Putman AI, Tredway LP, Clarke BB, Crouch JA (2018) Clarireedia: A new fungal genus comprising four pathogenic species responsible for dollar spot disease of turfgrass. Fungal biology 122:761–773

Sampson K (1933) The systemic infection of grasses by *Epichlöe typhina* (Pers.) Tul. Transactions of the British mycological society 18:30–47

Schardl CL (2001) Epichloë festucae and related mutualistic symbionts of grasses. Fungal genetics and biology 33:69–82

Schmit RM, Duell RW, Funk CR (1974) Isolation barriers and self-compatibility in selected fine fescues. Pages 9–17 *in* Roberts EC, ed. Proceedings of the second international turfgrass research conference. Madison, WI: American Society of Agronomy, Crop Science Society of America

Schwartz BM, Kenworthy KE, Engelke M, Genovesi AD, Quesenberry KH (2009) Heritability estimates for turfgrass performance and stress response in Zoysia spp. Crop science 49:2113–2118

Senseman SA (2007) Herbicide handbook: Lawrence, US: Weed science society of america.

Smiley RW, Dernoeden PH, Clarke BB (2005) Compendium of turfgrass diseases: American Phytopathological Society (APS Press)

Sprague GF, Tatum LA (1942) General vs. Specific Combining Ability in Single Crosses of Corn. Agron J 34:923–932

Turgeon AJ (1996) Turfgrass management. Upper Saddle River, NJ: Pretice Hall

Van Becelaere G, Miller JF (2004) Combining ability for resistance to Sclerotinia head rot in sunflower. Crop science 44:1542–1545

Vargas Jr JM, Golembiewski R, Detweiler AR (1992) Dollar spot resistance to DMI fungicides. Golf course management 60:50–54

Vogel KP, Pedersen JF (1993) Breeding systems for cross-pollinated perennial grasses *in* Janick J, ed. Plant breeding reviews

Walsh B, Ikeda SS, Boland GJ (1999) Biology and management of dollar spot (Sclerotinia homoeocarpa); an important disease of turfgrass. Hortscience 34:13–21

Watkins E, Hollman AB, Horgan BP (2010) Evaluation of alternative turfgrass species for low-input golf course fairways. Hortscience 45:113–118

Yue Q, Johnson-Cicalese J, Gianfagna TJ, Meyer WA (2000) Alkaloid production and chinch bug resistance in endophyte-inoculated chewings and strong creeping red fescues. Journal of chemical ecology 26:279–292

Zhang Y, Kang MS, Lamkey KRJAJ (2005) Diallel-Sas05. Agron J 97:1097-1106

Chapter 2

Maternal Inheritance and the presence of the *Epichlöe festucae* fungal endophyte in dollar spot disease resistance in hard fescue

INTRODUCTION

Hard fescue (*Festuca trachyphylla* (Hack.) Hack.) is a bunch type cool-season turfgrass that spreads by tillers. It has a low fertility requirement and has increased heat and drought tolerance (Beard, 1973; Wang et al., 2017a; Wang et al., 2017b) compared to other species in the fine fescues. Hard fescue is known for its needle-like leaves and high shoot density. It has good shade tolerance and is well adapted for low maintenance areas such as home lawns, parks and roadsides (Meyer et al., 1989; Ruemmele et al., 1995; Ruemmele et al., 2003). One aspect of hard fescue being popular in low maintenance areas is the mutualistic relationship it has with the *Epichlöe festucae* fungal endophyte and the increased tolerance to drought stress and recovery by the presence of the endophyte that it gives to its host (Saha et al., 1987). The occurrence of the endophyte and the defensive mutualism is documented in many different cool-season grasses in *Festuca* and *Lolium* genera (Clay, 1988). These fungal endophytes have been shown to improve resistance to disease and above ground feeding insects in fine fescues. Clarke et al. (2006) determined that the presence of the endophyte improved resistance to dollar spot, caused by *Clarireedia jacksonii* C. Salgado, L.A. Beirn, B.B. Clarke, & J.A. Crouch sp. nov. in hard, blue (*F. glauca* Vill.), Chewings (*F. rubra* L. subsp. *commutata* Markgr.-Dann.) and strong creeping red fescue (*F. rubra* L. subsp. *rubra*). In Chewings and strong creeping red fescue the presence of one strain of the endophyte has suppressed red thread disease caused by *Laetisaria fusiformes* (McAlp.) Burdsall (Bonos et al., 2005). Fungal endophyte presence in fine fescues has also been shown to reduce herbivory from above ground feeding insects (Saha et al., 1987). In 2012, a study by Ambrose and Belanger identified an antifungal protein gene, Efe-AfpA, that was potentially involved in disease resistance observed in strong creeping red fescue. Further studies confirmed the partially purified protein Efe-AfpA suppressed the growth of the dollar spot pathogen in a plate assay (Tian et al., 2017).

The *E. festucae* endophyte is transmitted vertically via the seeds of the infected plants (Schardl, 1996). The vertical transmission efficiency from the maternal plant to the progeny is not perfect, meaning not all seeds from an E+ mother will inherit the endophyte. A study by Saikkonen et al. (2010) demonstrated that the fungal infection was lost in some seedlings of the offspring in 40% of the *E. festucae* endophyte-infected maternal families in nature. In *E. coenophiala,* which infects tall fescue (*Festuca arundinacea* Schreb.), seed transmission has been reported at 96% efficiency (Bouton et al., 2002; Florea et al., 2016). If a seed did not get the endophyte transmitted from the maternal parent then that plant would not have the benefits that the endophyte provides such as dollar spot disease resistance. The fine fescues susceptibility to diseases is one drawback according to Ruemmele et al. (2003). Increasing the resistance to dollar spot, which is one of the most problematic diseases of turfgrass would greatly improve the utility of hard fescue.

Breeding for disease resistance in turf is a major objective of the Rutgers Turfgrass breeding program. The safest, most effective and least expensive way of controlling plant diseases is by the development and use of resistant cultivars (Agrios, 2005). Understanding the inheritance of disease resistance is key to developing resistant cultivars. The objectives of this study are to 1. determine the maternal and reciprocal effects of dollar spot resistance in hard fescue using a diallel cross and 2. determine if the presence of the endophyte is a major factor influencing resistance to dollar spot in hard fescue.

MATERIALS AND METHODS

Six parental clones were selected from germplasm in the Rutgers turfgrass breeding program that were from the 2008 fine fescue turf trial which had a severe dollar spot disease infection throughout the trial. Individual plants were sampled from the resistant and susceptible turf plots and all of those plants were tested for the presence of the *Epichlöe festucae* fungal endophyte using the Agrinostics Ltd. Phytoscreen Field Tiller Endophyte Detection Kits (Agrinostics Ltd. Co. Watkinsville, GA 30677 USA). The endophyte was present in all dollar spot resistant plants and no endophyte was present in all dollar spot susceptible plants. Six clones were selected from the tested material, three resistant (E+); R1 (A08-461-7), R2 (A08 517-18), and R3 (A08 532-13) and three susceptible (E-); S1 (A08-466-9), S2 (A08-500-9), and S3 (A08-512-23 S3) plants. Plants were clonally propagated and planted into a spaced-plant nursery in the fall of 2014 and allowed to vernalize over winter. In the spring of 2016 the six parental clones were brought into the greenhouse from the field and placed 1.5 M under

400 W high pressure sodium lights (PL. Light Systems, Beamsville, Ontario, Canada) to increase day length to 14-hours to encourage flower induction. The individual clones were moved in or out of the extended daylength lighting depending on their maturity to synchronize anthesis. Crosses were organized in all combinations; resistant by resistant, resistant by susceptible and susceptible by susceptible. A total of 15 controlled crosses were organized for a total of 30 crosses including reciprocals. Crosses were organized prior to anthesis and the crosses were isolated spatially at a distance of at least 30 m with various structures in between. A test for selfing was conducted by placing 3-4 panicles of each parental clone in glassine pollination bags during anthesis. No viable seed was harvested from any panicle that was inside the pollination bags for any of the parental clones therefor it assumed no selfing occurred for any crosses in this study. Plant inflorescences were manually tapped each morning from 05:30 am to 06:30 am each morning during anthesis to promote pollen movement between clones.

Seed from each clone was harvested, dried and threshed. Seeds were germinated in a growth chamber maintained at 25/15 C (day/night) with a 10 h photoperiod of 400 μmol m^{-2} s^{-1}. Eighty randomly selected individuals from each of the 30 individual crosses were then transplanted into plastic flats containing Pro-mix growing media. Thirty-two clones of each of the six parents were clonally propagated to a single tiller and transplanted into the above-mentioned plastic flats. All plants were grown for six weeks while receiving daily irrigation and bi weekly fertilization with MacroN 28-7-14 Sprayable Fertilizer, LESCO Inc., Cleveland, OH 44114. The field study was planted on October 16, 2015 into a mowed 30.5 cm spaced plant field trial at the Rutgers Plant Biology Research Farm in Adelphia, NJ. The soil type was a Freehold sandy loam (fine-loamy, mixed, active, mesic Typic Hapludults). A randomized complete block design was utilized with 4 replicate blocks. Each rep included 20 genotypes from each cross and eight clones of each parent. A one plant border was planted around the perimeter of the trial to minimize any border effects. Plants were fertilized with 10-10-10 (N-P-K) at a rate of 0.84 Kg N ha^{-1} on October 19, 2015 and again on December 6, 2015 with 15.5-0-0 (N-P-K) a rate of 0.56 Kg N ha^{-1} to aid in the establishment. Plots were irrigated daily for the first two weeks to avoid any drought stress during establishment. The following spring the plots were again fertilized with 15.5-0-0 (N-P-K) at a rate of 0.56 Kg N ha^{-1} on April 1, 2016. The plots were then mowed using a rotary mower at 10 cm during the growing season at a frequency of one to two times per week based on amount of plant growth as not to eliminate more than 1/3 of the plant tissue in a single mowing event.

Inoculum was prepared by combining 1500 cc of Kentucky bluegrass seed and 300 ml of water in half size (32.3 x 26.3 x 6.5 cm) aluminum foil catering pans (Sam's West, Inc. Bentonville, AR 72716). The seeds and water were mixed thoroughly to distribute the water and then the pans were covered with heavy aluminum foil. Each pan was autoclaved on a gravity cycle at 20 min sterilization time and 15 minute drying time two separate times allowing each pan to cool to the touch before second autoclave cycle. Once both cycles were completed and pans were at room temperature one 100 x 15 mm PDA petri dish containing mycelium of a single strain of *C. jacksonii* was sliced into approximately 3 mm cubes and added to the pan in a sterile laminar flow hood. Five strains of *C. jacksonii* were chosen to inoculate the tiller plots. Two strains (ADHF1, ADSC1) were isolated from symptomatic fine fescue turf from the Rutgers Plant Biology Research and Extension Farm in Freehold, NJ and the other 3 strains D19, SE16F4, and D15387, from other cool-season turfgrass hosts were obtained from B. B.Clarke. The pans were kept at room temperature and were then shaken every three days to distribute the mycelial growth throughout the pan. After about three weeks the contents of the pans were spread thinly on newspaper and dried. Once dried the inoculum was then sieved in a No. T slotted commercial sieve (Seedburo Equipment Company Chicago, IL 60607) to achieve a uniform size. All isolates were kept separate until ready to use when it was then homogenized and applied using a drop spreader. Grub damage was observed in nearby fields so an application of granular trichlorfon was applied at a rate of 14.65 g m^{-1} on June 15, 2016. Cyazofamid was also applied at 583 g a.i. ha^{-1} to prevent pythium disease on June 15, 2016. On July 5, 2016 a second application of inoculum was applied at 1 g m^{-1} due to humid conditions and heavy morning dew forecasted for the following week. Irrigation was applied mid-morning and late-afternoon to increase the leaf wetness period and encourage disease development. Once uniform disease pressure was throughout the plots, percent disease was rated on a visual scale from 0-100 percent, where 0 equals no disease and 100 equal all of the leaf tissue exhibiting dollar spot lesions. After the disease rating were completed, boscalid was applied at 385 g a.i ha^{-1} to control dollar spot on 25 August 2016. Plants were then fertilized on 23 September 2016 with 20-0-6 (N-P-K) fertilizer at a rate of 0.56 Kg N ha^{-1} and managed to encourage full recovery throughout the fall. The following spring, plots were fertilized wit 20-0-6 at 0.56 Kg N ha^{-1} to aid in the recovery and second year of data collection. On June 28, 2017 the plots were inoculated with the aforementioned dollar spot inoculum at a rate of 1.15 g m^{-1} and managed to encourage disease development by irrigating mid-morning and late-afternoon to increase the leaf wetness period. Percent dollar spot disease ratings were then taken on the visual scale previously mentioned for the second year on August 12, 2016.

Upon completion of the field trial progenies from the endophyte containing maternal lines (R1, R2, and R3) that had more than 20 percent dollar spot infection averaged over the 2 year study were then tested for the presence of the endophyte using a DNA based PCR technique which utilized primers that only amplified *Epichlöe* specific DNA (R. Wang, unpublished data). This was conducted to determine if the susceptibility to dollar spot of the progeny from a resistant mother was due to the endophyte not being transmitted from the maternal parent. To reduce the total number of samples and quickly determine where the endophyte was not present, leaf tissue from up to nine individual plants was pooled into a single tube. In previous testing (data not shown) of the primer set it was determined that as little as 6% E+ tissue in a sample would show a positive test. Since it has been demonstrated that the presence of the endophyte does suppress dollar spot in hard fescue and that the endophyte is vertically transmitted in hard fescue the test for presence of the endophyte was conducted only in progeny from a resistant E+ maternal parent that had greater than 20% dollar spot averaged over the two year study. If a progeny from a resistant E+ mother did not get greater than 20% dollar spot it was assumed the endophyte was inherited by those progeny. Two factors were the reasoning for this; 1) the previously reported relationship of resistance to dollar spot provided by the presence of the fungal endophyte and 2) the results of testing the individual plants when selecting the parents for the study where we found only E+ individual plants from resistant plots and E- individuals from susceptible plots.

All data analysis was conducted using combined averages across all the four replications. The combining ability analysis was done to calculate maternal effects for parent plants based on method 3 which is for one set of parents and their reciprocals and using model 1 used for fixed effects based on (Griffing, 1956).

$$X_{ijk} = u + g_i + g_j + S_{ij} + r_{ij} + 1/bc \sum\sum e_{iikl}$$

Where:

$i,j = 1,......,p$; $k = 1,......., b$; $l = 1, ..., c.$

X_{ijk} = percent dollar spot disease ratings of *ij*-th cross in the *k*-th block, u = population mean, g_i = general combining ability (GCA) effect of the *i*-th parent, g_j = GCA effect of the *j*-th parent, s_{ij} = specific combining ability (SCA) effect for *ij*-th cross, r_{ij} = reciprocal genotypic effect such that $r_{ij} = -r_{ji}$ and e_{ijkl} = error effect peculiar to the ijklth observation. The restrictions are $\sum g_i = 0$, $\sum_{i \neq j} sij = 0$ (for each j). Data was analyzed using DIALLEL-SAS05 (Zhang et al., 2005). Although this analysis computes the combining abilities for parental clones and the heritability estimates, those genetic effects are confounded with the presence of the *E. festucae* fungal endophyte, so it is not truly indicative of the genetic effects of dollar spot resistance in the plants, so that data is not presented. This method, though, does provide estimates for maternal effects of parental clones which is presented below. Significance of the maternal effects were calculated by comparing the progeny means of reciprocal crosses and is accomplished using a two-sample t test using data obtained from a diallel cross (Kitchens, 1998). The maternal effects of the analysis will be informative with the addition of the endophyte presence to better understand the relationship the endophyte has with disease resistance.

RESULTS AND DISCUSSION

Parent and Progeny Response to Dollar Spot

The resistant and susceptible parents used for the diallel crosses had significant differences in the response to dollar spot disease. All three resistant parents had significantly less disease than the susceptible parents. The overall dollar spot percent disease means for the resistant parents were 0.7, 0.5 and 1.6, while the susceptible parents had dollar spot percent disease means 53.5, 79.1, and 47.9 over the two year study (LSD 5.8) Table 2.1. The resistant parents had no statistical differences between them with the average percent disease not being more than 1.6 percent infection for the study. All susceptible parents were statistically different from each other. This demonstrates that there were greater degrees of susceptibility with the S2 parent being the most susceptible averaging 79.1 percent infection. Additionally, it indicates there is some variability in dollar spot resistance that is due to genetics (not just endophytes). The progeny means of all the crosses ranged from 2.8 to 77.2 for percent dollar spot disease in the study. These progeny means fall between the parental means. The disease infection for each year following the inoculation was uniform across the trial. A picture of a resistant parent and susceptible parent with mycelium and infection present planted side by side is shown in Figure 2.1. When you plot the mid-parent means against the progeny means the data points fall into four distinct groups (RxR, RxS, SxR, and SxS) as shown in Figure 2.2.

Maternal and Non-maternal Effects

Maternal effects of dollar spot resistance for the diallel crosses were significant in this study (Table 2.1). The maternal effects of resistant parents reduced disease by 19.6 % (R1), 17.4 % (R2) and 17.5% (R3), while the maternal effects of the susceptible parents significantly increased disease by 18. 2 % (S1), 21.6% (S2) and 14.7% (S3). This indicates that the maternal parent influences dollar spot response in the progeny. Interestingly, S2 had the highest maternal effect and also had the highest percent disease (Table 2.1).

The analysis of the reciprocals is listed in Table 2.1. For all of the resistant by susceptible crosses (where the resistant parent was the mother), the mean percent disease was 11% or below. For the susceptible by resistant crosses (where the susceptible parent was the mother), the mean percent disease was 55% or above (Table 2.1). One cross, R1 x R3, did not have a significant reciprocal effect meaning that each of those maternal parents contributed similarly. The remaining R x R and S x S crosses did have a significant reciprocal effect but their changes on percent disease ranged from 3.3, 3.7, -3.6 and -6.8. Those changes were minimal considering a disease scale from 0-100. One cross, S1xS2 had a significant reciprocal effect and an estimated percent change of -13.1. This can be explained by the large difference in susceptibility of those two parents with S1 having 53.5 percent disease and S2 79.1 percent disease.

Epichloe festucae fungal endophyte test

Individual progeny from a resistant mother that had an average percent disease greater than or equal to 20 for the duration of the study was tested for the presence of the *Epichloe festucae* fungal endophyte. The hypothesis was that these progeny did not have the maternally transmitted endophyte and that the absence of the endophyte was the reason those plants were now susceptible to dollar spot. The results from the endophyte testing showed any individual plant that had a percent disease average over approximately 40 did not have the endophyte present (Table 2.3). The endophyte is maternally transmitted and the strong maternal influence found in the maternal effects analysis strongly indicates that the presence of the endophyte is a major factor contributing to dollar spot resistance. This supports the results found by Clarke et al. (2006) that found the presence of the *E. festucae* fungal endophyte suppresses dollar spot disease in hard fescue. For the pools of individual plants that had between 20 and 40 percent disease, a band presence indicated that one or more of these lines tested positive for the endophyte. Many lines in these pools had one disease rating that was much higher compared to the other two ratings. One particular rating date in 2016 that was somewhat higher that the other rating dates where disease pressure was intense. There could have been localized environmental factors that increased disease pressure that was more than the endophyte could suppress. Future studies could look at inoculum load and environmental conditions to see if there is a point at which the endophyte mediated resistance is decreased from severe disease epidemics.

These results give the Rutgers turfgrass breeding program a better understanding of dollar spot resistance in hard fescue and the important influence that the *E. festucae* fungal endophyte has in that resistance. These results will enable more efficient breeding and selection of material to use in the development of improved dollar spot resistant cultivars. The least expensive, most effective and safest way of controlling a plant disease is the use of resistant cultivars (Agrios, 2005). This endophyte mediated suppression is a key focus of turfgrass breeders to develop resistant cultivars.

REFERENCES

Agrios GN (2005) Plant pathology. 5th edn: Elsevier Acad. Press.

Ambrose KV, Belanger FC (2012) SOLiD-SAGE of endophyte-infected red fescue reveals numerous effects on host transcriptome and an abundance of highly expressed fungal secreted proteins. PloS one 7:e53214

Beard JB (1973) Turfgrass: Science and culture. Englewood Cliffs, NJ: Prentice-Hall, Inc.

Bonos SA, Wilson MM, Meyer WA, Funk CR (2005) Suppression of Red Thread in Fine Fescues Through Endophyte-Mediated Resistance. Applied turfgrass science

Bouton JH, Latch GCM, Hill NS, Hoveland CS, McCann MA, Watson RH, Parish JA, Hawkins LL, Thompson FN (2002) Reinfection of Tall Fescue Cultivars with Non-Ergot Alkaloid–Producing Endophytes. Agron. J. 94:567-574.

Clarke BB, White JF, Hurley RH, Torres MS, Sun S, Huff DR (2006) Endophyte-mediated suppression of dollar spot disease in fine fescues. Plant disease 90:994–998

Clay K (1988) Fungal endophytes of grasses- a defensive mutualism between plants and fungi. Ecological society of america 69:10-16

Florea S, Phillips TD, Panaccione DG, Farman ML, Schardl CL (2016) Chromosome-End Knockoff Strategy to Reshape Alkaloid Profiles of a Fungal Endophyte. Genes genomes genetics 6:2601-2610

Griffing B (1956) Concept of general and specific combining ability in relation to diallel crossing systems. Australian journal of biological sciences 9:463–493

Kitchens LJ (1998) Exploring statistics: A modern introduction to data analysis and inference: Duxbury Press Pacific Grove, CA:

Meyer WA, Funk CR (1989) Progress and benefits to humanity from breeding cool-season grasses for turf. Pages 31–48 *in* Sleper DA, Asay KH, Pedersen JF, eds. Contributions from breeding forage and turf grasses. Madison, WI: Crop Science Society of America

Ruemmele BA, Brilman LA, Huff DR (1995) Fine fescue germplasm diversity and vulnerability. Crop science 35:313–316

Ruemmele BA, Wipff JK, Brilman L, Hignight KW (2003) Fine-leaved Festuca species. Pages 129–174 Turfgrass biology, genetics, and breeding. Hoboken, NJ, USA: John Wiley & Sons, Inc.

Saha DC, Johnsoncicalese JM, Halisky PM, Vanheemstra MI, Funk CR (1987) Occurrence and significance of endophytic fungi in the fine fescues. Plant disease 71:1021–1024

Saikkonen K, Wäli PR, Helander M (2010) Genetic Compatibility Determines Endophyte-Grass Combinations. PloS one 5:e11395

Schardl CL (1996) Epichloë species: fungal symbionts of grasses. Annual review of phytopathology 34:109-130

Tian Z, Wang R, Ambrose KV, Clarke BB, Belanger FC (2017) The Epichloë festucae antifungal protein has activity against the plant pathogen Sclerotinia homoeocarpa, the causal agent of dollar spot disease. Scientific reports 7:5643

Wang J, Burgess P, Bonos SA, Meyer WA, Huang B (2017a) Differential Physiological Responses and Genetic Variations in Fine Fescue Species for Heat and Drought Stress. Journal of the American society for horticultural science 142:367

Wang J, Juliani HR, Jespersen D, Huang B (2017b) Differential profiles of membrane proteins, fatty acids, and sterols associated with genetic variations in heat tolerance for a perennial grass species, hard fescue (Festuca Trachyphylla). Environmental and experimental botany 140:65-75

Zhang Y, Kang MS, Lamkey KRJAJ (2005) Diallel-Sas05. Agron J 97:1097-1106

Table 2.1 Dollar spot disease response of parents and progenies, in a diallel cross between six hard fescue (*Festuca trachyphylla*) parents evaluated in a field trial in 2016 and 2017.

Parent/Cross	Disease Severity (%)	Maternal effect	Standard Error	t Value	Pr > |t|
R1	0.7	-19.6	0.55	-35.8	<.0001
R2	0.5	-17.4	0.54	-32.1	<.0001
R3	1.6	-17.5	0.55	-32.0	<.0001
S1	53.5	18.2	0.54	33.6	<.0001
S2	79.1	21.6	0.55	39.6	<.0001
S3	47.9	14.7	0.54	27.0	<.0001
R1 x R2	2.8	-6.8	1.22	-5.6	<.0001
R2 x R1	15.3				
R1 x R3	8.6	1.9	1.25	1.5	0.1359
R3 x R1	5.9				
R2 x R3	9.8	-3.6	1.22	-3.0	0.003
R3 x R2	16.6				
R1 x S1	2.9	-35.2	1.22	-29.0	<.0001
S1 x R1	68.8				
R1 x S2	14	-32.6	1.22	-26.7	<.0001
S2 x R1	77.2				
R1 x S3	9.5	-25.3	1.22	-20.8	<.0001
S3 x R1	62				
S1 x R2	67.7	32.7	1.22	26.9	<.0001

Parent/Cross	Disease Severity (%)	Maternal effect	Standard Error	t Value	Pr > |t|
R2 x S1	7.1				
S1 x R3	70.2	33.1	1.22	27.2	<.0001
R3 x S1	7.6				
S2 x R2	66.7	25.3	1.22	20.8	<.0001
R2 x S2	17.3				
S2 x R3	69.2	33.5	1.23	27.2	<.0001
R3 x S2	6.6				
S3 x R2	74.7	32.4	1.22	26.7	<.0001
R2 x S3	9.9				
S3 x R3	55.1	22.9	1.22	18.8	<.0001
R3 x S3	11.6				
S1 x S2	35.9	-13.1	1.22	-10.7	<.0001
S2 x S1	59				
S1 x S3	54.5	3.3	1.22	2.7	0.0072
S3 x S1	48				
S2 x S3	65.8	3.7	1.22	3.0	0.0025
S3 x S2	58				
LSD at α =0.05	5.8				

Table 2.2 Analysis of variance for dollar spot resistance in a diallel cross between six hard fescue (*Festuca trachyphylla*) parents evaluated in a field trial in 2016 and 2017.

Source of Variation	DF	Mean Square	
Year	1	11976.18	***
Rep (Year)	6	5436.85	***
Cross	35	113545.91	***
Cross x Year	35	1634.94	***
GCA[1]	5	239020.839	***
SCA[2]	15	10407.219	***
GCA*Year	5	1591.425	*
SCA*Year	15	855.84	
Maternal SS	5	494341.345	***
Non-maternal SS	10	7820.266	***
Maternal*Year	5	4703.101	***
Non-maternal*Year	10	1274.996	***
ERROR	210	559.628	

*Significant at the 0.05 probability level.

***Significant at the 0.001 probability level.

[1] GCA general combining ability.

[2] SCA specific combining ability.

Table 2.3 Test for presence of endophyte in progeny in a diallel cross between six hard fescue (*Festuca trachyphylla*) parents evaluated in a field trial in 2016 and 2017.

Plant	Cross	overall mean	Endophyte	
98-8†	R1 x S2	0.0	E+	†
107-13	R1 x S3	0.0	E+	†
57-9	R2 x R1	0.0	E+	†
55-6	R2 x S1	0.0	E+	†
26-10	R2 x S2	0.0	E+	†
78-17	R2 x S2	0.0	E+	†
46-6	R2 x R3	0.0	E+	†
19-2	R3 x S3	0.0	E+	†
18-1	R3 x R2	0.0	E+	†
104-20	R3 x R2	1.7	E+	†
130-19	R1 x S2	21.7	Band Present indicating E+ presence in sample	‡
13-2	R1 x R2	21.7		‡
75-6	R1 x R2	21.7		‡
75-10	R1 x R2	21.7		‡
15-3	R1 x R3	21.7	Band Present indicating E+ presence in sample	‡
19-9	R3 x S3	21.7		‡
60-8	R3 x R2	21.7		‡
37-4	R1 x R3	23.3		‡
37-9	R1 x R3	23.3		‡
117-8	R2 x S3	23.3		‡
117-13	R2 x S3	23.3		‡
79-5	R3 x S1	23.3		‡
79-11	R3 x S1	23.3		‡
84-11	R3 x S2	23.3	Band Present indicating E+ presence in sample	‡
54-12	R1 x S1	25.0		‡
57-19	R2 x R1	25.0		‡
31-7	R2 x S1	25.0		‡
46-18	R2 x R3	25.0		‡
99-12	R3 x R1	25.0		‡
84-9	R3 x S2	25.0		‡
19-4	R3 x S3	25.0		‡
18-10	R3 x R2	25.0		‡
1-4	R1 x S2	26.7	Band Present indicating E+ presence in sample	‡
30-19	R1 x S3	26.7		‡

Plant	Cross	overall mean	Endophyte	
107-4	R1 x S3	26.7		‡
107-20	R1 x S3	26.7		‡
126-20	R3 x R1	26.7		‡
84-10	R3 x S2	26.7		‡
19-8	R3 x S3	26.7		‡
19-13	R3 x S3	26.7		‡
129-12	R3 x S3	26.7		‡
129-18	R3 x S3	26.7	Band Present indicating E+ presence in sample	‡
77-11	R2 x S3	28.3		‡
18-9	R3 x R2	28.3		‡
107-3	R1 x S3	30.0		‡
37-7	R1 x R3	30.0		‡
78-8	R2 x S2	30.0		‡
24-3	R2 x R3	30.0		‡
46-19	R2 x R3	30.0		‡
20-8	R3 x S1	30.0		‡
104-12	R3 x R2	30.0		‡
119-13	R1 x R3	31.7		‡
57-18	R2 x R1	31.7		‡
55-15	R2 x S1	31.7		‡
38-3	R2 x S3	31.7	Band Present indicating E+ presence in sample	‡
18-18	R3 x R2	31.7		‡
41-4	R1 x S2	33.3		‡
15-9	R1 x R3	33.3		‡
27-10	R2 x R1	33.3		‡
21-20	R2 x S3	33.3		‡
117-1	R2 x S3	33.3		‡
126-5	R3 x R1	33.3		‡
56-14	R3 x S1	33.3		‡
19-12	R3 x S3	33.3	Band Present indicating E+ presence in sample	‡
107-6	R1 x S3	35.0		‡
13-1	R1 x R2	35.0		‡
103-2	R2 x R3	35.0		‡
61-8	R3 x R1	35.0		‡
41-7	R1 x S2	36.7		‡
98-9	R1 x S2	36.7	Band Present indicating E+ presence in sample	‡
107-7	R1 x S3	36.7		‡

Plant	Cross	overall mean	Endophyte	
55-9	R2 x S1	36.7		‡
26-7	R2 x S2	36.7		‡
26-8	R2 x S2	36.7		‡
18-19	R3 x R2	36.7		‡
60-13	R3 x R2	36.7		‡
60-18	R3 x R2	36.7		‡
100-6	R2 x S2	37.5		‡
50-9	R3 x S2	37.5		‡
55-8	R2 x S1	38.3		‡
38-19	R2 x S3	38.3	Band Present indicating E+ presence in sample	‡
90-13	R3 x S3	38.3		‡
18-11	R3 x R2	38.3		‡
61-20	R3 x R1	40.0		‡
18-6	R3 x R2	40.0		‡
60-9	R3 x R2	40.0	E-	‡
110-12	R1 x S1	41.7	E-	‡
107-16	R1 x S3	41.7	E-	‡
15-15	R1 x R3	41.7	E-	‡
46-1	R2 x R3	41.7	E-	‡
19-5	R3 x S3	41.7	E-	‡
110-5	R1 x S1	43.3	E-	‡
107-5	R1 x S3	43.3	E-	‡
119-7	R1 x R3	43.3	E-	‡
127-12	R2 x R1	43.3	E-	‡
121-19	R2 x S1	43.3	E-	‡
38-13	R2 x S3	43.3	E-	‡
102-9	R3 x S2	43.3	E-	‡
78-10	R2 x S2	45.0	E-	‡
46-9	R2 x R3	45.0	E-	‡
89-14	R2 x R3	45.0	E-	‡
102-3	R3 x S2	45.0	E-	‡
127-14	R2 x R1	46.7	E-	‡
55-10	R2 x S1	46.7	E-	‡
81-20	R1 x S3	48.3	E-	‡
107-14	R1 x S3	48.3	E-	‡
55-16	R2 x S1	48.3	E-	‡
18-13	R3 x R2	49.7	E-	‡
15-10	R1 x R3	50.0	E-	‡

Plant	Cross	overall mean	Endophyte	
18-3	R3 x R2	50.0	E-	‡
104-2	R3 x R2	50.0	E-	‡
104-7	R3 x R2	50.0	E-	‡
85-4	R3 x R2	50.0	E-	‡
130-17	R1 x S2	51.7	E-	‡
81-18	R1 x S3	51.7	E-	‡
38-1	R2 x S3	51.7	E-	‡
18-12	R3 x R2	53.0	E-	‡
129-13	R3 x S3	53.3	E-	‡
104-8	R3 x R2	53.3	E-	‡
130-2	R1 x S2	55.0	E-	‡
26-5	R2 x S2	55.0	E-	‡
78-12	R2 x S2	55.0	E-	‡
24-13	R2 x R3	55.0	E-	‡
1-7	R1 x S2	58.3	E-	‡
46-13	R2 x R3	58.3	E-	‡
1-6	R1 x S2	60.0	E-	‡
81-3	R1 x S3	60.0	E-	‡
85-8	R3 x R2	60.0	E-	‡
26-14	R2 x S2	63.0	E-	‡
100-10	R2 x S2	63.3	E-	‡
81-13	R1 x S3	64.7	E-	‡
98-3	R1 x S2	65.0	E-	‡
103-6	R2 x R3	65.0	E-	‡
56-15	R3 x S1	65.0	E-	‡
81-11	R1 x S3	66.3	E-	‡
46-17	R2 x R3	66.7	E-	‡
85-15	R3 x R2	66.7	E-	‡
76-12	R1 x R3	68.3	E-	‡
27-13	R2 x R1	68.3	E-	‡
95-6	R2 x R1	68.3	E-	‡
38-8	R2 x S3	68.3	E-	‡
24-12	R2 x R3	68.3	E-	‡
52-19	R3 x S3	68.3	E-	‡
104-13	R3 x R2	68.3	E-	‡
45-4	R2 x S2	70.0	E-	‡
104-14	R3 x R2	71.7	E-	‡
98-4	R1 x S2	73.3	E-	‡

Plant	Cross	overall mean	Endophyte	
98-5	R1 x S2	73.3	E-	‡
98-12	R1 x S2	73.3	E-	‡
57-5	R2 x R1	73.3	E-	‡
78-11	R2 x S2	73.3	E-	‡
38-16	R2 x S3	74.7	E-	‡
57-16	R2 x R1	75.0	E-	‡
57-17	R2 x R1	75.0	E-	‡
52-20	R3 x S3	75.0	E-	‡
98-6	R1 x S2	76.7	E-	‡
45-7	R2 x S2	76.7	E-	‡
45-9	R2 x S2	76.7	E-	‡
78-2	R2 x S2	76.7	E-	‡
100-9	R2 x S2	76.7	E-	‡
100-16	R2 x S2	76.7	E-	‡
98-14	R1 x S2	78.3	E-	‡
27-8	R2 x R1	78.3	E-	‡
52-11	R3 x S3	78.3	E-	‡
26-9	R2 x S2	79.7	E-	‡
57-20	R2 x R1	81.7	E-	‡
56-7	R3 x S1	81.7	E-	‡
56-9	R3 x S1	81.7	E-	‡
55-5	R2 x S1	83.0	E-	‡
26-20	R2 x S2	83.0	E-	‡
57-10	R2 x R1	83.3	E-	‡
57-11	R2 x R1	83.3	E-	‡
57-12	R2 x R1	85.0	E-	‡
78-1	R2 x S2	88.0	E-	‡

† Randomly selected resistant progeny
‡ Progeny from a resistant E+ maternal parent that averaged more than 20% dollar spot disease.
* Lines separating each group of nine or samples indicate pooled samples. Multiple plants pooled to quickly determine if no endophyte is present

Figure 2.1 Dollar spot mycelium and of hard fescue (*Festuca trachyphylla*)symptoms on a resistant (Left) and susceptible parent (Right) in a 6 parent diallel cross spaced-plant field trial at the Rutgers Plant Biology Research and Extension Farm in Freehold, NJ.

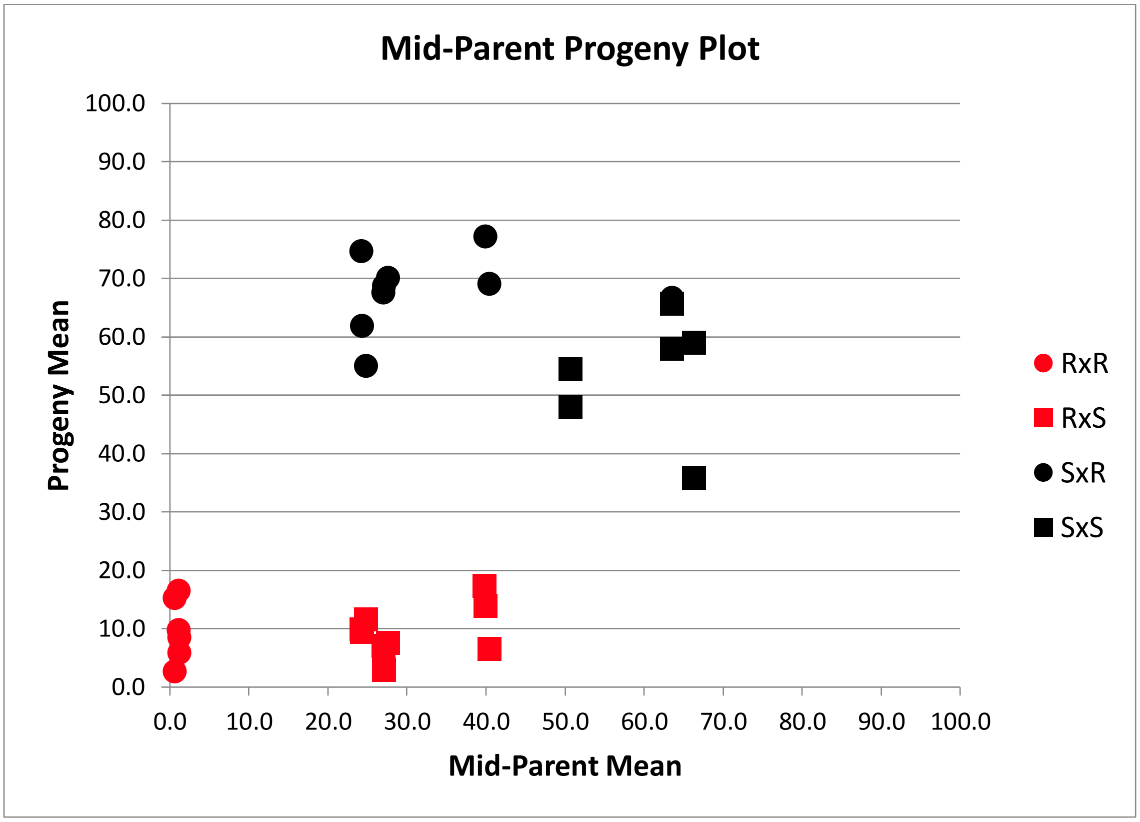

Figure 2.2 Mid-parent-progeny plot of six hard fescue parents crossed in a diallel mating design evaluated for dollar spot resistance in 2016 and 2017.

Chapter 3

Evaluation of mesotrione tolerance levels and ^{14}C-mesotrione absorption and translocation in three fine fescue species

INTRODUCTION

The fine fescues (*Festuca* spp.) are a group of cool-season turfgrasses that are adapted to cool, dry, shaded environments. This group of species are tolerant of infertile, acidic soils and drought conditions (Beard 1973; Hanson and Juska 1969; Turgeon 1996). They also exhibit the better performance under lower fertility levels compared to other cool-season turfgrasses (Ruemmele et al. 2003). These traits make the fine fescues a good choice for low maintenance turf. Fine fescues a good choice for low maintenance turf due the above mentioned traits. The different species of fine fescues are divided into two major groups, the red fescue (*Festuca rubra*) complex and the sheeps fescue (*Festuca ovina*) complex. Within the red fescue complex there are rhizomatous or creeping growt h habits as well as non-rhizomatous or bunch-type growth habits. The sheeps fescue complex contains only non-rhizomatous growth habits. The three species that are most commonly utilized for turfgrass are hard fescue (*Festuca trachyphylla* (Hack.) Hack.), Chewings fescue [*Fesctuca rubra* ssp. *commutata* (Thuill.) Nyman], and strong creeping red fescue (*Festuca rubra* ssp. *rubra* Gaudin). Hard fescue is in the sheeps fescue complex and both Chewings and strong creeping red fescue are in the red fescue complex (Ruemmele et al., 2003). Chewings and hard fescue are both hexaploid (2n = 42) and strong creeping red fescue is an octaploid (2n = 56) (Ruemmele et al.

2003). Weed control during establishment is critical for the planted species to grow without the competition from invasive species (Beard 1973). Currently, there are limited options for control of broadleaf and grassy weeds during establishment for fine fescues. Previous efforts in breeding fine fescues for increased tolerance to herbicides have been successful using a recurrent selection method. Herbicide tolerance development in hard fescue has been demonstrated before with the non-selective herbicide glyphosate. ‘Aurora Gold’ is an advanced-generation synthetic cultivar derived from ‘Aurora’ hard fescue after using five cycles of phenotypic recurrent selection over a 10-yr period following direct applications of glyphosate at 0.8 to 1.6 kg/ha (Hart et al., 2005). A study conducted by McCullough et al. in 2015 determined the mechanism of resistance to glyphosate in ‘Aurora Gold’ hard fescue was due to less target site inhibition. An aminotriazole tolerant Chewings fescue cultivar ‘Countess’ was developed using recurrent selection (Johnston and Faulkner 1986). This provided a selective control of *P. annua* in but unfortunately aminotriazole became a restricted use chemical not long after its release.

Mesotrione is a 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibiting herbicide. It works by inhibiting the HPPD enzyme which converts tyrosine to plastoquinone and α-tocopherol in carotenoid biosynthesis (Beaudegnies et al. 2009). In susceptible species, HPPD inhibition results in damage to cell membranes from free radicals. Visual symptoms are foliar bleaching and tissue necrosis (Lee et al. 1998; McCurdy et al. 2009). Mesotrione provides effective pre- and early postemergence control of many problematic broadleaf and grassy weeds including annual bluegrass (*Poa annua* L.). Mesotrione is currently only labeled for use on mature fine fescue plants (Anonymous, 2008). Mesotrione is utilized at seeding for tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.), and Kentucky bluegrass (*Poa pratensis* L.) with little to no turfgrass injury and control of problematic weeds such as *P. annua* (Askew and Beam 2002; Dernoeden et al. 2008).

The development of fine fescue cultivars with improved tolerance levels to mesotrione could improve weed control during establishment. In addition to current breeding efforts, it will be important to understand the physiological behavior of mesotrione in various fine fescue species to identify the mechanisms associated with enhanced tolerance levels. The objectives of this research were to quantify the differential tolerance levels of three fine fescue species to mesotrione, and determine differences in mesotrione behavior associated with injury potential.

MATERIALS AND METHODS

Plant Material

The same nine individual plants selected, also referred to as lines, due to being identical genotypes which were propagated vegetatively were used in the rate titration and ^{14}C absorption and translocation experiments. This was done to eliminate any effects from genotypic differences in the plant material that would have been present by using different individuals from the same population. Three lines each of Chewings, hard and strong creeping red fescue were used in all experiments. The individual plants were selected from a spaced plant nursery at the Rutgers University turfgrass breeding program research farm in Freehold, NJ 07728. The nursery from which the individual plants were selected contained the progenies of a single generation of breeding for increased tolerance to mesotrione. Plants were selected based on a range of visual injury responses following multiple applications of mesotrione. One plant of each species were selected with no bleaching, moderate bleaching and severe bleaching of foliar tissue. This was done to include a range of injury response for each species. All nine plants were vegetatively propagated by tillers and were maintained for a rate titration study and an absorption and translocation study.

Mesotrione Rate Titration Study

Rate titration experiments were conducted at Rutgers University in New Brunswick, NJ 08901. Nine individual plants of Chewings, hard and strong creeping red fescue were divided into individual tillers and a single tiller was planted in 3.8 cm diameter and 20.5 cm deep “Cone-tainers” (Stuewe and Sons, Inc., Corvallis, OR). Soil was a mixture of sand and peat moss (80:20 v/v). The experiment was conducted in a growth chamber set for 25/15 C (day/night) with a 10 h photoperiod of 400 μmol m^{-2} s^{-1}. Plants were kept in the growth chamber for 3 weeks to acclimate before treated. Irrigation was applied as needed to promote growth and plants were fertigated bi-weekly (MacroN 28-7-14 Sprayable Fertilizer, LESCO Inc., Cleveland, OH 44114). Plants were allowed to reach 7 to 10 tillers before treatment. The treatments for this experiment were eleven rates of mesotrione (0, 17.5, 35, 70, 140, 280, 560, 1121, 2242, 4483, and 8966 g a.i.

ha^{-1}), all of which included 0.25% v/v non-ionic surfactant (Activator 90, Loveland Products, Inc., Greeley, CO 80632). All treatments were applied in a spray chamber set to deliver 260 L ha^{-1}. Cone-tainers were randomized every 2 days to minimize any chamber effects.

Absorption and Translocation Experiment

Absorption and translocation experiments were conducted at the University of Georgia in Griffin, GA 30223. Three plants of each from three fine fescue species (Chewings fescue, hard fescue, and strong creeping red fescue) were established from plugs in the greenhouse. Individual tillers were then transplanted in Cone-tainers with 3.8 cm diameters and 20 cm depths in a greenhouse set for 23/17 C (day/night). Soil was a mixture of sand and peat moss (80:20 v/v). Irrigation was applied as needed to promote growth and pots were fertigated weekly (MacroN 28-7-14 Sprayable Fertilizer, LESCO Inc., Cleveland, OH 44114). Plants were allowed to develop 4 to 7 new tillers, and were selected for treatments based on size and population uniformity.

Root Absorption of ^{14}C-mesotrione

Plants were removed from greenhouse pots, roots were rinsed to remove soil, and plants were grown hydroponically in a 10 L plastic tank filled with a half-strength Hoagland solution (Hoagland and Arnon 1950). Grasses were placed through holes in the plastic lid that facilitated root submergence in the solution. The tank was covered with aluminum foil to shield roots from light and then placed in a growth chamber (Percival Scientific, Inc. 505 Research Drive, Perry, IA 50220) set for 24/14 C (day/night) with a 12 h photoperiod of 350 μmol m^{-2} s^{-1}. An aquarium pump (Shkerry AuqaH, Shanghai Uni-Aqua Co., Ltd, Chang Shou Road, Shanghai 200042, China) was used to provide oxygen to the solution.

After one week, tap water was added to the tank to bring the volume back to 10 L. The tank was then spiked with a total of 83 kBq of ^{14}C-mesotrione plus 1 μM of nonlabeled mesotrione. Plants were harvested at 72 h after treatment (HAT) and roots were blotted dry with paper towels. Roots were separated from shoots with shears and samples were oven-dried for 7 d at 40 C. Samples were then oxidized for 2 min in a biological oxidizer (OX-500, R. J. Harvey Instrument Corp., 11 Jane St., Tappan, NY 10983) and radioactivity was quantified with liquid scintillation spectroscopy (LSC, Beckman LS 6500®, Beckman Coulter Inc., Fall River, MA 02720). Absorption was determined by dividing the radioactivity recovered by sample dry weight. Translocation was determined by dividing the ^{14}C recovered in shoots by the total radioactivity in the plant (roots + shoots).

Foliar Absorption of ^{14}C-mesotrione

Grasses were established with aforementioned materials and methods. Grasses selected for treatments were at a 4 to 7 tiller growth stage and placed in the aforementioned growth chamber. Grasses were acclimated in the growth chamber for 72 h and irrigated as needed to prevent wilting.

Before treatments, the second fully expanded leaf on a selected tiller was covered with Parafilm (Bemis Company Inc., Neenah, WI 54956). A broadcast treatment of mesotrione was then applied at 0.28 kg ha^{-1} with a CO_2-pressured sprayer calibrated to deliver 187 L ha^{-1}. Immediately after the broadcast application, parafilm was removed from the second fully expanded leaf and two 1 μL droplets of ^{14}C-mesotrione were applied at 165 Bq each with a 10 μL syringe. Formulated mesotrione was added to the spotting solution at 1.5 $\mu g\ \mu L^{-1}$ to simulate droplets of spray solution. A nonionic surfactant (Activator 90, Loveland Products, Inc., Greeley, CO 80632) was added to the broadcast treatment and radiolabeled solution at 0.25% v/v to facilitate droplet deposition on the leaf surface. Methods for the foliar absorption and translocation were conducted as was previously described by Yu and McCullough (2016).

Plants (roots + shoots) were harvested at 24 or 96 HAT. The treated leaf was excised from shoots with shears and rinsed in a 20 mL glass scintillation vial with 10 mL of methanol. The base of the leaf was held with forceps and rinsate was applied towards the leaf tip with a 5 mL pipette. This methodology completely removed adsorbed ^{14}C from leaves in pilot experiments. Roots were then separated from shoots with shears and samples were oven-dried at 40 C for 7 d.

Samples were combusted with the aforementioned oxidizer and methods. The entire plant was oxidized from the 24 h harvest. Plant parts (treated leaf, nontreated shoots, and roots) were oxidized separately at the 96 h harvest to quantify translocation of radioactivity. Foliar absorption was quantified by dividing the total radioactivity recovered by the total ^{14}C applied. Translocation was determined by dividing radioactivity recovered in plant parts (treated leaf, nontreated shoots, or roots) from the total radioactivity recovered in the plant. Methanol from leaf rinsate was evaporated from vials in a fume hood, 20 mL of scintillation fluid was then added to vials, and the adsorbed radioactivity was quantified with LSC.

Experimental Design and Data Analysis

The mesotrione rate titration experiment was conducted as randomized complete block design with four replications and was conducted twice. Visual percent injury ratings were taken at 10, 13, 16, and 21 DAT. A log-logistic regression model was fitted to the data and I_{50} values and 95% confidence intervals calculated as outlined by Seefeldt et al. (1995). Foliar and root absorption experiments were conducted as completely randomized designs with five replications, and both experiments were repeated. Data were subjected to analysis of variance with the General Linear Model procedure in SAS (SAS v. 9.3, SAS Institute Inc., Cary, NC 27513). Means were separated with Fisher's Protected LSD test at $\alpha = 0.05$. Experiment by treatment interactions were not dtected, and thus results were pooled over runs.

RESULTS AND DISCUSSION

Rate Titration Experiment

The I_{50} (mesotrione rate that caused 50% injury) and 95% confidence interval was calculated for each line at the 16 DAT rating date (Table 3.1, Fig. 3.1, 3.2, 3.3). This date was selected because the highest injury symptoms detected were on this time point. The hierarchical rank of species for mesotrione tolerance from highest to lowest was: hard fescue > Chewings fescue > strong creeping red fescue.

Differential tolerance levels to mesotrione were detected among lines within each species (Fig. 3.4, 3.5, 3.6). The I_{50} values for hard fescue lines were >8966, 8276 and 6632 g ha^{-1} for H3, H2 and H1, respectively (Table 3.1). This is concurrent with observations made in the field to broadcast applications made to the breeding germplasm nursery in the initial screen and the treatment of the first generation germplasm. There were a greater number of hard fescue plants in the germplasm with less injury compared to Chewings and strong creeping red fescue which had more plants with bleaching injury. The I_{50} values for the Chewings fescue lines were 4329, 3861, and 3106 g ha^{-1} for the C3, C2, and C1, respectively. Strong creeping red fescues had the most injury and the I_{50} values measured 3670, 1507, and 1323 g ha^{-1} for the S2, S3, and S1 lines, respectively. The wide range of I_{50} levels indicated that there was some tolerance tolerance to mesotrione present in the germplasm. Having variation and higher tolerance present in the germplasm is an indication that the level can be increased using recurrent selection based on previous research published increasing fine fescue tolerance to other herbicides.

Absorption and Translocation Experiment

Total recovery in foliar absorption experiments was 94 % (±1.8 SE) of the applied radioactivity. There was no significant effect of line and there was no significant interaction of species by line for foliar absorption (Table 2.). There was also no effect for species, line, or species by line interaction for translocation of the foliar applied ^{14}C-mesotrione herbicide. There was a significant effect of species for absorption at both 24 ($p < 0.0001$) and 96 HAT ($p < 0.0001$). Chewings fescue absorbed the highest percentage of applied ^{14}C-mesotrione compared to hard fescue and strong creeping red fescue. Foliar absorption for all species at 96 HAT was higher than the levels at 24 HAT. For 24 HAT, hard fescue absorbed 3.1%, strong creeping red fescue absorbed 6.1% and Chewings fescue absorbed 14.2% of the applied ^{14}C labeled mesotrione. At 96 HAT, hard fescue absorbed 3.5%, strong creeping red fescue absorbed 6.9% and Chewings fescue absorbed 19.3% of the applied ^{14}C labeled mesotrione herbicide. Low levels of foliar uptake in hard fescue, compared to the other species in this experiment, may be associated with higher tolerance levels to broadcast mesotrione applications observed in the rate titration but there are other factors not tested in this study like metabolism and binding site affinity that need to be evaluated. Low foliar absorption of mesotrione in fine fescue could be associated with leaf surface morphology such as the thin, rolled nature of the leaves which may limit retention of spray droplets but further studies would be needed to determine if the absorption in fine fescues are due to these traits.

There was a significant interaction of species and line ($p = 0.0045$) for root absorption (Table 3). For Chewings fescue, the C1 line (most susceptible) absorbed 38% more ^{14}C labeled mesotrione herbicide than the C2 and C3 lines with greater tolerance levels. Similarly, the S1 lines of strong creeping red fescue recovered 33% more radioactivity (Bq g^{-1}) from root absorption than the more tolerance lies, S2 and S3. The greater absorption of the root applied ^{14}C labeled mesotrione in the most susceptible line of Chewings and strong creeping red fescue do correlate but further studies are needed to determine if the differences in root absorption observed in this study are causing the greater bleaching injury to those individual plants. For hard fescues, differences detected among lines for root absorption had dissimilar trends to tolerance levels noted in the rate titration experiment. Hard fescue generally had the best tolerance levels among the three species and root absorption does not appear to be associated with trends in injury potential for hard fescue based on this data. The main effect of species was significant ($p = 0.0067$) for translocation of root-absorbed radioactivity, while no significant effect of line or interaction of species and line was detected (Table 4). Strong creeping red fescue and Chewings fescue translocated 58 and 56% of absorbed ^{14}C to shoots, respectively, while hard fescue only translocated 44%. Perhaps reductions in acropetal movement of radioactivity from root absorbed ^{14}C-mesotrione in hard fescues are associated with reduced bleaching and injury potential compared to the more susceptible species, Chewings and strong creeping red fescue but further studies into the fate and binding affinity of the herbicide once absorbed into the plants is needed before that can be concluded.

Implications for breeding mesotrione-tolerant fine fescues

In this study the fine fescues had a wide range of tolerance to mesotrione. The hard fescues had I_{50} values that ranged from greater than 16x to 11.8x of the high label rate of 560 g ha^{-1} of mesotrione, the Chewings fescues had I_{50} values that ranged from 7.7x to 5.5x, and strong creeping red fescues had I_{50} values that ranged from 6.5x to 2.4x. This demonstrated that after one generation of breeding for increased tolerance to mesotrione there was a wide range of tolerance levels from in the nine individual plants selected. With the results from this study we were encouraged that we could increase the tolerance of fine fescues to mesotrione using recurrent selection as it had been done with glyphosate in hard fescue (Hart et al., 2005) and aminotriazole in Chewings fescue (Johnston and Faulkner 1986). Less foliar uptake of mesotrione may be associated with enhanced tolerance of hard fescue to broadcast applications compared to Chewings and strong creeping red fescues but further studies are needed to determine the fate and binding affinity of the absorbed herbicide before any conclusions about the mechanism of increased tolerance can be made. Root uptake appears to be less consequential for tolerance levels among species evaluated, but could have a stronger association with injury potential among lines of individual species. Reductions in acropetal movement after root uptake could also be associated with enhanced tolerance levels of fine fescues, such as hard fescue, compared to more susceptible species. Further research is needed to evaluate differential levels of metabolism and target site inhibition of mesotrione in fine fescues.

Recurrent selection and breeding efforts will continue following the testing of these individual plants from the first generation. The overall goal is for tolerance to be increased to a level where mesotrione applications can be made at seeding without reducing or slowing establishment and not causing bleaching injury in the seedlings. Mesotrione tolerant Chewings, hard and strong creeping red fescue cultivars would greatly increase the utility of these grasses because it would provide an option to control *Poa annua* and many other problematic grassy and broadleaf weeds during establishment.

REFERENCES

Anonymous (2008) Tenacity herbicide label. Syngenta Crop Protection, Inc.: Greensboro, NC: 8 p

Askew S, Beam J (2002) Weed management in cool-season turf with mesotrione. Page 129 *in* Proceedings of the Annual Meeting-Northeastern Weed Science Society. Cambridge, MA: Northeastern Weed Science Society

Beard JB (1973) Turfgrass: Science and culture. Englewood Cliffs, NJ: Prentice-Hall, Inc.

Beaudegnies R, Edmunds AJF, Fraser TEM, Hall RG, Hawkes TR, Mitchell G, Schaetzer J, Wendeborn S, Wibley J (2009) Herbicidal 4-hydroxyphenylpyruvate dioxygenase inhibitors—a review of the triketone chemistry story from a Syngenta perspective. Bioorg Med Chem 17:4134–4152

Dernoeden PH, Kaminski JE, Fu JJH (2008) Selective creeping bentgrass control in Kentucky bluegrass and tall fescue with mesotrione and triclopyr ester. HortScience 43: 509-513.

Johnston DT, Faulkner JS (1986) Countess and Duchess-Aminotriazole-tolerant cultivars of Chewings fescue and browntop bent. Journal Sports Turf Research Institute 62:217.

Hart SE, Derr JF, Lycan DW, Rose-Fricker C, Meyer WA (2005) Increased glyphosate tolerance in ‘Aurora Gold’hard fescue (Festuca longifolia). Weed technology 19:640–646

Hanson AA, Juska FV (1969) Turfgrass Science. Madison, Wisconsin: American Society of Agronomy. 715 p

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Journal circular. 2nd edn. Calif Agric Exp Stn 347:32 p

Lee DL, Knudsen CG, Michaely WJ, Chin HL, Nguyen NH, Carter CG, Cromartie TH, Byron HL, Shribbs JM, Fraser T (1998) The structure–activity relationships of the triketone class of HPPD herbicides. Pestic Sci 54:377-384

McCurdy JD, McElroy JS, Kopsell DA, Sams CE (2009) Mesotrione control and pigment concentration of large crabgrass (*Digitaria sanguinalis*) under varying environmental conditions. Pest Manag Sci 65:640-644

McCullough PE, Yu J, Shilling DG, Czarnota MA (2015) Physiological basis for glyphosate tolerance in hard fescue and perennial ryegrass cultivars. Crop Sci. 55:2352-2358

Ruemmele BA, Wipff JK, Brilman L, Hignight KW (2003) Fine-leaved Festuca species. Pages 129-174 *in* Turfgrass biology, genetics, and breeding. Hoboken, NJ: John Wiley & Sons, Inc.

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose response relationships. Weed Technol. 9:218-227

Turgeon AJ (1996) Turfgrass management. 4th ed. Upper Saddle River, NJ: Pretice Hall 406 p

Yu J, McCullough PE (2016) Growth stage influences mesotrione efficacy and fate in two bluegrass (*Poa*) species. Weed Technol. *30*(2), 524-532

Table 3.1 Herbicide concentrations to cause 50% (I_{50}) injury and 95% confidence intervals (CI) for a rate titration from 0-8966 g ai ha^{-1} of mesotrione herbicide on three lines of Chewings fescue, hard fescue, and strong creeping red fescue at 16 days after treatment (DAT) in a growth chamber experiment.

Species	Line		I_{50}	95% CI
			------------g ai ha^{-1}------------	
Chewings fescue	C1	a †	3106	2626 - 3712
	C2	ab	3861	3117 - 4728
	C3	b	4329	3922 - 4763
Hard fescue	H1	a	6632	5533 - 8405
	H2	ab	8276	7389 - >8966
	H3	b	>8966	NA‡
Strong creeping red fescue	S1	a	1323	1085 - 1646
	S2	b	3670	3117 - 4307
	S3	a	1507	1190 - 1926

† Lines within species followed by the same letter are not considered statistically different according to Fisher’s protected LSD at α 0.05.

‡ Unable to calculate 95% CI due to I_{50} being greater than the highest rate in the experiment

Table 3.2 Foliar absorption and translocation of ^{14}C labeled mesotrione herbicide on three lines each of Chewings fescue, hard fescue, and strong creeping red fescue at 24 and 96 hours after treatment (HAT) in a growth chamber experiment.

Species	Absorption		Translocation
	24 HAT	96 HAT	96 HAT
	----------% of ^{14}C applied----------		% of ^{14}C absorbed
Chewings fescue	14.2	19.3	31.9
Hard fescue	3.1	3.5	26.0
Strong creeping red fescue	6.1	6.9	26.7
$LSD_{0.05}$	3.6	4.3	NS
Species	*	*	NS
Line	NS	NS	NS
Species × line	NS	NS	NS

* Indicates a significant difference at α=0.05

NS not significant at $\alpha = 0.05$

Table 3.3 Root absorption of ^{14}C labeled mesotrione herbicide on three lines of Chewings fescue, hard fescue, and strong creeping red fescue in a growth chamber experiment.

Species	Line	Absorption
		Bq/g dry wt
Chewings fescue	C1	202.3
	C2	143.6
	C3	150.0
	$LSD_{0.05}$	49.8
Hard fescue	H1	134.3
	H2	177.24
	H3	146.8
	$LSD_{0.05}$	36.6
Strong creeping red fescue	S1	179.7
	S2	135.3
	S3	134.6
	$LSD_{0.05}$	32.6
	Species	NS
	Line	*
	Species × line	*

* Indicates a significant difference at α=0.05

NS not significant at α = 0.05

Table 3.4 Translocation of root absorbed of ^{14}C labeled mesotrione herbicide on three lines of Chewings fescue, hard fescue, and strong creeping red fescue in a growth chamber experiment.

Species	Translocation
	Percentage of ^{14}C translocated
Chewings fescue	55.5
Hard fescue	43.6
Strong creeping red fescue	57.5
$LSD_{0.05}$	9.1
Species	*
Line	NS
Species × line	NS

* Indicates a significant difference at $\alpha=0.05$

NS not significant at $\alpha = 0.05$

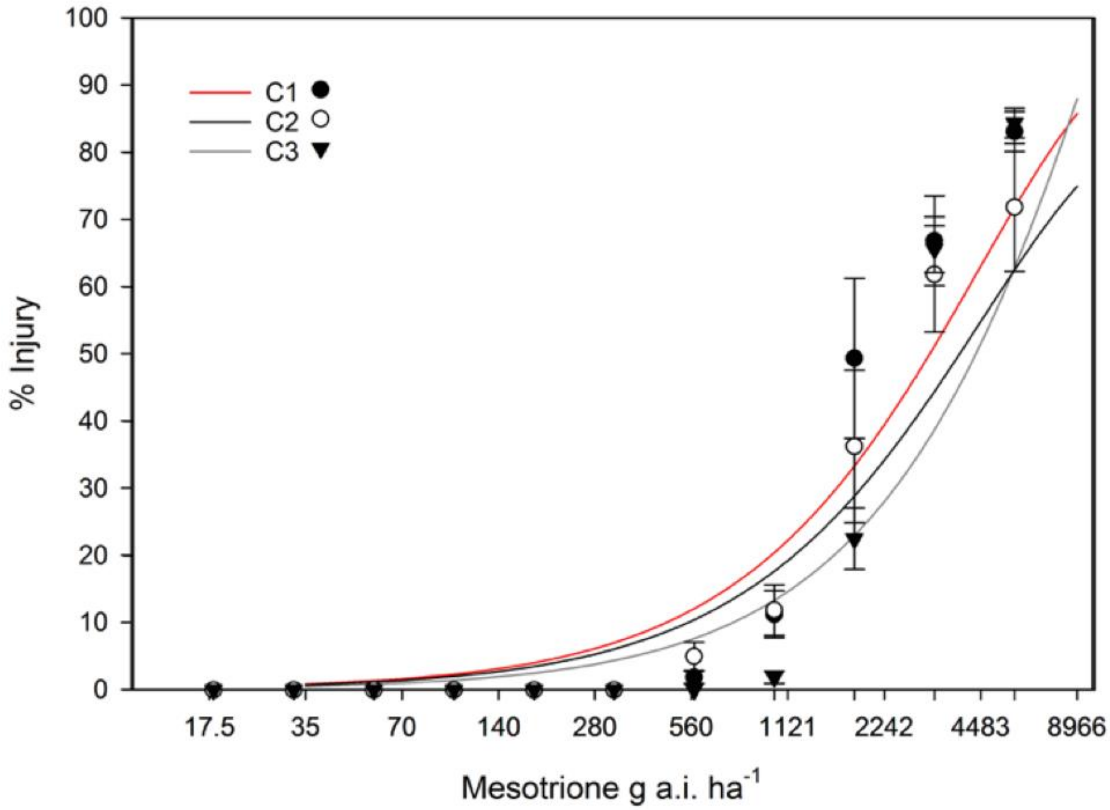

Figure 3.1 Foliar percent injury response curves of three lines (C1, C2, C3) of Chewings fescue (*Festuca rubra* subsp. *commutata*) to eleven rates of mesotrione 16 days after treatment (DAT) in a growth chamber experiment.

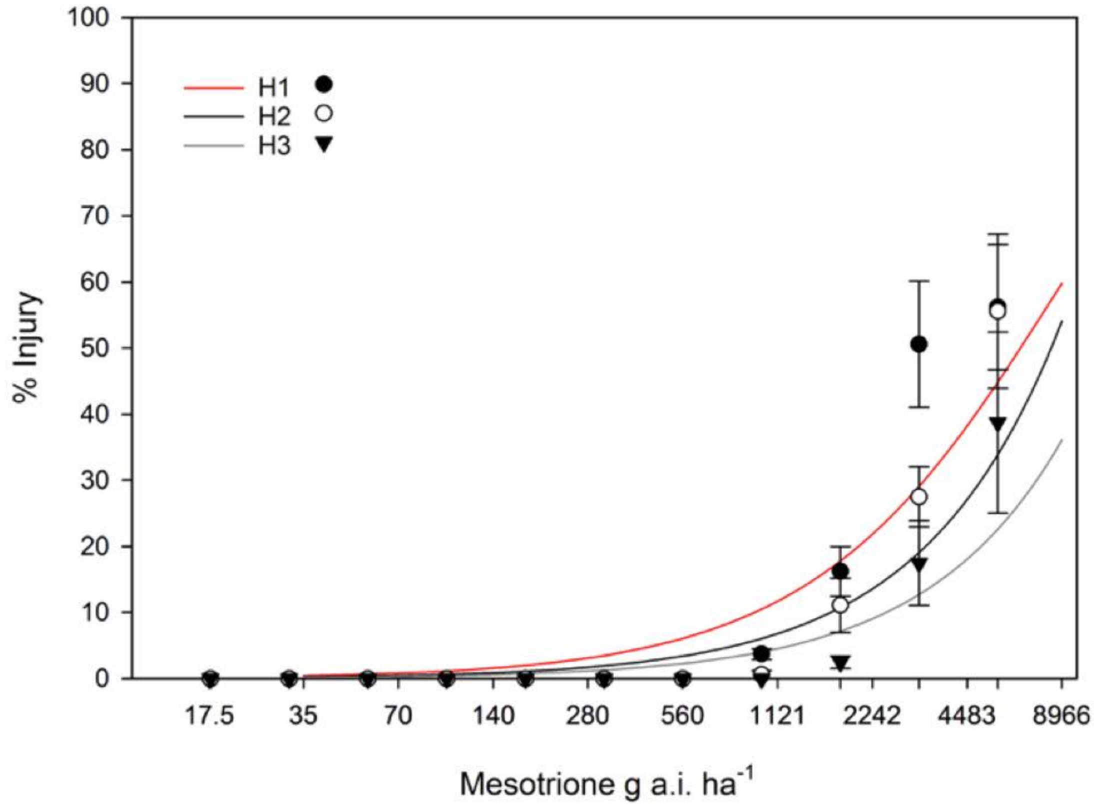

Figure 3.2 Foliar percent injury response curves of three lines (H1, H2, H3) of hard fescue (*Festuca trachyphylla*) to eleven rates of mesotrione 16 days after treatment (DAT) in a growth chamber experiment.

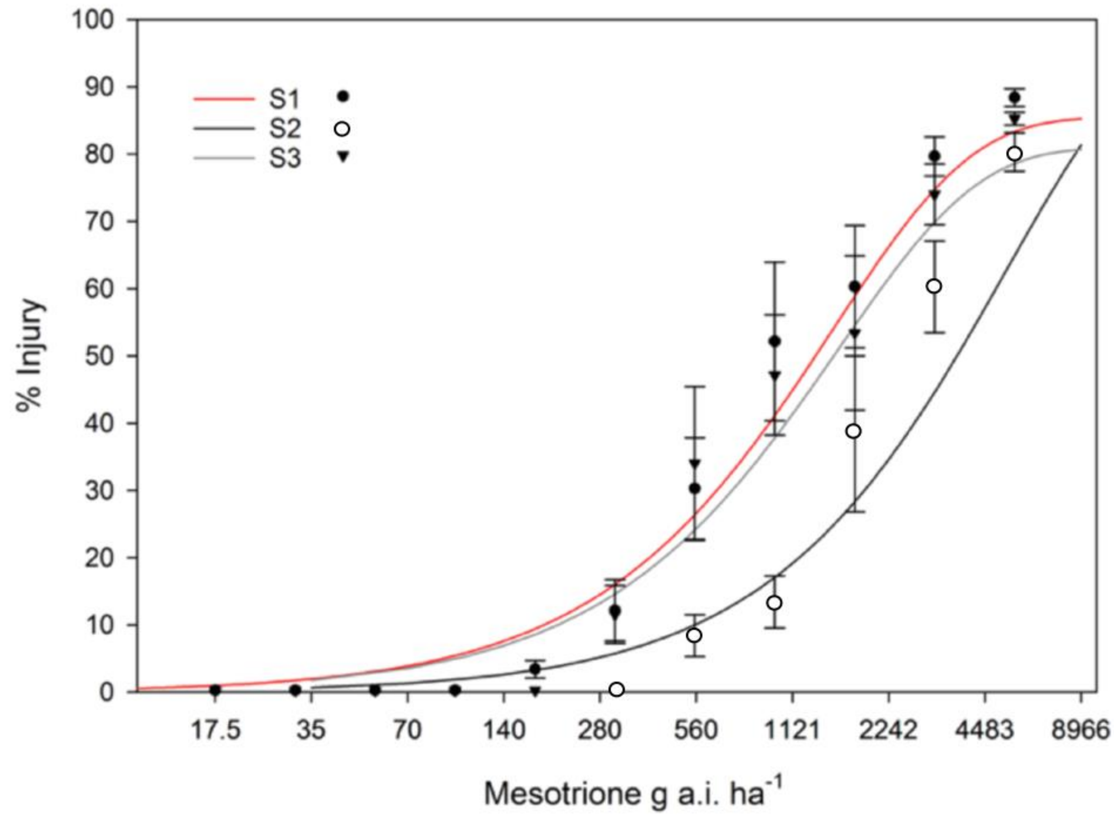

Figure 3.3 Foliar percent injury response curves of three lines (S1, S2, S3) of strong creeping red fescue (*Festuca rubra* subsp. *rubra*) to eleven rates of mesotrione at 16 days after treatment (DAT) in a growth chamber experiment.

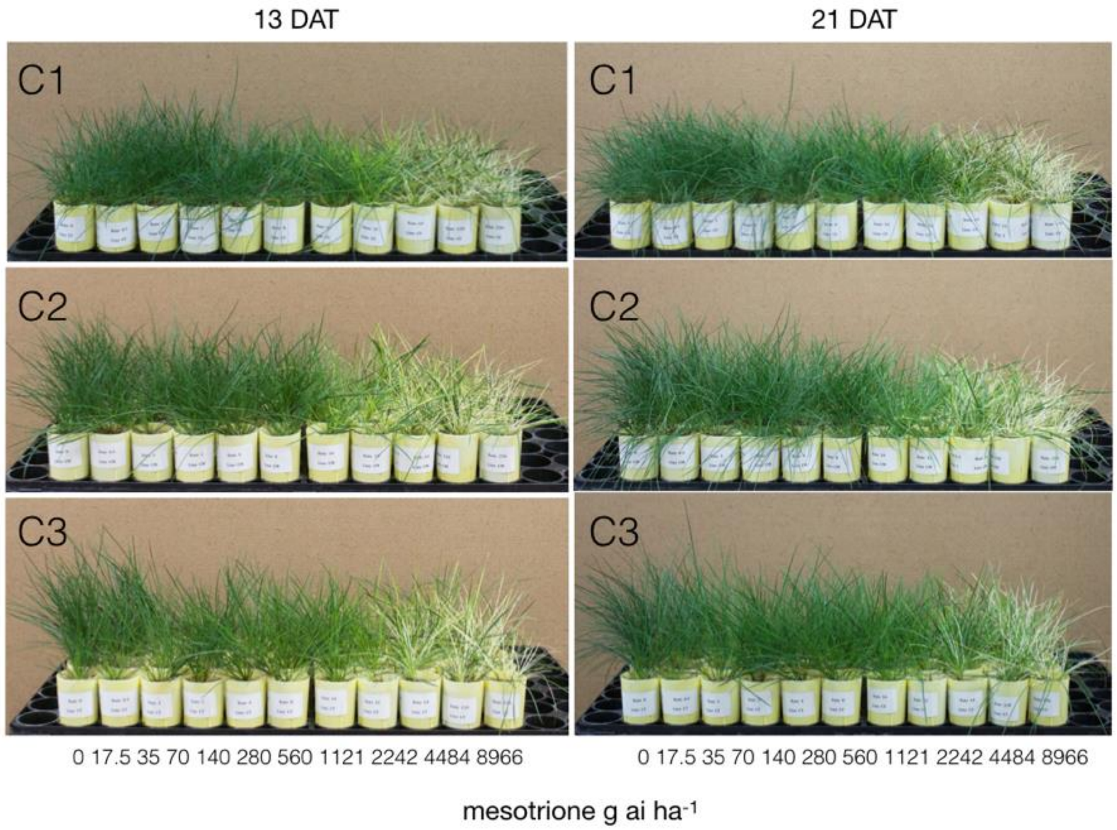

Figure 3.4 Foliar injury symptoms at 13 and 21 days after treatment of three lines (C1, C2, C3) of Chewings fescue (*Festuca rubra* subsp. *commutata*) treated with eleven rates of mesotrione in a growth chamber experiment.

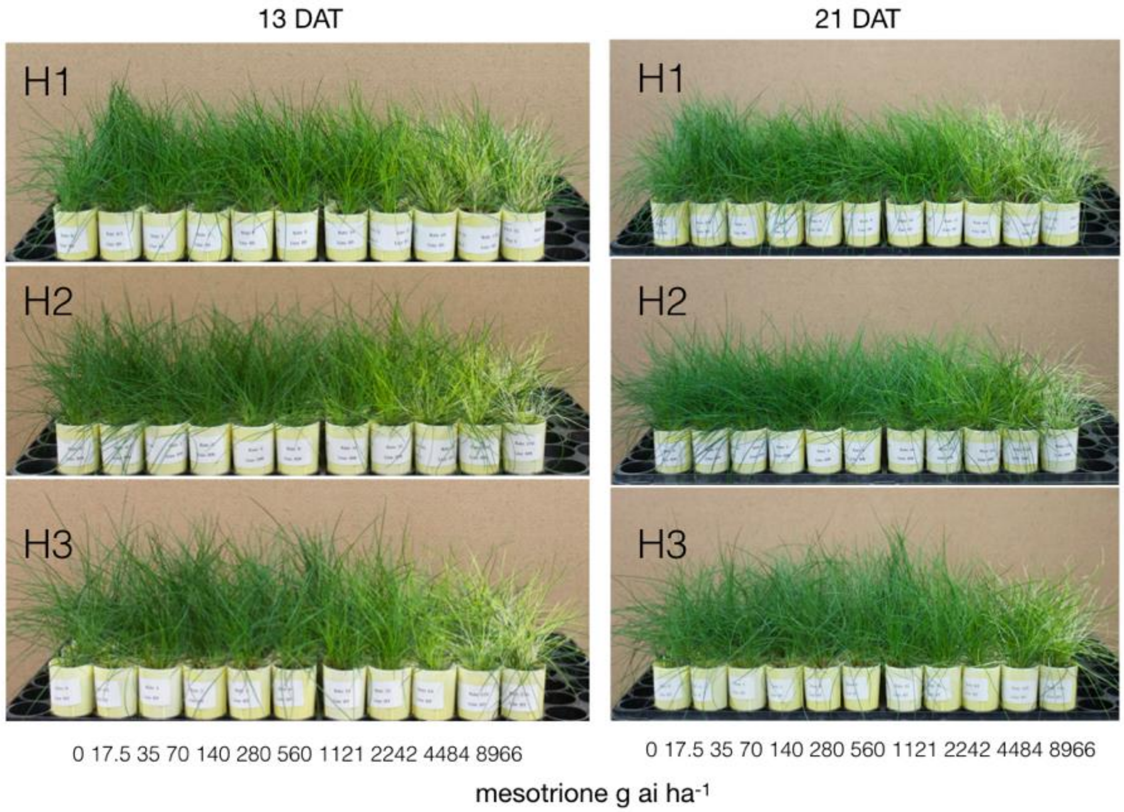

Figure 3.5 Foliar injury symptoms at 13 and 21 days after treatment of three lines (H1, H2, H3) of hard fescue (*Festuca trachyphylla*) treated with eleven rates of mesotrione in a growth chamber experiment.

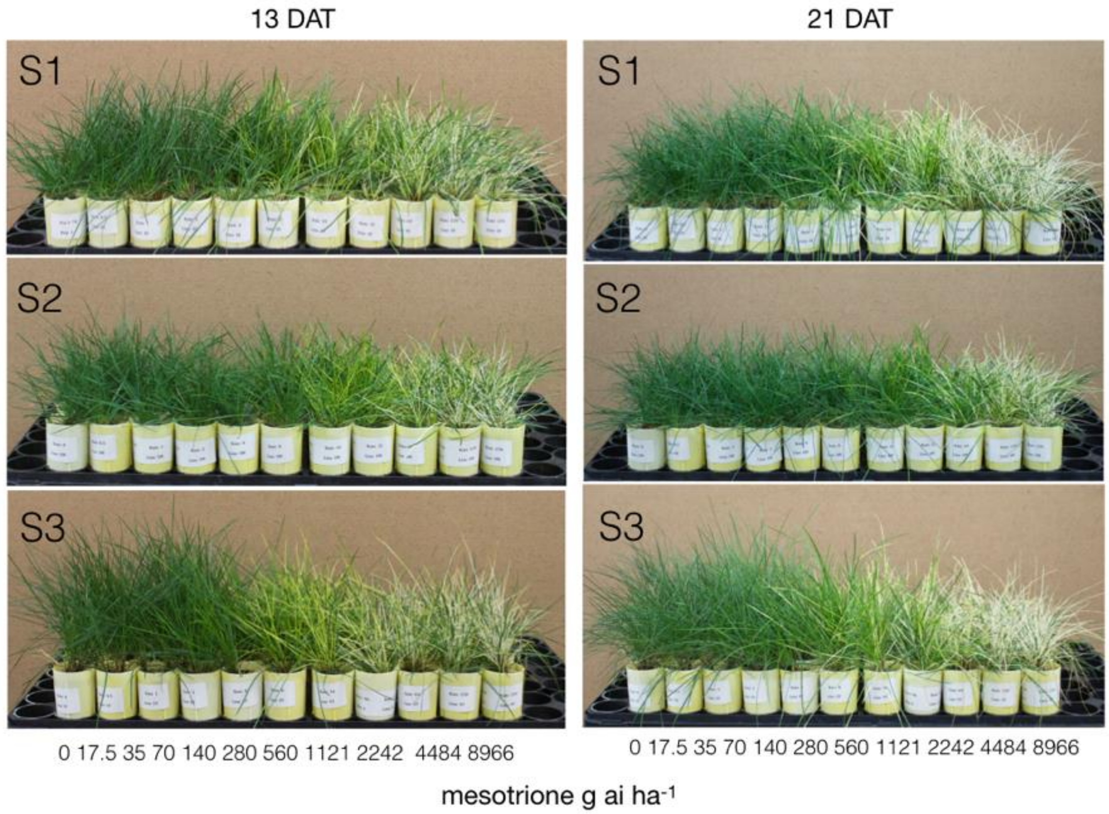

Figure 3.6 Foliar injury symptoms at 13 and 21 days after treatment of three lines (S1, S2, S3) of strong creeping red fescue fescue (*Festuca rubra* subsp. *rubra*) treated with eleven rates of mesotrione in a growth chamber experiment.

Chapter 4

Breeding and Evaluation of Fine Fescues for Increased Tolerance to Mesotrione

INTRODUCTION

The fine fescues (*Festuca* spp.) are a group of cool-season turfgrass species that have needle-like fine leaf texture and are well adapted to cool humid regions of the world. They are adapted to infertile, acidic soils, shade and drought (Beard, 1973; Hanson et al., 1969; Turgeon, 1996). This group of species does well under lower fertility compared to other cool-season grasses (Ruemmele et al., 2003). The fine fescues have been found in a wide range of habitats from beaches, dunes, coastal rock, cliffs, salt marshes, meadows and grasslands (Pavlick, 1985). These traits make them good choices for low maintenance areas (Beard, 1973; Meyer et al., 1989; Turgeon, 1996). Once established these species need few inputs to maintain a good turf stand.

Currently there are very few options for weed control during seeding and establishment for fine fescues. Weed control before and during establishment is an important component to successfully establishing a healthy stand of cool-season turfgrass (Beard, 1973; Musser and Perkins, 1969). Weeds compete for light, water, and nutrients and usually have a much faster establishment and growth rate than fine fescues. The use of a selective pre-emergent control herbicide would allow the fine fescues to establish without the competition and encroachment from weeds. Having safe, selective, pre-emergent control of problematic weeds like *Poa annua* in fine fescues would increase the ability to successfully establish these low maintenance grasses. Mesotrione is an HPPD inhibiting herbicide that selectively controls many monocot and dicot weeds at seeding in many cool-season turfgrasses. Currently mesotrione is labeled for use in many cool-season turfgrasses at the rates of 280-560 g a.i. ha^{-1} It is not currently labeled for use in fine fescues at seeding or for use in seed blends that contain more than 20% fine fescue (Anonymous, 2008). Mesotrione can have several negative impacts on fine fescues. Phototoxicity (bleaching of leaf tissue) is a commonly associated with the use of mesotrione on fine fescue (Williams et al., 2009). This can significantly impact the ability of newly seeded fine fescue areas to establish and survive. In addition, mesotrione has also been shown to reduce germination of fine fescue seeds. The objective of this research was to: 1) utilize a recurrent selection technique (Vogel and Pedersen, 1993) to develop mesotrione tolerant Chewings, hard and strong creeping red fescue and 2) conduct field trials to compare the new selections to commercially available cultivars and experimental germplasm not selected for tolerance to mesotrione.

MATERIALS AND METHODS

First generation

Selections were made from a spaced plant nursery that had been sprayed with three applications of mesotrione at a rate of 560 g a.i. ha-1 + 0.25% v/v non-ionic surfactant (Activator 90, Loveland Products, Inc., Greeley, CO 80632) at four week intervals. Plants that had no bleaching injury response to those applications were noted and those plants the following spring grouped by phenotype and flowering time and moved into crossing blocks prior to anthesis.

In total, 189 plants were moved in May of 2013 into seven different crossing blocks; two hard fescue, three Chewings and two strong creeping red fescue. Individual plants were harvested when seed was mature, dried, threshed and a composite for each block was made using seed from each plant in the block.

A field trial for the first generation of selection were planted September of 2013 and September of 2014 at the Rutgers Plant Biology Research and Extension Station in Adelphia, NJ. The turf trial included a replicated section with each crossing block composite, commercially available cultivars, and experimental germplasm that had not undergone mesotrione selection. A non-replicated section was also included in the 2013 trial which included single plot progenies from each maternal parent. Plots were 0.9 x 1.5 m in size with a 15mm unseeded border and were sown at a rate of 17.9 g m^{-1}. A randomized complete block design was utilized with three replications. The seeding date for each trial was September 9, 2013 and September 18, 2014 respectively. The 2014 trial was a repeat of the 2013 but only included the replicated entries. Applications of mesotrione + 0.25% v/v non-ionic surfactant were made at sowing at a rate of 420 g a.i. ha^{-1} and followed by an application of 280 g a.i. ha^{-1} + 0.25% v/v non-ionic surfactant 28 days after seedling emergence to the entire field trial area. Trials were maintained at a 6.35 cm mowing height with rotary mower to avoid excessive accumulation of clippings. Each trial received 24.4 kg ha^{-1} of N at seeding and received 48.8 kg ha^{-1} of N over two application dates the first year of the trial. Both trials were irrigated to prevent severe drought stresss. Visual ratings were taken for establishment, injury through the first 12 weeks after planting and quality for a two year period after the trial was planted. Each visual rating utilized a 1-9 scale, where 9 = the best establishment, least amount of injury, and highest turf quality, respectively. Data were subjected to analysis of variance with the General Linear Model procedure in SAS (SAS v. 9.3, SAS Institute Inc., Cary, NC 27513). Means were separated with Fisher's Protected LSD test at $\alpha = 0.05$.

Second Generation

Selections were made from plots with the least mesotrione injury and best turf quality ratings in the single plot progeny section of the 2013 trial. Tillers were taken and individual plants planted in a spaced plant nursery and allowed to establish. In total there were 6,840 plants planted in the nursery; 3,456 hard fescues, 2,616 Chewings fescues, and 768 strong creeping red fescues. Four applications of mesotrione at 560 g a.i. ha^{-1} at four week intervals were made followed by an application at 700 g a.i. ha^{-1} two weeks after the previous application. Bleaching injury symptoms began to appear three to five days following the last application so plants having no injury were documented. The following spring plants that exhibited no injury and had good turf quality with no disease were by phenotype and flowering time and moved into crossing blocks prior to anthesis. In total 290 plants were moved into eight individual crossing blocks; two Chewings, four hard; and two strong creeping red fescue. Individual plants were harvested, dried, threshed and a composite for each block was made using seed from each plant in the block.

The second generation (2015) trial included both the replicated section and single plot progeny as describe for the 2013 trial above. The turf trial was seeded on August 26, 2015. In this trial, plots were 1.2 m x 1.8 m plots with a 15mm unseeded border and were sown at a rate of 17.9 g m^{-}1. The larger plots were utilized so herbicide strip treatments could be applied. The strips included a 0.6 m strip of 420 g a.i. ha^{-1} rate of mesotrione and a 0.6 m strip of non-treated control. An example of two plots with each strip is shown in Figure 4.1. Each treatment was applied at sowing and repeated 4 weeks after seedling emergence with a CO_2-pressured sprayer calibrated to deliver 280 L ha^{-1}. A randomized complete block design was used for this study with three replications. Ratings were taken as previously described for injury and turf quality. An additional rating evaluating the negative impact to establishment from the mesotrione treatment was also included. This was a comparison of the mesotrione strip to the non-treated portion of the turf plot. Ratings were on a 1-9 scale, where 9 = no negative effect on establishment from mesotrione and 1 being complete inhibition of establishment. Data were subjected to analysis of variance with the General Linear Model procedure in SAS (SAS v. 9.3, SAS Institute Inc., Cary, NC 27513). Means were separated with Fisher's Protected LSD test at $\alpha = 0.05$.

Third generation

Selections from the single plot progeny section of the 2015 trial were made based on mesotrione-induced injury, effect of mesotrione on establishment, and overall turf quality for the following year. In total, 7,692 individual plants were planted in the fall of 2016; 3,900 hard fescues, 2,112 Chewings fescues, and 1,680 strong creeping red fescues. Two applications of mesotrione at a rate of 840 g a.i. ha-1 + 0.25% v/v non-ionic surfactant at 2 week interval followed by an application of mesotrione at a rate of 1120 g a.i. ha-1 + 0.25% v/v non-ionic surfactant one week following the previous spray. Injury was recorded and plants were selected using the parameters listed above the following spring for use in crossing blocks as previously described. In total, 314 plants were selected to use in 14 individual crossing blocks; five Chewings fescues, five hard fescues and four strong creeping red fescues. The third generation field trial was seeded on 5 September 2018. Plots were 0.9 x 1.5 m is size with a 15 mm unseeded border and were sown at a rate of 17.9 g m^{-1}. A randomized complete block design was utilized with three replications for the replicated entries followed by a block of the single, unreplicated progeny of each maternal line of the crossing blocks. Replicated plots had three 0.5 m wide strip treatments; 0.5 m of a 560 g a.i. ha^{-1} rate of mesotrione + 0.25% v/v non-ionic surfactant, 0.5 m of untreated control and 0.5 m of an 1120 g a.i. ha^{-1} mesotrione rate + 0.25% v/v non-ionic surfactant. Each treatment was applied at sowing and at 4 weeks after seedling emergence with a CO_2-pressured sprayer calibrated to deliver 280 L ha^{-1}. Plots were rated for injury and establishment inhibition as a comparison to the untreated control section of the same plot. The rating was on a 1-9 scale, where 9 = no injury or reduction in establishment and 1 = no establishment or all bleached tissue. Ratings were taken separately for the 560 g a.i. ha^{-1} and 1,120 g a.i. ha^{-1} rate strips. Data were subjected to analysis of variance with the General Linear Model procedure in SAS (SAS v. 9.3, SAS Institute Inc., Cary, NC 27513). Means were separated with Fisher's Protected LSD test at α = 0.05.

RESULTS AND DISCUSSION

First generation field trial

The injury ratings for the first generation trials ranged from 5.8 to 2.0 (LSD = 1.0). Both of the first generation mesotrione selected hard fescue (MEH1 Composite and MEH2 Composite) selections were in the top statistical grouping for having the lowest injury with ratings of 4.5 and 4.3, respectively (Table 4.1). The mesotrione selected selections of Chewings fescue (MEW1 Composite, MEW2 Composite and MEW3 Composite) exhibited light to moderate injury with ratings of 4.8, 5.8, and 5.0 respectively. The mesotrione selected selections of strong creeping red fescue (MES1 Composite and MES2 Composite) exhibited moderate injury with ratings of 3.7 and 3.3 respectively. Each of the mesotrione selected composites had injury ratings that were near the top of the ratings for each of their species group. Establishment for the trial had a range from 5.8 to 1.4 (LSD = 1.0). The two mesotrione selected hard fescues (MEH1 Composite and MEH2 Composite) were somewhat slower to establish therefor the establishment ratings were relatively poor with ratings of 2.8 and 3.0 respectively. The three mesotrione selected Chewings (MEW1 Composite, MEW2 Composite and MEW3 Composite) had good establishment overall with ratings of 5.2, 5.3, and 4.9 respectively. The mesotrione selected strong creeping red fescues

(MES1 Composite and MES2 Composite) had establishment ratings of 3.2 and 3.6 respectively. The strong creeping red fescues did not establish as quickly as that species usually does and it was suspected that the mesotrione applications could have been a factor.

The turf quality was analyzed for each year (Year 1 and Year 2) and overall for both years (Table 4.2). Plots that were poor to establish in general had poor turf quality ratings for the first year but some of those had much better second year turf quality which indicates that there could have been lasting resisdual effects from the mesotrione treatments that needed to be examined in the next generation field trial. Overall the first generation mesotrione selected selections had acceptable turf quality or a turf quality rating that was better than the species average.

Second generation field trial

Overall the performance of the second generation mesotrione selected selections were better than the first generation within each species. The effect on establishment rating ranged from 8.0 to 1.3 (LSD = 1.0) (Table 4.3). The second generation mesotrione selected selections of hard fescue (TEH2 Composite, TEH3 Composite,TEH1 Composite, and TEH4 Composite) had the least effect on establishment by mesotrione and were the top rated in the test with ratings of 8.0, 7.7, 7.7, and 7.3 respectively. This was much higher than the first generation hard fescues which were 6.3 and 6.0. The second generation mesotrione selected Chewings selections had little negative effect from mesotrione at establishment and had better establishment than the two first generation Chewings. The second generation strong creeping red fescues had greatly improved establishment compared to the first generation; TR1 Composite = 6.0 and TR2 Composite = 5.0 compared to the first generation MES2 Composite of 3.3.

The four second generation mesotrione selected selections of hard fescue (TEH4 Composite, TEH2 Composite,TEH3 Composite, and TEH1 Composite) were the least injured in the trial with ratings of 8.2, 8.2, 8.0, and 7.8 respectively (Table 4.3). The mesotrione selected selections of Chewings fescue (TW2 Composite and TW1 Composite) had very little injury with ratings of 7.7 and 7.0 respectively compared to the first generation MEW1 Composite and MEW2 Composite which rated 6.2 and 5.0. All of the second generation hard and Chewings selections were in the top statistical grouping for injury. The second generation mesotrione selected strong creeping red fescues (TR1 Composite and TR2 Composite) were not in the top statistical group but only had moderate injury (6.0 and 4.3) and were better than the first generation strong creeping red fescue MES2 Composite which rated a 4.0.

Turf quality was affected by the mesotrione applications in some of the more sensitive entries so ratings were taken for both the control strips and mesotrione treated strips separately for the first year. The turf quality for the year one non-mesotrione treated control ranged from 2.3 to 5.9 with an LSD of 0.9 (Table 4.4). For the mesotrione treated strip, turf quality ranged from 2.0 to 6.0 with an LSD of 1.1. The mesotrione selected second generation and first generation entries all maintained acceptable turf quality ratings, with all of the second generation entries being in the top statistical group, excluding TR1 Composite which was just 0.1 less than the cutoff for statistical significance.

By the second year there were no visual effects from the mesotrione treatments so whole plots were rated for turf quality (Table 4.4). The turf quality ratings for the second year of the trial ranged from 2.6 to 6.6 with an LSD of 0.9. The top statistical grouping for the second year turf quality ratings included all of the four second generation mesotrione selected selection hard fescues and MEH2 Composite from the first generation entries. Progress was being made increasing the tolerance to mesotrione from the first generation to the second generation while maintaining and in many cases improving the turfgrass quality.

Third generation field trial

The third generation trial included a rating for each of the two mesotrione rates applied and evaluated bleaching injury as well as any negative effect on establishment. There were also tall fescue and Kentucky bluegrass included in this study to compare the mesotrione selected fine fescue material with species which are on the mesotrione label and considered safe to use at seeding. One application of the 1,120 g a.i. ha^{-1} rate is the maximum amount allowed annually on the mesotrione label so the subsequent application at four weeks after seedling emergence would be out of compliance with the label. The range of the injury and effect on establishment rating for the 560 g a.i. ha^{-1} rate was from 1.4 to 9.0 with an LSD of 0.9 (Table 4.5). The top statistical grouping for the 560 g a.i. $ha^{-}1$ rate included all of the third generation entries. The HTB5 Composite was the best performing entry in the trial for injury and effect on establishment; for the 1120 g a.i. $ha^{-}1$ rate, the rating was from 1.0 to 8.3. with an LSD of 1.3. The top statistical grouping of the 1,120 g a.i. $ha^{-}1$ rate included all of the third generation hard fescues, all of the third generation Chewings fescues, and the third generation strong creeping red fescue STB1 Composite which ranked 5^{th} overall in each rating. This was interesting and exciting to see because from generation one to generation two the strong creeping red fescues had the least amount of improvement.

These results demonstrate that utilizing a recurrent selection method is an effective way to increase the tolerance of fine fescues to mesotrione. After three generations, selections of hard, Chewings and strong creeping red fescues had equivalent or better tolerance to mesotione than tall fescues and Kentucky bluegrasses which are on the label for safe use at seeding. These third generation selections will continue to be tested for their turf quality performance and to ensure there are not any long term adverse effects of mesiotrione on these species. If they continue to do well in testing, the third generation selection fine fescues could be safely treated with mesotrione at seeding in the future to establish low maintenance turf free from weed competition during establishment, which is one of the most critical times for weed control.

REFERENCES

Anonymous (2008) Tenacity herbicide label. Pages 8. Greensboro, NC: Syngenta Corp Protection

Beard JB (1973a) Cool season turfgrasses. Pages 54-131 Turfgrass: Science and Culture. Englewood Cliffs, NJ: Prentice-Hall

Hanson AA, Juska FV, Burton GW (1969) Species and Varieties. Pages 715 Turfgrass science. Madison, Wisconsin: American Society of Agronomy

Meyer WA, Funk CR (1989) Progress and benefits to humanity from breeding cool-season grasses for turf. Pages 31–48 *in* Sleper DA, Asay KH, Pedersen JF, eds. Contributions from breeding forage and turf grasses. Madison, WI: Crop Science Society of America

Musser HB, Perkins AT (1969) Guide to Seedbed Preparation. Pages 462-473 *in* Hanson AA, Juska FV, eds. Turfgrass science. Madison, Wisconsin: American Society of Agronomy

Pavlick LE (1985) A new taxonomic survey of the Festuca rubra complex in northwestern North America, with emphasis on British Columbia. Phytologia 57:1–17

Ruemmele BA, Wipff JK, Brilman L, Hignight KW (2003) Fine-leaved Festuca species. Pages 129-174 Turfgrass biology, genetics, and breeding. Hoboken, NJ, USA: John Wiley & Sons, Inc.

Turgeon A (1996) Turfgrass management. Upper Saddle River, NJ: Pretice Hall

Vogel K, Pedersen JF (1993) Breeding systems for cross-pollinated perennial grasses. *in* Janick J, ed. Plant breeding reviews

Williams MW, Johnston WJ, Yenish JP, Miltner ED, Golob CTJITSRJ (2009) Glasshouse evaluation of pre-plant and at planting application of mesotrione on perennial ryegrass and Chewings fescue. 11:1237-1245

Table 4.1 Establishment and mesotrione† injury ratings of first generation mesotrione selected selections, experimental selections and commercially available cultivars of fine fescue in two field trials planted in September 2013 and September 2014 at the Rutgers Plant Biology Research and Extension Station in Adelphia, NJ.

Injury Rank	Entry	Species	Establishment[1]	Injury[2]
1	MEW2 COMP*	Chewings	5.3	5.8
2	Seabreeze GT	slender creeping	4.4	5.2
3	MEW3 COMP*	Chewings	4.9	5.0
4	4-12FF-1	strong creeping	5.8	5.0
5	MEW1 COMP*	Chewings	5.2	4.8
6	Radar	Chewings	4.9	4.8
7	7W4 COMP	Chewings	4.2	4.7
8	4-12FF-2	strong creeping	4.4	4.7
9	MEH1 COMP*	hard	2.8	4.5
10	Daisy	sheeps	2.5	4.5
11	2-10FRR-12	strong creeping	4.2	4.5
12	2-10FRR-13	strong creeping	4.2	4.5
13	5-12FF-5	strong creeping	4.5	4.5
15	PPG-FRC 113	Chewings	4.0	4.3
16	PPG-FRC 115	Chewings	4.6	4.3
17	PPG-FRC 107	Chewings	4.8	4.3
14	MEH2 COMP*	hard	3.0	4.3
18	4-12FF-BULK	strong creeping	5.2	4.3
19	4-12FF-5	strong creeping	5.2	4.3
20	Fairmont	Chewings	4.8	4.2
23	PPG-FRC 114	Chewings	5.0	4.0
25	Shadow II	Chewings	3.9	4.0
26	SR5130	Chewings	4.4	4.0
27	08-5FCE+	Chewings	3.5	4.0
28	Ambrose	Chewings	2.8	4.0
29	7W2 COMP	Chewings	3.8	4.0
21	TE1 COMP	hard	2.3	4.0
22	BM2 COMP	hard	1.9	4.0
24	2-10FRR-8	strong creeping	4.6	4.0
30	PPG-FRR 111	strong creeping	4.5	4.0
31	4-12FF-3	strong creeping	4.9	4.0
36	7W3 COMP	Chewings	3.6	3.8
32	H575 COMP	hard	2.3	3.8

Injury Rank	Entry	Species	Establishment[1]	Injury[2]
33	PSG TH3	hard	3.4	3.8
34	7H3 COMP	hard	2.4	3.8
35	TE2 COMP	hard	1.7	3.8
37	FT6 COMP	strong creeping	2.8	3.8
38	5-12FF-8	strong creeping	4.4	3.8
39	FF2	strong creeping	4.3	3.8
40	4GRP	strong creeping	3.5	3.8
47	Compass	Chewings	4.9	3.7
41	H573 COMP	hard	2.3	3.7
42	Reliant IV	hard	2.3	3.7
43	7H1 COMP	hard	2.8	3.7
44	PPG-FL 107	hard	2.8	3.7
49	Seafire	slender creeping	3.0	3.7
45	MES1 COMP*	strong creeping	3.2	3.7
46	Navigator II	strong creeping	4.4	3.7
48	CRF-11-4A	strong creeping	4.2	3.7
50	Shademaster III	strong creeping	3.2	3.7
51	5-12FF-4	strong creeping	2.7	3.7
54	4SHR-CH	Chewings	4.5	3.5
52	Predator	hard	1.9	3.5
53	Cardinal	strong creeping	4.1	3.5
55	FT2 COMP	strong creeping	3.0	3.5
56	Kent	strong creeping	4.3	3.5
58	7W1 COMP	Chewings	3.9	3.3
57	PPG-FL 106	hard	2.4	3.3
59	7C3 COMP	strong creeping	3.3	3.3
60	2-10FRRBULK	strong creeping	4.1	3.3
61	MES2 COMP*	strong creeping	3.6	3.3
62	SR5250	strong creeping	4.0	3.3
63	OR 126	strong creeping	3.8	3.3
64	PPG-FRR103	strong creeping	4.4	3.3
65	FT1 COMP	strong creeping	3.1	3.3
66	PSG 5RM	strong creeping	2.9	3.3
67	BRSO	strong creeping	4.3	3.3
68	5-12FF-6	strong creeping	3.8	3.3
69	BRSG	strong creeping	4.1	3.3
70	5-12FF-BULK	strong creeping	3.6	3.3
82	PSG 50C3	Chewings	1.7	3.2
85	Windward	Chewings	3.6	3.2
71	7H4 COMP	hard	3.1	3.2
72	PPG-FL 108	hard	3.7	3.2
73	H571 COMP	hard	2.2	3.2
74	7H2 COMP	hard	1.8	3.2

Injury Rank	Entry	Species	Establishment[1]	Injury[2]
76	7H6 COMP	hard	3.1	3.2
77	Soilguard	hard	1.9	3.2
75	Marco Polo	sheeps	3.8	3.2
80	Sealink	slender creeping	3.7	3.2
78	2-10FRR-6	strong creeping	4.3	3.2
79	S571 COMP	strong creeping	3.4	3.2
81	Jasper II	strong creeping	4.2	3.2
83	FT5 COMP	strong creeping	3.0	3.2
84	Gibraltar Gold	strong creeping	3.4	3.2
86	4CRD-8	strong creeping	3.5	3.2
87	Oracle	strong creeping	2.8	3.2
91	4CHY	Chewings	3.6	3.0
89	Oxford	hard	1.4	3.0
88	Blueray	blue hard	2.4	3.0
90	2-10FRR-4	strong creeping	4.2	3.0
92	FT3 COMP	strong creeping	2.5	3.0
93	4CRD-P	strong creeping	3.5	3.0
94	Beacon	hard	3.0	2.8
95	Spartan II	hard	3.5	2.8
96	FRR 62	strong creeping	2.8	2.8
97	Gibraltar	strong creeping	3.6	2.8
98	FT4 COMP	strong creeping	2.5	2.7
99	Miser	strong creeping	2.3	2.5
100	4BND	hard	3.4	2.3
101	Bighorn GT	sheeps	1.9	2.3
102	Azure	sheeps	1.8	2.0
		LSD @ α 0.05	1.0	1.0

* First generation mesotrione selected selection

† Mesotrione applications at sowing (420 g a.i. ha^{-1} + 0.25% v/v non-ionic surfactant) and four weeks after seedling emergence (280 g a.i. ha^{-1} + 0.25% v/v non-ionic surfactant)

[1] Rated on 1-9 scale with 9 = no reduction in establishment and 1= complete inhibition of establishment

[2] Rated on 1-9 scale with 9 = no injury and 1= completely bleached tissue

Table 4.2 Two year overall and individual turfgrass quality ratings of first generation mesotrione selected selections, experimental selections and commercially available cultivars of fine fescue in two field trials planted in 2013 and 2014 at the Rutgers Plant Biology Research and Extension Station in Adelphia, NJ.

Rank	Entry	Species	Overall Quality ‡	Year 1 Quality ‡	Year 2 Quality ‡
1	PSG TH3	hard	5.7	5.5	5.9
2	H575 Composite	hard	5.5	5.1	6.1
3	MEH1 Composite*	hard	5.5	5.4	5.6
4	PPG-FL 108	hard	5.5	5.3	5.7
5	MEH2 Composite*	hard	5.4	5.3	5.6
6	7H1 Composite	hard	5.4	5.3	5.6
7	BM2 Composite	hard	5.4	5.2	5.7
8	7H4 Composite	hard	5.3	4.9	5.8
9	TE1 Composite	hard	5.3	4.9	5.8
10	7H3 Composite	hard	5.3	4.9	5.8
11	4BND	hard	5.3	4.9	5.7
12	H573 Composite	hard	5.2	4.7	5.8
13	PPG-FL 107	hard	5.2	4.9	5.5
14	Reliant IV	hard	5.2	4.8	5.7
15	MEW1 Composite*	Chewings	5.2	5.8	4.4
16	PPG-FL 106	hard	5.2	4.7	5.8
17	TE2 Composite	hard	5.1	4.7	5.7
18	Marco Polo	sheeps	5.1	5.1	5.2
19	Beacon	hard	5.1	4.6	5.7
20	Spartan II	hard	5.1	4.8	5.5
21	Blueray	blue hard	5.1	4.7	5.5
22	7H2 Composite	hard	5.0	4.6	5.6
23	MEW2 Composite*	Chewings	5.0	5.5	4.4
24	Predator	hard	5.0	4.4	5.7
25	H571 Composite	hard	4.9	4.3	5.6
26	MEW3 Composite*	Chewings	4.9	5.2	4.4
27	Radar	Chewings	4.8	5.2	4.4
28	7H6 Composite	hard	4.8	4.6	5.1
29	Soilguard	hard	4.6	4.2	5.0

Rank	Entry	Species	Overall Quality ‡	Year 1 Quality ‡	Year 2 Quality ‡
30	Oxford	hard	4.6	4.0	5.3
31	Bighorn GT	sheeps	4.6	4.3	5.0
32	PPG-FRC 114	Chewings	4.5	4.8	4.3
33	PPG-FRC 115	Chewings	4.5	4.8	4.2
34	7W3 Composite	Chewings	4.5	4.7	4.2
35	7W1 Composite	Chewings	4.5	4.7	4.1
36	Fairmont	Chewings	4.4	4.7	4.2
37	PPG-FRC 113	Chewings	4.4	4.5	4.3
38	PPG-FRC 107	Chewings	4.4	4.8	4.0
39	7W4 Composite	Chewings	4.3	4.8	3.8
40	Shadow II	Chewings	4.3	4.5	4.2
41	SR5130	Chewings	4.2	4.4	4.1
42	Sealink	slender creeping	4.2	4.7	3.7
43	2-10FRR-8	strong creeping	4.2	4.2	4.2
44	Seabreeze GT	slender creeping	4.1	4.6	3.5
45	08-5FCE+	Chewings	4.0	4.2	3.8
46	Compass	Chewings	4.0	4.4	3.7
47	Daisy	sheeps	4.0	3.8	4.3
48	Ambrose	Chewings	4.0	4.2	3.8
49	7W2 Composite	Chewings	4.0	4.3	3.6
50	4SHR-CH	Chewings	4.0	4.3	3.7
51	MES1 COMP*	strong creeping	4.0	3.8	4.2
52	2-10FRRBulk	strong creeping	4.0	4.0	3.9
53	2-10FRR-6	strong creeping	3.9	3.8	4.2
54	4CHY	Chewings	3.9	4.2	3.7
55	MES2 Composite*	strong creeping	3.8	3.8	3.9
56	Azure	sheeps	3.8	3.5	4.2
57	2-10FRR-12	strong creeping	3.8	3.7	3.9
58	2-10FRR-4	strong creeping	3.8	3.7	4.0
59	S571 Composite	strong creeping	3.8	3.7	3.9
60	Navigator II	strong creeping	3.8	3.8	3.7
61	SR5250	strong creeping	3.7	3.7	3.8
62	7C3 Composite	strong creeping	3.7	3.5	3.9
63	2-10FRR-13	strong creeping	3.7	3.7	3.8
64	PPG-FRR 111	strong creeping	3.7	3.8	3.6
65	Cardinal	strong creeping	3.7	3.7	3.7
66	Jasper II	strong creeping	3.7	3.7	3.7

Rank	Entry	Species	Overall Quality ‡	Year 1 Quality ‡	Year 2 Quality ‡
67	Seafire	slender creeping	3.7	3.9	3.4
68	OR 126	strong creeping	3.7	3.7	3.6
69	CRF-11-4A	strong creeping	3.6	3.7	3.4
70	PPG-FRR103	strong creeping	3.6	3.6	3.5
71	4-12FF-Bulk	strong creeping	3.5	3.9	3.2
72	FRR 62	strong creeping	3.5	3.4	3.7
73	PSG 50C3	Chewings	3.5	3.4	3.6
74	Windward	Chewings	3.4	3.6	3.3
75	Kent	strong creeping	3.4	3.6	3.2
76	FT5 Composite	strong creeping	3.4	3.3	3.6
77	4-12FF-3	strong creeping	3.4	3.7	3.1
78	5-12FF-8	strong creeping	3.4	3.6	3.2
79	Shademaster III	strong creeping	3.4	3.4	3.4
80	FT2 Composite	strong creeping	3.4	3.4	3.5
81	BRSG	strong creeping	3.4	3.6	3.2
82	BRSO	strong creeping	3.4	3.5	3.3
83	Gibraltar	strong creeping	3.4	3.4	3.3
84	FF2	strong creeping	3.3	3.5	3.2
85	PSG 5RM	strong creeping	3.3	3.3	3.4
86	4-12FF-1	strong creeping	3.3	3.6	3.0
87	FT6 Composite	strong creeping	3.3	3.2	3.5
88	FT4 Composite	strong creeping	3.3	3.2	3.5
89	Miser	strong creeping	3.3	3.2	3.5
90	FT3 Composite	strong creeping	3.3	3.0	3.6
91	4-12FF-2	strong creeping	3.3	3.6	2.9
92	4-12FF-5	strong creeping	3.3	3.6	2.9
93	Gibraltar Gold	strong creeping	3.2	3.2	3.3
94	4CRD-8	strong creeping	3.2	3.3	3.1
95	4CRD-P	strong creeping	3.2	3.2	3.3
96	5-12FF-6	strong creeping	3.2	3.1	3.3
97	FT1 Composite	strong creeping	3.2	3.0	3.4
98	5-12FF-5	strong creeping	3.2	3.4	2.9
99	Oracle	strong creeping	3.0	3.0	3.0
100	4GRP	strong creeping	3.0	3.0	3.0
101	5-12FF-Bulk	strong creeping	3.0	2.9	3.1
102	5-12FF-4	strong creeping	2.4	2.2	2.8
103	7 Seas	Chewings	1.4	1.3	1.5

Rank	Entry	Species	Overall Quality ‡	Year 1 Quality ‡	Year 2 Quality ‡
		LSD @ α 0.05	0.5	0.7	0.5

* First generation mesotrione selected selection

† Mesotrione applications at sowing (420 g a.i. $ha^{-}1$ + 0.25% v/v non-ionic surfactant) and four weeks after seedling emergence (280 g a.i. $ha^{-}1$ + 0.25% v/v non-ionic surfactant)

‡ Quality rated on a 1-9 scale with 9 = best turfgrass quality

Table 4.3 The mesotrione effect on establishment and mesotrione injury ratings of first and second generation mesotrione selected selections, experimental selections and commercially available cultivars of fine fescue in a field trial planted in 2015 at the Rutgers Plant Biology Research and Extension Station in Adelphia, NJ.

Rank	Entry	Species	Effect on Establishment † 1	Injury Average † 2
1	TEH2 Composite**	hard	8.0	8.2
2	TEH4 Composite**	hard	7.3	8.2
3	TEH3 Composite**	hard	7.7	8.0
4	TEH1 Composite**	hard	7.7	7.8
5	TW2 Composite**	Chewings	7.0	7.7
6	Minimus	hard	5.7	7.2
7	TW1 Composite**	Chewings	5.3	7.0
8	MEH1 Composite*	hard	6.3	6.2
9	Chariot	hard	5.3	6.2
10	Firefly	hard	4.0	6.2
11	MEW1 Composite*	Chewings	7.0	6.2
12	Sword	hard	5.0	6.0
13	TR1 Composite**	strong creeping	6.0	6.0
14	MEH2 Composite*	hard	6.0	5.5
15	FH2 Composite	hard	4.7	5.3
16	FH3 Composite	hard	5.3	5.3
17	Beacon	hard	4.0	5.3
18	Radar	Chewings	5.0	5.3
19	MEW2 Composite*	Chewings	7.3	5.0
20	FH4 Composite	hard	4.7	4.8
21	PPG-FRC 113	Chewings	4.7	4.8
22	Compass	Chewings	4.3	4.7
23	Blueray	blue hard	4.3	4.3
24	TR2 Composite**	strong creeping	5.0	4.3
25	FW2 Composite	Chewings	4.7	4.2
26	FR3 Composite	strong creeping	4.0	4.2
27	FH1 Composite	hard	3.7	4.0
28	PPG-FO 102	sheeps	3.0	3.2
29	Ambrose	Chewings	2.3	3.2

Rank	Entry	Species	Effect on Establishment † [1]	Injury Average † [2]
30	Marvel	strong creeping	3.0	3.2
31	Lighthouse	slender creeping	3.0	3.0
32	PPG-FRT 101	slender creeping	3.7	3.0
33	PPG-FRR 111	strong creeping	3.0	3.0
34	Garnet	strong creeping	2.3	2.8
35	FR1 Composite	strong creeping	2.3	2.5
36	FR2 Composite	strong creeping	2.0	2.5
37	Navigator II	strong creeping	2.3	2.5
38	Pathfinder	strong creeping	2.7	2.5
39	FR4 Composite	strong creeping	2.3	2.3
40	Cardinal	strong creeping	2.7	2.3
41	MES2 Composite*	strong creeping	3.3	2.0
42	SR5250	strong creeping	1.7	1.8
43	Predator	hard	2.3	1.5
44	PPG-FL 106	hard	2.0	1.3
45	Audubon	strong creeping	1.3	1.2
46	Reliant IV	hard	1.7	1.0
47	Rescue 911	hard	1.3	1.0
48	Azure	blue	1.3	1.0
		LSD @ α 0.05	1.0	2.1

* First generation mesotrione selected selection

** Second generation mesotrione selected selection

† 420 g a.i. ha^{-1} rate applications at sowing and four weeks after seedling emergence + 0.25% v/v non-ionic surfactant

[1] Rated on 1-9 scale with 9 = no reduction in establishment and 1= complete inhibition of establishment

[2] Rated on 1-9 scale with 9 = no injury and 1= completely bleached tissue

Table 4.4 Turf quality ratings of first and second generation mesotrione selected selections, commercially available cultivars and experimental selections in a field trial planted in September 2015 at the Rutgers Plant Biology Research and Extension Station in Adelphia, NJ.

	Entry	Species	2016 Quality mesotrione strip † ‡	2016 Quality untreated strip ‡	2017 Quality ‡
1	TEH2 Composite**	hard	5.7	5.6	6.3
2	TEH4 Composite**	hard	5.8	5.7	6.4
3	TEH3 Composite**	hard	5.9	5.8	6.5
4	TEH1 Composite**	hard	6.0	5.9	6.1
5	TW2 Composite**	Chewings	5.2	5.2	4.8
6	Minimus	hard	5.0	5.0	5.0
7	TW1 Composite**	Chewings	5.2	5.1	4.6
8	MEH1 Composite*	hard	5.5	5.4	5.4
9	Chariot	hard	5.1	5.0	5.2
10	Firefly	hard	4.7	4.7	5.4
11	MEW1 Composite*	Chewings	5.5	5.4	4.8
12	Sword	hard	4.9	4.9	5.0
13	TR1 Composite**	strong creeping	5.0	4.9	3.7
14	MEH2 Composite*	hard	5.7	5.9	6.3
15	FH2 Composite	hard	4.8	5.0	5.8
16	FH3 Composite	hard	4.5	4.8	5.3
17	Beacon	hard	4.9	5.0	5.3
18	Radar	Chewings	4.7	4.6	4.4
19	MEW2 Composite*	Chewings	5.3	5.1	4.5
20	FH4 Composite	hard	5.3	5.2	6.1
21	PPG-FRC 113	Chewings	5.0	4.9	4.8
22	Compass	Chewings	4.1	4.1	3.6
23	Blueray	blue hard	4.0	4.1	3.9
24	TR2 Composite**	strong creeping	5.5	5.4	4.1
25	FW2 Composite	Chewings	5.5	5.4	5.0
26	FR3 Composite	strong creeping	4.7	4.8	4.4
27	FH1 Composite	hard	5.3	5.4	6.6
28	PPG-FO 102	sheeps	3.2	3.3	3.6

	Entry	Species	2016 Quality mesotrione strip † ‡	2016 Quality untreated strip ‡	2017 Quality ‡
29	Ambrose	Chewings	4.1	4.2	3.7
30	Marvel	strong creeping	3.6	3.9	3.1
31	Lighthouse	slender creeping	2.7	2.6	2.6
32	PPG-FRT 101	slender creeping	4.8	5.0	5.0
33	PPG-FRR 111	strong creeping	4.3	4.2	3.8
34	Garnet	strong creeping	2.6	3.4	3.0
35	FR1 Composite	strong creeping	4.1	4.4	4.8
36	FR2 Composite	strong creeping	4.4	4.9	4.7
37	Navigator II	strong creeping	3.4	4.0	3.3
38	Pathfinder	strong creeping	3.1	3.4	3.1
39	FR4 Composite	strong creeping	4.6	4.8	4.8
40	Cardinal	strong creeping	3.4	3.7	3.2
41	MES2 Composite*	strong creeping	4.8	5.2	3.7
42	SR5250	strong creeping	2.5	3.2	2.9
43	Predator	hard	3.8	4.1	5.2
44	PPG-FL 106	hard	2.6	3.5	4.6
45	Audubon	strong creeping	2.0	3.3	3.3
46	Reliant IV	hard	3.4	3.9	5.5
47	Rescue 911	hard	2.0	2.3	3.1
48	Azure	blue	2.0	2.7	3.5
		LSD @ α 0.05	1.1	0.9	0.9

* First generation mesotrione selected selection

** Second generation mesotrione selected selection

† 420 g a.i. ha^{-1} rate applications at sowing and four weeks after seedling emergence + 0.25% v/v non-ionic surfactant

‡ Quality rated on a 1-9 scale with 9 = best turfgrass quality

Table 4.5 Injury ratings of first, second, and third generation mesotrione selected selections, experimental selections, and commercially available cultivars of fine fescues, tall fescues and Kentucky bluegrasses in a field trial planted in September 2018 at the Rutgers Plant Biology Research and Extension Station in Adelphia, NJ.

Rank	Cultivar	Species	560 g a.i. ha^{-1} † ‡	1120 g a.i. ha^{-1} † ‡
1	HTB5 Composite***	hard	9.0	8.3
2	HTB4 Composite***	hard	8.9	8.3
3	TEH3 Composite**	hard	8.9	8.3
4	TEH2 Composite**	hard	9.0	8.1
5	STB1 Composite***	strong creeping	8.8	8.1
6	WTBT1 Composite***	Chewings	8.9	7.9
7	WTB2 Composite***	Chewings	8.9	7.9
8	Padre II	tall fescue	8.5	7.9
9	WTBT3 Composite***	Chewings	8.9	7.7
10	WTU5 Composite***	Chewings	8.9	7.7
11	Talladega	tall fescue	8.3	7.6
12	Bordeaux	Kentucky bluegrass	8.3	7.5
13	HTN3 Composite***	hard	8.9	7.5
14	STU2 Composite***	strong creeping	8.5	7.5
15	HTB2 Composite***	hard	8.7	7.4
16	WTB4 Composite***	Chewings	8.6	7.3
17	Champagne	Kentucky bluegrass	8.1	7.1
18	HTB1 Composite***	hard	8.7	7.1
19	Gladiator	hard	8.7	7.0
20	Hot Rod	tall fescue	8.5	7.0
21	TW2 Composite**	Chewings	8.3	6.9
22	Sword	hard	8.3	6.7
23	STB3 Composite***	strong creeping	8.3	6.7
24	TR1 Composite**	strong creeping	7.9	6.7
25	STN4 Composite***	strong creeping	8.5	6.6
26	Blue Note	Kentucky bluegrass	7.5	6.5
27	Windward	Chewings	8.0	6.5
28	Ambrose	Chewings	8.1	6.3
29	Marvel	strong creeping	7.3	5.5
30	MEW1 Composite*	Chewings	6.9	4.4

Rank	Cultivar	Species	560 g a.i. ha^{-1} † ‡	1120 g a.i. ha^{-1} † ‡
31	Fairmont	Chewings	7.6	4.3
32	Navigator II	strong creeping	6.8	4.2
33	MEH2 Composite*	hard	5.1	3.3
34	MEH1 Composite*	hard	5.5	2.5
35	SR5250	strong creeping	2.6	1.3
36	Garnet	strong creeping	1.4	1.0
		LSD @ α 0.05	0.9	1.3

* First generation mesotrione selected selection

** Second generation mesotrione selected selection

*** Third generation mesotrione selected selection

† applications at sowing and four weeks after seedling emergence + 0.25% v/v non-ionic surfactant rate

‡ Rated on 1-9 scale with 9 = no injury or reduction in establishment and 1= major reduction in establishment and completely bleached tissue

Figure 4.1 Example of mesotrione treated strip (bottom) and a non-mesotrione treated strip (top). Note bleaching injury and reducing establishment in a non mesotrione selected turf plot (left) and a mesotrione selected selection (right) exhibiting no injury or reduction in establishment.