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EVALUATION AND BREEDING OF FINE FESCUES FOR LOW MAINTENANCE

APPLICATIONS

by

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ABSTRACT OF THE DISSERTATION

EVALUATION AND BREEDING OF FINE FESCUES FOR LOW MAINTENANCE APPLICATIONS

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Fine fescues (*Festuca* spp.) are being bred for low-maintenance turfgrass applications. One of the major limitations to the widespread use of fine fescue is summer patch susceptibility and traffic tolerance. *Magnaporthiopsis poae* (Landschoot & Jackson), is the long known causal organism of summer patch, however recent research has found a new species, *Magnaporthiopsis meyeri-festucaae* (Luo & Zhang) from the diseased roots of fine fescue turfgrasses exhibiting summer patch symptoms. Breeding for improved tolerance to summer patch is critical but in order to do so a better understanding of the pathogen(s) is necessary. During 2017 and 2018, isolates of *M. meyeri-festucaae* were compared to isolates of *M. poae* through plant-fungal interaction in growth chamber experiments and *in vitro* fungicide sensitivity assays with penthiopyrad, azoxystrobin, and metconazole. In the plant-fungal interaction experiments, *M. poae* was shown to exhibit higher levels of virulence than *M. meyeri-festucaae*; however, certain isolates of the two species were ranked equal. In the fungicide sensitivity assays, an isolate of *M. meyeri-festucaae* was shown to be 9.5 times more tolerant to azoxystrobin

than a *M. poae* isolate. These results indicate that *M. meyeri-festuciae* may be involved with summer patch symptoms of fine fescue under field conditions and should be considered along with *M. poae* when breeding for tolerance and developing best management strategies for controlling summer patch disease in fine fescue.

Genetic resistance is an important control strategy and could reduce fungicide use. This study determined narrow-sense heritability of summer patch resistance in hard fescue (*F. brevilipa* R. Tracey) and evaluated inheritance characteristics of summer patch disease resistance. Inheritance characteristics such as heterosis were determined by evaluating the disease severity of progeny from crosses between resistant and susceptible hard fescue clones. Parental clones and progenies from crosses were established in a field trial in a randomized complete block design and inoculated with an isolate of both *M. poae* and *M. meyeri-festuciae* applied at a rate of 3 cc per plant of prepared inoculum. Differences in progeny means between crosses were observed. Progeny from resistant × resistant crosses had less disease severity than resistant × susceptible and susceptible × susceptible crosses. Medium narrow-sense heritability estimates support the idea that additive gene action plays a role in disease resistance and that summer patch resistance is possibly quantitatively inherited.

To better understand the tolerance to wear and traffic replicated field studies were established in North Brunswick, NJ and St. Paul, MN, and each included 157 Chewings fescue (*F. rubra* ssp. *commutata* Gaudin; syn. *F. rubra* ssp. *fallax* (Thuill.) Nyman), 155 hard fescue, and 149 strong creeping red fescue (*F. rubra* L. *rubra* Gaudin) genotypes. Wear tolerance was evaluated in North Brunswick and traffic tolerance was evaluated in St. Paul during 2015 and 2016 using different simulators to determine both plant

performance and broad-sense heritability estimates for wear and traffic tolerance. Broad sense heritability estimates for the three species when calculated on a clonal basis was between 0.69 and 0.82 for wear tolerance in the North Brunswick location and between 0.49 and 0.60 for traffic tolerance in the St. Paul location. On a single plant basis, broad sense heritability estimates for the three species were between 0.31 and 0.45 for wear tolerance in the North Brunswick location and 0.09 and 0.12 for traffic tolerance in St. Paul. However, this research does indicate that improvement of wear and traffic tolerance in fine fescues is possible through recurrent breeding methods based on selection of replicated clonally propagated genotypes rather than selection of single individual plants of a population. This was the first study to determine the genetic effects of wear and traffic tolerance in any turfgrass species.

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Chapter 3, entitled Comparison of a New Fungus, *Magnaporthiopsis meyeri-festucae*, with *Magnaporthiopsis poae*, Associated with Summer Patch-Like Disease of Fine Fescue Turfgrass, has sections published in *Mycologia* (2018) 109(5):780-789 and remaining sections will be submitted to *Plant Disease*. Chapter 5, entitled Heritability of Simulated

Wear and Traffic Tolerance in Three Fine Fescue Species, was published in Horticultural Science (2018) 53(4):416-420.

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1. INTRODUCTION

The turf breeding program at Rutgers has had a rich and productive history of producing cultivars for the turf industry. Recently, economic and social issues have driven the need for plant breeders to develop new cultivars that address a different range of conditions. As the world becomes more environmentally conscious the turf industry will need innovative solutions so that turf landscapes utilize less pesticides and fresh water. The Rutgers University turf program has a goal to not only aid New Jersey growers, but those around the world as well. Cultivar development and selection has shifted in the past few years from finding the best quality genotypes under high levels of management to also identifying those with traits that improve performance while reducing inputs, including irrigation, herbicides, or labor. The following studies include research aimed at identifying and solving a subset of the future needs of the turf industry as a whole.

Fine fescues (*Festuca spp.*) are currently being developed for use as low-maintenance turfgrasses. Fine fescues are known for their shade, drought, and cold tolerance, and adaptation to infertile and acidic soils (Carroll, 1943; Hanson et al., 1969; Beard, 1973; Dernoeden et al., 1994; Reiter et al., 2017). They also have lower water use rates than other cool-season species such as Kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.); along with the ability to perform well in low-input management situations (Newell and Gooding, 1990; Kenna and Horst, 1993). However, a serious drawback of these species are their susceptibility to summer patch and lack of traffic and wear tolerance.

Summer patch is a destructive root disease of Kentucky bluegrass and fine fescues traditionally caused by *Magnaporthiopsis poae* (Kobayashi et al., 1995). In fine fescues symptoms appear in early summer as patches of wilted and thinned turf around 3-8 cm in diameter, and progress into patches up to 30 cm or larger (Smiley et al., 2005). In prolonged heat stress and drought these patches can coalesce into large irregular shaped areas of completely blighted turf. Summer patch is observed to be most severe on sunny, poorly drained and compacted sites during hot and wet years. Heat stress plays an important role in development, and drought stress can intensify the disease once severe root rot has occurred. Summer patch has been observed on hard, strong creeping red, and Chewings fescues with interspecific levels of resistance. It should be noted that hard fescue is the most severely impacted by the disease and due to this all hard fescues are considered susceptible whereas genotypes of the other species can show very little to no disease. (NTEP, 2008). Therefore, hard fescues have been the focal point for ongoing disease resistance studies.

Historically, fine fescues have been considered less traffic/wear tolerant than other turf species (Shearman and Beard, 1975; Minner and Valverde, 2005; Cereti et al., 2010), so use in high traffic areas has been discouraged. However, there is evidence of good wear tolerance with newer fine fescue cultivars compared to other turf species under reduced maintenance on golf course fairways (Watkins et al., 2010). Regardless, the fact remains turf managers still have an assumption that all fine fescue species have poor wear tolerance. Although most turfgrasses exhibit traffic tolerance there is a difference in levels of traffic tolerance between species. This has led to certain species predominately being used and studied for different sports and recreational activities.

Most traffic tolerance research in turfgrass systems has focused on higher maintenance situations such as athletic fields (Samaranayake et al., 2008; Carroll and Petrovic, 1991; Brosnan et al., 2005).

Therefore, the objectives of this study were to better understand the resistance of fine fescues to summer patch and the tolerance of fine fescues to traffic and wear to aid breeders and researchers for future studies.

2. LITERATURE REVIEW

2.1 Fine fescues

Fine fescues are a group of widely used cool-season turfgrass species utilized since the sixteenth century. The group contains at least seven species including hard fescue (*F. brevilipa* R. Tracey), Chewings fescue (*F. rubra* ssp. *commutata* Gaudin; syn. *F. rubra* ssp. *fallax* (Thuill.) Nyman), strong creeping red fescue (*F. rubra* L. *rubra* Gaudin), slender creeping red fescue (*F. rubra* L. ssp. *litoralis* (G. Mey.) Auquier), blue fescue (*F. glauca* Vill.), sheeps fescue (*F. ovina* L.), and hair fescue (*Festuca filiformis* Pourr.). However, the blue, sheeps, and hair fescues are rarely used compared to the others. The group is valuable for use in home lawns, recreational turf, and low maintenance turf areas. Fine fescues are generally adapted to dry, shady, low pH conditions, and perform best in well drained soils but are not suited for saturated soil conditions (Murphy, 1996). In general, the fine fescues have poor heat tolerance and lack tolerance to excessive nitrogen fertilization during periods of high temperatures which can lead to disease (Meyer and Funk, 1989). A low rate of nitrogen fertilizer (454- 908g N 92.9 m⁻² yr⁻¹) is recommended annually because under high nitrogen, fine fescues typically develop thatch and become succulent and soft and are more susceptible to disease (Meyer and Funk, 1989). Fine fescues are normally less tolerant to wear also, when compared to other species like Kentucky bluegrass (*Poa prantensis* L. (Shearman and Beard, 1975). Some fine fescues contain endophytes (*Epichloë* spp.) which have been shown to improve resistance and tolerance to a variety of abiotic and biotic stresses including drought, chinch bugs (*Blissus* spp.), armyworms (*Spodeoptera mauritia* Boisduval), red thread (*Laetisaria fuciformis* McAlpine), and dollar spot (*Clarireedia*

jacksonii sp. nov.) (Bonos et al., 2002; Clarke et al., 2006; Funk et al. 1993; Saha et al., 1987).

The center-of-origin for fine fescues is central Europe, but fine fescues are also thought to be native to both Eurasia and North America and have become established in regions of Africa (Beard et al., 2014). Typically, they are found in temperate zones with a preference towards cooler rather than warmer climates. They are usually found in turf mixtures with other species including Kentucky bluegrass and are rarely found in monoculture except in shady areas. Germplasm expansion and genetic pools have increased with collections from areas of origin in Europe and Asia. Germplasm has also been obtained from naturally occurring stands along the eastern seaboard of the United States where colonists originally introduced the species to North America (Beard et al., 2014).

Breeding work within the group has been limited until recently. Modern breeding programs have gathered an increased amount of germplasm from around the world and released many new cultivars. However, there is still a need for the improvements in wear tolerance, heat and cold tolerances, improved sod-forming ability, seed yield, and seed viability. Improved disease resistance is needed for Pythium blight (*Pythium aphanidermatum* (Edson) Fitzp.), leaf spot (*Bipolaris sorokiniana* (Sacc.) Shoemaker), pink snow mold (*Microdochium nivale* (Fr.) Samuels & I. C. Hallet), take-all patch (*Gaeumannomyces graminis* (Sacc.) Arx & D.), summer patch (*Magnaportheopsis poae* Landschoot & Jackson), gray snow mold (*Typhula incarnate* Fr.), powdery mildew (*Blumeria graminis* (DC.) E. O. Speer), brown patch (*Rhizoctonia solani* Kühn), and dollar spot (*Clarireedia jacksonii* sp. nov.) (Meyer and Funk, 1989). Work by Hodges et

al. in 1975 indicated hard fescue was the least susceptible to dollar spot, which was variable among the three *F. rubra* species, and Peel in 1988 reported less red thread disease incidence with cultivars of hard and Chewings fescue than in strong and slender creeping red fescues., Compared to other turf species, all three *F. rubra ssp.* had greater snow mold resistance than *Agrostis spp.* at golf course fairway height in Wisconsin (Gregos et al., 2011).

Similar taxonomic and differentiating characteristics such as variable ploidy within the fine fescues have presented breeders with a major challenge. These include isolating factors such as chromosome number, anthesis date, and hours of pollen shedding. The first taxonomic work within the group was conducted by Hackel in 1882. His work had five subspecies based largely on leaf anatomy and tiller formation. Traditionally, the fine fescues have been separated into two categories or complexes with similar morphological characteristics. The two complexes are the *F. rubra* (red fescue) complex which contains strong creeping red fescue and Chewings fescue and the *F. ovina* complex which contains hard fescue and sheep fescue. These complexes are delineated by sheath and tiller characteristics. The *F. rubra* complex is described as having a sheath of young tiller-leaves fused into a tube almost to top with some or all tillers extravaginal. The *F. ovina* complex has a sheath of young tiller-leaves with at least the upper 40% with free, overlapping margins and all tillers intravaginal (Ruemmele et al., 2003). Also, the *F. rubra* complex typically has wider leaves than species in the *F. ovina* complex and have five or more epidermal ridges. Within *F. rubra* complex, growth habit is used to differentiate the two creeping red fescues (which contain rhizomes) from Chewings fescue (which lacks rhizomes and has a bunch type growth habit). As the name suggests,

strong creeping red fescue has stronger and longer rhizomes than slender creeping red fescue (Ruemmele et al., 2003). Chewings fescue is grouped into the *F. rubra* complex, but it has always been difficult to distinguish from both groups due to its bunch type growth habit suggesting it should fall in the *F. ovina* complex. Chewings fescue scientific name *commutata* translated from latin means “closely resembling another species” (Clifford and Bostock, 2007). It is characterized as being different from *F. ovina* species by thicker leaf blades, smooth culms, tubular leaf sheaths, and longer-awned lemmas (Ruemmele et al., 2003). The *F. ovina* complex tends to have a dark grayish green and sometimes a powdery blue color, whereas the *F. rubra* species tend to have a lighter to medium green color.

Chromosome counts provide important information for breeders in terms of crossing feasibility within the group. Using flow cytometry, Huff and Palazzo (1998) confirmed chromosome counts of fine fescue species. For fine fescues, the base number of chromosomes is seven ($2n=2x=14$). Within the group, plants have been found from diploid to octoploid ($2n=2x=56$). The group could be considered a cytospecies group which is defined as one with very similar species morphologically, but each with its own unique chromosome number due to ecological or geographical isolation. It is also worth noting that polyploids are often found in adverse areas due to their ability to possess wider adaptation due to a larger gene pool. The two-common species of hard, and Chewings are shown to be hexaploid ($2n=6x=42$). Hexaploids in the group are characterized as fine-leaved, dense, clump-forming types. Spreading types are reported to be mostly octoploid ($2n=8x=56$) or hexaploid. The spreading types are shown to be coarser leaved and rhizomatous. Creeping and spreading red fescues are often classified

into the same species (*F. rubra* L. sub sp. *rubra*) without regard to ploidy level. This ploidy difference could lead to reproductive isolation within the species due to sterility barriers. The sheeps fescues are shown to be a tetraploid ($2n=4x=28$) (Huff and Palazzo, 1998).

Along with ploidy, flowering differences may lead to the lack of interspecific crosses able to be utilized by breeders. The fine fescues have different flowering dates and hours of anthesis. Flowering dates can change each year due to environmental conditions, but the sequence of the species does not change. Hard and sheeps fescues are the first to flower in mid to late may at 41°N latitude. Chewings fescues precede the spreading-type fescues in flowering date but have some overlap in late May to early June (Schmit, 1974). The time of pollen shed within the species is most important and can hinder crossing even if flowering dates are manipulated in a breeding program by the use of daylength lighting. Chewings fescue initiates anthesis between 5-6:00 a.m., hard fescue sheds pollen prior to 8:00 a.m., while sheeps fescue shed pollen around 12:00 p.m., and spreading types shed pollen between 4:00 and 5:00 p.m. These timings are shown to be very consistent on bright, sunny days which favor pollen shedding during the flowering season. The differences in pollen shed are typically associated with speciation and isolation due to pollen viability being lost prior to another species hour of receptivity (Schmit, 1974).

Pollen grain longevity is measured by germinating percentages following anthesis. Hard and Chewings fescues have shown to have very short pollen viability of four to six hours (Schmit, 1974). This may be due to the fact their flowering times are closer together. To create species isolation these two species may be trying to reduce the

chance of crossing. The spreading type fescues are the most isolated group in that all pollen from the other species is not viable by early afternoon prior to the time these species flower, and their pollen is not viable the next morning when others are flowering (Schmit, 1974). Also, worth noting is that under field conditions grass pollen longevity is further reduced by high temperatures and high solar radiation that can be expected during late May and early June (Schmit, 1974). Chewings, hard, and sheeps fescues are therefore not reproductively isolated by hour of anthesis and pollen longevity and could feasibly be crossed in breeding programs if ploidy were similar.

All of the fine fescue species are adapted to a wide range of environments, but the species do have differences in terms of the environment they reside in or management practices they are subjected to. Fine fescues and primarily hard fescue have been identified as potential candidate species for immediate application in a low maintenance turf. Hard fescue has been shown to be drought resistant due to an extensive root system (Fry and Butler, 1989; Brar and Palazzo, 1995; Wang et al., 2018) and lower mowing and fertilization requirements compared to traditional species used in lawn and recreational sites while maintaining a higher quality of turf (Dernoeden et al., 1994). New cultivars have reduced vertical growth and improved disease resistance to red thread (*Laetisaria fusiformes* (McALp.) Burdsall), net blotch (*Drechslera dictyoides* F. Sp. Dreschs), and anthracnose (*Collectrichum graminicola* (Ces.) G.W. Wils.) (Tate et al., 2018).

However, one of its drawbacks is that it has shown to be very susceptible to summer patch caused by *Magnaporthiopsis poae*. Summer patch can cause severe damage to a turfgrass stand and require complete renovation. With the use of fungicides, summer patch can be controlled, but for a low maintenance situation the use of fungicides

and other pesticides is not realistic. Also, there is has been little management research performed on controlling summer patch specifically in the fine fescues.

2.2 Summer patch

Patch diseases have been a serious issue of turfgrasses since the late 1950s (Couch and Bedford, 1966). Symptoms of patch diseases tend to appear as distinct sunken zones of necrotic turf. A patch disease exhibiting symptoms started being reported with increased frequency in the northeastern United States during the early 1960s (Couch and Bedford, 1966). It was reported on bentgrass (*Agrostis spp.*), red fescue and Kentucky bluegrass during summer stress. This disease became to be known as Fusarium blight (*Fusarium roseum* and *F. tricinctum*). Throughout the next two decades the casual pathogen of this disease was disputed. Smiley and Fowler (1985) were able to reproduce symptoms similar to Fusarium blight syndrome on Kentucky bluegrass by inoculation experiments using isolates they identified as *Philaphora graminicola*. The group was unable to produce a teleomorph of their isolates and could not identify the organism, but they called the disease summer patch. Landschoot and Jackson (1989) produced an isolate of the species and determined that it was the anamorph of the previously undescribed species *Magnaporthe*. They named the new species *M. poae* and noted it was the casual pathogen of summer patch.

Summer patch is caused by an ascomycete fungus that is part of the *Magnaportheace* family. The family differs immensely in its morphology, life cycle, and nutritional mode. They occur on all plant parts and in terrestrial and aquatic environments, with the most research being conducted on the plant pathogens. (Luo et al., 2017) The pathogens include both economically important soil-borne and stem infecting

fungi that can colonize grass species in the genera *Poa*, *Festuca*, and *Agrostis* such as rice blast (*Magnaporthe oryzae*), take-all root rot (*Gaeumannomyces graminis*), and summer patch of turf. However, there are also endophytic species that have beneficial potential to plant hosts. (Luo et al., 2017)

Magnaporthaceae belongs to the single-family order *Magnaporthales* in the *Sordariomycetidae* and based on phylogenetic studies is closely related to *Diaporthales* and *Ophiostomatales*. (Luo et al., 2017). They are characterized morphologically by solitary and non-stromatic perithecia, which are partly submerged in host tissue, globose to subglobose with a long beak, brown to black, and with a glabrous surface. Their paraphyses are well developed, tapering gradually, with thin walls. Asci of the family are cylindrical to clavate, eight-spored, and with an apical ring. Their ascospores are smooth, fusoid to filiform, septate, biseriate to multiseriate within the ascus. Hyphopodia range from simple to lobed, when present.

Summer patch (caused by *M. poae*) is ectotrophic root-infecting pathogen and named for its patch like symptoms that occur in summer months. It generally begins to colonize turfgrass roots, rhizomes, and stolons once soil temperatures reach 18°C in the months of June to September (Smiley et al., 2005). The disease is difficult to control due to infection occurring 6-8 weeks prior to visual symptoms. Initial symptoms are rarely seen or visible until severe damage has been done to the below ground sections of the plant and begin to be noticed as what would be traditionally described as drought or heat stress symptoms. Normal symptoms of summer patch include black roots and tan to yellowish foliar color. Development of the disease occurs in 0.15 m to 1 m circular patches in the turf with severe cases much larger. The initial patches will be off color and

prone to wilt at a higher rate than surrounding turf (Smiley et al., 2005). As the disease progresses the turf will become a straw color and start to lose canopy integrity and collapse to the soil surface. Excessive nitrogen in the spring, potassium deficiencies, poor soil drainage, high soil pH, over-irrigation, excessive thatch accumulation, and soil compaction has been shown to encourage summer patch development. These management practices normally lead to poor root development which makes plants more susceptible to root diseases. For cultural control of summer patch, reducing soil pH and adopting deep infrequent irrigation practices are recommended. Encouraging a deep root zone is ideal to deal with summer patch infection. Summer patch has also been shown to be controlled or reduced by the source of a nitrogen fertilizer. It was proven that applying an acidifying fertilizer like ammonium sulfate fertilizer compared to one containing calcium nitrate reduced summer patch disease severity by up to 75 % (Hill et al., 2003). It has been proposed that the acidifying fertilizers reduced soil pH which could be the cause of the reduction in disease. If chemical control is needed, multiple fungicides have been shown to control summer patch disease. As with any pathogen a preventative plan is preferred. Fungicides that are used to control summer patch include the strobilurin fungicides azoxystrobin, fluoxastrobin, along with strobilurin mixes with demethylation inhibitors. These fungicides should be used as a preventative application in late spring to early summer when soil temperatures reach 18°C to 20°C (Smiley et al., 2005).

2.2.1 New developments of summer patch causal pathogens

Magnaportheopsis J. Luo & N. Zhang is a fungal genus typified by *M. poae* in *Magnaportheales* (Sordariomycetes, Ascomycota). Six species are currently accepted in

this genus which include *M. agrostidis*, *M. incrustans*, *M. maydis*, *M. panicorum*, *M. poae*, and *M. rhizophila*. They are characterized by black globose perithecia with a cylindrical neck, two-layered perithecial wall, clavate asci with a refractive apical ring, fusiform to fusoid and septate ascospores, simple hyphopodia, and Phialophora-like or harpophora-like anamorph. Species in this genus are mostly necrotrophic pathogens on grass roots.

Magnaporthales fungi contain several model species, and, accordingly, genomic work has been widely carried out on these fungi. Currently, nine genomes and 21 transcriptomes have been reported in *Magnaporthales* (Xu et al., 2014; Luo et al., 2015). In *Magnaporthiopsis*, the genome of *M. poae*, which is a model species of grass root pathogens, was published recently by Okagaki et al. (2015).

In New Jersey, during the summer of 2013 a possible new summer patch casual pathogen was isolated from diseased roots of hard fescue. Fungal isolates from these diseased roots were observed to have growth patterns slight variable to what would be expected from *M. poae*. This discovery led to preliminary studies that conclude with Luo et al. (2018) reporting a new *Magnaporthiopsis* species. Cultural characteristics were recorded from potato dextrose agar (PDA, BD) and corn meal agar (CMA, BD), and the color names of colonies followed Ridgway's nomenclature (Ridgway, 1912). Microscopic examinations, measurements, and photographs were taken from slides of fungi mounted in distilled water. This species has thin and fast-growing colonies on PDA, straight and phialidic conidiogenous cells, ellipsoidal conidia, as well as grass host, which fit well in the genus *Magnaporthiopsis*. However, complex and branched conidiophores, narrow, straight, and ellipsoidal conidia, and *Festuca* spp. hosts make it

distinct from other species. In addition, the relatively slow growth rate on PDA distinguishes this species from the other summer patch pathogen *M. poae*.

Thermal optimum for the growth of the new *Magnaporthiopsis* species and *M. poae* was investigated. A mycelial disc of 5 mm in diameter of each isolate on a Petri dish containing half strength PDA. After inoculation, the Petri dishes were incubated in the dark for 6 days under different thermal conditions: 4, 20, 25, 29, and 35 C for 6 days. Three isolates of the possible new species were used in the growth rate test, and consisted of AG2, SCR11, and H43 which all showed a similar growth pattern. They grew well at 20 to 30 C but not at 4 C, and had the maximum radial growth at 29 C. As a reference, the summer patch fungus, *M. poae* (isolates C13 and M47) was tested as well. Significant slower ($p < 0.05$) growth rates were observed in the new species compared to *M. poae* at 20, 25, 29, and 35 C after 6 days of inoculation.

The new fungal isolates fell into the subclade of *Magnaporthiopsis*, from which a new species is recognized and distinct from other species in the genus. Based on the molecular phylogeny together with morphological, biological and ecological characteristics, one new species is thereby proposed named *Magnaporthiopsis meyeri-festucae*.

2.3 Turfgrass wear

Among the groups of grasses, turfgrasses are unique in the aspect that they will maintain cover under mowing and other forms of traffic. Their dense root and canopy systems have made them ideal for maintaining soil integrity and limiting displacement along with adding a cushioning effect that aids in withstanding traffic. These properties

have led to a long-standing human tradition of recreation and sports that humans have developed on turfgrass areas.

Turfgrass traffic is made up of two components: wear and compaction (soil surface disruption) (Beard, 1973). Wear stress includes tissue tearing, tissue bruising, and tissue removal resulting from horizontal forces, while surface soil disruption includes soil compaction and displacement as a result of vertical forces (Vanini et al., 2007). Therefore, it is important to distinguish between the two types of traffic when developing a turfgrass. Although most turfgrasses exhibit traffic tolerance there is a difference in levels of traffic tolerance between species. This has led to certain species predominately being used and studied for different sports and recreational activities. Most traffic tolerance research in turfgrass systems has focused on higher maintenance situations such as athletic fields (Samaranayake et al., 2008; Carroll and Petrovic, 1991; Brosnan et al., 2005). Typically, turfgrass researchers would rank perennial ryegrass as the most traffic tolerant species followed by tall fescue, Kentucky bluegrass, and then fine fescues. Within the fine fescues, hard fescue is considered more wear tolerant than Chewings and strong creeping red fescue (Shearman and Beard, 1975; Minner and Valverde, 2005; Cereti et al., 2010)

Traffic tolerance becomes very complicated depending of the activity, duration, and timing of the traffic being applied to the turfgrass. Other abiotic and biotic factors can aid or limit the performance of a turfgrass with respect to traffic. Studies have been instrumental in providing turf managers with information regarding species or cultivar selection for wear and traffic tolerance. Early studies showed large variability between species, more recent advanced and larger studies have shown there is still a great deal of

variability within a certain species (Shildrick et al., 1983; Bonos et al., 2001; Cross et al., 2103). Work has been done on bentgrass, bermudagrass, fine-leaf fescue, tall fescue, ryegrass, and Kentucky bluegrass. Grimshaw et al. (2018) showed through heritability estimates that a reasonable increase in wear tolerance through breeding was possible in the fine fescues. The NTEP program has additionally added to the knowledge of traffic tolerance. Along with the included wear studies within NTEP, the program also evaluates turfgrass cultivars from all major species across a variety of locations for turfgrass quality. Quality has been shown to be correlated to wear tolerance (Bonos et al., 2001). For example, in the 2010 National perennial ryegrass test the top three entries for wear tolerance were also the within the top 20 for overall turf quality (NTEP, 2010). However, this is not the case in all species and can be related to density or texture as these are a component of turf quality ratings.

An issue that these studies have illucidated is that there is an inconsistency of cultivar performance across different experiments and with the use of varying wear/traffic simulators (discussed below). Because wear is a component of traffic it would be assumed that a traffic tolerant cultivar would also be wear tolerant, but this is not always the case. In addition to the type of traffic or wear there is the issue of time of year in which the traffic/wear is applied. Chen et al. (2016) showed leaf bruising, which is indicative of wear, was more intense in summer and fall versus spring. This evidence suggests studies performed at varying times of the year could affect the overall tolerance/ or perception of tolerance of a given genotype.

2.3.1 Evaluation of Wear in Turfgrasses

Researchers have developed many ways in which to simulate traffic in research trials. For a more accurate understanding of the components of wear and traffic, devices had to be made that differentiated between wear and soil compaction. During any activity one form of traffic stress will be more predominate. It is believed that wear will be dominate on sandy soils or soils below field capacity and soil compaction will be dominate on finer soils when heavy loads are applied. Examples of machines designed to impart different forms of traffic include the Rutgers Wear Simulator (RWS) (Park et al., 2016), a 0.8-m-wide wear simulator constructed from a modified walk-behind power broom (Sweepster, LLC, Dexter, MI) as described by Bonos et al. (2001). This machine applies wear without causing soil compaction. Another is a custom-built golf cart traffic simulator towed behind a turf utility vehicle which had been used successfully in previous studies to simulate traffic (Horgan et al., 2007; Watkins et al., 2010). The traffic simulator consisted of two 454-kg traffic units on an axle containing five golf cart tires. This simulator imparts both wear and soil compaction to the turf thus simulating golf cart traffic of two golfers, and equipment on pneumatic tires (Alderman, 2016)

2.3.2 Wear tolerance in tine fescues

Historically, fine fescues have been considered less traffic/wear tolerant than other turf species (Shearman and Beard, 1975; Minner and Valverde, 2005; Cereti et al., 2010). In older trials dating to the 1970's and 1980's, Chewings and slender creeping red fescues gave better performance than strong creeping fescues to wear stress (Shildrick, 1976b; Shildrick et al., 1983). Shildrick (1975) also evaluated turf mixtures under wear stress and revealed that perennial ryegrass mixtures containing Chewings fescue were less affected by wear stress than mixtures containing strong creeping red fescue. The

wear tolerance from these studies was summarized in Shildrick (1984) as: poor to good for both Chewings and slender creeping red fescues, bad to good for hard fescue, and bad to poor for strong creeping red fescue.

Since that time, new cultivars from breeding efforts have led to improvements in wear tolerance across the fine fescue species, but there is still a similar trend across the species with hard and Chewings fescues, having the best wear tolerance, and strong creeping red fescue having the poorest wear tolerance among fine fescue species (Bonos et al., 2001; Watkins et al., 2012; Cross et al., 2013; Grimshaw et al., 2018). Most often, past wear tolerance research indicated that Chewings fescues is the top performer among fine fescues (Shildrick, 1976b; Shildrick et al., 1983; Bonos et al., 2001, Newell and Wood, 2003; Cereti et al. 2010; Watkins et al., 2010, 2012). In experiments with modern cultivars or germplasm, there are occurrences where hard and slender creeping red fescues exhibited top wear and traffic tolerance among fine fescues or similar to Chewings (Watkins et al., 2010; Cross et al., 2013; Chen et al, 2016; Grimshaw et al., 2018).

Researchers at Rutgers University and the University of Minnesota have conducted trials to evaluate, screen potential mechanisms, and develop breeding techniques related to improved traffic and wear tolerance (Watkins et al., 2012; Chen et al., 2016; Chen and Murphy, 2017; Grimshaw et al., 2018). Researchers discovered more effective methods of improving wear and traffic tolerance in fine fescues, such as using a phenotypic recurrent selection program with replicated clonally propagated genotypes rather than selection of single individual plants of a population as reported by Grimshaw et al. (2018).

Overall, there are documented differences in wear tolerance among the fine fescue species. New cultivars have continued to demonstrate improvement and comparable wear tolerance to other cool-season species when grown in cool climatic zones and under low-input conditions. Future studies and breeding efforts should continue to focus on improving wear tolerance of fine fescues so that options for trafficked, low-maintenance areas are available.

This dissertation will focus on work aimed at the improvement of fine fescue turfgrasses in areas of traffic/wear tolerance and summer patch resistance. Chapters 3 and 4 will explore summer patch causal pathogens and heritability in hard fescue, while chapter 5 will aim to provide insight on the traffic and wear tolerance of three fine fescue species. Currently we have some knowledge on fine fescues in terms of management and breeding but there are many unanswered questions when it comes to these underutilized species.

3. COMPARISON OF A NEW FUNGUS, MAGNAPORTHIOPSIS MEYERI-FESTUCAE, WITH MAGNAPORTHIOPSIS POAE, ASSOCIATED WITH SUMMER PATCH-LIKE DISEASE OF FINE FESCUE TURFGRASS

3.1 Introduction:

Magnaporthiopsis J. Luo & N. Zhang is a fungal genus typified by *M. poae* in *Magnaporthales* (*Sordariomycetes*, *Ascomycota*). A new *Magnaporthiopsis* species, *M. meyeri-festucaae*, was discovered from the diseased roots of fine fescue turfgrasses. It is described and illustrated based on phenotypic characteristics and DNA sequences of SSU, ITS, LSU, *MCM7*, *RPB1* and *TEF1* genes (Luo et al., 2018). The symptomology of the plants exhibited similar signs as plants suffering from summer patch infection, but *M. meyeri-festucaae* needs to be proven through inoculation experiments to be pathogenic on hard fescue (*Festuca brevipila*) and Kentucky bluegrass (*Poa pratensis*) to be considered a new summer patch causal pathogen (Luo et al. 2018). Hard fescues are currently being developed for use as low- maintenance turfgrasses, and are known for their shade, drought, and cold tolerance, and adaptation to infertile and acidic soils (Carroll, 1943; Hanson et al., 1969; Beard, 1973; Dernoeden et al., 1994; Reiter et al., 2017). They also have lower water use rates than other cool-season species such as Kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.); along with the ability to perform well in both low-input and high input situations (Newell and Gooding, 1990; Kenna and Horst, 1993). As the demand to reduce water, fertilizer, and pesticide inputs rises, the demand will also rise for more low-input turfgrass options, such as hard fescues. However, a serious drawback of this species is its susceptibility to summer patch. Summer patch is a destructive root disease of Kentucky bluegrass and hard fescue

traditionally caused by *Magnaporthiopsis poae* (Kobayashi et al., 1995). In fine fescues, symptoms appear in early summer as patches of wilted and thinned turf around 3-8 cm in diameter, and progress into patches up to 30cm or larger (Smiley et al., 2005). In prolonged heat stress and drought these patches can coalesce into large irregular shaped areas of completely blighted turf which can occur in fine fescues with their severe lack of tolerance. Summer patch is observed to be most severe on sunny, poorly drained and compacted sites during hot and wet years. Heat stress plays an important role in development, and drought stress can intensify the disease once severe root rot has occurred. The pathogen can spread along roots, crown, and stem tissue at around 3 cm per week. The disease can be reduced by cultural practices that relive stress and promote healthy root development including raising mowing height, using slow-release fertilizers that lower soil pH to below 6.0, aerification, and improving drainage, along with the use of acropetal penetrant fungicides (Smiley et al., 2005).

The discovery of a possible new casual pathogen adds complications to current research being conducted in breeding and related best management strategies. Researchers at Rutgers University have noticed a greater intensity of summer patch like symptoms in research plots, even with the use of fungicides (Murphy, unpublished). A new pathogen with higher levels of pathogenicity or fungicide tolerance could lead to these observations. In the case of resistance to fungicides, it has been shown that some soilborne pathogens express cytochrome and alternative oxidase respiratory pathways enabling them to produce ATP in the presence of strobilurin fungicides (Joseph-Horne et al., 1998, 1999).

The objectives for this project were to 1) confirm *M. meyeri-festuca* as a summer patch casual pathogen of turfgrasses through Koch's postulates 2) determine the level of virulence of *M. meyeri-festuca* as compared to *M. poae*, and 3) determine if *M. meyeri-festuca* and *M. poae* are tolerant/resistant to fungicides traditionally used to control summer patch in turfgrass stands.

3.2 Material and methods

3.2.1 Fungal isolation

Fine fescue turfgrasses, including hard fescue (*Festuca brevipila*), strong creeping red fescue (*Festuca rubra* var. *rubra*), and Chewings fescue (*Festuca rubra* var. *falax*) that showed summer patch-like symptoms were sampled from Horticultural Farm 2 at Rutgers University in New Brunswick, New Jersey in 2013 and 2016. Within 24 h after collection, the roots were rinsed in tap water to remove soil particles on the surface and cut into about 5 mm long segments. The segments were then surface sterilized with 95% ethanol for 30 sec, followed by 2 min in 0.6% sodium hypochlorite (NaOCl), 2 min in 70% ethanol and two rinses in sterile distilled water. The sterilized segments were placed on malt extract agar (MEA, BD) with 0.07% lactic acid and incubated at room temperature. Fungal cultures were isolated and purified by subculturing from emergent hyphal tips. To confirm the species of the isolates from *M. meyeri-festuca* and *M. poae* the protocols used in Zhang et al. (2011) and Luo and Zhang (2013) were followed for DNA extraction, PCR amplification, and sequencing of small subunit (SSU), internal transcribed spacer (ITS), and large subunit (LSU) of ribosomal RNA genes, DNA replication licensing factor (MCM7), the largest subunit of RNA polymerase II (RPB1), and translation elongation factor 1- α (TEF1) genes.

3.2.2 Inoculation experiments to fulfill Koch's postulates for *M. meyeri-festuceae*

Inoculation procedures for fulfillment of Koch's postulates were adopted and modified from those used by Landschoot and Jackson (1990), Elliott (1991), and Tredway (2006). Inoculum was prepared by sterilizing 300 ml of oat (*Avena sativa*) seeds and 150 ml of deionized water in a glass jar. One Petri dish (90 mm diameter) of the *Magnaporthiopsis meyeri-festuceae* isolate FF2 was cut into small pieces (approximately 1 cm²) and added to the sterilized oat seeds, and the mixture was incubated at 29 C for 40 days.

Inoculations were carried out in 3.8 by 20 cm conetainers (Stuwe & Son, Tangent, OR, USA), which were filled with 75 ml of sterilized sand (Premium Play Sand[®], The QUIKRETE[®] Companies, Atlanta, GA, USA), five inoculated oat seeds to provide an inoculum layer in soil profile, and an additional 28 ml of sterilized sand. Each conetainer was seeded with 0.024 g of hard fescue or 0.014 g of Kentucky bluegrass seed. The control units were constructed in the same manner; however, the oat seed in those was not inoculated with the fungus.

The prepared conetainers were placed in light- and temperature-controlled growth chambers for 11 weeks. Growth chambers had 12 hr photoperiods with the day temperature of 29 C and night temperature of 23 C. Relative humidity was maintained at approximately 70%. Plants were fertilized every 14 days to promote growth and watered to maintain adequate soil moisture. After 11 weeks, plants were removed from the growth chambers and evaluated for foliar and root characteristics.

Treatment factors for this study were fungus and turfgrass cultivar. There were two levels of fungus (inoculated and uninoculated) and four levels of turfgrass cultivar ('Beacon' hard fescue, 'Predator' hard fescue, 'Baron' Kentucky bluegrass, and 'Midnight' Kentucky bluegrass). Beacon and Predator were included for confirmation of Koch's postulates on hard fescue, and Baron and Midnight were included to test if the host range of the fungus extended beyond hard fescue. The treatments were positioned as a 2×4 factorial in a completely randomized design with three replicates of each treatment combination. Containers were repositioned three times per week within a growth chamber to increase homogeneity of environmental conditions within the growth chamber. The experiment was repeated in a second growth chamber.

Evaluation parameters included shoot and root health, root length, and shoot and root dry weight. Shoot health was visually assessed based on a rating scale from 1-5, where 1 = tan to yellowish brown color of leaf tissue, 2 = yellowish green color of leaf tissue, 3 = light green color of leaf tissue, 4 = medium green color of leaf tissue, and 5 = dark green color of leaf tissue. Root health was visually assessed based on a rating scale from 1-6, where 1 = no root tissue present, 2 = severe discoloration of roots, 3 = moderately severe discoloration of roots, 4 = moderate discoloration of roots, 5 = slight discoloration of roots, and 6 = no discoloration of roots. Root length was determined by measuring the length (mm) of the longest root in a given sample. Shoot and root weights were determined by drying and weighing shoot and root material from each sample. Prior to drying the root tissue for weighing, 5 mm of the middle root profile for each plant sample was collected and used to re-isolate the fungus (Fig. 3.1). Significant treatment effects

were identified by subjecting the data to analysis of variance via the General Linear Model procedure in SAS v. 9.4 (SAS Institute Inc., Cary, NC).

3.2.3 Plant-fungal isolate interaction between *M. poae* and *M. meyeri-festuca*

Inoculation procedures for this study were adopted and modified from previous work (Landschoot and Jackson, 1990; Elliott, 1991; Tredway, 2006). Seven isolates of *Magnaportheopsis meyeri-festuca* and three isolates of *Magnaportheopsis poae* were used in the plant-fungal interaction experiment. Inoculum was prepared by sterilizing 300 ml of oat (*Avena sativa*) seeds and 150 ml of deionized water in a glass jar. One Petri dish (90 mm diameter) of each isolate was cut into small pieces (approximately 1 cm²) and added to the oat seed, and the mixture was then incubated at 29 °C for 40 days.

Inoculations were carried out in 3.8 cm by 20 cm conetainers, which were filled with 75 ml of sterilized sand (Premium Play Sand®, The QUIKRETE® Companies, Atlanta, GA, USA), five inoculated oat seeds were added to provide an inoculum layer in the soil profile, and an additional 28 ml of sterilized sand was placed on top. Each conetainer was seeded with hard fescue (Beacon, Predator) at 9.7 g m⁻² and Kentucky bluegrass (Baron) at 5.7 g m⁻². The control units were constructed in the same manner; however, the oat seed in those was not inoculated with the fungus. The experiment was a completely randomized design with three replications. Containers were randomly moved every two weeks to prevent growth chamber effects. The experiment was conducted twice in the same growth chamber.

The prepared conetainers were placed in light- and temperature-controlled growth chambers for 16 weeks. Growth chambers had 12 hr photoperiods emitting 375 μmol · m⁻²

$2 \cdot s^{-1}$ of light, with the day temperature of 29 °C and night temperature of 23 °C. Relative humidity was maintained at approximately 70%. The containers were transferred to a growth chamber and misted twice per day to encourage rapid seed germination. After germination, the turf was maintained in the growth chamber by irrigating twice daily and with a complete nutrient solution containing nitrogen at 106.23 mol·m⁻³, phosphorus at 10.41 mol·m⁻³, and potassium at 111.03 mol·m⁻³. After 16 weeks, plants were removed from the growth chambers and evaluated for shoot and root characteristics. Roots were washed in water to remove excess sand and debris.

Evaluation parameters included shoot and root health and shoot and root dry weight. Shoot health was visually assessed based on a rating scale from 1 to 5, corresponding to the percentage of leaf tissue exhibiting chlorosis or dieback and root health or dieback. (1 = 100%, 3 = 50%, and 5 = 0%). Root health was visually assessed based on a rating scale from 1-4, where 1 represented small, discolored roots and 4 represented large, white or translucent colored roots. Shoot and root weights were determined by drying at 50 °C for one week and weighing shoot and root material from each sample. Mean separation of shoot quality, root quality was conducted by paired *t*-test with Bonferroni correction at a familywise α level of 0.05. Shoot and root weights were converted to retained weight percentage based on control weight.

3.2.4 In vitro fungal-fungicide interactions of *M. poae* and *M. meyeri-festuciae*

Fungicides used in the in-vitro experiment were azoxystrobin (Heritage; Syngenta Crop Protection Inc., Greensboro, NC), metconazole (Tourney; Valent USA Corp., Washington D.C.), and penthiopyrad (Velista; Syngenta Crop Protection Inc.,

Greensboro, NC). Azoxystrobin is a systemic, broad-spectrum fungicide that was first introduced in 1998 for the prevention and control of diseases of turf and ornamentals, transplants of fruit and nut trees, and vegetable and herb plants. Metconazole is a sterol biosynthesis inhibitor fungicide that works by inhibiting demethylation and other processes in sterol biosynthesis. The fungicide may be used for disease control in turfgrass on golf courses, lawns and landscape areas around residential, institutional, public, commercial and industrial buildings, parks, recreational areas, athletic fields and sod farms. Penthiopyrad is a carboxamide fungicide that inhibits fungal respiration by binding to mitochondrial respiratory complex II. It is a water-dispersible granular fungicide for turf disease control on golf courses, residential, institutional, commercial and municipal lawns, park and recreation areas, and sod farms.

For *in vitro* experiments, the two fungi (FF2, *M. meyeri-festucae*, and C11, *M. Poae*) were grown on potato dextrose agar (PDA; Sigma, St. Louis, MO) for 1 week. Three-millimeter diameter disks from the growing edge of the fungal colony were transferred to the center of fresh PDA amended to result in a range of $\mu\text{g a.i. ml}^{-1}$ of the following fungicides: azoxystrobin which was evaluated at 0.001, 0.01, 0.1, 1, 10 $\mu\text{g a.i. ml}^{-1}$ and the strobilurin fungicides at each concentration was amended with salicylhydroxamic acid (SHAM) at 100 $\mu\text{g ml}^{-1}$ to prevent the fungi from using an alternative respiration pathway for growth, Metconazole was evaluated at 0.001, 0.01, 0.1, 0.2, 0.5, 0.8, 1.0 and 2.0 $\mu\text{g a.i. ml}^{-1}$, and penthiopyrad was evaluated at 0.01, 0.1, 1.0, 10.0, 50.0, and 100.0 $\mu\text{g a.i. ml}^{-1}$. Agar was amended by preparing 200 ml of PDA and adding 2 ml of sterile acetone or the appropriate fungicide dilution which were dissolved in acetone to achieve the desired concentration of active ingredients brought up

to 2 ml in sterile syringes. Fungicides were added to the PDA after autoclaving and once the media had cooled to approximately 55°C. Twenty milliliters of medium were dispensed into each of three replicate 90-mm diameter plastic petri plates. Two perpendicular lines were drawn on the bottom of each petri plate and the fungal disk was placed at the intersection of the two lines. The diameter of the fungus was measured once on each of the two perpendicular lines drawn on the plate once the fungus on the control plates reached the edge of the plate (up to 14 days). The experiment was conducted twice with three replications. For comparison of the efficacy of different fungicides, root dry weight was converted to percentage of untreated control, and fitted to a log-logistic model described by Seefeldt *et al.* (1995), given by the formula:

$$f(x | b, c, d, e) = c + (d - c) / (1 + e^{b(\log x - \log e)})$$

where root dry weight, $f(x)$, is a conditional function of application rate x . ED_{50} , which is denoted by e , is the effective dose to reduce colony diameter by 50%. Parameter d and c define the range of response variables (100 and 0, respectively). Comparisons of the ED_{50} values between the two species were carried out by paired t test, and data visualization was performed using R package ‘ggplot2’ (Wickham, 2009).

3.3 Results

3.3.1 Inoculation experiments to fulfill Koch’s postulates for *M. meyeri-festuceae*

Inoculation experiments in temperature- and light-controlled growth chambers resulted in disease symptoms and fulfillment of Koch’s postulates. Assessments for pathogenicity confirmation included visual ratings of shoot and root material, measurements of root length, and shoot and root weights. The fungus was recovered from all inoculated samples, and a representative set were used for identification via ITS

sequencing. Morphological characteristics of recovered strains fit well with the type strain FF2 that was used for inoculation, and all ITS sequences match that of the original strain with 100% identity.

Shoot health of the plants in this study ranged from 1 to 4, and the overall average shoot health was 2.6. Mean shoot health was independently and significantly affected by cultivar ($p = 0.0022$) and fungus ($p = 0.0106$). Midnight (mean = 3.3), Predator (mean = 2.8), and Beacon (mean = 2.8) had significantly greater shoot health than Baron (mean = 1.8) did. With respect to the main effect fungus, shoot health was significantly reduced in inoculated samples compared to uninoculated samples (Table 3.1). The shoot weight, across all samples, averaged 958 mg and ranged from 70 to 2450 mg. Shoot weight was not affected by the factors included in this study.

Root health ranged from 2 to 6, and the overall average root health was 4.3. As with shoot health, mean root health was independently and significantly affected by cultivar ($p = 0.0197$) and fungus ($p < 0.0001$). With respect to root health, Midnight (mean = 5.0) was the best, Baron (mean = 4.1) and Predator (mean = 3.8) were the worst, and Beacon (mean = 4.3) was similar to all cultivars. Inoculated samples had significantly lower root health than uninoculated samples (Table 3.1). The overall mean root length was 168.2 mm, and the values ranged from 45 to 205 mm. Root length was significantly affected by the main effect fungus ($p = 0.0007$). The root length of inoculated samples was 14% shorter than that of uninoculated samples (Table 3.1). Root weight ranged from 60 to 1590 mg, and the average across all samples was 681 mg. Root weight was also significantly affected by fungus ($p = 0.0386$). The root weight of

inoculated samples was 26% less than the root weight of uninoculated samples (Table 3.1).

3.3.2 Plant-fungal isolate interaction between *M. poae* and *M. meyeri-festucaae*

Inoculation experiments of both *M. meyeri-festucaae* and *M. poae* resulted in disease symptoms in both hard fescue and Kentucky bluegrass. Overall *M. poae* exhibited an overall higher level of shoot symptomology than *M. meyeri-festucaae* (Tables 3.2 and 3.3). However, individual isolates could not be separated based on species. Assessments for pathogenicity confirmation included visual ratings of shoot and root material, and dry shoot and root weights. Shoot quality of the plants in this study ranged from 1 to 5, and the overall average health of above ground biomass was not significantly different for Baron, Beacon, and Predator. Shoot health was significantly reduced in certain inoculated samples compared to uninoculated samples with *M. meyeri-festucaae* isolate FF2 consistently ranking least pathogenic and *M. poae* isolate C11 ranking most pathogenic (Table 3.2). For Baron, the least severe isolates were *M. meyeri-festucaae* isolates FF2 (4.66) and SCR12 (4.33) and the most severe isolates were *M. poae* isolates C11 (1.50) and C13 (1.83) (Table 4.1). For Beacon, the least severe isolates were *M. meyeri-festucaae* isolates FF2 (4.16) and SCR12 (4.16) and the most severe isolates *M. poae* isolates C11 (1.33) and C13 (1.83) (Table 3.2). For Predator, the least severe isolates were *M. meyeri-festucaae* isolates FF2 (4.16) and SCR12 (3.66) and the most severe isolates were *M. poae* isolate C11 (2.33) and *M. meyeri-festucaae* isolate SCR11 (2.66) (Table 3.2). To examine the shoot weights, shoot weight was studied as a percent of the uninoculated control. This transformation of the data removed the inherent differences in the general increased biomass observed in the roots of Baron versus the

two hard fescues. With this variance removed, the isolates can be more easily separated, statistically. The shoot weight average for Baron, Beacon, and Predator was 0.63g, 0.49g, and 0.47g, respectively (not shown). With respect to the main effect isolate, shoot weight was significantly reduced in certain inoculated samples compared to uninoculated samples in Baron and Predator (Table 3.3). For Baron, the highest retained shoot weights were obtained for *M. meyeri-festuca* isolates W15-22 (104%) and FF2 (92%) and the isolates with the lowest retained shoot weights were *M. poae* isolates C11 (36%) and C13 (36%) (Table 3.3). For Beacon, the highest retained shoot weights were *M. meyeri-festuca* isolates FF2 (82%) and SCR12 (72%) and the lowest retained shoot weights were *M. poae* isolates C11 (21%) and C13 (43%) (Table 3.3). For Predator, the highest retained shoot weights were obtained for *M. meyeri-festuca* isolates FF2 (113%) and W15-22 (98%) and the isolates with the least shoot weights were *M. poae* isolate C13 (53%) and *M. meyeri-festuca* isolate SCR9 (59%) (Table 3.3).

Root quality of the plants in this study ranged from 1 to 4. Overall *M. poae* exhibited an overall higher level of root symptomology than *M. meyeri-festuca* (Tables 3.4 and 3.5, Figs. 3.1 and 3.3). However, individual isolates could not be separated based on species but again *M. meyeri-festuca* isolate FF2 consistently ranked least pathogenic and *M. poae* isolate C11 ranked most pathogenic (Table 3.4). The overall average root health for Baron, Beacon, and Predator was 3.03, 2.47, and 2.24, respectively with Baron's root quality being significantly higher from the two hard fescues.

Root health was significantly reduced in certain inoculated samples compared to uninoculated samples (Table 3.4). For Baron, the least severe isolates were *M. meyeri-festuca* isolates FF2 (3.66), W15-22 (3.5), and SCR12 (3.5) and the most severe isolates

were *M. poae* isolates C11(1.66) and C13 (1.83) (Table 3.4). For Beacon, the least severe isolates were *M. meyeri-festucaae* isolates FF2 (3.00) and H21(3.00) and the most severe isolates were *M. poae* isolates C11(1.00) and C13(1.66) (Table 3.4). For Predator, the least severe isolates were *M. meyeri-festucaae* isolates FF2 (3.33), W15-22 (2.33), and SCR12 (2.33) and the most severe isolates were *M. poae* isolates C13 (1.50), C11 (1.66) and *M. meyeri-festucaae* isolate SCR9 (1.66) (Table 3.4). To examine the roots further, root weight was studied as a percent of the uninoculated control. This transformation of the data removed the inherent differences in the general increased biomass observed in the roots of Baron versus the two hard fescues. With this variance removed, the isolates can be more easily separated, statistically. With respect to the main effect isolate, root weight was significantly reduced in certain inoculated samples compared to uninoculated samples in Baron. For Baron, the highest root weights were found for *M. meyeri-festucaae* isolates FF2 (130%) and H21 (78%) and the lowest root weights were observed for *M. poae* isolate C13 (26%) and C11 (20%) (Table 3.5). For Beacon, the highest root weights were *M. meyeri-festucaae* isolates FF2 (122%) and SCR12 (101%) and the lowest root weights were *M. poae* isolates C11 (14%) and C13 (26%) (Table 3.5). For Predator, the highest root weights were *M. meyeri-festucaae* isolates FF2 (94%) and W15-22 (74%) and the lowest root weights were *M. meyeri-festucaae* isolate SCR9 (33%) and *M. poae* isolate C13 (31%) (Table 3.5). *M. poae* isolates tend to be more severe in the reduction of root weights compared to *M. meyeri-festucaae* (Figure 3.1, 3.2, and 3.3), and in the cases of Baron and Beacon were significantly different. However, in the case of Predator, *M. meyeri-festucaae* isolate SCR9 was more severe in the reduction of root weight than two isolates of *M. poae*, and the two species were not significant from each other. In studies

conducted evaluating heat and drought stress in fine fescues, Predator was found to be more tolerant to these factors than other fine fescues (Wang et al. 2017). Due to the fact summer patch symptomology is increased under drought and heat, this could play a role in why Predator is considered more summer patch resistant under field conditions. In this study, drought and heat were not a factor, if this study were conducted under drought and/or heat conditions, it may lead to insight why Predator performed differently from the other two entries.

3.3.3 In vitro fungal-fungicide interactions of *M. poae* and *M. meyeri-festucaae*

ED₅₀ values between *M. meyeri-festucaae* and *M. poae* isolates in response to metconazole, azoxystrobin, penthiopyrad fungicides were determined through fungal colony diameter measurements. For azoxystrobin and metconazole, there was a significant ($p < 0.05$) effect of species on the efficacy of the two fungicides. In the case of penthiopyrad, there was no difference between the two species (Table 3.6). For azoxystrobin, the isolate of *M. meyeri-festucaae* exhibited an ED₅₀ of 0.0095 ug a.i. ml⁻¹, whereas the isolate of *M. poae* exhibited an ED₅₀ of 0.0010 ug a.i. ml⁻¹. This represents a 9.5-fold increase in tolerance to azoxystrobin by the *M. meyeri-festucaae* isolate (Table 3.6 and Figure 3.5). For metconazole, the isolate of *M. meyeri-festucaae* exhibited an ED₅₀ of 0.14 ug a.i. ml⁻¹, whereas the isolate of *M. poae* exhibited an ED₅₀ of 0.18 ug a.i. ml⁻¹. This represents a slight but significant increase in tolerance to metconazole by the *M. poae* isolate (Table 3.6 and Figure 3.5). These results suggest a difference in fungicide sensitivity between the species.

3.4 Discussion

To date, the fungus *Magnaporthe poae* (syn. *Magnaporthe poae*) is the only confirmed pathogen associated with summer patch disease in fine fescues and other cool-season turfgrasses (Landschoot and Jackson, 1989; Luo and Zhang, 2013). This study reports a new fungal species of *Magnaporthe* associated with summer patch-like symptoms on fine fescue that shows different morphology, growth rate, and genetics compared to *M. poae*. The results of this study confirmed the pathogenicity of *M. meyeri-festuca* on hard fescue and Kentucky bluegrass in the growth chamber and its association with fine fescue grasses at a research farm in New Jersey.

Summer patch disease continues to be a severe detriment to the widespread use of hard fescues. Through breeding and management research, summer patch became less destructive on Kentucky bluegrass (Smiley et al., 2005), and with continued work, researchers are optimistic the same can be accomplished in hard fescue. The discovery of a new summer patch causal pathogen *M. meyeri-festuca* adds a layer of complication to this goal. The results from this study show there is justification for including both *M. meyeri-festuca* and *M. poae* in future experiments targeted to resolve the summer patch issue in hard fescues. In plant-fungal interaction experiments, it was shown that both species and multiple isolates can cause summer patch symptoms on Kentucky bluegrass and hard fescue cultivars (Tables 3.2, 3.3, 3.4, and 3.5). The foliar symptoms appeared faster on plants inoculated with *M. poae*, and was shown in figures 3.2, and 3.3 to be more pathogenic overall as a species when comparing root effects. Since this pathogen infects mainly the roots of plants and from data collected here root weight seems to be a critical component of evaluating this disease in the growth chamber. However, in future field experiments researchers have to rely on mostly shoot visual ratings to evaluate

summer patch disease progression (Landschoot, 1995). This leads to researchers needing the most virulent isolate possible to be used in future experiments to create severe shoot symptoms. Other, continued work could be done to determine the timing of infection between the two species. There is a possibility that the two species are more active or complete their life cycles at varying times of the year. This difference in life cycles also could be a cause for reduced fungicide control and increased severity of summer patch symptoms seen in field studies. However, the fungicide sensitivity experiment revealed the possibility of differential tolerance levels to certain fungicides and could also result in the reduced efficacy under field conditions. Pathogens have been shown to have the ability to express cytochrome and alternative oxidase respiratory pathways enabling them to produce ATP in the presence of strobilurin fungicides (Joseph-Horne et al., 1998, 1999). The *M. meyeri-festuca* isolate was shown to have a 9.5-fold increase in tolerance to azoxystrobin, a strobilurin fungicide.

Overall, this study gives researchers the knowledge that *M. meyeri-festuca* and *M. poae* must be included in work to improve hard fescues. It is important to note growth chamber studies cannot entirely match the disease progression associated with field studies. In the field inoculations are made long in advance of disease symptomology and any number of factors can contribute to disease severity. It is still unclear the role each plays in the development and persistence of the disease, along with if the two interact in any manner. A multitude of future studies could help reduce the complexity in studying summer patch. Predator was mentioned before as having increased heat and drought tolerance and could possibly be more summer patch resistant because of this characteristic. Studies conducted to test hard fescue genotypes for their

correlation between heat and drought tolerance and summer patch tolerance would be critical. An increase in knowledge for all areas of fine fescue management will be necessary for the successful management of summer patch disease.

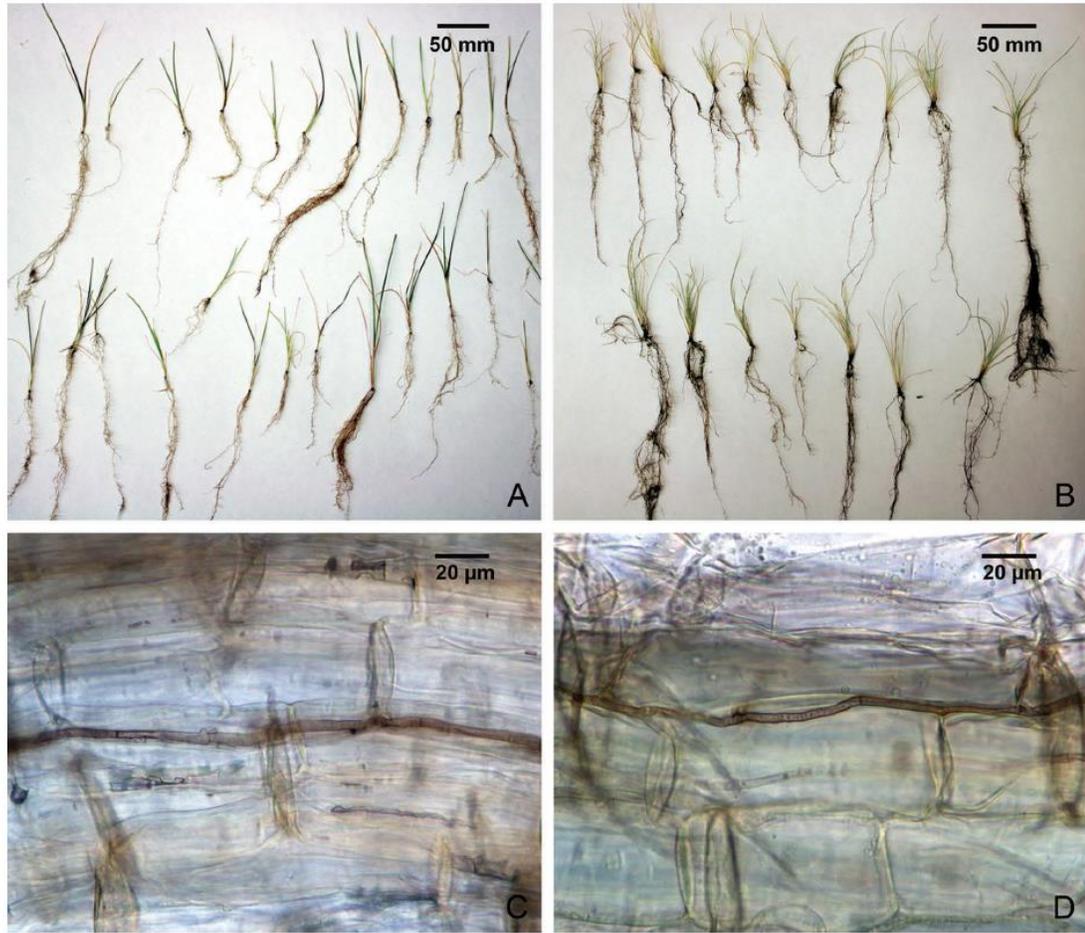


Figure 3.1 - *Magnaporthiopsis meyeri-festuciae* diseased roots (B) and uninoculated roots (A), and fungal hyphal growth on infected roots (C, D) taken from roots of hard fescue inoculated in conetainer growth chamber experiments.

Table 3.1: Mean shoot health, root health, root length, shoot weight, and root weight of ‘Beacon’ hard fescue, ‘Predator’ hard fescue, ‘Baron’ Kentucky bluegrass, and ‘Midnight’ Kentucky bluegrass inoculated and uninoculated with *Magnaporthiopsis meyeri-festuca* isolate FF2 evaluated in conetainer growth chamber experiments.

Fungus	Shoot health ^a	Root health ^b	Shoot weight ^d	Root length ^c	Root weight ^e
Inoculated	2.3 b ^f	3.3 b	985	155.2 b	580 b
Uninoculated	3.0 a	5.3 a	931	181.2 a	780 a

^a Shoot health, visual rating scale from 1-5 (1 = tan to yellowish brown color of leaf tissue, 2 = yellowish green color of leaf tissue, 3 = light green color of leaf tissue, 4 = medium green color of leaf tissue, and 5 = dark green color of leaf tissue)

^b Root health, visual rating scale from 1-6 (1 = no root tissue present, 2 = severe discoloration of roots, 3 = moderately severe discoloration of roots, 4 = moderate discoloration of roots, 5 = slight discoloration of roots, and 6 = no discoloration of roots)

^c Root length as determined by measuring the length (mm) of the longest root in a given sample

^d Shoot weight, determined by drying and weighing foliar material from a given sample

^e Root weight, determined by drying and weighing root material from a given sample

^f Within each column, means followed by different letters are significantly different according to Fisher’s LSD test ($\alpha = 0.05$)

Table 3.2: Shoot quality of plants of hard fescue and Kentucky bluegrass inoculated with isolates of *Magnaporthiopsis meyeri-festuca* and *M. poae* evaluated in container growth chamber experiments in 2018.

Baron				Beacon				Predator			
Isolate ^x	Shoot Qty ^y	STD ^z	GRP [†]	Isolate ^x	Shoot Qty ^y	STD ^z	GRP [†]	Isolate ^x	ShootQty ^y	STD ^z	GRP [†]
Control	4.50	0.83	ab	Control	4.67	0.51	a	Control	4.67	0.51	a
FF2	4.67	0.51	a	FF2	4.16	1.16	ab	FF2	4.16	1.32	ab
SCR12	4.33	1.21	abc	SCR12	4.16	1.32	ab	SCR12	3.66	1.36	ab
SCR9	3.50	1.04	abc	SCR9	3.83	1.47	abc	H21	3.16	1.32	ab
H21	3.33	0.81	abcd	W15-22	3.66	1.50	abc	H43	3.16	0.75	ab
M47	3.00	0.89	bcde	SCR11	3.50	1.22	abc	SCR9	3.00	1.26	ab
SCR11	3.00	0.89	bcde	H21	3.16	0.98	abcd	W15-22	3.00	0.89	ab
W15-22	3.00	0.63	bcde	M47	2.83	1.16	abcd	M47	2.83	0.75	ab
H43	2.83	0.75	bcde	H43	2.33	1.03	abcd	C13	2.66	0.81	ab
C13	1.83	0.41	cde	C13	1.83	0.75	bcd	SCR11	2.66	0.81	ab
C11	1.50	0.54	de	C11	1.33	0.51	cd	C11	2.33	0.51	b

^x Isolates of *M. meyeri-festuca* and *M. poae* (**Bold**) ranked in order of least severe to most

^y Shoot Quality Rating (5-1) 5: Healthy shoot, 1: necrotic or dead tissue

^z Standard deviation

[†] Grouping: Isolates with different letters indicate significance at p-value=0.05

Table 3.3: Shoot weight as percentage of control of hard fescue and Kentucky bluegrass plants inoculated with isolates of *Magnaporthiopsis meyeri-festuca* and *M. poae* evaluated in conetainer growth chamber experiments in 2018.

Baron				Beacon				Predator			
Isolate ^x	Shoot % ^y	STD ^z	GRP [†]	Isolate ^x	Shoot % ^y	STD ^z	GRP [†]	Isolate ^x	Shoot % ^y	STD ^z	GRP [†]
W15-22	104	85	a	FF2	82	17	a	FF2	113	18	a
FF2	92	59	a	SCR12	72	21	ab	W15-22	98	27	ab
SCR12	88	57	a	W15-22	70	25	ab	SCR12	93	13	ab
H21	74	14	a	H21	70	21	ab	H43	92	44	ab
M47	66	25	a	SCR11	68	28	ab	H21	82	34	ab
SCR11	59	14	a	SCR9	66	46	ab	SCR11	75	23	ab
SCR9	59	9	a	M47	66	42	ab	M47	71	15	ab
H43	50	8	a	H43	50	23	ab	C11	64	17	ab
C11	36	13	ab	C13	43	31	ab	SCR9	59	19	b
C13	36.	13	b	C11	21	16	b	C13	53	21	b

^x Isolates of *M. meyeri-festuca* and *M. poae* (**Bold**) ranked in order of least severe to most

^y Shoot Weight as percentage of control

^z Standard deviation

[†] Grouping: Isolates with different letters indicate significance at p-value=0.05

Table 3.4: Root quality of hard fescue and Kentucky bluegrass plants inoculated with isolates of *Magnoporthiopsis meyeri-festuca* and *M. poae* evaluated in conetainer growth chamber experiments in 2018.

Baron				Beacon				Predator			
Isolate ^X	Root Qty ^Y	STD ^Z	GRP [†]	Isolate ^X	Root Qty ^Y	STD ^Z	GRP [†]	Isolate ^X	Root Qty ^Y	STD ^Z	GRP [†]
Control	4.00	0.00	a	Control	3.83	0.40	a	Control	3.83	0.40	a
FF2	3.63	0.51	a	FF2	3.00	0.89	ab	FF2	3.33	1.03	ab
SCR12	3.50	0.83	a	H21	3.00	0.89	ab	SCR12	2.33	0.81	bc
W15-22	3.50	0.83	a	SCR11	2.83	0.75	ab	W15-22	2.33	0.81	bc
H21	3.33	0.51	a	W15-22	2.83	0.98	ab	M47	2.16	0.40	bc
SCR11	3.16	0.98	a	M47	2.50	0.83	ab	H21	2.00	0.89	bc
M47	3.00	0.89	ab	SCR12	2.50	0.54	ab	H43	2.00	0.63	bc
SCR9	3.00	0.00	abc	SCR9	2.16	0.75	ab	SCR11	1.83	0.75	c
H43	2.66	0.81	abc	H43	1.83	0.75	bc	C11	1.66	0.51	c
C13	1.83	0.75	abc	C13	1.66	0.51	bc	SCR9	1.66	0.51	c
C11	1.66	0.81	bc	C11	1.00	0.00	bc	C13	1.50	0.54	c

^X Isolates of *M. meyeri-festuca* and *M. poae* (**Bold**) ranked in order of least severe to most

^Y Root Quality- Rating (4-1) 4: Healthy root, 1: necrotic or dead tissue

^Z Standard deviation

[†] Grouping: Isolates with different letters indicate significance at p-value=0.05

Table 3.5: Root weight as percentage of control weight of hard fescue and Kentucky bluegrass plants inoculated with isolates of *Magnaporthiopsis meyeri-festuca* and *M. poae* evaluated in conetainer growth chamber experiments in 2018.

Baron				Beacon				Predator			
Isolate ^x	Root Wt% ^y	STD ^z	GRP [†]	Isolate ^x	Root Wt% ^y	STD ^z	GRP [†]	Isolate ^x	Root Wt% ^y	STD ^z	GRP [†]
FF2	130	47	a	FF2	122	68	a	FF2	94	42	a
H21	78	39	ab	SCR12	101	37	ab	W15-22	74	34	ab
SCR12	74	30	ab	W15-22	96	34	ab	SCR12	71	18	ab
SCR9	74	31	ab	SCR11	86	44	abc	SCR11	58	18	ab
SCR11	73	46	ab	SCR9	81	53	abc	H21	47	28	b
W15-22	66	29	ab	H21	74	34	abc	M47	44	14	b
M47	62	29	b	H43	68	34	abc	H43	44	17	b
H43	58	24	b	M47	53	30	abc	C11	34	11	b
C13	26	10	b	C13	26	17	bc	SCR9	33	21	b
C11	20	6.0	b	C11	14	11	c	C13	31	10	b

^x Isolates of *M. meyeri-festuca* and *M. poae* (**Bold**) ranked in order of least severe to most

^y Root Weight as percentage of control

^z Standard deviation

[†] Grouping: Isolates with different letters indicate significance at p-value=0.05

Table 3.6: Analysis of Penthiopyrad, Azoxystrobin, and Metconazole tolerance of isolates of *Magnaporthiopsis meyeri-festuca*, and *M poae* grown on amended PDA.

Herbicide	Species	ED50 (ppm)	Ratio (M.mf/M.p)	P-Value^z
Penthiopyrad	<i>M. meyeri-festuca</i>	22.43	1.15	0.743
	<i>M. poae</i>	19.41		
Azoxystrobin	<i>M. meyeri-festuca</i>	0.0095	9.5	8.9e-05*
	<i>M. poae</i>	0.0010		
Metconazole	<i>M. meyeri-festuca</i>	0.14	0.77	0.006*
	<i>M. poae</i>	0.18		

^z Significant* if below P-value below 0.05

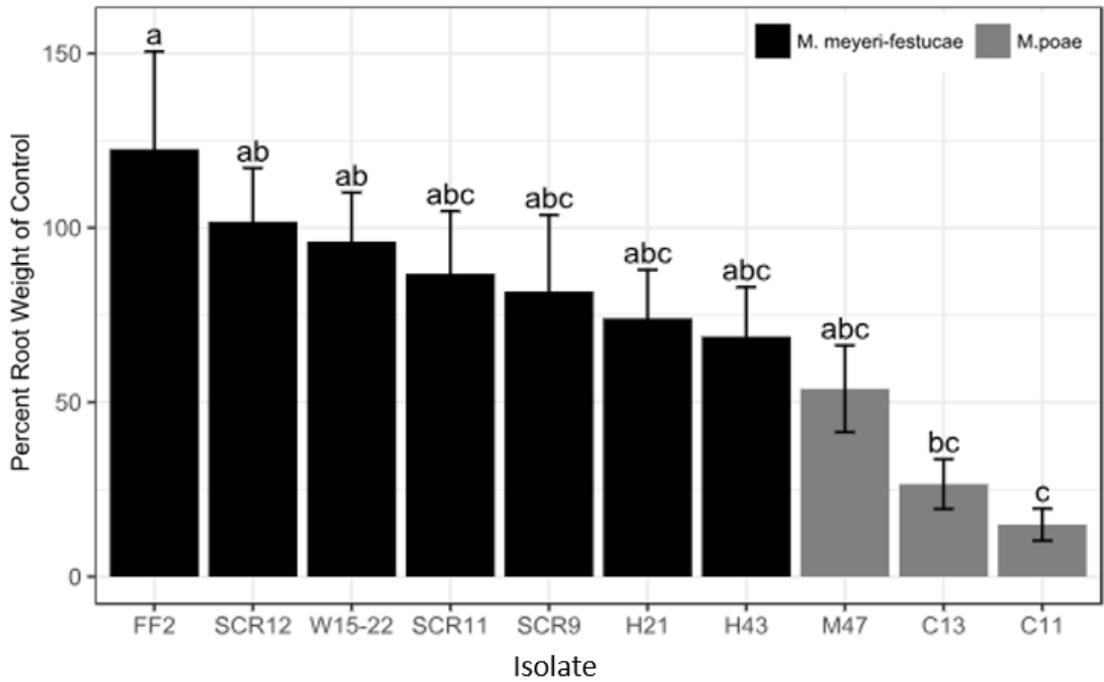


Figure 3.1: Percent dry root weight (g/g x100) of Beacon hard fescue infected with either *M. meyeri-festuca* or *M. poae* isolates compared to un-inoculated control evaluated in container growth chamber experiments in 2018.

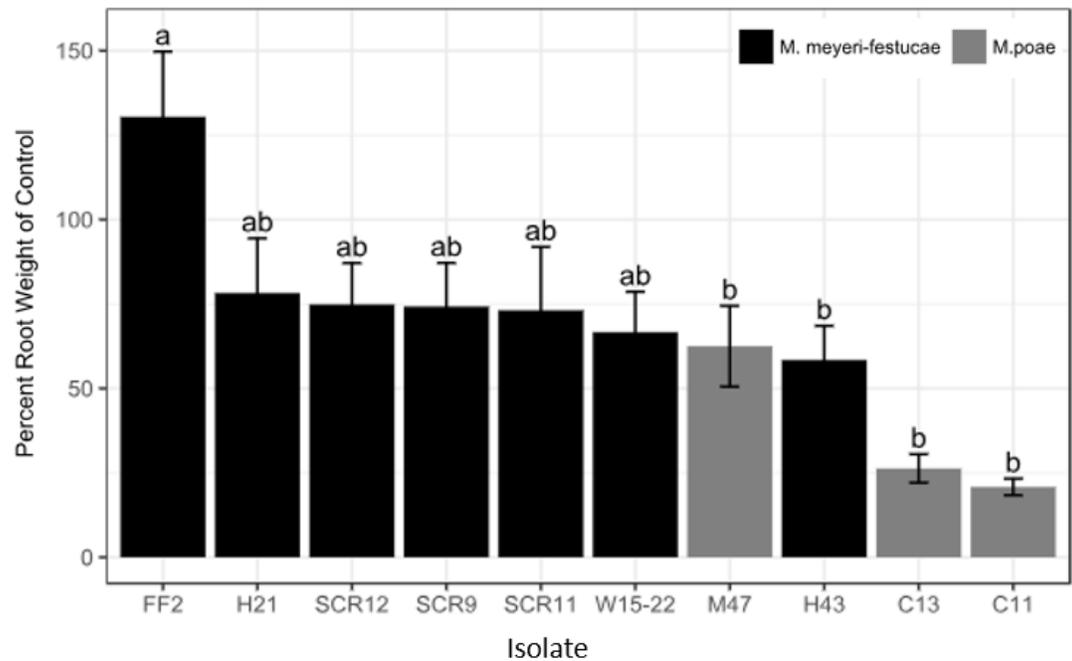


Figure 3.2: Percent dry root weight (g/g x100) of Baron Kentucky bluegrass infected with either *M. meyeri-festuca* or *M. poae* isolates compared to un-inoculated control evaluated in conetainer growth chamber experiments in 2018.

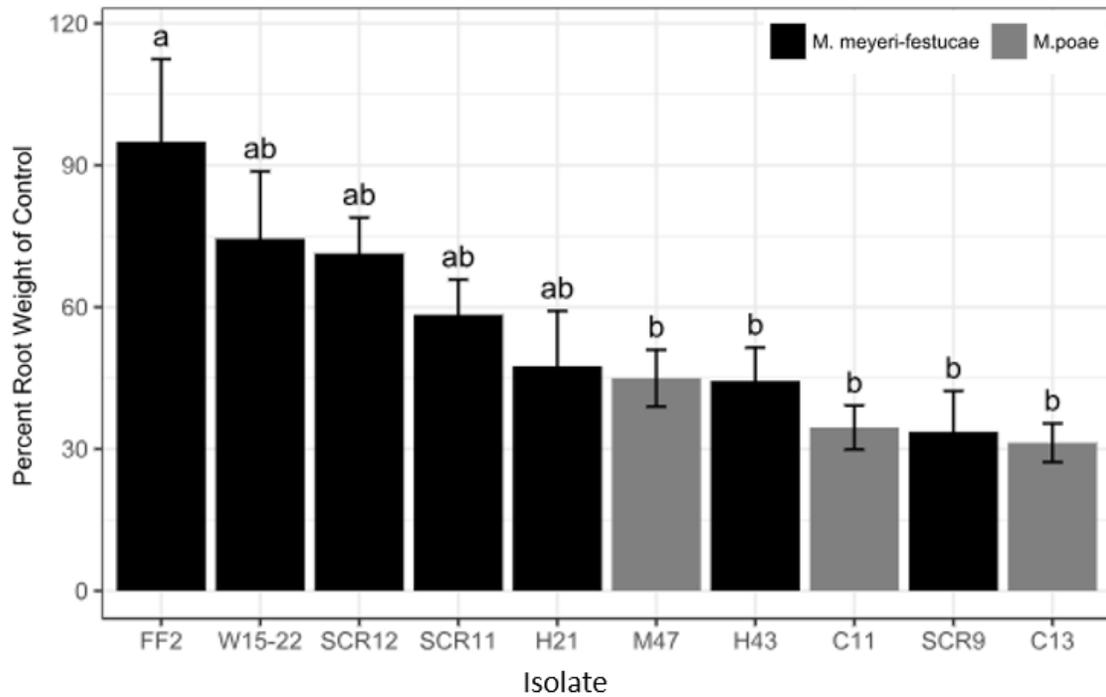


Figure 3.3: Percent dry root weight (g/g x100) of Predator hard fescue when infected with *M. meyeri-festucaae* or *M. poae* isolates compared to un-inoculated control evaluated in container growth chamber experiments in 2018.



Figure 3.4: Images of the Beacon hard fescue plants (A: Control), (B) colonized by isolates of *M. poae* (C) colonized by isolate of *M. meyeri-festucae* (SCR9) and (D) colonized by isolate of *M. meyeri-festucae* (H43) evaluated in container growth chamber experiments in 2018.

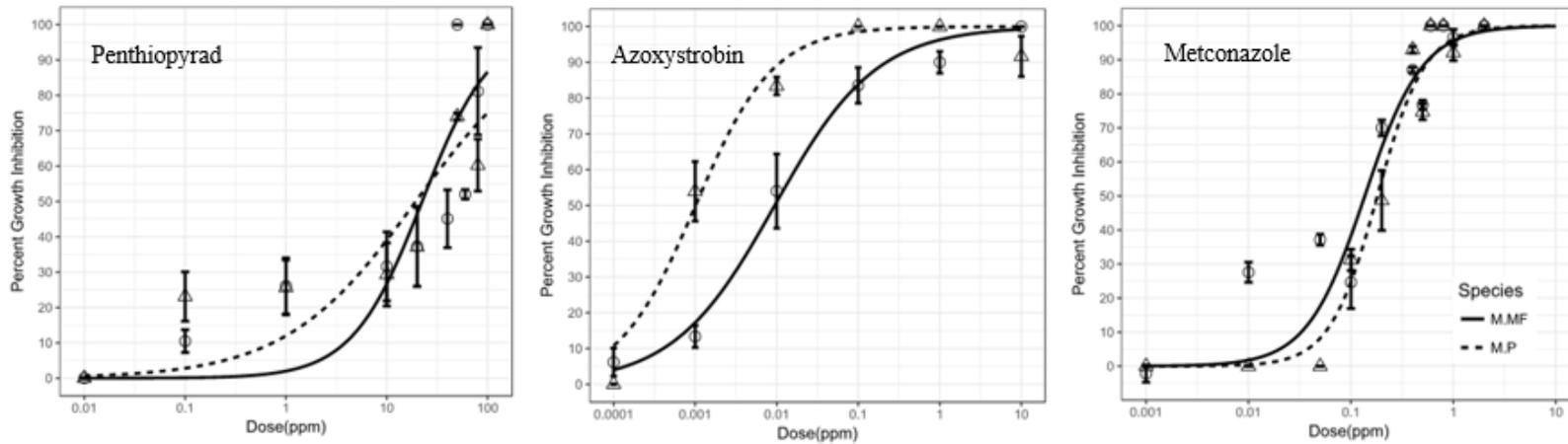


Figure 3.5: ED50 curves of Penthiopyrad, Azoxystrobin, and Metconazole tolerance of isolates of *M. meyeri-festuca*, and *M poae* grown on amended PDA.

4. NARROW-SENSE HERITABILITY OF SUMMER PATCH RESISTANCE IN HARD FESCUE

4.1 Introduction

Communities and local governments around the country are currently focusing on reducing environmental effects and reducing the costs of recreational and homeowner landscapes. One of the main areas of concern is maintaining turfgrass areas in a more sustainable way by reducing irrigation inputs, mowing frequency and pesticides. Currently research is being conducted in all facets of turfgrass breeding to develop turfgrass cultivars that can be managed under lower maintenance. Fine fescues and primarily hard fescue (*Festuca brevipilia*) has been identified as a potential candidate species for immediate application. Hard fescue has been shown to be drought resistant due to extensive root numbers. (Fry and Butler 1989; Brar and Palazzo, 1995) and require less mowing and fertilization compared to traditional species used in lawn and recreational sites while maintaining a higher quality of turf (Dernoeden et al., 1994). Recently, hard fescue has also been shown to have the best heat tolerance of the fine fescue species (Xu et al., 2018).

Hard fescue is part of the *Festuca ovina* complex, which also includes the sheep and blue fescues. The hard fescues are normally characterized by greener shoots with high density, with a somewhat tufted growth habit (Meyer and Funk, 1989). New cultivars have reduced vertical growth and improved disease resistance to red thread (*Laetisaria fusiformes*), net blotch (*Drechslera dictyoides*), and anthracnose (*Collectrichum graminicola*). However, one of its drawbacks is susceptibility to summer patch (*Magnaporthiopsis poae*). Summer patch can cause severe damage to a

turfgrass stand and require complete renovation. With the use of fungicides summer patch can be controlled, but for a low maintenance situation the use of fungicides and other pesticides is undesirable.

Summer patch caused by *Magnaporthiopsis poae*, *Magnaporthiopsis meyeri-festuca* (Luo & Zhang, 2013; Luo et al., 2018) which are ascomycete fungi are ectotrophic and root-infecting and part of the *Magnaportheace* family which includes both economically important soil-borne and stem infecting fungi that can colonize grass species in the genera *Poa*, *Festuca*, and *Agrostis*. The authors included *M. meyeri-festuca* in this study because it was discovered from the diseased roots of hard fescue turfgrasses. It is described and illustrated based on phenotypic characteristics and DNA sequences of *SSU*, *ITS*, *LSU*, *MCM7*, *RPB1* and *TEF1* genes (Luo et al., 2018). The symptomology of the plants exhibited similar signs as plants suffering from summer patch infection, and *M. meyeri-festuca* was proven through inoculation experiments to be pathogenic on hard fescue and Kentucky bluegrass (*Poa pratensis*) (Luo et al. 2018). Summer patch is named for its patch like symptoms that occur in summer months and generally begins to colonize turfgrass roots, rhizomes, and stolons once soil temperatures reach 18.3°C in the months of June to September. The disease is difficult to control due to infection occurring 6-8 weeks prior to visual symptoms. Initial symptoms are rarely seen or visible until severe damage has been done to the below ground sections of the plant and begin to be noticed as what would be traditionally described as drought or heat stress symptoms. Normal symptoms of summer patch include black roots and tan to yellowish foliar color. Development of the disease occurs in 0.15m to 1m circular patches in the turf with severe cases much larger. The initial patches will be off color and

prone to wilt at a higher rate than surrounding turf. As the disease progresses the turf will become a straw color and start to lose canopy integrity and collapse to the soil surface. Excessive nitrogen in the spring, potassium deficiencies, poor soil drainage, over-irrigation, excessive thatch accumulation, and soil compaction has been shown to encourage summer patch development (Smiley et al., 2005). For cultural control of summer patch, reducing soil pH and deep infrequent irrigation are recommended. Encouraging a deep root zone is ideal to deal with summer patch infection. Summer patch has also been shown to be affected by the nitrogen source of fertilizer. It was shown that applying an ammonium sulfate fertilizer compared to one containing calcium nitrate reduced summer patch disease severity by up to 75 % (Hill et al., 2003). The ammonium-based nitrogen source reduced soil pH which could be the mechanism behind the reduction in disease. If chemical control is needed, multiple fungicides can control summer patch disease including the strobilurin fungicides azoxystrobin, fluoxastrobin, along with strobilurin mixes with demethylation inhibitors (Smiley et al., 2005).

For breeding purposes knowledge on the inheritance of summer patch resistance in hard fescue is important to accurately establish a selection procedure. Traits can exhibit qualitative or quantitative inheritance. A qualitative trait is one controlled by one or few genes and phenotypes can fall into distinct groups such as white versus purple flowers, and dwarf versus tall plants. Qualitative traits are usually affected very little by environmental factors. Quantitative traits are defined by a continuum of phenotypes rather than distinct groups. An easy example of a quantitative trait is yield which is measured in tons per hectare, or another quantitative unit of measure. The environment, such as soil fertility, rainfall, and heat units, has greater effects on quantitative traits than

qualitative traits and can cause vast differences in phenotypes of a trait such as yield. Quantitative traits are studied by measures of central tendency such as mean and variance and controlled by the joint action of many genes (Bernardo, 2002). Heritability is a way to partition the variance associated with the phenotype. Heritability contains two main factors that result in a phenotype, a genetic effect and an environmental effect. Breeders can only manipulate genetic factors and therefore a trait with a large genetic component is desired. The larger the value of heritability the more quickly and less intensively improvement of a trait can be made. (Nyquist and Baker, 1991). The genetic component of heritability is made up of additive, dominant, and epistatic effects. Additive effects are due to the average, per-copy effects of each allele. A large additive genetic component allows breeders to make gains by selection of a desired trait.

Broad sense heritability estimates, denoted by H , contain all three genetic factors, while narrow-sense estimates, denoted by h_2 contain only additive components. However, narrow-sense estimates are typically more intensive as they involve the use of reciprocal crosses and parent-progeny regression of F_2 populations. Broad sense heritability can be calculated using clonal replicates located in multiple locations to maximize the environmental variation (Poehlman and Sleper, 1995). Broad sense estimates can be helpful in determining selection efficiency.

Heritability has not been estimated for summer patch tolerance in any turfgrass species, however, heritability has been determined for a variety of traits in other turfgrass species. These include phenotypic traits of zoysiagrass (*Zoysia* sp.) in response to stresses (Schwartz et al., 2009), and dollar spot (*Clariireedia jacksonii* sp. nov.) resistance in creeping bentgrass which were 0.79 for narrow sense and 0.90 for broad sense

heritability (Bonos, 2006; Casler et al., 2003). However, these results for dollar spot represent a foliar disease which may not be as complex as a root pathogen. Genetic resistance to root pathogens has been rarely studied (Bruehl, 1983). Reasons for the lack of studies are due to the nature of soil-borne pathogens. Soil-borne disease problems are usually observed in terms of their seedling and mature stand effects. With soil-borne pathogens in food crops there are problems connecting yield loss with the disease incidence associated with that loss. Root pathogen identification is difficult compared to foliar pathogen evaluation because 1) symptoms are not directly visible since the roots and crown tissue have to be extracted from the soil, 2) above-ground symptomology such as necrosis, yellowing, stunting, and wilt are indistinct and can be caused by any number of biotic and abiotic factors, and 3) more than one pathogen could possibly be present in the soil at any time. (Singleton, 2012.) Furthermore, the soil presents a distinct challenge in that with new molecular tools researchers are discovering thousands of distinct microorganisms being in the soil. Bruehl (1983) described work associated with understanding soil-borne pathogens genetic resistance to be working in “shades of gray not black and white”, and to their knowledge any study “resembling the precise knowledge of the genetics of resistance exist”. Studies involving soil-borne pathogens involve multitude of processes within the plant versus a specific resistance gene. For example, Pythium root rot (*Pythium* spp.) of sugarcane (*Saccharum* L.) involves the vigor of hybrids to overcome disease, and when Pythium root rot infects barley (*Hordeum* L.) it involves the balance between vegetative and reproductive processes (Bruehl, 1983). In terms of the study being conducted here, the mechanisms for why certain turfgrasses are more summer patch resistant are not known. (Luo et al., 2018).

Therefore, this study is the beginning stages of understanding this disease. The objectives of this study were to 1) determine the narrow-sense heritability estimates of summer patch resistance in hard fescue and 2) evaluate the inheritance characteristics of summer patch disease resistance of pseudo F₂ progeny from crosses between fixed resistant and susceptible clones.

4.2 Materials and methods

4.2.1 Crosses and field trial establishment

Three resistant and three susceptible hard fescue parents were selected from a preliminary trial planted August 2013 at the New Jersey Agricultural Experiment Station (NJAES) located in Freehold, NJ (Table 1). These parents were selected based on their resistance characteristics to the pathogen *Magnaporthiopsis poae*. Resistant parents were the top performing entries in this trial while susceptible parents were chosen from the lowest performing selections that had not died from summer patch symptoms. Each parent was clonally propagated for a total of 12 replications in the fall of 2014 and vernalized in a field nursery during the winter of 2014-2015. Controlled crosses between resistant and susceptible parents were made in spring of 2015 using a diallel mating design. Parents were forced to flower by bringing them into the greenhouse maintained between 18 C- 21 C and exposing them to 18h days using supplemental high sodium lamp lighting receiving a DLI of 17 mol/m²/day. Prior to anthesis, parents were matched and isolated to prevent cross contamination. To make sure no self-pollination occurred within the controlled crosses, a single plant was isolated. Both parents, within the controlled crosses, were used as male and female and pollen was transferred by tapping inflorescences with a bamboo stick.

In May of 2015, seed was harvested from each parent and dried at 45°C for 1 week and threshed and placed in cool storage for six months. During the winter of 2015/2016, seed was then sown into a tray, and 100 seedlings were randomly selected to be representative of a single cross. Once the seedlings were 4 months old a field experiment was established at the Rutgers Horticultural Farm 2, North Brunswick, NJ in May of 2016. The soil type is a Nixon loam (fine-loamy, mixed, mesic Typic Hapludults). The mature seedlings along with 24 replicates of the parents were then planted in a randomized complete block design with 25 progeny from each parent in each block on 31cm spacing between plants. The plants were maintained as needed for irrigation (twice-weekly) and fertilizer (19-0-9 granular N-P-K fertilizer, two applications of 2.44 g m⁻² N) in the spring to promote summer patch development and mowed twice a week with a Toro Groundsmaster mower (Toro Corporation, Bloomington, MN) at a height of 6.4cm. Other pathogens and biotic stresses were controlled with chemical applications. Another trial with the same exact clones was planted in a different location on the same farm in the spring of 2017 using clonal replications from the first trial location.

During the first year, inoculum of summer patch was prepared by growing two isolates (AG2, FF2) of *M. meyeri-festuciae* on sterilized oat seed. These isolates were obtained from Rutgers University Horticultural Farm 2 located in New Brunswick, NJ from fine fescue turf. The prepared inoculum was applied by placing three cubic centimeters of oat seeds into the planting hole below the seedling. However, no symptoms developed in year 1, so in the spring of 2018, fields were inoculated with a mixture of both a *M. meyeri-festuciae* isolate (SCR9) and a *M. poae* isolate (C11).

4.2.2 Evaluation

Summer patch symptoms appeared three months after inoculation (only at one location) in 2018 and plants were evaluated both visually and with Normalized Difference Vegetation Index (NDVI). Visual rating occurred two times during August 2018 using a 1-to-10 rating scale: 10 represented 0% diseased turf, 9 represented approximately 1-10% diseased turf, 8 represented approximately 10 to 20% diseased turf, 7 represented approximately 20 to 30% diseased turf, 6 represented approximately 30 to 40% diseased turf, 5 represented 40 to 50% diseased turf, 4 represented approximately 60 to 70% diseased turf, 3 represented approximately 70 to 80% diseased turf, 2 represented approximately 90% diseased turf, and 1 represented 100% diseased turf or a dead plant. The NDVI is a common vegetation index from which researchers can exploit the differences in spectral reflectance between the red and NIR wavelengths. NDVI reports a value between 0 and 1 with a larger number being a healthier plant. NDVI has been proposed as an objective alternative to the more traditional, subjective method of visually estimating turfgrass quality (Bell et al., 2002; Keskin et al., 2008). This is because significant correlations have been observed between NDVI and turfgrass visual quality in several studies (Trenholm et al., 1999; Bell et al., 2002; Fitz-Rodriguez and Choi, 2002; Jiang and Carrow, 2005, 2007; Lee et al., 2011) All data analysis was conducted on percent disease and NDVI.

4.2.3 Statistical analysis

Narrow-sense heritability was determined from midparent versus progeny regression analysis where the slope of the regression line (b) is equal to h^2 . Mid-parent and progeny means utilized in the analysis are presented in Figure 5.1 and 5.2. The

standard error of the estimate and F tests of the regression equation were analyzed using ggplot in R software. Heterosis, the comparison between progeny and mid-parent means, and maternal effects, the comparison of progeny of reciprocal crosses, were evaluated for significance with the two-sample t test.

4.3 Results and Discussion

4.3.1 Summer patch reaction of parents and progeny

Significant differences were observed between resistant and susceptible parent plants used for the crosses (Table 4.1). Parent 8 and 10 exhibited the least summer patch disease of the parental clones for both NDVI and visual ratings. Parents 10, 8, 7, and 6 were significantly more resistant than parents 5, and 11 in both ratings. Individual “selfed” crosses of the parents yielded no progeny, indicating that seed production by accidental self-pollination was believed to be infrequent, and indicated a high level of self-incompatibility among these hard fescue clones. Most grasses, including hard fescue, are considered to have a high level of self-incompatibility, so these results are consistent with the breeding behavior of hard fescue.

Certain progeny from particular crosses exhibited high levels of summer patch resistance, which is promising for breeding purposes. For example, when parent 8 is crossed with parent 10 (the two most resistant parents) the progeny exhibit a visual rating higher than any parent (Table 4.1), and when parent 8 was used in a resistant by susceptible cross (8x6) the progeny exhibit both a higher visual rating and NDVI rating than any parent (Table 4.1 and 4.2).

An interesting observation from this dataset, evident in Figures 4.1 and 4.2, is that progeny populations are exhibiting a non-normal distribution, where the progeny either

exhibit a resistant phenotype or a completely susceptible phenotype with no phenotypes in between. A good example of this distribution is evident in the cross of parents 11x7 in figure 4.1. The initial explanation of this is that there is dominant gene (or genes) responsible for summer patch resistance in hard fescue. If this is true, then the narrow sense heritability estimates would not be applicable since narrow-sense heritability estimates additive gene action in the population so it would violate the assumptions of the model. However, these results could also be an artifact of evaluating summer patch disease on individual spaced plants, rather than a larger stand of turf. We look forward to evaluating these crosses for another year in order to confirm whether this response is biologically significant for the inheritance of summer patch resistance in hard fescue.

4.3.2 Narrow-sense heritability estimate:

Narrow-sense heritability estimates based on mid-parent progeny regression analysis were medium at (0.56 ± 0.03 , visual rating [Fig. 4.3]; 0.37 ± 0.03 , NDVI [Fig. 4.4]) These estimates are similar for the two methods, but the differences seen can be deduced from the limitations of each data collection method. The visual method performed by the rater considers the entire plant and the rater has the ability to rate a plant on specific symptomology observed over the entire plant or take into account overall plant health. The NDVI method allows for more resolution since it is on a continuous scale versus the raters 1 to 10 scale, and so it is completely quantitative. However, the NDVI takes measurements from a single point on the plant and cannot take into account the entire plant health.

4.3.3 Heterosis effects:

Progeny means of some of the controlled crosses for 2018 in both NDVI and visual ratings had no significant difference in progeny mean and mid-parent mean (Tables 4.2 and 4.3) which would indicate no heterosis, but half did have significant heterosis. Most of the cases (but not all) where heterosis was seen involved progeny having higher means than parents in susceptible by susceptible crosses. The heterosis observed in multiple crosses could be due to lack of uniformity of infection of these progeny raising their mean above the parents. There were also cases in which resistant by resistant crosses were showing heterosis with progeny showing a lower resistance than the parents. Out of the resistant parents (clone 7) showed higher susceptibility than parents 10, and 8. In a resistant by resistant cross with parent 7, the progeny could be exhibiting susceptibility closer to that of parent 7. However, it is important to point out instances where a susceptible by resistant cross was made and heterosis was seen due to the progeny exhibiting a mean in the direction of the resistant parent. These include crosses 8x11, 8x5, 6x8, 11x8, 5x10, 5x8 in Table 4.2, and 8x11, 6x8, 11x10, 11x8 in Table 4.3. In these cases, the progeny had significantly higher means than the mid-parent mean which suggests the resistant parent is having a strong effect on the progeny mean, in what could be described as a dominant effect. This effect is being shown regardless whether the resistant parent is the maternal or paternal line, indicating resistance can be delivered by pollen. In Figures 4.1 and 4.2 the distribution of progeny graphs show a clustering of progeny towards the resistant parent in these same crosses.

These data indicate the possibility of a genetic component associated with summer patch resistance in hard fescue. Additionally, the medium narrow-sense heritability estimate (0.56, 0.37) reported in this study indicated that some of the genetic

variation can be attributed to additive gene effects. It should be noted that the estimate reported is specific for this population and the particular environments sampled. This is supported by the fact that progeny from resistant \times resistant crosses had significantly less disease severity than resistant \times susceptible and susceptible \times susceptible crosses. The observation of non-continuous distribution of disease severity phenotypes among progeny reported here possibly show support for a low number of major genes. However, this is somewhat surprising considering the complexity of summer patch symptomology and lower to medium heritability estimates. In other studies, conducted on nonspecific resistance to nonspecialized pathogens breeding has been difficult (Labonte et al. 2015). In some cases, such as stalk rot (*Diplodia zeae*) in maize (*Zea mays*) resistance to root pathogens is seldom high and is difficult to transfer into commercially useful plants (Kappelman and Thompson, 1966). Lack of effective implantation of resistance to these root pathogens is usually due to the complexities of the resistance, and the fact that pathologists cannot develop sufficient precision in testing and evaluation methods to identify individual genes and their effects, so results are difficult to analyze genetically (Bruehl, 1983).

A significant difference between mid-parent means and progeny means indicates the presence of heterosis and provides evidence for the presence of dominant or nonadditive genetic effects. Ongom (2010) studied the inheritance of Fusarium root rot (*Fusarium solani* f. sp Phaseoli) in common bean (*Phaseolus vulgaris* L.) showed high narrow-sense heritability estimates of 0.76 and 0.86 indicating selection could lead to improvement. However, heterosis for resistance was negative in a majority of their crosses. In only two of their crosses was the progeny mean higher than the most resistant

parent, while most of their crosses the progeny was more resistant than the worse parent. They reported that root rot was conditioned by two or more loci displaying partial-dominance and epistasis, suggesting that even with high heritability estimates inheritance of resistance was complex. In the hard fescue crosses reported here, heterosis was seen in resistant by resistant, resistant by susceptible, and susceptible by susceptible crosses. For the resistant by resistant crosses, progeny means were observed to be lower than parental means. These results could indicate a complexity in summer patch resistance and that parents 10 and 8 could be exhibiting an optimal genotype for hard fescue resistance to summer patch. When a cross was made from these two parents the reshuffling could lead to lower performance of the offspring overall. In the case of parent number 7 heterosis in crosses could be caused by a partial susceptibility within the genotype. For, resistant by susceptible crosses we are seeing heterosis due to progeny exhibiting means similar to the resistant parent or higher than the parental mean. These results give the appearance of dominance effects for summer patch resistance. It is possible that since hard fescues are hexaploids the offspring are receiving three dominant alleles from the resistant parent. This could suggest susceptibility to summer patch in hard fescue could be caused by a low frequency of resistant alleles in breeding populations. It is impossible to rule out at this point that there could be a lack of disease development and consistency within the trial and these non-uniform distributions will become more uniform as more data is collected. For susceptible by susceptible crosses heterosis is being caused by progeny means being higher than parental means. Heterosis was seen in crosses involving parent 6 which was had showed higher resistance to summer patch than parents 5, and 11. Parent 6 could potentially have offspring that are

slightly more resistant and again due to the possibility of disease progression that is not completely uniform allowing for progeny to exhibit resistance.

In summary, the results reported here, show evidence that summer patch resistance can be inherited in hard fescue, which has not been shown before. Heterosis results indicate that dominant gene effects may be important for resistance/susceptibility in particular hard fescue plants. Half or slightly more than half of the crosses (Tables 4.2, and 4.3) depending on the rating method were observed to exhibit heterosis, thus the authors at this point cannot rule out that summer patch could be quantitatively or qualitatively inherited and presently more data needs to be collected to determine the extent of genetic control seen. The method used of regression of progeny means by parental means is also highly influenced by dominant effects and can influence the analysis. Due to the complexity of summer patch resistance it will be important to see two or more years consistency in the symptomology to confirm if our screening method is effective. Only, then will the authors be able to appropriately evaluate the data at hand with less speculation. In the review by Bruehl (1983) they spoke of the issues with how to effectively design a methodology for evaluating soil-borne pathogens which allows a researcher to have a high level of certainty that the symptomology seen is due to their experimental method. This is the first report documenting the importance of parent selection in improving summer patch resistance in hard fescue.

Table 4.1: Summer patch disease severity of six parental clones used in controlled crosses generated in 2015. Plants were established in mowed spaced-plant trial in the spring of 2017 at North Brunswick, NJ, inoculated with *Magnaporthiopsis poae* and *meyeri-festuca* in May 2017 and 2018 and evaluated for disease in 2018.

Parent ¹	NDVI			Parent ¹	Visual Rating		
	NDVI ²	Std	Group ³		Rating ⁴	Std	Group ³
10(R)	0.642	0.065	a	8 (R)	7.375	0.518	a
8 (R)	0.635	0.054	a	10 (R)	6.625	0.744	a
7 (R)	0.599	0.107	ab	7 (R)	6.375	1.506	a
6 (S)	0.480	0.179	abc	6 (S)	3.875	1.458	ab
11(S)	0.380	0.119	bc	5 (S)	2.750	1.389	b
5 (S)	0.333	0.105	c	11 (S)	2.375	0.744	b

¹ Parental hard fescue clones were established in a mowed spaced plant trial and evaluated for 1 years (2018) after inoculation. R, resistant; S, susceptible.

Figure 4.1: Population distribution of pseudo F2 population means of crosses between resistant and susceptible hard fescue genotypes evaluated for summer patch resistance by NDVI in 2018. Parents and progeny were established in mowed spaced-plant trial in the spring of 2017 at North Brunswick, NJ, inoculated with *Magnaporthiopsis poae* and *meyeri-festucaae* in May 2017 and 2018. Dashed line indicates maternal parent average, and solid line indicates paternal parent average. Paternal parents on Y-axis, maternal parent on X-axis.

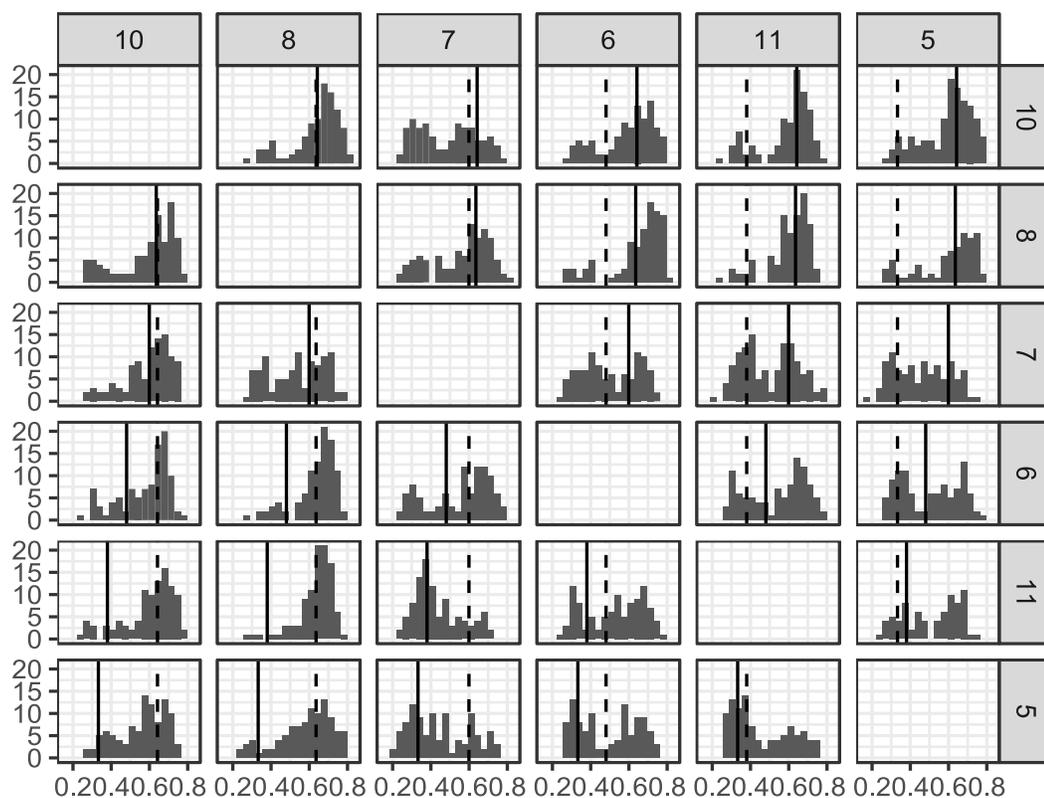


Figure 4.2: Population distribution of pseudo F2 population means of crosses between resistant and susceptible hard fescue genotypes evaluated for summer patch resistance by visual ratings in 2018. Parents and progeny were established in a mowed spaced-plant trial in the spring of 2017 at North Brunswick, NJ, inoculated with *Magnaporthiopsis poae* and *meyeri-festucaae* in May 2017 and 2018. Dashed line indicates maternal parent average, and solid line indicates paternal parent average. Paternal parents on Y-axis, maternal parent on X-axis.

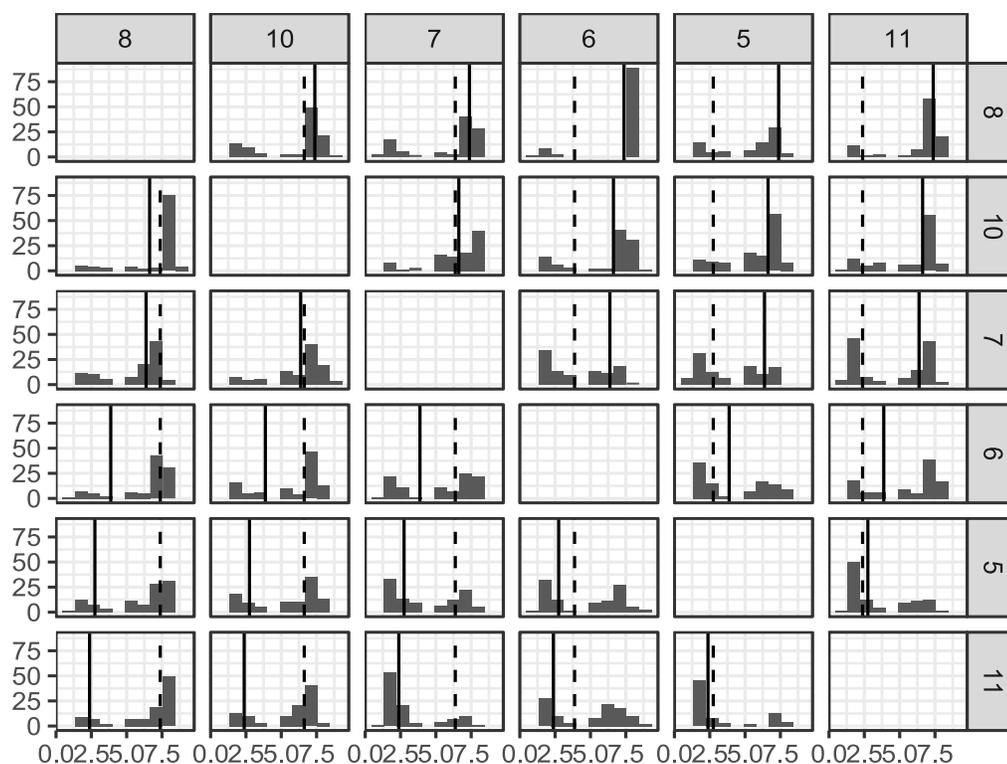


Figure 4.3: Mid-parent-offspring regression of pseudo F2 population means regressed on the mid-parent value from 15 crosses between six hard fescue parents evaluated by visual ratings for summer patch resistance. Parents and progeny were established in mowed spaced-plant trial in the spring of 2017 at North Brunswick, NJ, inoculated with *Magnaporthiopsis poae* and *meyeri-festucaae* in May 2017 and 2018 and evaluated for disease in 2018. $Y = 2.66 + 0.56X \pm 0.03$, $R^2 = 0.09$

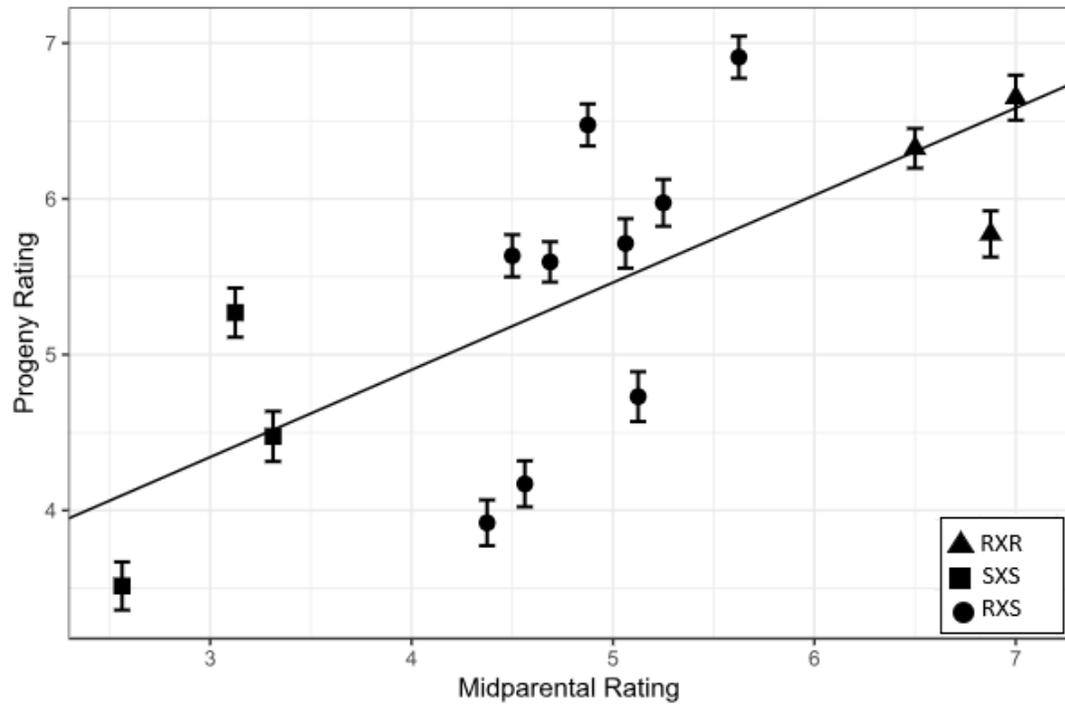


Figure 4.4: Mid-parent-offspring regression of pseudo F2 population means regressed on the mid-parent value from 15 crosses between six hard fescue parents evaluated by NDVI for summer patch resistance. Parents and progeny were established in mowed spaced-plant trial in the spring of 2017 at North Brunswick, NJ, inoculated with *Magnaporthiopsis poae* and *meyeri-festucaae* in May 2017 and 2018 and evaluated for disease in 2018. $Y = 0.36 + 0.37X \pm 0.03$, $R^2 = 0.04$

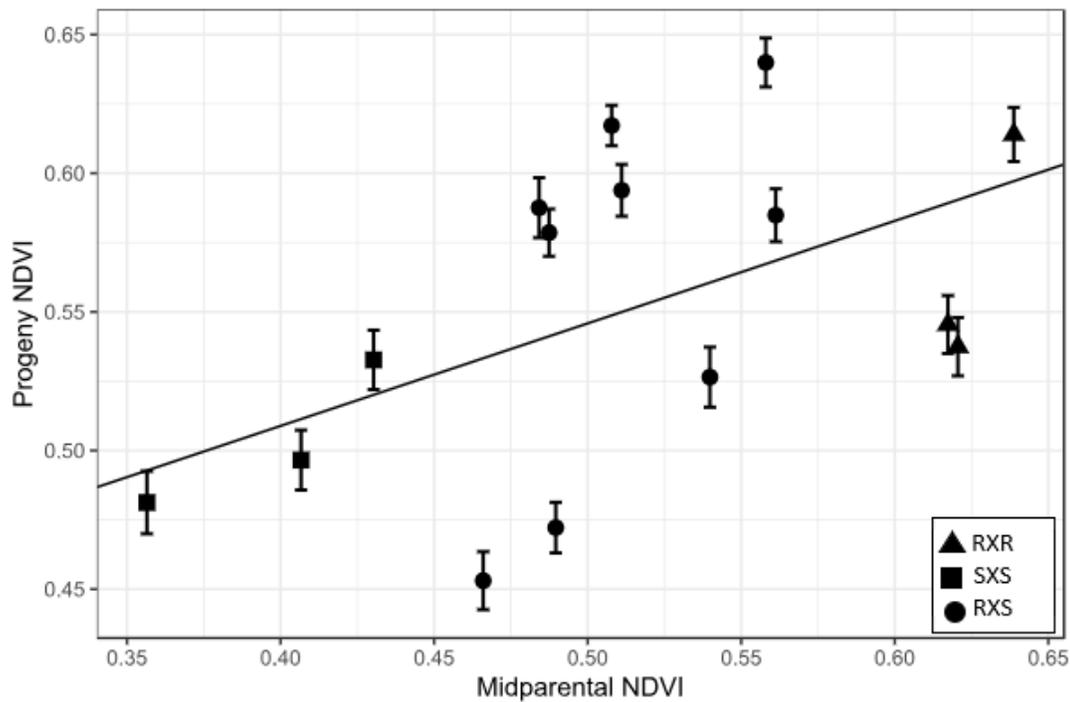


Table 4.2. Description of controlled crosses between summer patch resistant and susceptible creeping hard fescue clones assessed by NDVI including heterosis calculations^a

Female x male ^b	Number of Progeny	Pseudo F ₂ progeny mean NDVI	Mid-parent mean NDVI	Heterosis <i>t</i> test ^c	df	<i>P</i> value ^d
10*8 (RxR)	100	0.58882	0.63875	-2.4226	52.974	0.01886
10*7 (RxR)	100	0.59024	0.62056	-1.1987	25.425	0.2417
10*6 (RxS)	100	0.57647	0.56125	0.3737	18.526	0.7129
10*11 (RxS)	100	0.59979	0.51106	2.055	18.312	0.05443
10*5 (RxS)	100	0.55939	0.48744	1.5354	17.395	0.1427
8*10 (RxR)	100	0.63913	0.63875	0.01988	41.932	0.9842
8*7 (RxR)	100	0.53482	0.61731	-3.251	30.775	0.00279
8*6 (RxS)	100	0.63327	0.55800	1.9227	17.451	0.07099
8*11 (RxS)	100	0.63356	0.50781	3.0718	16.594	0.00706
8*5 (RxS)	100	0.57959	0.48419	2.067	18.144	0.0533
7*10 (RxR)	100	0.48464	0.62056	-5.0482	31.968	1.73E-05
7*8 (RxR)	100	0.55607	0.61731	-2.3517	33.762	0.02466
7*6 (RxS)	100	0.54882	0.53981	0.21495	20.406	0.8319
7*11 (RxS)	100	0.43570	0.48963	-1.3066	18.013	0.2078
7*5 (RxS)	100	0.44770	0.46600	-0.4018	19.048	0.6923
6*10 (SXR)	100	0.59327	0.56125	0.7795	19.157	0.4452
6*8 (SXR)	100	0.64661	0.55800	2.2025	19.429	0.03989
6*7 (SXR)	100	0.50411	0.53981	-0.8632	19.395	0.3986
6*11 (SXS)	100	0.52488	0.43031	2.2615	19.755	0.03517
6*5 (SXS)	100	0.49132	0.40669	1.9631	19.692	0.06392
11*10 (SXR)	100	0.58788	0.51106	1.7825	18.177	0.09137
11*8 (SXR)	100	0.60085	0.50781	2.2442	17.446	0.03806
11*7 (SXR)	100	0.50138	0.48963	0.28388	18.227	0.7797
11*6 (SXS)	100	0.54049	0.43031	2.6309	19.87	0.01607
11*5 (SXS)	100	0.45760	0.35650	3.191	24.553	0.00385
5*10 (SXR)	100	0.59392	0.48744	2.2862	16.978	0.03536
5*8 (SXR)	100	0.59819	0.48419	2.4156	19.771	0.02552
5*7 (SXR)	100	0.45842	0.46600	-0.1678	18.451	0.8686
5*6 (SXS)	100	0.50180	0.40669	2.2122	19.483	0.03907
5*11 (SXS)	100	0.51279	0.35650	4.8106	26.805	5.16E-05

a) Parents and progeny were established in mowed spaced-plant trial in the spring of 2017 at North Brunswick, NJ, inoculated with *Magnaporthiopsis poae* and *meyeri-festuca* in May 2017 and 2018 and evaluated for disease in 2018.

b) S = summer patch susceptible, R = summer patch resistant, selected as R or S from clonally replicated field trials evaluated in 2014.

c) Heterosis effects were tested by comparing pseudo F₂ population means with mid-parent means using a two-sample *t* test. Highlighted columns denote significant *T*-test for heterosis.

d) Significance at $P < 0.05$.

Table 4.3. Description of controlled crosses between summer patch resistant and susceptible creeping hard fescue clones assessed by visual ratings including heterosis calculations^a

Female x male ^b	# of Progeny	Pseudo F ₂ progeny mean disease rating	Mid-parent mean disease rating	Heterosis <i>t</i> test ^c	df	<i>P</i> value ^d
10*8 (RxR)	100	6.07	7.00	-3.33	64.809	0.001459
10*7 (RxR)	100	6.24	6.50	-0.765	28.169	0.4506
10*6 (RxS)	100	5.71	5.25	0.925	21.818	0.3648
10*11 (RxS)	100	5.48	4.50	1.615	18.27	0.1234
10*5 (RxS)	100	5.42	4.68	1.207	19.49	0.2419
8*10 (RxR)	100	7.23	7.00	0.898	50.904	0.3735
8*7 (RxR)	100	5.60	6.87	-3.61	27.749	0.001186
8*6 (RxS)	100	6.47	5.62	1.522	18.962	0.1446
8*11 (RxS)	100	6.49	4.87	2.325	17.95	0.03198
8*5 (RxS)	100	5.99	5.06	1.358	18.424	0.1909
7*10 (RxR)	100	6.41	6.50	-0.26	28.531	0.7937
7*8 (RxR)	100	5.95	6.87	-2.44	36.083	0.01994
7*6 (RxS)	100	5.30	5.12	0.326	22.912	0.7475
7*11 (RxS)	100	3.22	4.37	-1.87	17.876	0.07785
7*5 (RxS)	100	4.37	4.56	-0.31	19.339	0.7607
6*10 (SXR)	100	6.24	5.25	1.977	22.433	0.06041
6*8 (SXR)	100	7.35	5.62	3.104	19.009	0.005836
6*7 (SXR)	100	4.16	5.12	-1.85	20.54	0.07893
6*11 (SXS)	100	4.92	3.12	4.402	30.141	0.0001242
6*5 (SXS)	100	4.63	3.31	2.996	28.258	0.005643
11*10 (SXR)	100	5.79	4.50	2.116	18.602	0.04805
11*8 (SXR)	100	6.46	4.87	2.307	17.201	0.03375
11*7 (SXR)	100	4.48	4.37	0.168	18.968	0.8687
11*6 (SXS)	100	5.62	3.12	6.183	29.023	9.63E-07
11*5 (SXS)	100	3.56	2.56	2.968	32.972	0.005553
5*10 (SXR)	100	5.74	4.68	1.779	17.394	0.09281
5*8 (SXR)	100	5.35	5.06	0.413	18.969	0.6843
5*7 (SXR)	100	3.97	4.56	-0.96	18.73	0.3499
5*6 (SXS)	100	4.32	3.31	2.316	27.135	0.02833
5*11 (SXS)	100	3.45	2.56	2.402	44.442	0.02055

a) Parents and progeny were established in mowed spaced-plant trial in the spring of 2017 at North Brunswick, NJ, inoculated with *Magnaporthiopsis poae* and *meyeri-festucaae* in May 2017 and 2018 and evaluated for disease in 2018.

b) S = summer patch susceptible, R = summer patch resistant, selected as R or S from clonally replicated field trials evaluated in 2014.

c) Heterosis effects were tested by comparing pseudo F₂ population means with mid-parent means using a two sample t test. Highlighted text denotes a significant T-test for heterosis.

d) Significance at *P* < 0.05.

5. HERITABILITY OF SIMULATED WEAR AND TRAFFIC TOLERANCE IN THREE FINE FESCUE SPECIES

5.1 Introduction

Historically, there has been a high consumer expectation for turfgrass quality and performance in home lawns and landscapes leading to greater fertilizer use and water consumption over the years (Osmond and Hardy, 2004). Interest in conserving natural resources has grown within both the public sector and various political groups (Hamrick, 2016). As restrictions on pesticide and water use on turfgrass landscapes become more prevalent, there will be increased demand for sustainable solutions. Yue et al. (2017) showed that many consumers are environmentally conscious and would be willing to pay more for low-maintenance grasses, which suggests that turfgrass breeders should devote more resources to the development of low-input turfgrass species.

Fine fescues (*Fescue* spp.) are considered to be among the best low-maintenance cool-season turfgrasses (Bonos and Huff, 2013; Dernoeden et al., 1994) and provide a number of benefits for low-input turf uses. Fine fescues are generally adapted to dry, shady, low pH conditions, and perform best in well drained soils that are not saturated (Rummele et al., 2003; Bertin et al., 2007). Fine fescues can develop dense, fine turf cover requiring low amounts of water, fertilizer and pesticides.

Three of the fine fescue species most widely used for turfgrass areas are strong creeping red fescue (*F. rubra* L. subsp. *rubra*), Chewings fescue (*F. rubra* L. subsp. *fallax* (Thuill.) Nyman), and hard fescue (*F. brevipilia* Tracey). Strong creeping red fescue has a rhizomatous growth habit which aids in recovery when leaf tissue is severely damaged, however it does not have high levels of drought tolerance when compared to

other fescue species such as hard fescue (Smith et al., 2010) and does not produce turf as dense as Chewings and hard fescues. Strong creeping red fescue is also more susceptible to dollar spot (caused by *Sclerotinia homoeocarpa* F.T. Benn), red thread (caused by *Laetisaria fuciformis* (McAlpine) Burds.) and leaf spot diseases (caused by *Bipolaris* spp.) than Chewings and hard fescues (Han et al., 2003; Shortell et al., 2005). Chewings fescue is a bunch type grass able to withstand lower mowing heights than most other fine fescues, allowing it to be used on golf courses (Watkins et al., 2010). Hard fescue, also a bunch-type grass, has been shown to perform the best out of all the fine fescue species in low- maintenance situations and usually maintains its dark green color longer in drought conditions (Cortese et al., 2011) especially on sandy, well drained soils. However, in heavier soils, hard fescue can be severely impacted by summer patch disease (caused by *Magnaportheopsis poae* Landschoot & N.Jackson).

For low-input turfgrasses to be useful they also need to meet the demands required of turf landscapes, such as recreational uses in home lawns, parks, or athletic fields. This includes the ability to withstand and maintain quality under wear and traffic conditions. Turfgrass traffic tolerance is made up of two components: wear and compaction (soil surface disruption) (Beard, 1973). Wear stress includes tissue tearing, tissue bruising, and tissue removal resulting from horizontal forces, while surface soil disruption includes soil compaction and displacement as a result of vertical forces (Vanini et al., 2007). Therefore, it is important to distinguish between the two types of traffic when developing a turfgrass. Most traffic tolerance research in turfgrass systems has focused on higher maintenance situations such as athletic fields (Samaranayake et al., 2008; Carroll and Petrovic, 1991; Brosnan et al., 2005).

Wear or traffic tolerance is a critical need in fine fescue development, especially since fine fescues are known for their poor performance under wear or traffic conditions. They lack traffic tolerance and have very slow recuperative ability compared to other cool-season turfgrasses (Bonos et al., 2013). In fact, they are often not used in high traffic areas. This attribute was also found to be the most important to consumers (Yue et al., 2017), so it is important to improve wear/traffic tolerance in fine fescues that otherwise perform at acceptable levels under low-maintenance. A few recent studies have shown fine fescues have the potential to perform well under wear in low-maintenance situations (Cortese et al., 2011; Horgan et al., 2007; Watkins et al., 2010). Additionally, considerable genetic variability in wear tolerance (Bonos et al., 2001; Cross et al., 2013) or traffic tolerance (Chen et al., 2014) has been observed within the fine leaf fescues under medium maintenance management. However, no research has been conducted to characterize the genetic contribution of wear or traffic tolerance in any turfgrass species.

Understanding the genetic and environmental effects on the phenotypic expression of a trait is useful in order to develop a breeding strategy to improve the trait. Heritability determines the amount of variability in the phenotype that is due to genetic and environmental factors. Heritability calculations give a measure of the genetic component controlling the phenotype, so the larger the value of heritability the more quickly and less intensively improvement of a trait can be made (Nyquist and Baker, 1991). Breeders can only manipulate genetic factors and therefore a large genetic component is desired over a large environmental component which cannot be influenced by selection. Since heritability estimates are not known for traffic or wear tolerance in any turfgrass species, the objective of this study was to estimate the variance components

and broad-sense heritability of wear and traffic tolerance in three of the most widely used fine fescue species.

5.2 Materials and methods

5.2.1 Plant propagation and field trial establishment

Individual genotypes of Chewings fescue (157 total), hard fescue (155) and strong creeping red fescue (149) were selected from improved breeding material from the turfgrass breeding program at Rutgers University and the New Jersey Agricultural Experiment Station and from several commercial cultivars. Breeding material consisted of individual plants selected from several hundred half-sib progeny turf plots that exhibited superior performance in mowed turf plots; seed used to establish these turf plots originated from numerous isolated polycrosses. Commercial cultivars (four of each of the three fine fescue species) were represented by twelve individual genotypes. In total, one-third of the genotypes in this study originated from commercial cultivars and the remaining were from breeding material. Single tillers were propagated from each genotype and then transplanted to 6.4- by 6.4-cm cell flats filled with BX potting media (Pro-Mix HP, K.C. Shafer, York, PA). After these plants reached the multiple tiller stage (approximately two months), each individual plant (clone) was split evenly into two ramets. One ramet of each clone was separated equally into 6 vegetative replicates and maintained under greenhouse conditions for six months. The other ramet was sent to University of Minnesota where it was split into six vegetative replicates as above. This provided enough plant material to establish two field trials in randomized complete block designs with six replicates at each location (described below).

5.2.2 Wear tolerance trial

The wear tolerance trial was conducted on a Nixon loam (fine-loamy, mixed, mesic Typic Hapludults) at the Rutgers Turfgrass Research Facility at Horticultural Farm #2 in North Brunswick, NJ. The trial was seeded to Stellar GLR perennial ryegrass (*Lolium perenne*) maintained with 16-0-8 granular N-P-K fertilizer (2.44 g N m⁻²) in the fall of 2013. Plants were transplanted into the field within the established ryegrass stand in June of 2014 on 38 cm spacing with a tubular turf plugger (Turf-Tec Intl., Tallahassee, FL). Supplemental watering was applied as needed to ensure establishment and avoid severe drought stress. Fungicides (cyazofamid, and boscalid) were applied monthly from May to August to preventatively control diseases so that wear responses were not confounded by disease presence. The trial was maintained at 7.62 cm mowing height. Prior to wear applications, the ryegrass stand and fine fescues were treated with fluazifop herbicide (1000g a.i. ha⁻¹) every six weeks during the growing season to selectively retard the growth of the perennial ryegrass while at the same time not injuring the fine fescue clones. This allowed the fine fescue clones to grow to their full potential without competition from the perennial ryegrass ground cover since fine fescues are resistant to fluazifop herbicide effects. It also allowed full exposure of the fine fescues to wear/traffic forces. Wear was applied using the Rutgers Wear Simulator (RWS) (Park et al., 2016), a 0.8-m-wide wear simulator constructed from a modified walk-behind power broom (Sweepster, LLC, Dexter, MI) as described by Bonos et al. (2001) (Fig. 6.1a). This machine applies wear without causing soil compaction. Treatments consisted of two passes of the wear simulator applied once a week for six weeks (12 passes in total) from mid-September through October of 2015 and 2016.

5.2.3 Traffic tolerance trial

The traffic tolerance trial was conducted on a Waukegan silt loam (fine-silty over sandy, mixed, mesic Typic Hapludoll) at the Turfgrass Research, Outreach, and Education Center at the University of Minnesota in St. Paul, MN. The trial was planted into established “Champ GQ” perennial ryegrass, maintained with Sustane 15-3-9 granular N-P-K fertilizer (2.44 g N m⁻²), irrigated at establishment, and sprayed with clopyralid herbicide for control of broadleaf weeds. This trial was maintained at 7.62 cm mowing height and treated with fluazifop to retard the ryegrass prior to traffic treatment, similar to those methods described above for the wear tolerance trial. Fine fescue clones were trafficked with a custom-built golf cart traffic simulator towed behind a turf utility vehicle which had been used successfully in previous studies to simulate traffic (Horgan et al., 2007; Watkins et al., 2010). The traffic simulator consisted of two 454-kg traffic units on an axle containing five golf cart tires. This simulator imparts both wear and soil compaction to the turf thus simulating golf cart traffic of two golfers, and equipment on pneumatic tires (Alderman, 2016) (Fig. 6.1b). Traffic was applied 3 d each week beginning mid-September through October of 2015 and 2016. Plots received two passes of traffic on each day for a total of six passes of traffic each week (36 passes total).

5.2.4 Wear/Traffic assessment

Assessment of wear/traffic quality was made during October/November of 2015 and 2016. Turf quality was based on a 1-10 scale with 10 = no effect, 1= clone death. The turf quality rating contained elements of density, and bruising or discoloration of plant tissues. A visual guide of the quality rating was developed to allow raters at both locations to have a uniform rating scale. At the North Brunswick location diameters (cm) of clones were taken before and after wear application to evaluate reduction in size of

plants due to wear. Diameters were determined by measuring across the clone from edge to edge of the base of the plant using a ruler.

5.2.5 Statistical analysis

Due to differences in the type of wear (NJ) or traffic (MN) that was applied, data were analyzed and presented separately for wear and traffic. Data were subjected to analysis of variance (ANOVA). Wear and traffic quality mean separation was achieved by a paired t test ($\alpha=0.05$) with Bonferroni method for correction of multiple comparison. Broad-sense heritability for wear or traffic tolerance was determined by means of variance components calculated from expected means squares from the ANOVA. Data from each year were analyzed separately using the *lme4* version 1.1-12 in R with all sources of variation considered as random effects. Wear (and traffic) performance was analyzed as a randomized complete block for one year or split-plot in time for two years. Variance components were obtained by a model summary function in *lme4* package. Estimates of heritability were determined and their standard errors (SE) were determined using a simulation method by refitting the model 1000 times using parametric bootstrap samples by applying the *simulate* and *refit* functions of the *lme4* package to estimate the distribution of heritability (Yousef et al., 2015).

Broad sense heritability was calculated on a clonal mean basis (H_c) as well as on a single-plant basis (H_{sp}) using the following formula for traits evaluated on

$$\text{one date: } H_c = \frac{\sigma_g^2}{\sigma_p^2} = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

$$\text{and on multiple dates: } H_c = \frac{\sigma_g^2}{\sigma_p^2} = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{dg}^2}{D} + \frac{\sigma_{gr}^2}{R} + \frac{\sigma_e^2}{RD}}$$

$$H_{sp} = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{dg}^2 + \sigma_{gr}^2 + \sigma_e^2}$$

where σ_g^2 equals the variance of genotypes, σ_p^2 equals the phenotypic variance, σ_{dg}^2 equals the variance of dates \times genotypes, σ_{gr}^2 equals the variance of genotypes \times replications, σ_e^2 equals the error variance, D equals the number of dates, and R equals the number of replications (Yousef et al., 2015).

5.3 Results and discussion

5.3.1 Species response to wear

At North Brunswick, hard fescue received the highest average wear quality rating of (4.1 and 4.9, for 2015 and 2016 respectively), followed by Chewings fescue (4.0 and 4.6), and strong creeping red fescue (4.0 and 4.3) (Table 5.1). These results were similar to those found in other studies when full turf plots were worn (Cross et al., 2013; Smith et al., 2010), but contrasted with Bonos et al. (2001) who reported that strong creeping red fescues performed better than hard fescue under simulated wear. Cross et al. (2013) suggested that change in the ranking of species was due to increased breeding and selection work performed with hard and Chewings fescue during the early 2000's. In our trial, post-treatment quality ratings were higher in 2016 than 2015 and had a more uniform distribution (data not shown). Additionally, there were no significant differences between species in 2015 but in 2016, hard fescue was greater than Chewings fescue which was greater than strong creeping red fescue. This would indicate that plants that were more mature and larger in size (Table 5.2) generated a greater resolution in wear quality within the species and suggesting that evaluation of fine fescues on mature plants is preferable. Indeed, studies have shown that different morphological states (Dowgiewicz, 2009) and physiological changes due to season of wear (Park et al., 2010; Chen et al., 2016; Murphy and Ebdon, 2013) can cause changes in wear tolerance of

grasses. This study was conducted in the fall; The fall season was chosen for this study because Chen et al. (2016) showed that fine fescues subjected to traffic in the fall showed increased leaf bruising or discoloration of leaf tissue. The response of fine fescues to wear in different seasons could change evaluation parameters, species differences and the effectiveness of selection.

5.3.2 Species response to traffic

In St. Paul, the average traffic quality ratings for 2015 and 2016 were as follows: hard fescue received the highest average rating of (4.5, 4.7), followed by strong creeping red fescue (4.3, 4.6) and Chewings fescue (4.2, 4.8) (Table 5.1). Although, there was significance between the species for traffic tolerance in MN it was not consistent between years and in fact, Chewings fescue was significantly better than hard fescue in 2016, while strong creeping red fescue had the lowest traffic tolerance. Not surprisingly, we found very poor correlation for entry performance between the two sites (r -values = 0.24421; $P = 0.001$), indicating it may be difficult to identify clones with both wear and traffic tolerance. It is realized that growing environment may also play a role in the species and clone performance at the different locations.

Other researchers have found cultivars performed differently for traffic tolerance based on location (NTEP, 2009a/b; Murphy and Ebdon, 2013). The two machines used in our study applied traffic /wear to the turf in different ways. Brosnan et al. (2005) proposed that wear differences for an entry could be due to methods used to apply wear (i.e. simulator), duration of wear, and the intensity of wear. Greater traffic intensity has been shown to be required to change the ranking between species more so than between cultivars of the same species (Bourgoin and Mansat, 1981). This makes selection for

traffic and wear tolerance more difficult as it is affected by a combination of methods used to impose wear, the evaluation procedure, and the wear intensity (Murphy and Ebdon, 2013). In the case of this study, as with all heritability studies, the heritability estimates can only be truly applied to the population and study the estimates were derived from. However, the estimates can give researchers an idea as how to implement the knowledge gained and methods that might be useful to improve selection for wear and traffic tolerance.

5.3.3 Broad-Sense heritability

Moderate to high broad-sense heritability estimates (0.69-0.82) for wear tolerance in fine fescues were calculated from the North Brunswick location (Table 5.3).

Heritability estimates of wear tolerance on a clonal basis were 0.69 ± 0.04 for hard fescue, 0.73 ± 0.05 for Chewings fescue, and 0.82 ± 0.03 for strong creeping red fescue (Table 5.3). On a single plant basis, the estimates were 0.35 for hard fescue, 0.31 for Chewings fescue, and 0.45 for strong creeping red fescue. The broad-sense heritability estimates for traffic tolerance in St. Paul were considerably lower (0.49-0.60) (Table 5.4). Heritability estimates for traffic tolerance on a clonal basis were 0.49 ± 0.09 for hard fescue, 0.57 ± 0.07 for Chewings fescue, and 0.60 ± 0.06 for strong creeping red fescue. On a single plant basis, the estimates were 0.09 for hard fescue, 0.12 for Chewings fescue, and 0.11 for strong creeping red fescue.

Heritability estimates for wear tolerance were higher and less variable than the heritability estimates for traffic tolerance (Table 5.5). Data for wear tolerance from the North Brunswick location followed a more standard bell curve than data from the traffic tolerance trial at St. Paul which was less evenly distributed. This could have been due to

variability in the different machines used to impose wear and traffic as well as the soil differences throughout the field that may have affected the amount of soil compaction and related stresses that the traffic simulator may have caused.

Genetic improvement through breeding has made an impact on fine fescue performance, but it seems to have less of an impact than in other turf species. This may be due to less breeding effort given to the fine fescues but more likely by the complexity of the genomes present in fine fescues: hard and Chewings fescue are hexaploids ($2n=6x=42$) whereas the strong creeping red fescues are typically octoploids ($2n=8x=56$) (Huff and Palazzo, 1998).

This is the first report of heritability estimates for wear or traffic tolerance in any turfgrass species. The heritability estimates reported here suggest that selection for wear tolerance in fine fescues is possible as the variation observed was mainly due to genetic effects (Table 5.3, 5.4). This research supports the findings of Cross et al. (2013) who showed that wear tolerance had increased in a fine fescue breeding program after years of general quality selection. The low single plant heritability we found suggests that selection for wear tolerance should be performed on replicated vegetative clones to account for the environmental variation associated with wear tolerance. For species such as fine fescues that can be vegetatively propagated, clonal replications are more simply obtained compared to species for which replicated progeny must be used, but breeders will still be limited by field space and time constraints to evaluate replications. This is even more important for selection for traffic tolerance. Heritability estimates were relatively low for traffic tolerance (0.49-0.60) indicating that the environment has a strong effect on the observed variation. There are more factors involved in traffic than in

wear so the added components (i.e. associated soil compaction) could be the cause of the lower heritability estimates observed. Some progress may occur, but the improvements may take longer, and environmental effects should be controlled as much as possible in order to increase traffic tolerance among fine fescues. Interestingly, though regardless of whether wear or traffic was applied, the strong creeping red fescues exhibited the highest heritability estimates indicating that selection would be most effective in this species. This is important since strong creeping red fescues were shown in both locations (Table 5.1) to have the lowest tolerance to both wear and traffic; in essence, selection would be most effective where progress is needed most.

For a trait as complex as wear or traffic the heritability estimates are in a range similar to estimates found for disease resistance in other turfgrasses, though lower than estimates for dollar spot in creeping bentgrass (*Agrostis stolonifera*) ($H = 0.90$) (Bonos et al., 2003) and gray leaf spot (*Magnaporthe grisea*) in perennial ryegrass ($H = 0.92$) (Bonos et al., 2004) which are under strong genetic control. Nevertheless, given the complexity of wear and traffic tolerance, this research is promising to breeders in that improvements should be possible for wear and traffic tolerance in the fine fescues.

5.4 Conclusions

Although fine fescues currently make up a small percentage of the overall turfgrass seed industry (USDA, 2012), as environmental stewardship increases, and natural resources become scarcer, these low-input species will see an increasing role in sustainable turfgrass management. Through increased breeding efforts and the continued increase in knowledge of the inheritance of important traits in these grasses, the usefulness of these species in the industry will improve. Interestingly, our findings as

shown by the variances obtained for “clone” (Tables 5.3, 5.4) illustrate the presence of a significant amount of genetic variation among different fine fescue genotypes for wear tolerance (Table 5.3) and somewhat less for traffic tolerance (Table 5.4). Moderate to high wear heritability estimates indicate that a phenotypic recurrent selection program using replicated genotypes should be an effective tool in breeding for wear tolerance in fine fescue. Traffic tolerance showed lower heritability estimates and will have less response to breeding efforts. Traffic type and machinery used may influence selection and environmental conditions will need to be closely examined to determine the proper techniques to develop cultivars that have more wear and traffic tolerance and can be used in a wider range of applications and environments. From the results presented in this study the use of a wear simulator reduces variation involved with selection and may increase the improvement of the fine fescues as compared to a simulator which imparts both aspects of traffic.

Table 5.1. Wear and traffic quality means of fine fescues in New Brunswick, NJ and St. Paul, MN in 2015 and 2016 post wear and application. Plants were established in mowed spaced-plant trial in the spring of 2014.

NJ		
Species	Wear Quality 2015 ⁺	Wear Quality 2016 ⁺
Hard	4.1 ^a	4.9 ^a
Chewings	4.0 ^a	4.6 ^b
Strong Creeping Red	4.0 ^a	4.3 ^c
MN		
Species	Traffic Quality 2015 ⁺	Traffic Quality 2016 ⁺
Hard	4.5 ^a	4.7 ^b
Chewings	4.2 ^c	4.8 ^a
Strong Creeping Red	4.3 ^b	4.6 ^b

⁺Any two means within a column not followed by the same letter are significantly different at $P \leq 0.05$.

Table 5.2: Diameters of fine fescues in New Brunswick, NJ in 2015 and 2016 prior and post wear application with net change. Plants were established in mowed spaced-plant trial in the spring of 2014.

2015			
Species	Pre-Wear Diameter (cm)	Post-Wear Diameter (cm)	Change (+/- cm)
Hard	12.7	11.7	-1.0
Chewings	15.0	15.0	-1.3
Strong Creeping Red	15.3	14.3	-1.0
2016			
Species	Pre-Wear Diameter (cm)	Post-Wear Diameter (cm)	Change
Hard	23.1	17.6	-5.5
Chewings	24.8	20.8	-4.0
Strong Creeping Red	26.4	20.9	-5.4

Table 5.3: Analysis of variance and broad sense heritability estimates (H) of wear quality of fine fescue entries evaluated in North Brunswick, New Jersey in 2015 and 2016. Plants were established in mowed spaced-plant trial in the spring of 2014.

Hard					
Source	df	MS	F Value	P>F	Variance
Clone	157	13.11	16.3557	<0.0001	0.76
Rep	5	10.31	12.8579	<0.0001	0.03
Year	1	841.80	1049.9255	<0.0001	0.90
Clone x rep	768	1.02	1.2696	<0.0001	0.53
Clone x year	155	3.94	4.9099	<0.0001	0.53
Residuals	1207	0.80			0.80
$H_c = 0.69 \quad H_{sp} = 0.35$					
Chewings					
Source	df	MS	F Value	P>F	Variance
Clone	159	8.13	8.8323	<0.0001	0.50
Rep	5	23.60	14.7873	<0.0001	0.04
Year	1	214.50	233.1647	<0.0001	0.23
Clone x year	157	2.21	2.3998	<0.0001	0.22
Residuals	1559	0.92			0.92
$H_c = 0.73 \quad H_{sp} = 0.31$					
Strong Creeping Red					
Source	df	MS	F Value	P>F	Variance
Clone	162	9.43	17.0675	<0.0001	0.69
Rep	5	2.30	4.1689	<0.0001	0.01
Year	1	39.62	71.6965	<0.0001	0.04
Clone x rep	728	0.74	1.3363	0.0119	0.09
Clone x year	149	1.56	2.8281	<0.0001	0.18
Residuals	733	0.55			0.55
$H_c = 0.82 \quad H_{sp} = 0.45$					

H_c = Broad-sense heritability on clonal basis

H_{sp} = Broad-sense heritability on single plant basis

Table 5.4: Analysis of variance and broad sense heritability estimates (H) of traffic quality of fine fescue entries evaluated in St. Paul, Minnesota in 2015 and 2016. Plants were established in mowed spaced-plant trial in the spring of 2014.

Hard					
Source	df	MS	F Value	P>F	Variance
Clone	158	1.39	2.4529	<0.0001	0.05
Rep	5	7.44	13.1764	<0.0001	0.02
Year	1	18.56	32.8715	<0.0001	0.02
Clone x year	158	0.74	1.3053	0.008	0.03
Residuals	1522	0.56			0.57
$H_c = 0.49$ $H_{sp} = 0.09$					
Chewings					
Source	df	MS	F Value	P>F	Variance
Clone	156	1.61	3.0826	<0.0001	0.08
Rep	5	9.24	17.7249	<0.0001	0.03
Year	1	142.13	272.6123	<0.0001	0.15
Clone x year	156	0.69	1.3313	0.006	0.03
Residuals	1512	0.52			0.52
$H_c = 0.57$ $H_{sp} = 0.12$					
Strong Creeping Red					
Source	df	MS	F Value	P>F	Variance
Clone	150	1.30	2.4712	<0.0001	0.07
Rep	5	11.05	20.9483	<0.0001	0.04
Year	1	36.32	68.8390	<0.0001	0.04
Residuals	1578	0.53			0.52
$H_c = 0.60$ $H_{sp} = 0.11$					

H_c = Broad-sense heritability on clonal basis

H_{sp} = Broad-sense heritability on single plant basis

Table 5.5: Broad sense heritability estimates (H) of wear (NJ) and traffic (MN) quality of all species and locations. Plants were established in mowed spaced-plant trial in the spring of 2014.

Species	Location	Clone	Single Plant
Chewings	New Jersey	0.73 ± 0.05	0.31
Hard	New Jersey	0.69 ± 0.04	0.35
Strong Creeping Red	New Jersey	0.82 ± 0.03	0.45
Chewings	Minnesota	0.57 ± 0.07	0.12
Hard	Minnesota	0.49 ± 0.09	0.09
Strong Creeping Red	Minnesota	0.60 ± 0.06	0.11

Figure 5.1: Comparisons of wear (New Jersey – left) and traffic (Minnesota – right) simulators used to evaluate fine fescue species for wear and traffic tolerance.



6. CONCLUDING REMARKS

This dissertation investigated several aspects concerning the use of fine fescue as a low maintenance turfgrass for home lawns and public areas within the northern United States. When this work was initiated, information on abiotic and biotic stress tolerance of fine fescues was limited. Fine fescues have not had the same intensity of research conducted on them as some of the more prominent turfgrass species. Also, with this limited knowledge the research evolved with a new discovery of a fungus causing summer patch symptoms in fine fescues. Prior to this work, summer patch was believed to be mainly caused by a single pathogen known as *Magnaporthiopsis poae*. In the first study we discovered, characterized, and proved through Koch's postulates that another species could also cause similar symptomology. This new species was named *Magnaporthiopsis meyeri-festucaae* derived from its host and the name of Dr. William A. Meyer. This new species led to following studies within this dissertation and an entirely new focus on management and breeding practices for fine fescues.

Another study was designed to compare the virulence of *Magnaporthiopsis meyeri-festucaae* compared to *Magnaporthiopsis poae*. After we discovered the new pathogen, many other isolates were collected. For use in the breeding and management program it was deemed necessary to find isolates that would consistently lead to severe symptomology of summer patch to be used in future studies. From this work some isolates were found to be comparable to *M. poae* but several were less severe than *M. poae*. Another objective was to see if *M. meyeri-festucaae* had differing levels of fungicide tolerance. It had been observed that in certain areas fungicides seemed to lack efficacy to control summer patch. This worked showed an instance where an isolate of

M. meyeri-festuciae exhibited a 9.5-fold increase in tolerance to azoxystrobin fungicide. This work will need to be continued further to determine if *M. meyeri-festuciae* needs to be controlled differently than *M. poae*.

The next investigation in this dissertation determined the heritability of summer patch resistance in hard fescue. The goal of this work was to explore how breeding techniques could lead to improved summer patch resistance. Summer patch is a very complex trait and evidence is shown that resistance does have a genetic component. The study shows hard fescues selected for summer patch resistance exhibited lower levels of disease symptomology than plants chosen for susceptibility. Through the use of a diallel crossing scheme and comparison of progeny and parents, medium narrow-sense heritability estimates were calculated which support the idea that additive gene action plays a role in disease resistance and that summer patch resistance is possibly quantitatively inherited. But the frequency distributions of progeny from crosses and heterosis results suggest that dominance may also be involved in summer patch resistance. Also, for further analysis evidence of consistent response in multiple years is needed to confirm the screening method is consistent and uniform. This study is incomplete and further data needs to be collected to validate the data collected in 2018.

The final study in this dissertation was to determine broad sense heritability estimates for hard fescue, Chewing's fescue, and strong creeping red fescue. Heritability on a clonal basis was between 0.69 and 0.82 for wear tolerance in the North Brunswick location and between 0.49 and 0.60 for traffic tolerance in the St. Paul location. On a single plant basis, broad sense heritability estimates for the three species were between 0.31 and 0.45 for wear tolerance in the North Brunswick location and 0.09 and 0.12 for

traffic tolerance in St. Paul. However, this research does indicate that improvement of wear and traffic tolerance in fine fescues is possible through recurrent breeding methods based on selection of replicated clonally propagated genotypes rather than selection of single individual plants of a population. Intensive breeding efforts are going to be needed to elevate the level of wear tolerance in fine fescue to an acceptable level.

If fine fescues are to become a successful group of turfgrass species in the US, gains in knowledge, particularly information that will affect breeding methods, will have to be made. Most likely, fine fescue cultivars will be developed for use in targeted locations. The varying species may end up being useful depending on the goal of each site. While this body of work provides a foundation that has allowed for the development of new management practices, breeding strategies, and improved germplasm for the utilization of fine fescues, work in these areas should be continued to allow for the development and release of improved fine fescues.

For future researchers studying fine fescues, more studies will need to be performed, especially in the area of stress tolerances. The work conducted with summer patch illustrates the complexities of understanding the interaction of two or more living organisms. Effective protocols for field evaluation need to be tested to confirm screening is affective for genetic testing. More investigation needs to go into the possible connections of fine fescue summer patch resistance and tolerances to heat and stress. All, of these stresses are theorized to be controlled by a multitude of morphological differences and physiological processes. As we gain better understanding of all these characteristics then fine fescues can truly be utilized.

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