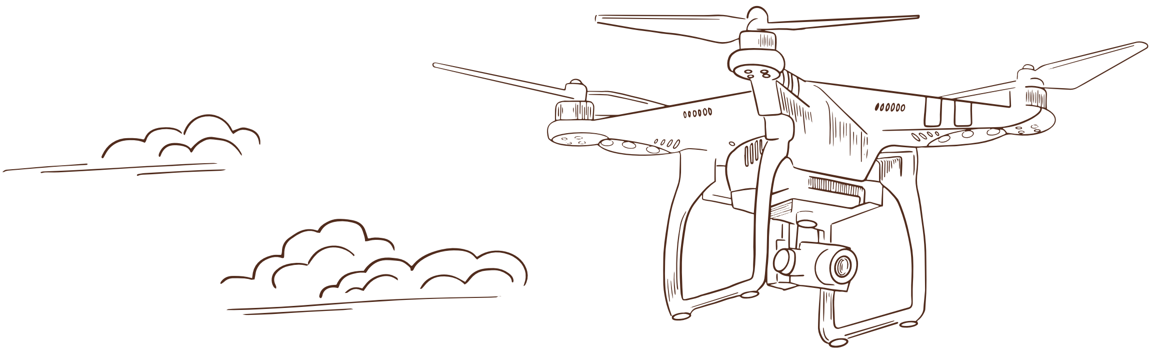

Towards Strategies to Manage Weeds in Turf

without

Herbicides

Daniel Hahn

Propositions

1. Because of its different growth habit tall fescue should not be part of the *Festuca* complex.
   (this thesis)

2. Allelopathy is not just a general weed suppression mechanism rather it is a weed species selection process.
   (this thesis)

3. Coral reefs will disappear by the end of the century due to climate change.

4. The optimal amount of animal protein in the human diet is 0%.

5. Legislative changes are driven by public opinion not data.

6. Lack of teaching basic life skills in school creates insecure young adults.

Propositions belonging to the thesis, entitled

Towards strategies to manage weeds in turf without herbicides

Daniel Hahn
Wageningen, 6 December 2021

Towards strategies to manage weeds in turf without herbicides

Daniel Hahn

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This research was conducted under the auspices of the C.T. de Wit Graduate School for Production Ecology and Resource Conservation

Towards strategies to manage weeds in turf without herbicides

Daniel Hahn

Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Monday 6 December 2021
at 1:30 p.m. in the Aula.

Daniel Hahn

Towards strategies to manage weeds in turf without herbicides,
136 pages

PhD thesis, Wageningen University, Wageningen, NL (2021)
With references, with summary in English

ISBN: 978-94-6395-968-1
DOI: https://doi.org/10.18174/553303

Abstract

The sportsturf industry in Europe is moving toward non-chemical broadleaf weed management. This thesis explored building a framework for alternative weed management strategies in turfgrass areas. Most importantly, I focused on the strategy of using suitable turfgrass species to maintain vigorous, dense turfgrass that is competitive against weeds. In light of climate change and stricter regulations regarding water, fertilizer and pesticide use, practitioners recently opted to use low input species such as *Festuca* spp. We therefore investigated the growth interfering capacity of 27 cultivars from five *Festuca* species (Chewings fescue [*F. rubra L.* ssp. *fallax* (Thuill.) Nyman], slender creeping red fescue [*F. rubra* L. ssp. *littoralis* (G.Mey.) Auquier] strong creeping red fescue [*F. rubra L. ssp. rubra Gaudin],* hard fescue [*F. brevipila Tracey]* and tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.]) against three common broadleaf turfgrass weeds, namely clover (*Trifolium repens* L.), daisy (*Bellis perennis* L.) and yarrow (*Achillea millefolium* L.). In a climate chamber, 60 *Festuca* seeds were placed on water agar in a series of plastic containers for 30 days. Thirteen days after sowing, twenty weed seeds were introduced to each container, and germination and root length of those weeds was recorded. Interference by presence of *Festuca* species did not affect weed seed germination, but a pronounced negative effect on weed root growth was observed, with reductions of up to 85%. Clover was most severely affected in the presence of tall fescue, whereas all fescue species caused a similar reduction in root length for yarrow. Within most *Festuca* species we observed cultivar differences in growth interfering capacity. Weed species used in this experiment differed in their susceptibility to interference by fescue, with yarrow being more sensitive to growth interference by *Festuca* cultivars than clover. Daisy was most sensitive, and due to high mortality rates the species was removed from the experimental analysis. While we conducted the growth chamber screening, we also sowed a field trial with six cultivars representing each species used in the growth chamber experiment and four weed treatments including clover, daisy, yarrow and a mixture of these species in a randomized block design replicated by year. Weather conditions varied between years and caused different results, however cultivar Musica (Chewings fescue) and Barpearl (slender creeping red fescue) were least affected by weed growth over both years and resulted in acceptable visual sward quality. Manual counting of weeds with a 100-point quadrat in the above-described experiment was time consuming and limited the number of recordings. We therefore collected aerial multispectral images and applied random forest model (RF) machine learning algorithms to quantify vegetation cover using image analysis. Object-based classification using spectral features from a previous segmented orthoimage resulted in highest classification accuracy to detect weeds with 99% accuracy and high agreement to point quadrat measurements on the ground. Particularly weeds with distinctive shape features, such as daisy, were clearly detectable and had a good agreement with ground measurements. We believe that development of an automated weed recognition tool would greatly improve scalability and quality of turf research in the future and would also have applications in the early detection of weed cover in amenity turf. We conclude that weed control without traditional herbicides requires defining the purpose of turfgrass areas, establishing threshold levels for control, management strategies to maintain dense turf cover, early detection of weed species, and alternative control measures such a mechanical removal or development of bioherbicides.

Keywords: growth interference, sustainable weed management, germination, remote sensing, turfgrass quality**.**

Contents

CHAPTER 1

General introduction

1.1 Ecological value of turfgrass

Grasses in general are a type of plant species that provides anthropogenic benefits, known as ecosystem services (Boyd and Banzhaf, 2007). Ecosystem services are divided into four subcategories: provisioning-, regulating-, supporting-, and cultural services (Figure 1.1). Provisioning services, are products derived from grasses such as sod and seed or food for livestock (Larson et al., 2016). Established grass swards regulate an ecosystem by reducing the heat in urban areas, filtering water or storing carbon (Monteiro, 2017). Also, grasses support ecosystems by contributing to soil formation and nutrient cycling (Kopp and Guillard, 2002; Wu and Bauer, 2012; Jiang et al., 2020). Lastly grass areas are aesthetically pleasing and provide cultural services, including areas for recreation (Monteiro, 2017).

Turfgrasses are a subcategory of grasses and have been defined in the broad sense as a group of graminaceous plant species that ideally coalescence into a dense sward, are maintained by anthropogenic practices such as regular mowing and, depending on their use, are further managed by fertilization, irrigation, cultural control (such as aeration) and pest control measures (Thompson and Kao-Kniffin, 2017; Monteiro, 2017; Brosnan et al., 2020b). Managed turfgrass areas worldwide include more than 700,000 athletic fields and 17,000 golf courses (Chawla et al., 2018).

The turfgrass industry provides income and employment to seed and sod producers, as well as owners and employees of sports facilities and municipal parks. Turfgrasses also serve municipalities by stabilizing road banks, and increasing homeowner property values (Figure 1.1) (Johnson et al., 2013). In the US alone 62 million acres of turfgrass areas are maintained, creating about 822,000 jobs (Chawla et al., 2018). Turfgrasses also provide cultural benefits through landscaping of public parks and squares in urban areas, which provides aesthetic value and space for recreation (Monteiro de Castilho et al., 2020). In urban environments, the presence, access and safety of turfgrass playing surfaces are even used as an indicator of the health of a city (Brosnan et al., 2020b). The European Union has reported an indirect connection between economic productivity of citizens in urban areas with access to green space, which increases human happiness and health (European Commission, 2018). Sports facilities in particular benefit society through the provision of tourism destinations, for example golf courses, and contribute to public health by providing areas intended for physical activity (Johnson et al., 2013; Brosnan et al., 2020b).

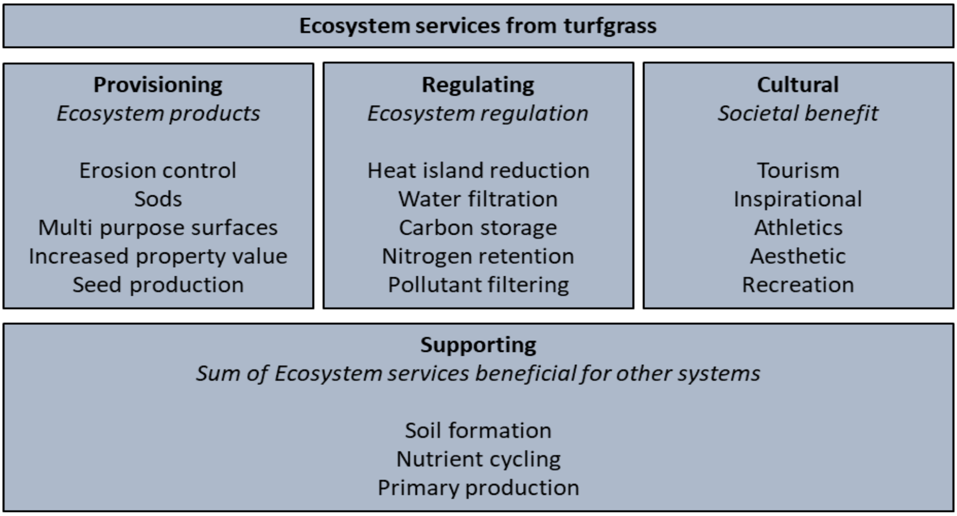

Fig 1.1. Ecosystem services provided by grasses. The figure is adapted from Thompson and Kao-Kniffin (2017) and Monteiro, (2017).

1.2 Broadleaf weeds interfere with benefits provided by turfgrasses

Weeds can be grouped according to morphological characteristics into grassy weeds, sedges (both Monocotyledon), and broadleaf weeds (Dicotyledon) (Uddin et al., 2010). Any plant species other than the desirable turfgrass species for a defined area is classified as a weed. Controlling weeds is necessary because some societal benefits associated with turfgrasses are negatively impacted by the presence of weed species. Sod production for example is estimated to be a 1.6 billion dollar industry and the invasion of weeds greatly reduces sod quality and achievable market prices (Brosnan et al., 2020c). Weed infestation in athletic fields reduces the aesthetic value and the function, such as playing quality, of turfgrass (Uddin et al., 2010). Broadleaf weeds have been identified as the most difficult weed in the sportsturf sector to control without herbicides in the Netherlands (N. Dokkuma, personal communication, September 1, 2017). For this reason, this dissertation will focus on this category of weeds. The presence of broadleaf weed species in turfgrass varies depending on the site characteristics and use of the turfgrass area. A survey conducted by Uddin et al. (2010) found that some species, such as slender amaranth (*Amaranthus viridis* L.), were only found in athletic fields, while common purslane (*Portulaca oleracea* L.) occurred on heavily irrigated sod farms with clay soils. However, some species, for example false buttonweed (*Borreria repens* D.C.), were found in all turf areas surveyed (Uddin et al., 2010). This suggests that some broadleaf species are widely adapted to various site conditions (Sit et al., 2007).

1.3 Synthetic herbicides and the shift towards alternative weed control strategies

Traditionally, weed management in turfgrass has relied heavily on herbicide use, as reflected by a review paper published in 2003, which found that 750 publications focused on chemical weed control in turfgrass and only 25 publications addressed cultural management to reduce weeds (Busey, 2003). A large majority of the turfgrass literature has been published in the US, where a broad range of herbicides is available for use in turfgrass. However, in many countries the amount of herbicide applied to amenity turf has drastically decreased in recent years. In the UK a 64% reduction was observed from 2006 to 2012, and on golf courses in Denmark a reduction of 82.7% was realized (DEFRA, 2018; K. Petersen, personal communication, 2018). There are several reasons for this shift towards reducing herbicide use in the turf sector.

For one, herbicide resistance due to overuse or a lack of active ingredient rotation has been the subject of over 40 turfgrass publications (Brosnan et al., 2020c; Heap, 2020). In most cases, *Poa annua* developed resistance to a variety of active ingredients such as simazine, prodiamine and pendimethalin (Heap, 2020). Also, broadleaf weeds such as buckhorn plantain (*Plantago lanceolata*) developed resistance to 2,4-D (Heap, 2020). As a result, scientists and practitioners have called for more research into developing novel modes of action (Brosnan et al., 2020a). However, the truth of the matter is that no significant new MOA has been discovered since the 1980's and this fact, combined with the increasingly rapid evolution of herbicide resistance, has led some researchers to suggest that almost all existing herbicides may be unusable by 2050 (Westwood et al., 2018).

Secondly, there is growing concern about health issues associated with the use/application of herbicides. Studies have reported health issues experienced by applicators of herbicide products, ranging from minor skin rashes to kidney disease and potential carcinogenic effects (Pimentel et al., 2013; Jayasumana et al., 2014; Sarwar, 2015). Turf specific literature on negative health effects of herbicides on non-target species includes one study that reported DNA damage of meadow voles (*Microtus pennsylvanicus*) living on golf courses in Ottawa, Canada (Knopper and Lean, 2004). A study conducted by Arcury-Quandt et al. (2011), in which ten golf course superintendents were interviewed, suggested that turf managers focus on machine and operator safety training and neglect pesticide safety training, which could be a reason for their misuse. In European countries such as Denmark and the Netherlands concerns about herbicides were raised after herbicidal metabolites were detected in groundwater wells and surface water used for drinking water (Kristoffersen et al., 2008; Malaguerra et al., 2012). Because of public health concerns, the European Union is promoting non-chemical weed control options to reduce the risk of indirect or direct contact to herbicides. Concerns over herbicides is therefore not a topic that is limited to the turf sector but extends to all herbicide usage. Most prominent is the concern over glyphosate, which was recently classified as a group 2A carcinogenic agent (Jayasumana et al., 2014; American Cancer Society, 2020). Regardless of this, glyphosate is the world's most used herbicide and its global use increased almost 250 fold from 3200 tons/year in 1974 to near 825,000 tons/year in 2014, with use expected to reach 920,000 tons/year by 2025 (Vandenberg et al., 2017; Landrigan and Belpoggi, 2018; Maggi et al., 2020). This underscores that despite environmental and health concerns, alternative control options are absent and there is a continued demand for herbicidal products to maintain productivity.

To reduce the risk and impacts of herbicides on human health and the environment, the European Union formulated a strategy (Directive 2009/128/EC) which requires member states to follow integrated pest management (IPM) strategies (European Parliament, 2009). The directive essentially promotes only using herbicides as a last means and favors the use of non-chemical control strategies to control weeds. The turf industry in many countries acted proactively, as exemplified by voluntary agreements reached by the Dutch turfgrass amenity sector, referred to collectively as the 'green deal', to phase out pesticides entirely by 2022 (Mansveld et al., 2016). However, the European turf sector still needs to develop non-chemical weed control strategies to secure the continued benefits of those ecosystem services provided by turfgrass that are most threatened by the presence of weeds.

1.4 Non-chemical broadleaf weed control strategies in turfgrass

Broadleaf weeds, like other weed species, are early colonizers, meaning that any openings in a turf sward are rapidly invaded by weed plants germinating from the natural seedbank. This process is also known as 'recruitment' (Busey, 2003; Abu-Dieyeh and Watson, 2007a; Turner et al., 2012). Once established, broadleaf perennial weeds can further spread vegetatively through rhizomes (for example *Achillea millefolium* L.) or stolons (for example *Trifolium repens* L.) depending on the species (Bourdôt, 1984; Chapman and Robson, 1992). Therefore, non-herbicidal management practices targeted at controlling broadleaf weeds include avoiding germination from the natural seedbank, reducing the ecological niche for plant establishment and growth, and applying direct control of mature plants with alternative products to herbicides or mechanical means.

Germination of broadleaf weed seeds from the seedbank is driven by the microsite, hence the biotic and abiotic conditions surrounding the seed (Turner et al., 2012). In newly seeded areas, the objective is to reduce the ecological niche for weed seed germination by establishing a dense turf rapidly (Beard, 1973; Watschke and Engel, 1994; Larsen and Fischer, 2005). In the absence of herbicides, management practices of established swards aim to improve the health of the desirable turfgrasses to prevent germination, recruitment and establishment of broadleaf weed species rather than targeting the seedbank, and to prevent cover loss due to abiotic or biotic stress (Busey, 2003; Larsen et al., 2004; Turner et al., 2012). Practitioners select desirable turfgrasses based on adaption to the local climate, which enhances resilience or resistance to biotic and abiotic stress (National Turfgrass Evaluation Program, 2020). However, the environmental footprint of turfgrass species is also an important factor, with slow growing/low input turfgrass species being the preferred choice. Species like *Festuca* spp., are often favored by practitioners because they require low inputs of water, nutrients and pesticides (Dernoeden et al., 1994; Arthur, 2003; Watkins et al., 2010). Turfgrass evaluation programs assess the performance or visual quality of turfgrass species, but assessments of competitiveness against weeds are not conducted. Currently, no reliable or affordable tools exist to objectively quantify weed cover in turfgrass trials, which leaves practitioners with little information about competitiveness of turfgrass cultivars against weeds. In turfgrass trials, researchers rely on visual scoring of total weed cover (Abu-Dieyeh and Watson, 2007b), using an intersected frame to either estimate percentage of weed cover within each section or record presence/absence of weeds underneath each intersection of a point-quadrat (Martelloni et al., 2019). Visual scoring is subject to bias, and point quadrat techniques are laborious and time consuming, limiting both the quality and scale of field research. In agricultural research conducted on vineyards, this problem was overcome by using unmanned aerial vehicles (UAV's) and analysis of multispectral images to detect bermudagrass (*Cynodon dactylon* L. Pers.) (Jiménez-Brenes et al., 2019). The challenge of weed detection in turfgrass is that vegetation is maintained at a short cutting height (small objects) and weeds blend well with the turfgrasses (similar pixel values). In agricultural settings, detecting weeds in row crops is arguably less challenging because any vegetation between the rows can be seen as a weed (crop row detection), and weeds are surrounded by soil, hence a clear difference exists between green vegetation and brown background (difference based on pixel values) (Louargant et al., 2018). Detecting weeds in turfgrass would help to better understand the competitiveness of some turfgrass species against weeds. Also, turfgrass managers need mapping tools to potentially spot treat weeds in the future, because spraying pre-emergence herbicides over large areas is no longer a legal option.

The underlying mechanism of growth interference of desirable turfgrass species by broadleaf weeds is resource competition for above ground light (Scott et al., 1984) as well as water, space, and nutrients below ground (Wilson, 1988; Casper and Jackson, 1997). While above ground competition is primary driven by one resource (light), below ground competition between turfgrass and weeds is more complex and poorly researched. Deep rooting turfgrass species and shallow rooting weed species competing for water could result in habitat partitioning and even less resource competition (Casper and Jackson, 1997). In the given example, a deeper rooting system would still favor turfgrass growth, because the deeper turfgrass root system is capable of absorbing more nutrients, which results in more shoot growth and tissue production leading to superior light competition (Casper and Jackson, 1997). Lane et al. (2019) observed that a fast germinating, rhizomatous species such as tall fescue [*Schedonorous arundinaceum* (Schreb.) Darbysh.] was less susceptible to establishment of kura clover (*Trifolium ambiguum*) than slow establishing bunch type species such as hard fescue (*Festuca brevipila* Tracy). The study by Lane et al. (2019) highlighted the challenge in identifying the main mechanisms of growth interference, as fertilizer regimes in this study "were likely on the low side of recommendation" and low nitrogen availability to turfgrass generally leads to high weed cover (DeBels et al., 2012). Apart from resource competition, growth interference mechanisms add to the complexity of turfgrass-weed interactions (Mahall and Callaway, 1992; Casper and Jackson, 1997). For example, *Festuca* species were reported to possess growth interfering potential against weed species in field settings and laboratory assessments through the production of allelopathic chemicals (Meta-Thyrosine) (Bertin et al., 2003a, 2007, 2009). The challenge that remains is to develop a method to screen for this growth interference potential among *Festuca* species and cultivars to help manage broadleaf weeds in turf settings without herbicides.

1.5 Research chapters in this thesis

In this thesis, I investigated some of the aforementioned concepts that collectively provide a framework that could be used to develop and implement alternative weed control strategies in turfgrass areas in the absence of herbicides. I investigated the natural ability of sustainable turfgrass species *Festuca* spp. (Watkins et al., 2010) to interfere with the germination and growth of three broadleaf weed species, *Bellis perennis* L., *Trifolium repens* L, and *Achillea millefolium* L, which are commonly found on turfgrass in the Netherlands. This investigation was conducted in a growth chamber experiment, as well as under field conditions. The field experiment also served as a test field to compare broadleaf weed cover estimations using traditional point-quadrat techniques to estimations by aerial multispectral images and a Pixel-, object- based random forest model classifier.

In the introductory chapter **(Chapter 1)** a sketch is given of the importance of turfgrass species and the ecological services they provide. This is followed by a description of the negative impact of broadleaf weeds on the functioning of turfgrass areas. Legislative bans on herbicides require the examination of alternative management options for these weeds. Various components of such alternative weed management strategies are introduced and investigations regarding these strategies and concepts are presented and discussed in the following chapters.

Chapter 2 gives a broad perspective of alternative weed management, including preventive measures based on general turfgrass management practices to avoid the weed problem as much as possible and direct control without herbicides. We identified the need to rethink management practices such as increasing fertilization rates to support the growth of desirable turfgrass species as a mechanism to outcompete weeds. We also examined several management practices, including overseeding, fertilization, mowing, and irrigation, and discussed how to optimize such practices to reduce weed invasion, establishment, and growth. Lastly, we reviewed options to control/remove existing weed stands by bioherbicides, organic products, and direct mechanical removal.

Chapter 3 presents the results of trials conducted to screen *27 Festuca* cultivars from five species for their ability to interfere with germination and growth of three common European broadleaf weeds (clover, daisy, and yarrow) in a growth chamber experiment under controlled environmental conditions. We placed *Festuca* seeds together with weed seeds on water agar, in plastic containers, and examined growth interference capabilities of *Festuca* on those weeds. We tested the hypothesis that *Festuca* species and cultivars differ in the ability to interfere with germination and growth of weed seed.

Chapter 4 presents the results of a field trial conducted to investigate the growth interference effect of *Festuca* cultivars on common broadleaf weed species. We sowed the fields at Barenbrug research station, Wolfheze, with six *Festuca* cultivars before we introduced weed treatments consisting of clover, daisy, yarrow, and a mixture of all three. This field trial was complementary to the growth chamber experiments described in chapter three and aimed to determine if observations made in a controlled environment could be replicated in the field. The overall objective was to investigate if *Festuca* cultivars differed in vigor and if the presence of cultivars influenced weed cover. We also examined which cultivar provided the best visual appearance when pre-emergent herbicides, a normal component of common sowing protocols, were not included.

In **Chapter 5** new methods for determination of weed cover were investigated. Since the use of point quadrat methods for the evaluation of weed cover is arduous and time consuming, it limits the number of genetic entries that can be included in field screening trials. We therefore explored options for speeding up the data recording in turfgrass trials with broadleaf weeds. We investigated an image analysis method to separate broad leaf weeds and grasses to replace visual scoring or point quadrat methods to estimate weed cover. We conducted our evaluations using the same field trial described in chapter 4 and took aerial multispectral images We then constructed an orthoimage which was segmented. Vegetation (grass, clover, daisy, yarrow) and non-vegetation (soils) in some of the segments were manually labelled to train a random forest model to label all segments. We drew polygons around each experimental plot and compared the performance of object-based image analysis (OBIA), pixel-based analysis and a combination of both with the current standard (point quadrat) to estimate weed cover. We also took individual images with a light box and estimated ground cover with a software (Turf Analyzer), which we compared to data obtained from the various image analysis approaches discussed above.

In **Chapter 6,** the general discussion, we discuss that policy in Europe is moving towards non-chemical broadleaf weed control, which comes with challenges how to control weeds in turfgrass areas in the future. We propose to redefine what a weed is and tolerance levels. We examine how weeds can be controlled starting from damaging the natural seedbank, selecting competitive turfgrass species to outcompete weeds and finally alternative strategies to remove weeds.

CHAPTER 2

Herbicide-free turfgrass management: optimizing maintenance practices to control weeds

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This chapter has been published as Hahn *et al*., 2020. Crop Science 60(5): 2204-2220.

Abstract

Bans on the use of synthetic herbicides require innovative management approaches to maintain the attractiveness and usability of turfgrass swards. Such measures should include the use of locally adapted cultivars that germinate and establish quickly, resulting in the densest possible stands. Additionally, a number of turfgrasses have been reported to produce allelopathic substances that inhibit growth of common turfgrass weeds. Mowing heights should be set to achieve maximum weed suppression while still providing acceptable quality for desired use. Sustainable turfgrass management programs have led to a reduction in fertilizer inputs, however, without the availability of herbicides, fertilization regimes need to be re-examined. The literature suggests that broadleaf weeds are reduced but never fully controlled when more N is applied; therefore, finding a balance between what is needed and what is environmentally safe and sustainable is critical. Organic herbicides include plant pathogens from the fungus *Phoma* and strains of the bacterium *Pseudomonas fluorescens*. Both can be used to control several weeds common to turfgrasses. Acetic acid has also been shown to have herbicidal activity, however it has limited residual activity and efficacy remains questionable on mature weeds. Thermal weed control can be used to sterilize a seedbank or spot treat existing weeds. Future turfgrass breeding programs could focus on understanding and enhancing the allelopathic potential of turfgrasses to outcompete weeds more effectively. Furthermore, more research should be directed at assessing the competitiveness of certain turfgrasses against weeds within the limitations of producing turfgrass areas of acceptable aesthetics and playing quality.

Keywords: mowing, fertilization, bioherbicides, allelopathy, turfgrass selection

2.1 Introduction

Weeds are plants that are defined as undesirable in a specified area, because of their ability to compete with native or desirable plant species (McCarthy, Murphy and Turgeon, 1994). Turfgrasses compete with weeds for nutrients, water and space below ground (Snaydon, 1971) and for light above ground (Holt, 1995). Moreover, the presence of weed species in turfgrasses reduces playing quality, aesthetic value and usability (Larsen et al., 2004; McCarthy et al., 1994; McElroy and Martins, 2013; Stewart-Wade et al., 2002). Weed species rapidly germinate in exposed soil (McElroy and Martins, 2013) caused by abiotic factors, such as vehicular or human wear, drought, shade, cold, as well as by biotic factors affecting turfgrasses, such as pest and disease damage (Brodie and Burton, 1967; Busey et al., 1982; Waddington et al., 1978). Established weed species have a lower wear tolerance compared to turfgrasses, which increases the risk of soil exposure on weed infested turfgrass areas (McElroy and Martins, 2013). In terms of their aesthetic appearance, weeds often stand out due to enhanced floral production or color differences compared to turfgrass (McCarthy et al., 1994). Additionally, some turfgrass weed species, such as dandelion (*Taraxacum officinale* Weber) and white clover (*Trifolium repens* L.), are too close to the soil to be removed by mowing and produce patches that disrupt uniformity and affect ball roll and lie in sports scenarios (Larsen et al., 2004).

Turfgrass areas infested with weeds are often considered a sign of mismanagement (Masin et al., 2005). However, weeds can establish even under optimal management (Busey, 2003). Nevertheless, weeds establish opportunistically, if managers fail to reduce overall weed densities to a minimum and maintain a dense turfgrass sward.

Herbicides are the predominant method to control weeds in turfgrass management, particularly in large areas, such as golf course fairways (Dahl-Jensen et al., 2014). No new herbicide mechanism of action (MOA) has been discovered since the 1980s and an ever increasing risk of herbicidal resistance could lead to virtually complete resistance to all conventional MOAs by 2050 (Westwood et al., 2018). In 2017, the Weed Science Society of America listed more than 300 commercially sold modes of action to control weeds in agriculture and turfgrass (WSSA, 2017). The use of herbicides in the United States golf industry increased by an average of approximately 2% between 2007 and 2015 (GCSAA, 2017), with regional differences; for example, golf courses located in transition zones increased herbicide use by 13% whereas North Central regions reduced use by 8% (GCSAA, 2017).

In the European Union, much stricter bans on herbicides have been established. The risk of herbicide resistance (Heap, 1997; De Prado and Franco, 2004), health risks associated with herbicide exposure (Karabelas et al., 2009) and environmental concerns (Stoate et al., 2009) have led to a restriction of synthetic herbicide products (Barzman and Dachbrodt-Saaydeh, 2011). Today, only 123 herbicides are approved for commercial use (European Commission, 2017), and strict bans have been introduced in the turfgrass sector. For example, in Germany only two herbicides, namely Banvel M and Nasalt (active ingredients Dicamba and MCPA, respectively), can be used on golf courses (Deutscher Golf Verband, 2017). In Great Britain, the maximum allowable overall load of herbicide active ingredients that may be applied to amenity turfgrass (public areas, residential lawns, sports field, golf courses etc.) was reduced from 1837 tons in 2006 to 671 tons in 2012, a 64% reduction (Defra, 2018). Denmark has set maximum permitted levels of pesticide use for designated areas on golf courses (European Commission, 2017). This has resulted in an 82.7% decrease in annual pesticide use on golf courses from 1998 to 2014 (K. Petersen, personal communication, 2018). In Holland, the turfgrass industry has agreed to accept a complete ban of all pesticides by 2020 (Dutch Ministries of Economic Affairs, I&M and BZK, 2017).

It is clear that the future of weed control in European turfgrass landscapes will be limited to non-chemical herbicide strategies. Few studies, other than those of Dahl-Jensen et al. (2014) and Silvertown et al. (2006), have investigated non-chemical management practices for weed suppression in amenity turfgrasses. In Scandinavia, most golf courses can cope with herbicide-free management, but Dahl-Jensen et al. (2014) found that weed densities severely increased after a period of around three years. Weed control is most effective under such circumstances when a variety of methods are used to support the growth of desirable species and reduce the fitness of unwanted weed species (Marble et al., 2015).

Weeds are early colonizers, and their persistence is dependent on their competitive ability against turfgrasses (Watschke et al., 1995). Non-chemical approaches to control weeds must therefore focus on reducing the availability of ecological niches in a turfgrass sward for weed colonization, germination, and establishment (Larsen et al., 2004). Maintaining dense turfgrass swards is primarily dependent on the genetic ability of turfgrasses to resist local abiotic- and biotic stresses (Watschke and Engel, 1994). Secondly, maintenance practices aimed at promoting turfgrass growth favor the competitive ability of turfgrasses to outcompete weeds (Dahl-Jensen et al., 2014). The following sections provide a perspective on amenity turfgrass management approaches focused on weed control without the use of herbicides.

Cultivars that germinate and establish quickly should be selected in newly seeded areas to reduce the chances of weed germination. The selected cultivars need to be adapted to the local climate to provide the densest turfgrass sward possible. Every year the British Society of Plant Breeders (BSPB) Turfgrass Seed manual and the National Turfgrass Evaluation Program (NTEP) rank the majority of turfgrass cultivars for different characteristics, such as visual merit, shoot density, live ground cover, resistance to abiotic and biotic stress, fineness of leaf, cleanness of cutting, disease resistance, color, and recovery (BSPB, 2018; National Turfgrass Evaluation Program, 2020). This allows turfgrass managers to select turfgrass cultivars that establish and become dense quickly for out competing weeds in seeded areas.

Selecting turfgrasses for resistance against local biotic (e.g. insects, diseases etc.) stresses can have a secondary effect on weed establishment over time (Busey, 2003). Davis (1958) recorded an outbreak of leaf blight [*Dreschlera poae* (Baudys) Shoemaker] in Kentucky bluegrass (*Poa pratensis* L.) which caused an outbreak of broadleaf weeds infestation. Waddington et al. (1978) attributed annual bluegrass (*Poa annua*) invasion in creeping bentgrass {*Agrostis palustris* Huds. [= *A.stolonifera* L. var. *palustris* (Huds.) Farw.]} swards to injury after dollar spot (*Sclerotinia homoeocarpa)* outbreaks. Brodie and Burton (1967) found a reduction in density of 'Tifgreen' [*Cynodon dactylon* (L.) Pers. X *C.transvaalensis* Burtt-Davy] under nematodes (*Belonolaimus longicaudatus* Rau) infestation and increased establishment of spotted spurge {*Euphorbia maculata* L. [=*Chamaesyce maculata* (L.) Small]} (Brodie and Burton, 1967).

Besides biotic stresses, abiotic stresses (e.g. drought, infertility etc.) can also increase weed densities in turfgrass swards. Perennial ryegrass (*Lolium perenne* L.) showed susceptibility to colonization by spotted spurge under reduced irrigation (Gibeault et al., 1985). In a trial for which 'Big Horn' blue fescue {*Festuca ovina* L. subsp. *glauca* (Lam.) W.D.J. Koch [= *Festuca ovina* var. *glauca* (Vill.) W.D.J. Hoch]} and 'Aurora' hard fescue {*Festuca longifolia* auct. Non Thuill. [= *F. trachyphylla* (Hack.) Krajina]} were kept without fertilizer and irrigation, the turfgrasses showed superior resistance to smooth crabgrass [*Digitaria ischaemum* (Schreb. Ex Schweigg.) Schreb. Ex Muhl.] and white clover infestation, compared to tall fescue (*Festuca arundinacea* Schreb.) cultivars (Dernoeden et al., 1994). The authors also found that tall fescue cultivars were poorer competitors against weeds under low cutting regimes compared to blue fescue and hard fescue.

Competitive growth studies between monocultures and cultivars in mixtures may provide insight into potential weed suppressing characteristics. In cool season turfgrasses, a mixture of turfgrasses under low maintenance regimes is generally more effective in controlling weeds than a single species sward (McKernan et al., 2001). Mixtures of Kentucky bluegrass and perennial ryegrass achieved 8% higher leaf area index (LAI) compared to monocultures of each single species, which may explain the superior weed suppression characteristics of such mixtures (Brede and Duich, 1984). Moreover, cultural management practices can be adjusted to favor certain species; however, dominance is a combination of the genotype and the environment. For example, long leaved perennial ryegrass is dominant over short leaved perennial ryegrass genotypes under infrequent cutting, because long leaved genotypes are better competitors for light (Hazard and Ghesquiere, 1995). Kentucky bluegrass can dominate over creeping red fescue (*Festuca rubra rubra*) at high levels of nitrogen (N) input, whereas creeping red fescue dominates at low levels of N (Juska et al, 1955). At low temperatures, mixtures of red fescue (*Festuca rubra* L. var. *littoralis* Vasey) and perennial ryegrass are capable of germinating more quickly than other species, potentially increasing their competitive ability over weeds (Larsen and Bibby, 2005). In mixtures of creeping bentgrass, red fescue and Kentucky bluegrass, the creeping bentgrass dominated when the turfgrass was maintained at close mowing heights (Davis, 1958).

A common approach in turfgrass management is to encourage the growth of turfgrasses requiring less inputs of valuable resources, which is critical for a sustainable management approach (Cisar, 2004). However, encouraging the use of low input species is only one aspect of sustainability. The most sustainable turfgrass species are those that provide the best performance for desired use year-round, as well as being adapted to local pest, disease, and weed problems.

2.2 Seeding and overseeding strategies

The choice of a proper seeding rate as well as the use of overseeding to increase turfgrass densities can provide a competitive edge over weeds (Parr, 1985). The benefits of planting newly constructed areas with higher than recommended seeding rates to suppress weeds have been reported. For example, Beard et al. (1980) showed that seeding Kentucky bluegrass at 90 kg ha^{-1}, instead of 45 kg ha^{-1}, reduced weed cover on average by 21% two months after seeding.

Overseeding strategies can successfully reduce weed populations if sufficient seed germination is achieved (Aamlid, 1992; Begon et al., 1996; Larsen et al., 2004; Larsen and Fischer, 2005). Harris (2008) found that overseeding at seeding rates ranging between 12.5 g m^2 and 100 g m^2 all provided denser swards with fewer weeds compared to not overseeding, if seeds successfully germinated. Overseeding can be integrated as a regular practice to increase the competitiveness of turfgrasses. Best practices to increase germination after overseeding and reduce weed pressure include overseeding during reduced weed growth, providing good seed-soil contact, and following a fertilizer regime (Vargas and Turgeon, 2004). During germination, sufficient phosphorous (P) must be present, whereas after establishment N is required to facilitate shoot and root growth (Christians, 2016; McVey, 1968).

Proper species selection for overseeding purposes is also crucial. Perennial ryegrass showed good germination rates regardless of whether or not water and fertilizer were applied (Dahl-Jensen, 2017; Elford et al., 2008). Additionally, perennial ryegrass germinates quickly and has good root and shoot competition against weeds (Haugland and Froud-Williams, 1999). Quick and successful establishment is important to outcompete weed seedlings for light and fertilizer (Jeangros and Noesberger, 1990; Snaydon and Howe, 1986). Frequent overseeding with high rates of perennial ryegrass appears to provide competition against perennial weeds over the short-term when weed cover is high and should be considered an important part of a weed management program for municipal turfgrass managers (Elford et al., 2008). Increasing overseeding rates in existing swards could be a long-term weed management strategy. The effect of overseeding might be especially noticeable on sparse turfgrass swards (Dahl-Jensen et al., 2014; Nyholt, 2010).

Another approach to aid turfgrass establishment in newly constructed and seeded areas is to use amendments. Products include Turfiber, alfalfa hay, oat straw, straw mulch, mat seeding, hydro mulch and other materials. These products increase moisture retention in the soil and suppress weed germination (Barkley et al., 1965; Hansford, 1981; Hensler et al., 2001; Shearman et al., 1979; Sowers and Welterlen, 1988). Emphasis must be placed on increasing germination success after overseeding using adequate machinery to place seeds at the right soil depth, provide uniform irrigation coverage, and adequate fertilization.

2.3 Allelopathic potential

The biological phenomenon of allelopathy has been effectively used in agriculture by producers to suppress weeds (Jabran et al., 2015). The capacity of turfgrasses to produce allelopathic substances that influence weed invasion has been reported for a variety of turfgrasses. Turfgrass roots are capable of preventing root invasion from competitor plants by producing leachates that can modify the soil matrix or lead to toxicity after absorption by competitor plants (Seigler, 2006). Thus, allelopathy can be described as a competition mechanism that could provide some control of weed species in turfgrass areas.

The application of aqueous leachates derived from perennial ryegrass, red fescue and Kentucky bluegrass to rooted cuttings of forsythia (*Forsythia intermedia* Spaeth.) reduced top growth of this woody plant (Fales and Wakefield, 1981). Perennial ryegrass showed allelopathic potential on clover, which was enhanced by crown rust (*Puccinia coronate* Corda f. sp. *lolii* Brown) infection of perennial ryegrass (Mattner and Parbery, 2001). Understanding the mechanisms of such interactions could lead to their improved effectiveness in the field and to the isolation of allelopathic compounds for biological herbicide formulations.

Peters and Luu (1985) observed tall fescue pastures free of other plant species and attributed the absence of other plants to the allelopathic potential of tall fescue. In bioassay trials, tall fescue exudates reduced seedling growth of rape (*Brassica nigra* L.), birdsfoot trefoil (*Lotus corniculatus* L.), red clover (*Trifolium pratensis* L.), and several other common turfgrass weeds (Rice, 1987; Luu et al., 1982; Peters and Zam, 1981). Clippings of creeping red fescue and colonial bentgrass (*Agrostis tenuis* Sibth.) reduced germination and early growth of white clover in container studies conducted by Norrington-Davies and Buckeridge (1994). Bertin et al. (2009) showed that weed suppression varied greatly among fescue cultivars. They found strong creeping red fescue cultivars and Chewings fescue cultivars to be most effective at weed suppression.

Allelopathy based breeding programs could be developed to select for allelopathic turfgrass varieties. A future approach might be to develop biological plant protection products from phytotoxic exudates. Putative compounds could be isolated from a methanol extract of root material and the chemical structure could be determined by high-resolution mass spectrometry, infrared spectroscopy and 1H NMR (Kato-Noguchi, 2003). Once allelopathic cultivars or species demonstrating strong competitiveness against weeds are identified, turfgrass managers should consider establishing these species over time.

2.4 Adjusting mowing practices and fertilization to increase competitiveness of turfgrass species

Mowing heights should be adjusted to a point where the turfgrass canopy becomes dense enough to suppress weed populations and short enough to provide good playing conditions. Cutting heights of turfgrasses can be adjusted throughout the year to stimulate either above or below ground growth. The same applies to fertilization applications. Fertilization can lead to quick or slow growth depending on the type of fertilizer being used. Fertilization can encourage above or below ground growth, depending on the application method, timing, and type of fertilizer. Practices such as aeration can generally increase infiltration rates, rooting depth and turfgrass health.

2.4.1 Mowing Practices

Increasing mowing frequency of turfgrasses results in less root mass, rhizome, and stolon production, but increased shoot density (Hull, 2000). The effect of mowing height on turfgrass weeds, such as crabgrass (*Digitaria* spp.), is well documented (Busey, 2003). Raising cutting heights has been found to reduce crabgrass densities over time in Chewings fescue [*F. rubra L.* ssp. *fallax* (Thuill.) Nyman] *(*Jagschitz and Ebdon, 1985), Kentucky bluegrass (Dunn et al., 1981; Niehaus, 1974), tall fescue (Dernoeden et al., 1993; Hall, 1980; Voigt et al., 2001) and fine fescue species (Dernoeden et al., 1998). Using variable mowing heights throughout the season had no effect on controlling crabgrass in tall fescue (Cropper et al., 2017). Therefore, using a consistently high mowing height might be most effective to increase competitiveness of turfgrasses against crabgrass.

Adams (1980) showed that annual bluegrass cover, in a perennial ryegrass dominated sward, decreased from 34% to 9% when the cutting height was raised from 1.25 cm to 7.5 cm. However, few studies exist that have investigated the relationship between mowing height and broadleaf weed densities. Fine fescue was reported to be more competitive over a mixture of broadleaf weeds (e.g. dandelion, white clover, crabgrass) when the mowing height was raised from 1.9 cm to 5.1 cm (Davis, 1958). Gray and Call (1993) found that raising the mowing height to 6 cm in tall fescue dominated turfgrass was successful in reducing blue violet (*Viola sororia* Willd).

Studies suggest that weed densities can generally be reduced but never fully controlled with only mowing and fertilization (Busey, 2003). The extent to which overall weed densities can be lowered is also dependent on the competitive ability of the turfgrass species composition. In a trial with different mowing heights, fertilizer rates and turfgrass species, DeBels et al. (2012) found 'Kenblue' Kentucky bluegrass had the highest weed encroachment regardless of fertilizer or mowing treatment.

Raising cutting heights usually increases competitiveness of desirable turfgrasses, however it is unclear if small changes within the limitations of usability has an effect on weed pressure. For example, golf fairways are usually maintained at heights ranging from 8 to 25 mm, but there are no studies that have examined whether a high over a low cutting height provides superior weed control. Common sense suggests that turfgrass should be kept as dense as possible throughout the year, hence cutting heights should be increased if turfgrass is sparse but can be lowered if turfgrass remains sufficiently dense. Furthermore, mowing height and frequency are dependent on the growth rate of the turfgrass. Factors that influence growth rates include genotype, climate, the growth medium and the management input of water and fertilizer. Therefore, it seems unlikely that weed pressure will be reduced by small changes in cutting height alone.

2.4.2 Handling of Clippings

Returning clippings to turfgrasses is thought to increase soil levels of carbon and N if microbial activity is sufficient to mineralize N (Haley et al., 1985; Law et al, 2017; Macdonald et al., 1989; Qian et al., 2003; Shepherd et al., 1996; Starr and DeRoo, 1981). Knot et al. (2017) and Qian et al. (2003) applied the century model and calculated that returning clippings can reduce a turfgrasses N requirement by 25% if it is 1-10 years old, by 33% if it is 11-25 years old, and more than 50% if it is older than 25 years. However, since additional nutrients can also be provided by increasing fertilizer rates, the decision to remove clippings is based more on aesthetic or financial considerations. The positive effects of returning nutrients derived from clippings to the system can be offset by the addition of weed seeds (Heckmann et al., 2000). Weed-related negative effects of returning clippings have been especially observed with annual weeds, which survive by producing large numbers of seeds. Clipping removal led to a 60% reduction of viable annual bluegrass seeds in a bentgrass dominated turfgrass sward (Gaussoin and Branham, 1989). Therefore, if the turfgrass sward is dominated by unwanted annual bluegrass, clippings removal should be considered to reduce the seedbank build up.

In contrast, clippings can contain allelochemical compounds, which have herbicidal activity (Akbari et al., 2015). Bertin et al. (2003b) and Wu et al. (2002) conducted studies in which these compounds were isolated and tested in pot and petri-dish experiments. Allelopathic compounds isolated from a mixture of perennial ryegrass, Kentucky bluegrass and particularly red fescue inhibited germination and seedling growth of prostrate pigweed (*Amaranthus blitoides* S. Wats) (Akbari et al., 2015). It is unclear if simply returning clippings after mowing has an allelopathic-based inhibitory effect on weeds, as allelopathic compounds interact with soil physical and chemical parameters, climate and other biotic factors such as microbial activity (Wu et al., 2002), all of which can influence allelopathic efficacy.

2.5 Fertilization

Fertilization changes botanical composition and reduces species richness through competitive exclusion but increases biomass in plant communities (DiTommaso and Aarssen, 1989; Gough et al., 2000, Grime, 1973; Templeton and Taylor, 1966). For example, tees, fairways, and greens are ideally dominated by only a few desirable turfgrass species to produce high quality surfaces (Bridges, 1994). Consequently, fertilization is a powerful tool to encourage dominance of desirable turfgrass species. In the absence of herbicides, turfgrass managers may need to reexamine some traditional cultural practices that have fallen out of favor in recent times due to budget constraints and environmental concerns. In particular, increased fertilization rates that have been shown to provide competitive edges to turfgrasses over weeds are now discouraged because of fertilizer costs, risk of groundwater leaching of nitrate, as well as surface runoff of excess fertilizer into receiving water bodies that lead to algal blooms, among other things.

Nitrogen is the most limiting nutrient for plant growth (Bowman et al., 1993). In aerated soils, plants acquire N mainly through inorganic forms of nitrate (NO^{3-}) and ammonium (NH^{4+}). Availability of N is subject to biotic and abiotic processes affecting the total pool of soil N. Minor portions of N are utilized in organic forms, i.e. amines and amino acids (Devienne-Barret et al., 2000; Lipson et al., 2001). Application of N to grassland systems changes the competitive ability of turfgrasses against weeds (Silvertown, 1987; Silvertown et al., 2006). Calhoun et al. (2005) reported that applying N to a mixture of Kentucky bluegrass, perennial ryegrass and red fescue at a rate of 150 kg ha^{-1} yr^{-1} reduced white clover density by 61 to 88 percent in a four-year study period. White clover was significantly reduced in tall fescue pastures when 135 kg N ha^{-1} yr^{-1} was applied (Rajaniemi, 2002). A turfgrass sward dominated by Kentucky bluegrass maintained with 196 kg ha^{-1} yr^{-1} of N exhibited better visual turfgrass quality with lower weed densities, compared to plots receiving no fertilizer (DeBels et al., 2012). Studies of Kentucky bluegrass, tall fescue, and Chewings fescue turfgrass swards showed that higher rates of N fertilizer reduced crabgrass densities significantly but did not result in full weed control (Dernoeden et al., 1993; Dunn et al., 1981; Jagschitz and Ebdon, 1985; Johnson, 1981; Johnson and Bowyer, 1982; Murray et al., 1983; Voigt et al., 2001). Johnson and Bowyer (1982) reported that Kentucky bluegrass plots receiving 300 kg N ha^{-1} yr^{-1} and 600 kg N ha^{-1} yr^{-1} had lower dandelion densities (approximately 30% and less than 10%, respectively) than plots that were not fertilized (40% dandelions). Other studies in which broadleaf weed composition was not given reported minimal weed densities when 300 kg N ha^{-1} yr^{-1} was applied to Kentucky bluegrass (Haley et al., 1985) or tall fescue- (Voigt et al., 2001) dominated turfgrass swards. The literature suggests that broadleaf weeds are reduced when more N is applied, therefore finding a balance between what is needed and what is environmentally safe and sustainable is critical.

Established turfgrass that is not over irrigated loses on average <5% of the N applied, when rates of 200-300 kg N ha^{-1} yr^{-1} are used (Barton and Colmer, 2006). Therefore, maintaining annual application rates of total N to turfgrass below the upper threshold of 300 kg N ha^{-1} yr^{-1} could be recommended. Such a high rate of N is rarely applied to any turfgrass area, hence if sensible turfgrass management approaches are used, such as frequent, low doses of N, the risk of environmental pollution of surface and groundwater is minimized, and the benefits of outcompeting weeds are maximized.

Unfortunately, N fertilization has also been shown to increase production of annual bluegrass, which outcompetes desirable turfgrass species (Dest and Guillard, 1987; Lodge and Lawson, 1993). Such a transition is a concern for turfgrass managers. Nam-Il et al. (2001) reported that annual bluegrass only requires 300 ppm N compared to bentgrass (1000 ppm N) to produce maximum shoot and root growth in a greenhouse study. Growth of both species, particularly root growth, is very responsive to N and to a lesser extent to P and potassium (K) levels (Nam-Il et al., 2001). However, the competitive advantage of annual bluegrass over other turfgrass species in N fertilizer trials is not conclusive. A minimal increase of 12% to 17% in annual bluegrass cover was observed in perennial ryegrass dominated turfgrass when N rates were increased from 0 to 352 kg N ha^{-1} yr^{-1} (Adams, 1980). Gaussoin and Branham (1989) observed an increase in annual bluegrass cover in one year out of three when 293 kg N ha^{-1} yr^{-1} was applied to a mixture of annual bluegrass and creeping bentgrass instead of 98 kg N ha^{-1} yr^{-1}. In contrast, Calvache et al. (2017) reported that changing N or P fertilizer rates had no effect on annual bluegrass densities in bentgrass and fescue dominated golf greens. The effect of N rates on the competitive ability of annual bluegrass might differ because of differences in soil type, category of fertilizer, method of application, or soil pH. For example, in a bentgrass and annual bluegrass mixture, foliar application of N favors annual bluegrass growth whereas granular applications increase bentgrass growth (O'Connor et al., 2018). Moreover, Lodge and Lawson (1993) reported that a stepwise increase in pH from 4.5 to 5.5, due the application of lime for one year, considerably increased annual bluegrass cover from 0% to 30% in a fescue/bentgrass dominated golf green.

For turfgrass managers it is often difficult to estimate minimum levels of N required to provide a turfgrass with a competitive edge over weeds. The approach used by agricultural producers could be followed in such cases. The critical N amount in agriculture is defined as the minimum concentration needed to achieve maximum plant growth. As plants mature, critical N concentrations decrease (Gastal and Lemaire, 2002). Turfgrass managers should focus on limiting N applications to minimum amounts needed to produce an acceptable level of playing quality or aesthetic appeal. Acceptable playing quality or aesthetic appeal can be measured objectively or defined by a combination of factors, such as human expectations, budget limitations and ecological considerations.

As the addition of N influences the competitive ability of turfgrasses over weeds, the dose and type of other nutrients added to a turfgrass area also influence turfgrass species composition. In a long-term parkland study, legumes established better in plots fertilized with P and K (>30% of ground cover), while turfgrasses dominated in plots fertilized with N (approximately 90% of ground cover) (Silvertown, 1987; Silvertown et al., 2006). Phosphorous and K fertilizer applied consistently at high rates favored annual bluegrass invasion into bentgrass swards (Goss et al., 1975; Kuo 1993a; Waddington et al., 1978). In low P soils that are acidic, bentgrass was found to outcompete annual bluegrass because it was more efficient at absorbing P under those conditions (Kuo et al., 1992). Consequently, macronutrients, such as P and K, should only be applied if soil levels are low, because annual bluegrass and broadleaf weeds such as dandelion are better competitors when soil levels of these minerals are high.

Other strategies to outcompete annual bluegrass include the application of sulfur to acidify the soil, and the reduction of N applications (Dest and Guillard, 1987; Goss et al., 1975). High levels of sulfur (168 kg S ha^{-1} yr^{-1}) lowered the pH of the soil, which in turn reduced P availability and annual bluegrass densities in creeping bentgrass swards (Goss, 1974).

Broadleaf weeds become somewhat less competitive when soil pH is lowered; this could potentially be achieved by the application of iron. Also, lowering the soil pH with long-term applications of ammonium sulfate led to reduced broadleaf weed populations and in some cases to weed-free turfgrass (Escrit and Lidgate, 1964; Thompson et al., 1995). In acidic soils, the application of calcium favors bentgrass growth over annual bluegrass (Kuo, 1993b). Furthermore, applying 1.68 kg ha^{-1} yr^{-1} of both iron and magnesium to bentgrass has been shown to suppress annual bluegrass by 65% (Bell et al., 1999); however, the pH must be maintained at an optimum level for turfgrass growth to maintain competitiveness.

Fertilization may not only affect growth of desired turfgrasses, but can also benefit broadleaf weeds in a stand. Dodd et al. (1994) and Tilman et al. (1999) documented that dandelion growth responded positively to K fertilization and increased 17 to 20 fold compared to non-fertilized controls in a mesotrophic grassland.

The timing of fertilizer applications is crucial to increase the ability of turfgrasses to outcompete weeds. For example, Kentucky bluegrass fertilized in spring and fall had significantly lower crabgrass densities than when fertilized in fall or summer (Dunn et al., 1981). Tall fescue fertilized in fall with two split applications each of 73 kg N ha^{-1} was more competitive against weeds compared to a single spring application of 73 kg N ha^{-1} (Hall, 1980). Fertilizer aids turfgrass growth and competitiveness, but neither fall nor spring fertilization had an effect on crabgrass germination levels (Turner and van Acker, 2014).

Placement of fertilizer may also be important in weed suppression. The application of fertilizer close to the roots has been shown to suppress weed growth in agronomic studies (Chauhan and Ahugho, 2013; Kirkland and Beckie, 1998; Mashingaidze et al., 2012). This weed suppressing effect of fertilizer placement has not been observed in landscape planting systems because of the ample availability of nutrients in the soil (Marble et al., 2015), but might have implications for turfgrass areas that are constructed on sand-based soil.

2.6 Bioherbicides

2.6.1 Fungi based herbicides

Phoma herbarum is a fungal plant pathogen that causes leaf spot on a wide range of host plants (Gilardi et al., 2017). The fungus produces the toxic metabolite 3-nitro-1,2-benzenedicarboxylic acid (3-nitrophthalic acid), that has herbicidal activity (Virkant et al., 2006). The efficacy of *P. herbarum* was tested on dandelion transplanted into an existing

Kentucky bluegrass sward (Neumann & Boland, 1999). Significantly higher rates of dandelion foliage infection were observed in plants inoculated with *P. herbarum* strains formulated with potato dextrose broth (PDB) together with either 5% durum semolina, 1% guar gum or 5% gluten flour compared to a PDB control, three weeks post inoculation. Field conditions reduce the efficacy of *P. herbarum* to infect dandelion (Schnick & Boland, 2004). The authors observed that in the spring-trial, foliage infection rates were lower because dandelion was able to outgrow the disease. Therefore, it is unclear if *P. herbarum* is capable of imposing a long-term fitness cost on dandelion.

Phoma macrostoma was isolated from Canada thistle [*Cirsium arvense* L (scop)] and caused chlorosis and bleaching of young leaves in broadleaf weeds, by producing phytotoxic macrocidins (Graupner et al., 2003; Graupner et al., 2006). Differences in efficacy of *P macrostoma* on broadleaf weeds were reported by Smith et al. (2013, 2015), with dandelion being most effectively controlled. Weeds have a very low risk of developing resistance to macrocidins isolated from *P. macrostoma* due to their diverse modes of action (Hubbard et al., 2016). According to Boerema et al. (2004), *P. macrostoma* is a weak and opportunistic pathogen, which enters host plants through wounds. *Phoma macrostoma* 94-44B was granted full registration in Canada to be sold and used for the control of a broad spectrum of broadleaf weeds found in turfgrass (Hynes, 2018). Mycelial fragments of *P. macrostoma* applied to soil caused photobleaching and death to dandelion but had no effect on monocotyledonous weeds (Bailey and Derby, 2001). For broadleaf weed control in turfgrass the *P. macrostoma* isolate 94-44B was formulated into a granule for soil applications to act as a pre and post emergence biological herbicide (Bailey et al., 2011). Field trials conducted at three locations (silt-loam, pH 7.1; sandy loam, pH 7.8; and silt loam pH 7) in Canada, with high dandelion densities reported an average reduction of dandelion densities ranging from 70-90% in summer and late fall. Product efficiency was further increased by 10-20% when N treatments were added (Bailey et al., 2013). Year to year efficiency of *P. macrostoma* was influenced by environmental conditions, soil type and organic matter content. Negative interactions between *P. macrostoma* and sulfate applications were observed, which reduced efficiency of dandelion control (Bailey et al., 2013). Currently no data are available on the efficiency of *P. macrostoma* to control other common turfgrass weeds.

Sclerotinia minor is the causal agent of lettuce drop (Wymore and Lorbeer, 1987). The fungus produces sclerotia that survives in the soil. *S. minor* can infest a wide range of host plants including common turfgrass weeds such as dandelion, white clover and broadleaf plantain (Harding and Raizada, 2015). The isolate IMI344141 was identified as suitable candidate for the production of bioherbicides (Briere et al., 2000). During pathogenesis oxalic acid accumulates in the host plant and synergistically enhances the effect of pectolytic enzymes (Marciano et al., 1983). Mycelia of *S. minor* require a susceptible host to persist in the environment, otherwise they decay after 10 days when applied to turfgrass areas (Watson, 2007). A Kentucky bluegrass sward treated with an inoculum of a *S. minor* strain, six times during the first year and four times during the following year, resulted in a reduction of 80.7% of dandelion (Riddle et al., 1991). A subsequent three-year field study showed that *S. minor* was as effective at controlling white clover, broadleaved plantain (*Plantago major*), birdsfoot trefoil and common ragweed (*Ambrosia artemisiifolia*) as the herbicide Killex (active ingredients: 2,4 D; mecoprop and dicamba) (Abu-Dieyeh and Waston, 2007a).

2.6.2 Bacterial based herbicides

Propagation of bacteria is less dependent on environmental influences compared to fungi (Li et al., 2003), and is therefore more rapid and more suitable for potential genetic modifications (Harding and Raizada, 2015; Johnson et al., 1996; Li et al, 2003). Several bacterial strains of *Pseudomonas fluorescens* were identified as agents to control annual bluegrass and certain graminaceous plants (Banowetz et al., 2008; Kenedy, 2016). Soil applied bacteria, such as *P. fluorescens* strain D7, produce a complex of a lipopolysaccharide and extracellular peptides, which inhibit root and shoot growth of annual bluegrass but no other turfgrass species (Gurusiddaiah, 1994; Kennedy, 2016). Furthermore, the *P. fluorescens* strain WH6 produces oxyvinylglycines and therefore inhibits germination of a broad range of plants (Banowetz et al., 2008). Fall applications of the bacterium led to establishment in the soil and propagation during cool temperatures similar to annual bluegrass root growth. Therefore, *P. fluorescens* has potential to be developed into a selective post-emergence soil spray to reduce the annual bluegrass seed bank in turfgrass swards (Kennedy, 2016). Johnston and Golob (2017) found that *P. fluorescens* applications to an annual bluegrass and Kentucky bluegrass golf fairway made in fall 2015 and spring 2016 were ineffective at controlling annual bluegrass. Currently *P. fluorescens* strain D7 is registered for the control of agricultural weeds but not annual bluegrass (Environmental Protection Agency, 2014).

Strains of the Gram-negative bacterium *Xanthomonas campestris* cause bacterial wilt on susceptible plants (Imaizumi et al., 1997). Bacteria of *X. campestris* enter host plants through natural openings such as stomata or wounds and multiply in intercellular spaces or the xylem.

Pathogenicity is caused by the injection of effector proteins or the manipulation of the plant transcriptome by mimicking transcriptional activators (Kay and Bonas, 2009). In Japan *X. campestris pv. Poannua* was formulated into a commercially available product (Nishino and Tateno, 2000). The bacterium is host specific for annual bluegrass and does not affect other turfgrasses (Fujimori, 1999; Zhou and Neal, 1995). In field tests, annual bluegrass abundance was reduced by 40% when *X. campestris pv. Poannua* formulated as a spray was repeatedly applied (Zhou & Neal, 1995). However, two to five weeks after discontinuing applications recovery occurred. Johnson et al. (1996) found that infection of annual bluegrass, in bermudagrass golf greens, only occurred when *X. campestris pv. Poannua* was applied with a surfactant and during mowing. Six monthly applications resulted in 70% control of annual bluegrass (Johnson et al., 1996).

2.7 Organic products with herbicidal mechanisms

Corn gluten meal derived from maize produces allelopathic chemicals such as Benzoxazinoids, which inhibit root growth, enzyme activity and germination of annual grassy weeds, and agricultural weed plant species such as okra (*Abelmoschus esculentus L.*) (Ayeni and Kayode, 2013; Christians, 1991; Jabran and Farooq, 2013). Other herbicidal activity is likely to be caused by multiple dipeptides, which have an inhibitory effect on weed seed germination (Baker and Grant, 2018; Unruh et al., 1997).

In greenhouse studies, corn gluten meal showed effective herbicidal activity on black medic (*Medicago lupulina L.*), buckhorn plantain (*Plantago lanceolata L.*), dandelion, crabgrass and other common turfgrass weeds (Baker & Grant, 2018). However, other studies question the claims of herbicidal activity and suggest that the N concentrations in corn gluten meal simply give turfgrasses a competitive advantage over weeds which allows them to reduce dandelion, crabgrass, and clover densities (Christians and Dant, 2005; John and DeMuro 2013; Patton and Weisberger 2012). In another study, liquid corn gluten meal showed no herbicidal effect on weeds in turfgrass trials (Lyons et al., 2015). The conclusion that can be drawn from these studies is that corn gluten meal can be used as an organic N source but not as an herbicidal alternative to control weeds in turfgrass settings.

Acetic acid is produced by aerobic bacteria, during the fermentation of ethanol containing plant material (Webber et al., 2005). Acetic acid applied to turfgrass causes the non-selective breakdown of foliage (Webber and Shrefler, 2006). In the United States, acetic acid products with less than 8% concentration do not have to be registered and are effective in controlling young weeds with 1-2 leaves (Webber and Shrefler, 2006). Improved control can be achieved by using higher rates of 20% acetic acid and increasing application volumes (Webber and Shrefler, 2006). The effectiveness of acetic acid in controlling weeds is also dependent on maximizing the area of contact with the foliage, which is determined by the growth habit of the weed and the method of product application (Evans et al., 2009).

2.8 Thermal weed control

Thermal weed control can be used to sterilize a seedbank or spot spray or burn existing weeds. Hoyle et al. (2012) documented good broadleaf and grassy weed but less nutsedge (*Cyperus esculentus* L.) control before tall fescue establishment when using an enclosed flaming system. The authors reported that six weeks after seeding tall fescue reached >60% establishment when flaming was applied in fall compared with summer. In agriculture, steaming soil with 70-100°C water steam, to a depth of 10 cm for 3-8 min is used to kill weed seeds in soil (Bond et al., 2003). During such a sterilization process, microorganism abundance is reduced for at least two months and the community function might change after recovery (Roux-Michollet et al., 2008). Steaming soil could be an alternative to pre-emergence herbicides to treat newly constructed turfgrass areas. For spot treatments, hot water was successfully used to reduce common broad- leaved dock (*Rumex obtusifolius* L.) by 80%, without causing damage to the soil structure (Latsch et al., 2017). Large patches of weeds can also be treated with solarisation. Solarisation requires covering patches of weed with plastic covers during favourable conditions. After some time, heat and solar radiation injures weeds but also dries out the seedbank (Horowitz et al., 1983). However, this method might only be appropriate for small turfgrass areas, because installing covers is labour intensive and covers need to be in place for several days. Other thermal weed control methods include freezing, electric currents, irradiation, microwave radiation and ultraviolet light.

Targeted mechanical or thermal weed control in large turfgrass areas would require high-resolution field scouting systems to initially detect weeds (Bell et al., 2013). In a next step, automated systems could be developed to remove the weeds. Weeds can be removed by laser treatment (Mathiassen et al., 2006) or hot water (95°C), which was more effective to spot treat weeds than flaming, steam, hot air and steel brushing (Kristoffersen et al., 2008).

2.9 Perspective on non-chemical weed control

A complete ban of herbicides will most likely require a change in perception of weeds and playing quality expectations. In cases where herbicides are banned, weeds may have to be treated only if they interfere with playing quality. In such occasions, threshold levels need to be established to determine which types of weeds need to be treated, in which playing areas, and at what infestation level (e.g. percentage of playing area covered by weeds). Treating weeds solely for aesthetic reasons will most likely no longer be an acceptable justification for legislators. If users of turfgrass areas still object to the presence of weeds for mainly aesthetic reasons, a stronger effort needs to be placed on convincing legislators as to why weeds interfere with the purpose of the area and why they need to be removed.

Future research should focus on assessing the competitiveness of certain turfgrass species and cultivars against weeds under different maintenance regimes and within the limitations of producing turfgrass areas of acceptable playing quality. The literature suggests that broadleaf weeds are reduced when more N is applied; therefore, finding a balance between what is needed and what is environmentally safe and sustainable is critical. In the context of achieving sustainability, increased attention has been placed on fertilization needed by turfgrass areas. The prevailing opinion of the public in general and legislators in particular has been that turfgrass in general and golf courses in particular are over fertilized (May et al., 2009), and for this reason Minimum Levels for Sustainable Nutrition (MLSN) could be used as guidelines (Woods et al., 2014). These guidelines were developed and published from a data set of over 16,000 soil samples, which were collected on turfgrass that was of acceptable quality (Pace Turf, 2014). However, since most of these soil samples were collected in the US, it might be fair to assume that herbicides were applied as part of the maintenance. Consequently, caution needs to be used when MLSN standards are applied but herbicides are not available for weed control.

In the future, the best approach for turfgrass managers might be to use remote sensing technology to detect deficiencies such as poor irrigation uniformity, soil compaction, and nutrient deficiencies. Improving overall plant health will encourage dense growth of turfgrasses and increases the competitive ability of turfgrasses to suppress weeds. Precision Turfgrass Management (PTM) might be a useful tool to address these issues. Unmanned aerial vehicles (UAVs) or autonomous ground-based sensing vehicles, equipped with cameras, multispectral sensors, remote sensors etc. can be used to collect data about turfgrass health, such as monitoring water stress, nutrient deficiencies, pest pressure and salinity (Carrow et al., 2010; Caturegli et al., 2016; Krum, Carrow and Karnok, 2010; Stowell and Gelernter, 2006). Spectral reflectance can provide information about leaf area index (LAI), chlorophyll content, biomass, drought stress and nutritional status (Agati et al., 2013; Caturegli et al., 2015a; Finke, 1992; Foschi et al., 2009; Rossi et al., 2010). Satellite remote sensing can also be used to assess N status of turfgrasses spatially and in real time (Caturegli et al., 2015b). In the future, NDVI sensors would ideally be mounted to mowing units to collect data in near real time. These technologies may be able to detect deficiencies and poor quality or sparse turfgrass but are unable to pinpoint the cause of the inadequacies. For example, to determine optimal (or minimal) fertilizer requirements, soil sampling or leaf tissue analysis is still needed. Moreover, precision fertilization practices include zoning a golf course into site-specific management units (SSMUs) where areas with similar usages, soil compositions, topography, plant responses and microclimates are zoned (Krum et al., 2010). A composite soil sample could be taken from each SSMUs to analyze soil nutrient status which requires less sampling compared to grid sampling (Carrow, 2010; Ikenaga and Inamura, 2008, Johnson et al., 2001; Shaner et al., 2008). A sensible approach might be to use soil sampling SSMUS to measure the soil pool of rather immobile nutrients, such as P and K (Giehl and Wirén, 2014), and to use remote sensing techniques on a more frequent basis to measure mobile nutrient status, such as N.

The most effective method of removing existing weeds in turfgrass swards without the use of synthetic herbicides might be to spot spray with hot water or high concentrations of non-selective biological products such as acetic acid. Host specific biological products based on fungi or bacteria are only available in selective countries around the world and their efficiency is questionable. Much effort is ongoing to increase the efficacy of these products and they might become viable options to synthetic herbicides in the future. Thorough testing including controlled screenings and field trials are needed to provide information about the host-specific persistence of biological products in changing environments over time. The development of robotic machines to detect and remove weeds might be one of the more promising approaches to control weeds in the future. Detecting and removing weeds in dense turfgrass swards is challenging but currently under development.

Sustainable turfgrass management will become increasingly important for budgetary and environmental reasons. However, approaches that address both objectives (cutting cost and environmental protection) are not always possible as they can be mutually exclusive. Therefore, if synthetic herbicides (or pesticides in general for that matter), are being banned from use on turfgrass areas, future research is needed to develop maintenance strategies that include MLSN guidelines that focus on outcompeting weeds and on the prevention of turfgrass pests in general.

2.10 Acknowledgments

Financial support of this report was provided by the Turfgrass University Research Foundation (TuRF), Nederlandse Golf Federatie (NGF), Nederlands Vereniging van Golfaccommodaties (NVG), and Nederlands Greenkeepers Associatie (NGA). The authors are also grateful for the constructive and insightful comments provided by the anonymous reviewers; they greatly improved the manuscript.

CHAPTER 3

Investigating *Festuca* species interference with germination and growth of clover (*Trifolium repens* L.), daisy (*Bellis perennis* L.) and yarrow (*Achillea millefolium* L.), established on water agar in controlled environments

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Abstract

Herbicide restrictions in the European union requires alternative strategies for broadleaf weed control in turfgrass. In recent years, *Festuca* species were identified for their allelopathic potential to interfere with growth of weed species. This study was designed to investigate the growth inhibitory effect of 27 Festuca cultivars selected from five *Festuca* species, including Chewings fescue [*F. rubra L.* ssp. *fallax* (Thuill.) Nyman], slender creeping red fescue [*F. rubra* L. ssp. *littoralis* (G.Mey.) Auquier] strong creeping red fescue [*F. rubra L. ssp. rubra Gaudin],* hard fescue [*F. brevipila Tracey]* and tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.]) Sixty grass seeds from each *Festuca* cultivar were placed in plastic containers with agar, allowed to establish for 13 days before 20 weed seeds from the species clover (*Trifolium repens* L.), daisy (*Bellis perennis* L.) and yarrow (*Achillea millefolium* L.), were placed in between the grass seeds. Daisy was highly sensitive to the presence of *Festuca* and was therefore not included in the analysis meant to find differences between interference potential of *Festuca* species. For clover and yarrow, full germination percentage (FGP) and mean germination period (MGP) were not affected by *Festuca* species or individual cultivars. At 30 DAS biomass of *Festuca* species was recorded as well as root length of clover and yarrow. Tall fescue species reduced clover root length by 71.6% compared to controls, which was significantly more compared to strong creeping red fescue (*F. rubra L. ssp. rubra Gaudin)*, hard fescue (*F. brevipila Tracey)* and Chewings fescues [*F. rubra L.* ssp. *fallax* (Thuill.) Nyman] which did not differ among each other and averaged 58.7% reduction compared to controls. Slender creeping red fescue [*F. rubra* L. ssp. *littoralis* (G.Mey.) Auquier] caused the smallest reduction in clover root length with 44.5%. Cultivar effects on clover roots differed between reductions of 24.8% (Cathrine, FRT) and 81.7% (Regenerate, FA). For yarrow, results differed with no significant differences among species and a strong general root length reduction of at least 75% compared to controls. Cultivar effects ranged from reductions of 62.9% (Samanta, FRT) up to 91.8% (Barcesar, FA). For both clover (-.264***) and yarrow (-.181**) negative correlations were found between *Festuca* biomass and root length of the weeds, suggesting that at least part of the inhibiting effect was directly related to *Festuca* biomass. We conclude that differences in interference potential between cultivars within species are at least as important as differences between species. Furthermore, clear differences in sensitivity were observed among weed species. Clover seems a suitable species for growth interference studies with *Festuca*, as results between species and cultivars varied more significantly compared to yarrow and daisy.

Keywords: fine fescues, hard fescues, tall fescues, allelopathy, broadleaf weeds, growth chamber, full germination percentage, mean germination period, root length

3.1 Introduction

Increasingly strict bans on herbicide use in amenity turf require alternative weed control strategies to provide aesthetic turf and acceptable playability of surfaces, particularly for sports turf (Larsen et al., 2004). One component of such strategies is the establishment of turfgrass species that possess an inherent ability to suppress weed species. An important characteristic of such turfgrass species is the ability to close the turfgrass canopy rapidly after sowing, thereby reducing the rate of weed seedling emergence (Islam and Kato-Noguchi, 2016; Masin and Macolino, 2016). After germination, desirable turfgrass species must be strong competitors for light, nutrients, water and space to maintain dominance over weed species (Snaydon and Howe, 1986; Holt, 1995). Such dominance is achieved by plant species that are best adapted to efficiently exploit limited resources and space (Weigelt and Jolliffe, 2003; Begon et al., 2007).

Among turfgrass species, a specific group within the genus *Festuca*, referred to as fine-leaved fescues, appear to perform well under the preferred low-external input conditions, of which the restrictions in herbicide use are an example. Fine-leaved fescues tolerate moderate shade and acidic soils (Bonos et al., 2006), are drought resistant (Fry and Huang, 2004), and require low inputs of water and nutrients (Dernoeden et al., 1994). Among the fine fescues, Chewings fescue [*F. rubra L.* ssp. *fallax* (Thuill.) Nyman] was identified as a suitable species for low-input golf course fairway management (Watkins et al., 2010). Along with fine fescues, tall fescues [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] are well adapted to southern European climates and are suitable as sustainable species for soccer fields in Italy (Grossi et al., 2004).

The weed suppressing ability of fescues was demonstrated in field experiments with 78 fine fescue cultivars from six species (Bertin et al., 2009). Particularly, cultivars from the red fescue complex (*Festuca rubra* L.) such as Chewings fescue and strong creeping red fescue (*F. rubra L. ssp. rubra Gaudin)* were found to be weed suppressive. Strong weed suppression of well performing cultivars was attributed to quick germination, rapid and dense canopy establishment as well as potential allelopathic interference (Bertin et al., 2009).

Allelopathy refers to the biological phenomenon whereby compounds produced and released into the environment by one plant cause beneficial or harmful effects on another plant (Inderjit and Del Moral, 1997; Rice, 2012). Plant-plant interference effects caused by the release of allelopathic chemicals are either direct or secondary, through microbial decomposition of plant materials (Inderjit and Weiner, 2001). Screening for allelopathy in soil environments, which closely resemble natural systems, has been attempted, but separating allelopathic effects from other mechanisms of interference under these conditions is challenging (Inderjit and Del Moral, 1997). Subsequent efforts have therefore focused on isolating allelopathic compounds using techniques such as bioassay-guided isolation (Duke, 2015). However, such approaches are time consuming and focus on identifying compounds that have herbicidal properties rather than identifying grass species that show natural weed suppression. With hard fescues (*F. brevipila Tracey)* and fine-leaved fescues, exudate extractions of donor plant root and shoot tissue were tested for seedling growth interference of receiver plants (curly cress, *Lepidium sativum* L.) (Bertin et al., 2003). Exudates from one hard fescue cultivar (Oxford) and two Chewings fescues (cvs. Sandpiper and Intrigue) exhibited high allelopathic potential in the laboratory assessment. Isolation of phytotoxic compounds from the Chewings fescue cultivar Intrigue identified m-thyrosin as the compound that interfered with growth of the indicator species (Bertin et al., 2007).

An alternative approach is to investigate the interference between donor and receiver plant under conditions where competition for resources is minimized, and the interference is largely driven by allelopathy (Bertin et al., 2003). To investigate the weed growth interference capabilities of fescues, donor plants, including three hard fescue and four Chewings fescue cultivars, were grown together with receiver plants, large crabgrass (*Digitaria sanguinalis*) and curly cress, on 0.8% water agar (80 ml) in plastic containers (Bertin et al., 2003). Root and shoot length of receiver plants were used as indicators of allelopathic potential of the donor plant. In the presence of all investigated fescue cultivars, mean root length of crabgrass and cress were reduced compared to the 'weed only' control, with root growth being more inhibited than shoot growth. The cultivar 'Intrigue' (Chewings fescue), which showed the strongest inhibitory effect, reduced root length of curly cress by 58% compared to the weed only control (Bertin et al., 2003).

The objective of this study was to determine the variability between and within *Festuca* species in their growth interference potential against broadleaf weed species. We investigated the influence of *Festuca* species on weed seed germination percentage, germination speed and root length. We further investigated whether differences in sensitivity amongst receiver species exist. For this we included three of the most problematic weeds in European turfgrass areas: clover (*Trifolium repens* L.), daisy (*Bellis perennis* L.), and yarrow (*Achillea millefolium* L.).

3.2 Materials and Methods

3.2.1 Plant material

In total 30 fescue (*Festuca* spp.) cultivars were selected from three sub-species including the red fescues and hard fescues from the fine fescue complex, and tall fescues. Selection criteria included species and cultivars commonly used on athletic fields and golf courses, and availability of seeds. Species included Chewings fescue (abbreviated as FRC), with cultivars Siskin, Barlineus, Ramona, Melitta, Livista, Annalena, Dancing and Musica, slender creeping red fescue [*F. rubra* L. ssp. *littoralis* (G.Mey.) Auquier] (abbreviated as FRT), with cultivars Nigella, Barcrown, Charlotte, Cathrine, Libano, Samanta, Barpearl and Baroyal, strong creeping red fescue (abbreviated as FRR), with cultivars Barisse, Rossinante, Sergei, Relevant, Livison, Mellori, Barjessica and Staybo, hard fescue (abbreviated as FRA), with cultivars Hardtop, Dumas 1 and Mentor and tall fescue (abbreviated as FA), with cultivars Regenerate, Melyane and Barcesar.

The experiment was conducted in April 2018 and replicated in January 2019 with new seeds for most of the cultivars. In 2019, fresh seeds of cultivars Sergei and Dancing were unavailable and therefore left-over seeds from the first experiment were used. These seeds had been stored in permanent darkness at room temperature (20°C).

Weed species included *Trifolium repens* L. (further referred to as clover), *Bellis perennis* L. (daisy) and *Achillea millefolium* L. (yarrow). These species are among the most common turfgrass weeds in Europe (N. Dokkuma, personal communication, 2017). All seeds were sterilized by placing them in a solution of 20% (v/v) sodium hypochlorite for 1 min, followed by rinsing with distilled water (Bertin et al., 2003a).

3.2.2 Growth Medium

In both experiments, purified agar with a working strength of 1%, moisture <7.5% and 'very low mineral' content (Oxoid purified Agar, Thermo Fisher Scientific, Waltham, MA) was mixed with Milli Q water (Milli-Q, Merck, Darmstadt, Germany) to produce 0.5% water agar. The mixture was then autoclaved at 120° for 2 hours. Three hundred ninety-six plant tissue containers (Sterivent High Container 107 x 94 x 96 mm, Duchefa-Biochemie, Haarlem, The Netherlands) were prepared with 100 ml of water agar each and cooled at room temperature for one day. We deviated from Bertin et al (2003), by using 100 ml of agar instead of 80 ml, to provide at least 10 mm of growing medium for root development.

3.2.3 Experimental set-up

Three hundred sixty containers (one for each of the 30 cultivars x three weed species x four replicates) were each seeded with 60 grass seeds by using an 8x8 matrix seeding template. No seeds were placed at any of the four corners of the matrix. We decided on 60 seeds per container based on recent published literature that reported the use of 50 seeds (Goatley et al., 2017: Giolo et al., 2019). Distance between individual grass seeds was 2 cm in all directions. Thirteen days after seeding of grass seeds (DAS), 20 weed seeds were placed randomly but always exactly in the middle of four neighboring grass seeds, representing a 3:1 ratio of grass seeds to weed seeds, similar to Bertin et al (2003). Following the recommendation of Bertin et al (2003), we chose to introduce the weed seeds 13 instead of seven days after sowing the grass seeds. A later introduction is thought to allow donor plants a longer period to produce potential allelopathic compounds. Another reason for the later introduction of weed seeds was because we also wanted to investigate the effect of *Festuca* species on weed seed germination. In addition, three containers for each of the four replications with only weed seeds were installed for all three weed species and used as control (three weed species x three containers x four replications = 36 control containers).

3.2.4 Climate Chamber

The climate chamber was set at 16/8 hours day/night cycle, with a corresponding temperature regime of +20 °C/ +10°C. Humidity was 70% and light intensity was 259 µmol m^2 s^{-1} photon flux density (PPFD) emitted from fluorescent tubes (Philips TL-D 58W/840 Reflex, Philips, Amsterdam, The Netherlands). Inside the climate chamber, two 1 m x 1.5 m tables were placed against opposite facing walls, with a walking lane of 80 cm in between. On each table, two replicates (blocks) were installed, one to the left and one to the right. Each of the four replicates was arranged in a matrix of six rows by 17 columns. Containers within each block were moved every four days to account for potential micro-climatic differences within the growth chamber. Settings of the second experiment were identical to those of the first experiment.

3.2.5 Measurements

Germination of grasses and weeds were recorded every four days after seeding. Germinated seeds were marked at the bottom of the plastic boxes. A seed was classified as germinated when the radical was visible. At 30 DAS, grass and weed seedlings were removed from the water agar and dried with paper towel. Total biomass of grasses within a container (g) and root length (cm) of individual weed plants were recorded. For root length, the total length of all roots was summed up.

Mean germination time (MGT) was adapted from Orchard (1977), to compute mean germination period (MGP). For the experiment four time periods were defined, each comprised of four-day periods, with measurements taken at the last day of each period.

$$MGP = \frac{\sum(1 \times n1 + 2 \times n2 + 3 \times n3 + 4 \times n4)}{N}$$

In this equation $n1$ = total number of seeds germinated in period one (1-4 days after sowing), $n2$ = total number of seeds germinated in period two (5-8 days after sowing), $n3$ = total number of seeds germinated in period three (9-12 days after sowing), $n4$ = total number of seeds germinated in period five (12-16 days after sowing) and N = total number of germinated seeds. For both grasses and weeds the final germination percentage (FGP), defined as the total percentage of seeds that germinated, was determined (Scott et al., 1984).

3.2.6 Data Analysis

An initial analysis for all *Festuca* and weed variables was conducted with experiment and interactions with experiment as random effects. Cultivars were treated as nested within *Festuca* species. Presented results were averaged over experiments. Adequacy of averaging across experiments was assessed by examining F-tests for interactions with experiment, by comparing cultivar means for the two experiments, and also by examining cultivar-control differences for both experiments. The Kenward-Roger denominator degree of freedom method was used to adjust standard errors and compute denominator degrees of freedom (Faes et al., 2009). A Tukey multiple comparison test was conducted to explore differences between species and cultivars within species. In order to explore the relationship among the dependent variables FGP, MGP, and biomass of *Festuca* and between *Festuca* biomass and weed FGP, MGP and root length, Spearman correlation coefficients (r) were computed, and P-values are reported. One replicate of cultivars Dumas 1 and Charlotte exhibited unexplainable long root growth and were noticeable different from all other entries. The studentized marginal residuals had magnitudes of greater than four. Consequently, these entries were identified as outliers and removed. Analyses were performed using SAS version 9.4 software (SAS, 2020) and significance was defined at $p \leq 0.05$.

3.3 Results

The cultivars Hardtop, Livista and Musica were removed from the experiment and analysis due to poor germination (<60%). Additionally, all experimental entries with daisy were removed, because in the presence of *Festuca* species daisy plants appeared photobleached and it was not possible to record root length.

3.3.1 Germination and biomass of Festuca species and cultivars

Full germination percentage (FGP) differed among *Festuca* species and among cultivars within these species (Table 3.1). The type of weed species did not influence FGP of species or cultivars. Therefore, in the subsequent analysis that explored species and cultivar characteristics, we combined the data and did not separate the analysis for either clover or yarrow.

Table 3.1. Analysis of variance of *Festuca* germination percentage (FGP) at 30 days after sowing, as obtained from a replicated growth chamber experiment (2018 and 2019).

Effect	Degrees of Freedom	F value	P value
Weed	1	0.31	0.5852
Species	4	5.73	0.0019
Species*Weed	4	0.70	0.5958
Cultivar (Species)	22	6.26	<0.001
Cultivar*Weed (Species)	22	1.09	0.4084

FGP of species ranged from 93.2% for hard fescue (FRA) to 86.4% for slender creeping red fescue (FRT) (Figure 3.1). Full germination percentage of strong creeping red fescue (FRR), (FRA) and Chewings fescue (FRC) was significantly higher compared to that of FRT, whereas FGP of tall fescue (FA) did not differ significantly from any of the other species.

Full germination percentage among cultivars varied from 96.8% (Barcrown, FRT) to 71.1% (Samanta, FRT) (Figure 3.1). Within all species, except for FRA, which was only represented by two cultivars, FGP of cultivars within species varied significantly.

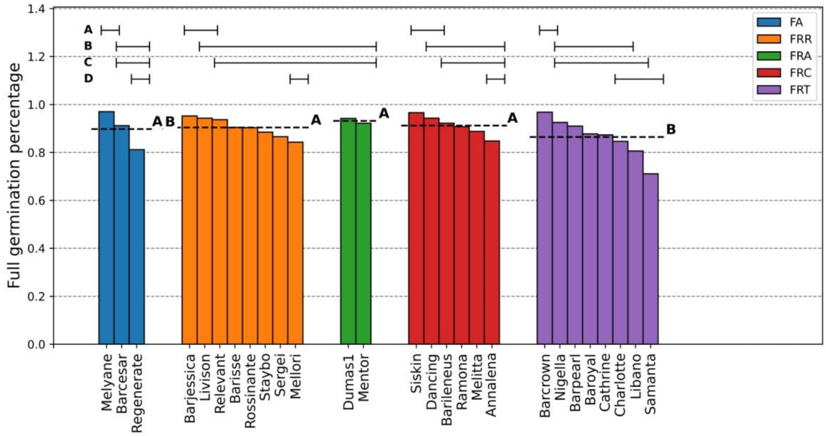

Figure 3.1. Full germination percentage (FGP) of 27 *Festuca* cultivars at 30 days after seeding, grouped according to species (FA, tall fescue; FRR, strong creeping red fescue; FRA, hard fescue, FRC, Chewings fescue; FRT, slender creeping red fescue). Dotted horizontal lines indicate species averages and solid lines indicate shared cultivar mean separation letters. Means sharing the same letter are not significantly different ($P<0.05$).

Mean germination period (MGP) did not differ among species and ranged from 1.6 for FRA to 1.9 for FA (data not shown). Significant differences were observed between individual cultivars (Table A1). FRR cultivars Livison and Sergei and FRC cultivars Melitta and Ramona had an MGP close to 1.5 or below and germinated significantly faster than Mellori (FRR) and Baroyal and Libano (both FRT), which had an MGP just above 2.

Biomass data for *Festuca* species and cultivars at 30 DAS was based on observations in containers including weeds (yarrow and clover), which were introduced at 13 DAS. Analysis revealed that weed species did not have a significant effect on *Festuca* biomass, although a *Festuca* species x weed species interaction was nearly significant (Table 3.2). Species and cultivars within species differed in the amount of developed biomass after 30 DAS (Table 3.2 & Figure 3.2).

Table 3.2. Analysis of variance of *Festuca* biomass at 30 days after sowing, as obtained from a replicated growth chamber experiment (2018 and 2019).

Effect	Degrees of Freedom	F value	P value
Weed	1	0.03	0.8878
Species	4	729.69	<0.001
Species*Weed	4	2.16	0.0731
Cultivar (Species)	22	8.22	<0.001
Cultivar*Weed (Species)	22	0.54	0.9580

Average biomass weight of FA (1022.3 mg) was significantly higher compared to all other species. Even though FRR was also significantly higher than the other three species, it produced less than 50% of the biomass of FA (488.8 mg). The other three species, FRA (435.8 mg), FRC (409.2 mg) and FRT (404.4 mg), did not differ significantly from each other (Figure 3.2).

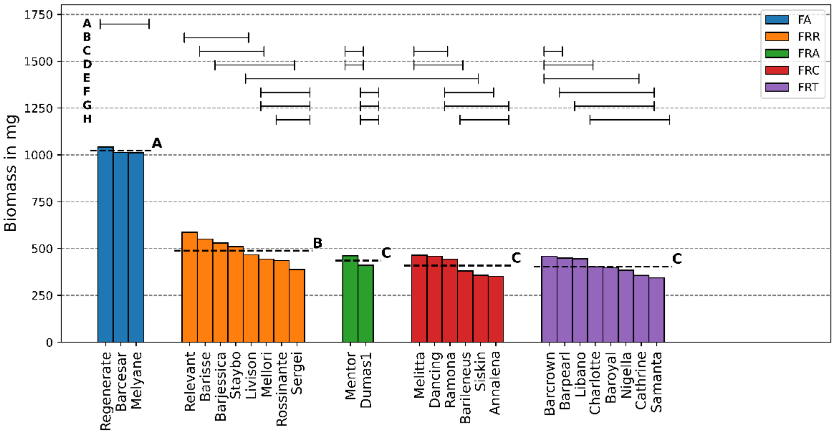

Figure 3.2. Biomass (mg/container) of 27 *Festuca* cultivars grown on water agar for 30 days in a controlled environment, grouped by species (FA, tall fescue; FRR, strong creeping red fescue; FRA, hard fescue, FRC, Chewings fescue; FRT, slender creeping red fescue). Data were averaged over weed species (clover, *Trifolium repens* L. and yarrow; *Achillea millefolium* L.) and two experiments. Dotted horizontal lines indicate species averages and solid lines indicate shared cultivar mean separation letters. Means sharing the same letter are not significantly different (P<0.05).

Differences in biomass among cultivars within species were observed for FRR, FRC and FRT, which were also the species represented with most cultivars. Within FRR, the cultivar Relevant (587.5 mg) developed most biomass, which was 33.9% more compared to the cultivar with the lowest biomass (Sergei, 388.1 mg). Within FRC and FRT the difference between the cultivars with the highest and lowest biomass was 24.4% and 25.1%, respectively.

Table 3.3. Spearman' correlation investigating the degree of association of 27 *Festuca* cultivars for biomass, full germination percentage (FGP) and mean germination period (MGP).

Variables	(1)	(2)	(3)
(1) Biomass	1		
(2) FGP	0.189**	1	
(3) MGP	0.125*	0.205***	1

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels.

A correlation analysis was conducted to investigate the association between germination and biomass. A higher germination percentage of *Festuca* was associated with a later mean germination period and more biomass (Table 3.3).

3.3.2 Festuca growth interference with clover

For clover controls, the FGP differed between experiments (P = 0.019). This is because FGP of clover in the replication of the experiment was 92.5%, compared to 97.1% in the first experiment. MGP did not differ between experiments (P= 0.48) as both averaged 1.0, indicating that all germinated seeds already germinated within four days. Similarly, no significant differences were observed for root length of clover controls (P = 0.84), which averaged 10.8 cm for the first and 10.9 cm for the second experiment.

For clover in the presence of *Festuca* species, no differences in FGP were observed among species. Clover FGP was not significantly different from that of the clover control (FGP= 94.8%) and ranged from 91.2% in the presence of FRR to 89.1% in the presence of FA (data not shown). Also, no cultivar differences were observed for clover FGP, which ranged from 94.4% (Charlotte, FRT) to 86.9% (Regenerate, FA). *Festuca* species nor individual cultivars within species influenced the MGP of clover. Similar to the control, the clover in presence of *Festuca* all scored a MGP of 1.0 (data not shown).

Clover root length was affected both by the presence of *Festuca* species (P< 0.001) and cultivars (P< 0.001). Clover root length in the presence of all species was significantly reduced compared to the control. Negative impacts on clover root length were strongest in the presence of FA, which reduced root length by 71.6% (3.1 cm) compared to controls (10.9 cm) (Figure 3.3). Between species FRR, FRA and FRC no significant difference was observed, with clover root length averaging between 4.4 cm (FRC) and 4.6 cm (FRA). Presence of FRT species interfered least with clover root length (6.02 cm), but still in this case root length was reduced with 44.5%.

In the presence of all cultivars, clover root length was significantly reduced compared to that of the control. In the presence of 20 out of 27 cultivars, clover root length was more than halved compared to the control. Among the seven cultivars with the lowest growth reducing effect on root length, five belonged to FRT. Within the group of cultivars leading to the shortest clover root length, the cultivars Relevant (2.6 cm, FRR), Ramona (2.3 cm, FRC), Rossinante (2.1 cm, FRR) and Regenerate (2.0 cm, FA) reduced clover root length below 3 cm. Significant differences between cultivars within species were present for FRR, FRC and FRT. Only within the species that were represented by just 2 of 3 cultivars (FA, FRA) cultivar differences were not observed.

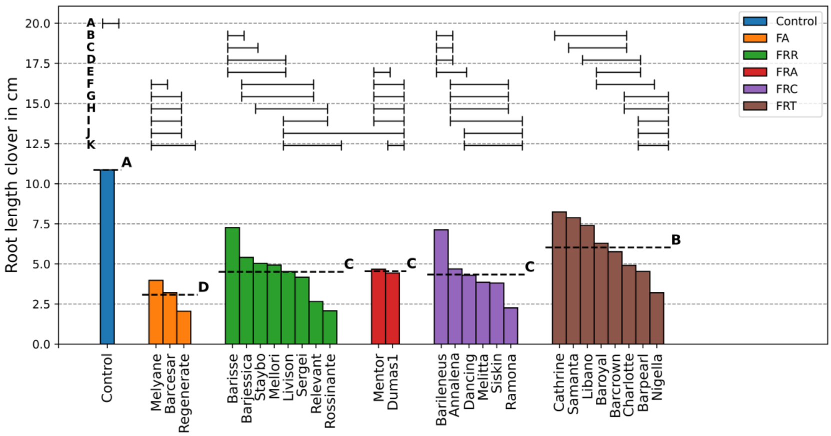

Figure 3.3. Root length of clover (*Trifolium repens* L.), grown together with 27 *Festuca* cultivars or as stand-alone control on water agar for 30 days in a controlled environment, grouped by species (FA, tall fescue; FRR, strong creeping red fescue; FRA, hard fescue, FRC, Chewings fescue; FRT, slender creeping red fescue) and experiments. Dotted horizontal lines indicate species averages and solid lines indicate shared cultivar mean separation letters. Means sharing the same letter are not significantly different (P<0.05).

A correlation analysis showed that biomass of *Festuca* had a significant negative association with root length of clover (Table 3.4), whereas FGP and MGP of clover did not have a significant association with *Festuca* biomass.

Table 3.4 Spearman' correlation investigating the degree of association between biomass of 27 *Festuca* cultivars and three clover (*Trifolium repens* L.) characteristics: full germination percentage (FGP), mean germination period (MGP), and root length.

Variables	Clover FGP	Clover MGP	Clover root length
Festuca biomass	n.s.	n.s.	-264***

***, Significant at the 0.001 probability levels.

3.3.3 Festuca growth interference with yarrow

Full germination percentage of yarrow controls did not differ (P= 0.27) between experiment one (96.7%) and experiment two (98.8%). For MGP, yarrow controls shortened from 1.2 in experiment one to 1.1 in experiment two (P <0.001). Also, the root length of yarrow controls differed significantly between experiments (P< 0.001); from 14.7 cm in experiment one to 17.2 cm in experiment two.

None of the *Festuca* species or cultivars had a significant effect on FGP or MGP of yarrow. For FGP species ranged from 97.3% (FRA) to 96.3% (FA) and cultivars ranged from 98.8% (Libano, FRT) to 94.3% (Samanta, FRT) (data not shown). MGP of all species was 1.1, whereas among the cultivars MGP ranged from 1.2 for Regenerate (FA) to 1.0 for Livison (FRR).

Yarrow root length was affected both by the presence of species (P< 0.001) and cultivars (P< 0.001). All *Festuca* species reduced yarrow root length with at least 75% compared to the control (15.9 cm). No significant differences in yarrow root length among species were observed, with root length ranging from 3.8 cm (FRT) to 2.3 cm (FA) (Figure 3.4).

Significant differences were observed among individual cultivars, though all cultivars generated a significant reduction in yarrow root length. Yarrow root length was least reduced with FRT cultivar Samanta (5.9 cm), but this reduction was still 63%. The reduction in root length of this cultivar was significantly lower than that of 14 cultivars that resulted in yarrow root length of 3 cm or lower. Presence of Barcesar (FA) led to yarrow root length of 1.35 cm, which was significantly different from seven species, with yarrow root lengths of 4.1 cm or longer.

Except for FRA and FRR, there were significant differences in growth interference among cultivars within species. FA cultivar Regenerate had less of an effect on yarrow root growth (4.13 cm) compared to Barcesar (1.34 cm). Also within FRC the two most extreme cultivars differed (Barileneus: 4.79 cm and Melitta: 1.98 cm). Within FRT, the extreme cultivars were Samanta (5.93 cm) and Barpearl (2.04 cm), but within this species more cultivar differences were observed.

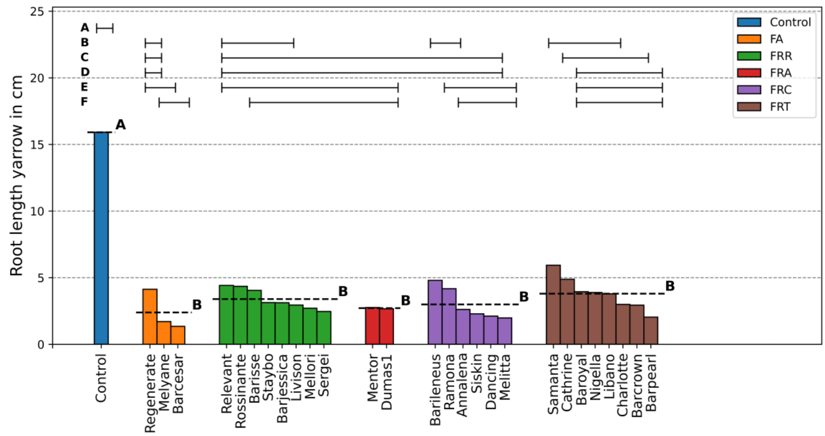

Figure 3.4. Root length of yarrow (*Achillea millefolium* L.), grown for 30 days in a controlled environment together with 27 *Festuca* cultivars or as a stand-alone control on water agar, grouped by species (FA, tall fescue; FRR, strong creeping red fescue; FRA, hard fescue, FRC, Chewings fescue; FRT, slender creeping red fescue) and experiments. Dotted horizontal lines indicate species averages and solid lines indicate shared cultivar mean separation letters. Means sharing the same letter are not significantly different (P<0.05).

A correlation analysis was conducted to investigate the association of *Festuca* biomass on yarrow FGP, MGP and root length (Table 3.5). *Festuca* biomass was negatively associated with MGP and weed root length, meaning that *Festuca* species with more biomass production were associated with an advanced germination of yarrow and reduced yarrow root length.

Table 3.5. Spearman' correlation investigating the degree of association of 27 *Festuca* cultivars for biomass on yarrow (*Achillea millefolium* L.), full germination percentage (FGP), mean germination period (MGP)and root length.

Variables	Yarrow FGP	Yarrow MGP	Yarrow root length
Festuca biomass	n.s.	-0.155*	-0.181**

*, ** Significant at the 0.05 and 0.01 probability levels.

3.4 Discussion

Resource competition and allelopathic interference are two important mechanisms of plant-plant interaction. Allocating the relative effect of each mechanism has been frequently attempted (Inderjit and Del Moral, 1997; He et al., 2012; Scavo et al., 2018), but to date, designing an experiment that convincingly separates effects of each has proven to be difficult. Since resource competition is prominent under field conditions, determining allelopathic potential is often attempted under laboratory conditions, with minimal supply of nutrients. Since resource competition cannot be completely excluded even under such experimental conditions, Breuillin-Sessoms et al. (2021) proposed to use the term "weed suppression potential". Observations on root length are commonly used to quantify interference potential (Bertin et al., 2003; Duke, 2015). It is this type of plant-plant interference study that we conducted.

In this study we examined the growth interference potential of five *Festuca* species including slender creeping red fescue [*F. rubra* L. ssp. *littoralis* (G.Mey.) Auquier], strong creeping red fescue (*F. rubra L. ssp. rubra Gaudin),* Chewings fescue [*F. rubra L.* ssp. *fallax* (Thuill.) Nyman] and tall fescues [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] on weeds, including clover (*Trifolium repens* L.), daisy (*Bellis perennis* L.) and yarrow (*Achillea millefolium* L). The number of cultivars tested for each species ranged from two (for hard fescue, referred to as FRA) to eight (strong creeping red fescue and slender creeping red fescue, referred to as FRR and FRT, respectively). Examining different cultivars allowed us to compare between and within species variability in interference potential. Differences in number of cultivars among species is not ideal for such an analysis but resulted from our intention to focus on cultivars commonly used in athletic fields and golf fairways. After growing grasses for 13 days in plastic boxes on water agar we introduced weed seeds and after 17 more days measured weed root length. We observed that all *Festuca* species had a significant effect on weed root growth. Compared to controls, average root length of clover was reduced by 56%, and yarrow root length was reduced by 79%. Data on daisy were excluded from further analysis because the presence of *Festuca* species caused bleaching of daisy after germination and root length was simply too short to be measured. Our results demonstrated that the extent of growth interference of weeds by *Festuca* is highly variable among weed species. This was also observed by Bertin et al (2003a), who showed root and shoot suppression of large crabgrass (*Digitaria sanguinalis*) in the presence of *Festuca* species of up to 80% and only up to 58% for curly cress (*Lepidium sativum* L.).

For clover root length, significant species differences were observed, with FA being the strongest suppressor and FRT being the weakest suppressor. Along with these species' differences, we observed significant differences among cultivars within FRC, strong creeping red fescue FRR, and FRT. Whereas no significant differences among *Festuca* species were observed for yarrow root length, significant differences were observed among cultivars within FA, FRC and FRT. This leads us to conclude that cultivar differences in interference potential are more prominent than species differences within *Festuca*. This suggests that when selecting a cultivar with high interference potential one is not necessarily restricted to a single *Festuca* species, as presence and variability in interference potential can be found in most of the species.

Differences in weed growth interference among *Festuca* cultivars were particularly pronounced regarding clover root length, resulting in a wide range of weed root suppression varying from 24.8% (Cathrine, FRT) up to 81.7% (Regenerate, FA), compared to controls. The average effect of *Festuca* cultivars on yarrow root length was stronger compared to the effect on clover, but varied less among cultivars, ranging from 62.9% (Samanta, FRT) to 91.8% (Barcesar, FA) compared to controls. Based on these findings, we suggest that clover is a better indicator species to screen for growth interference potential of *Festuca* species on weed characteristics such as root length. As mentioned before, we also used daisy in our experiments but removed all entries, because daisy plants appeared photo-bleached after germination, and root length and root length differences were difficult to determine. The high susceptibility of daisy to all *Festuca* cultivars makes it an unsuitable indicator species for identifying differences in interference potential.

One possible mechanism behind the observed growth interference of *Festuca* species and cultivars may simply be related to the growth potential of one species versus another. Higher biomass production implies a stronger metabolic activity, and proportional to metabolic activity the interference potential is generated. In fact, this hypothesis suggests that interference is not the result of specific compounds that are produced in higher quantity in one cultivar than the other, but results from more general compounds that are simply produced in a quantity proportional to growth rate. To investigate this premise, we determined the biomass of the different cultivars. Tall fescue (FA) produced the highest amount of biomass: just over two times more than the next highest producing species (FRA) and approximately 2.5 times more than the other three species. With clover species, the strongest reduction in root length was observed in the presence of FA, the highest biomass producer. However, with yarrow such a relation was not observed, as all species had an equal inhibitory effect on yarrow root length. Correlation analyses revealed that for both weed species *Festuca* biomass was negatively correlated to root length development of clover (-.264***) and yarrow (-.181**). This correlation suggests that metabolic activity, reflected in biomass, does influence root length inhibition. However, the relatively low correlation values also show that it is not the only responsible factor.

In addition to examining the effect of *Festuca* species and cultivars on weed root length, we investigated their influence on weed seed germination. For that, we modified the experimental design of Bertin et al. (2003a), on which our research was based. Following their recommendation, we increased the establishment period of grasses from 7 to 13 days, to allow production of more allelopathic compounds. Subsequently, we seeded the *Festuca* seeds in a matrix pattern with 2 cm between neighboring seeds. Weed seeds were placed in the center of individual cells, to shorten the distance between *Festuca* and weed seeds and increase the likelihood of weed seeds being in the sphere of influence of root exudates from the developing *Festuca* seedlings. Despite these modifications, we observed no *Festuca* species or cultivar effect on germination rate (FGP) or germination speed (MGP). This could indicate that either *Festuca* only influence weed growth processes but has no effect on their germination, or that, despite our modifications, the quantity of root exudates reaching the weed seeds was insufficient to express an effect. If the latter is the case, an alternative to the current experimental design may be needed to more adequately assess the effects of donor species on the germination of seeds of receiver plants. Vasilakoglou et al. (2005) for instance derived exudates from bermudagrass [*Cynodon dactylon* (L.) Pers] and johnsongrass [*Sorghum halepense* (L.) Pers] and demonstrated germination inhibition of corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.). A weakness of the parameter mean germination period (MGP), which we used to quantify germination speed, was the four day period we selected as the interval of observation. In the course of the experiment, we discovered that all germinated clover seeds had already germinated within four days (MGP=1), as did most of the germinated yarrow seeds (MGP=1 to 1.2). The interval of evaluation was thus insufficiently aligned to the objectives of our study and shorter observation intervals are recommended for future studies. However, our results suggest that any differences in germination speed would likely be relatively small and therefore not relevant.

The tendency to screen for allelopathic potential of cultivars under controlled conditions away from the complexity of the field has several benefits. However, it inevitably generates an important follow-up question: how relevant are the laboratory findings to the performance of the cultivars under field conditions? Effects as strong as those we observed under controlled conditions (root length reductions of over 90%) may not be detected under field conditions. What matters more is whether cultivar rankings established under controlled conditions are indicative of performance in the field. Published studies addressing this question are inconclusive. In a study on weed suppressive ability of rice cultivars, Olofsdotter et al. (1999) found some degree of correlation between laboratory results of root length reduction of barnyard grass [*Echinochloa crus-galli* (L.) Beauv.] and field performance of cultivars. On the other hand, Bertin et al (2003a) showed that cultivar Rescue 911 (hard fescue), which had minor effects on weed growth suppression of large crabgrass under controlled conditions, performed well in field trials (Bertin et al., 2003a, 2009). Weed suppressive ability in the field is the outcome of several processes, including the ability to compete for resources and allelopathic potential. In light of that, the current results can be considered a piece of the puzzle, indicating that allelopathic potential is present within *Festuca* species, and that this trait varies widely among cultivars within species.

CHAPTER 4

Assessing competitiveness of *Festuca* species established with white clover (*Trifolium repens* L.), daisy (*Bellis perennis* L.) and yarrow (*Achillea millefolium* L.) in field trials

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This chapter has been published as Hahn *et al*., 2021. Agronomy 11(11): 2226.

Abstract

Regulatory restrictions on herbicide use for managing turfgrass weeds has prompted the search for alternative control strategies. Fescue (*Festuca*) species were identified for their potential to interfere with growth of annual and perennial weeds. In a study conducted in 2018 and 2019 we tested six fescue cultivars from five different species for interference with growth of three common turfgrass weeds: white clover (*Trifolium repens* L., WC), daisy (*Bellis perennis* L.) and yarrow (*Achillea millefolium* L.). Fine and tall fescues were sown and grown in a field trial for 14 days before overseeding with different weeds. We recorded vigor and visual quality of grasses, weed cover, and vegetation cover regularly for 84 days. Differences in mean temperatures and precipitation between the two years of the study resulted in differences in growth of grasses and weeds, as well as in the extent of weed interference of fescue cultivars. Cultivars Musica (*F. rubra* L. ssp. *commutata* Gaudin) and Barpearl (*F. rubra* L. ssp. *littoralis*) slender creeping red fescue) were least affected by weed growth during both years, but there was overlap with other cultivars for the measured parameters. Melyane (*Schedonorus arundinaceus* (Schreb.) Dumort.) was deemed unsuitable for natural weed suppression because growth and vigor declined after first mowing, ultimately leading to unacceptable visual quality. Turfgrass visual scores were moderately negatively correlated to weed cover in both years. Future research should focus on *F. rubra* L. ssp. *rubra* Gaudin and *F. rubra* L. ssp. *littoralis* subspecies and identify the mechanisms used to interfere with weed growth.

Keywords: broadleaf weeds; growth interference; turfgrass vigor; visual quality; weed cover; vegetation cover.

4.1 Introduction

In some European countries, concerns over pesticide misuse have led to strict regulations, particularly in regards to herbicides used on amenity areas (Kristoffersen et al., 2008). In Denmark, metabolites of pesticides were detected in 40% of groundwater wells that supply drinking water to communities (Malaguerra et al., 2012). In the Netherlands, where 40% of all drinking water is derived from surface water, problems with pesticide contamination led to voluntary agreements with municipalities to reduce herbicidal use in amenity areas (Kristoffersen et al., 2008). Such voluntary agreements are also in place for athletic fields, as exemplified by the 'Green Deal' in The Netherlands (Mansveld et al., 2016). The 'Green Deal' was initiated to restrict the use of pesticides on amenity areas entirely; however an exemption period was granted until 2022, which allows the use of selected pesticides under strict conditions (Mansveld et al., 2016). In turf settings, herbicides are the most used pesticide in terms of product use (Meftaul et al., 2020). Herbicides are mainly applied to control broadleaf dicotyledonous weeds, such as white clover (*Trifolium repens*) or dandelion (*Taraxacum officinale*), which are the most problematic weeds in athletic fields (Raikes et al., 1994). In these areas, the main objective is to maintain a mono-stand of turfgrass species that produce aesthetically pleasing surfaces with a certain standard of playing quality (Busey, 2003; Larsen et al., 2004).

Weeds in athletic fields can be defined as unwanted species that interfere with the visual appearance and playing quality of these surfaces (Larsen et al., 2004). Weeds often establish in sparse areas as a result of abiotic/ biotic stress or management failures such as improper water management or fertilization (Masin et al., 2005; Pirchio et al., 2018). Once established, weeds compete with desirable turfgrass species for resources, namely water, light and nutrients, as well as space, such as in below ground root competition (Busey, 2003).

The requirement for desirable turfgrass species has shifted towards low-input species, to reduce the ecological footprint of amenity turfgrass areas (Braun et al., 2020; Hahn et al., 2020). Consequently, an ecological approach to weed management should focus on establishing sustainable turfgrass species that require low inputs of valuable resources, such as water and fertilizer, while providing a dense, healthy turf canopy that competes well against weeds (Ruemmele et al., 1995; Busey, 2003; Grimshaw et al., 2018; Pirchio et al., 2018).

Fine fescues (*Festuca* L. spp.) can establish a dense turf canopy with minimal inputs of water, fertilizer and pesticides (Grimshaw et al., 2018). These perennial fine-leaved turf species thrive in dry conditions but are also adapted to shade and low pH growing conditions (Grimshaw et al., 2018). Fescues can be grouped into two complexes, the red fescues (*Festuca rubra* L.) and the sheep fescues (*Festuca ovina* L.) (Stace, 1992; Braun et al., 2020). Within the red fescue species, strong creeping red fescue (*F. rubra* L. ssp. *rubra* Gaudin) and slender creeping red fescues [*F. rubra* L. ssp. *littoralis* (G. Mey.) Auquier] produce rhizomes (Stace, 1992), while Chewings fescue [*F. rubra* L. ssp. *commutata* Gaudin] exhibits a bunch type growth. Chewings fescue was identified as a species that provides acceptable quality on golf fairways under low- nitrogen inputs, defined as 4.9 grams of nitrogen per square meter, and is a superior species under reduced irrigation and low pesticide inputs compared to other fine fescue species and colonial bentgrass (*Agrostis capillaries* L., CL) mixtures (Horgan et al., 2007).

Hard fescue (*F. brevipila* Tracey) belongs to the sheep fescue complex, establishes slowly, and has a bunch type rooting system (Lane et al., 2019). Hard fescue has slower establishment vigor compared to creeping red fescue and Chewings fescue, and demonstrated less natural weed suppression (Bertin et al., 2009; Braun et al., 2020). Seedling vigor or establishment can be defined as the speed at which a plant develops from germination into a mature plant, and combines groundcover scores and plant height over time (Morris, 2020). Therefore a plant that shows higher vigor compared to another will develop more rapidly into a mature plant (Donart et al., 1973).

Tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.), is a stand-alone fescue complex, establishes quickly from seed (Lane et al., 2019) and can be easily separated from other turf species because of the wide leaf width of 4-18 mm. In comparison, *Festuca rubra* species have leaf widths of less than 2 mm (Braun et al., 2020). Additionally, tall fescues have a deep rooting system, making them one of the most drought resistant cool-season turfgrass species (Sun et al., 2013). Drought resistance combined with wear tolerance makes tall fescue one of the most used grass species for athletic fields in European transition zones (Pornaro et al., 2016).

Apart from being a sustainable turfgrass species, fescues have also demonstrated allelopathic potential (Bertin et al., 2003a, 2007, 2009). Fescues produce compounds which interfere with the growth of some neighboring plants, imparting them with natural weed suppression capabilities (Bertin et al., 2003a). A series of field studies of 78 fine-leaf fescue cultivars showed that three Chewings fescue cultivars and one hard fescue cultivar showed 'good' natural weed suppression capabilities (defined as more than 70% of weeds suppressed compared to a control) (Bertin et al., 2009).

Information is lacking on the prevalence of weed suppression capabilities among certain fescue complexes or species. Moreover, it is unknown if fescues interfere differently with the growth of different weed species. In this study, we investigated the extent of growth interference of white clover (*Trifolium repens* L., WC), daisy (*Bellis perennis* L.) and yarrow (*Achillea millefolium* L.), three common European broadleaf weeds, during the establishment phase of six fescue cultivars from five species. We also investigated if final grass vigor was negatively correlated to weed establishment, if better visual scores could be attributed to low weed cover or high vigor and lastly, if quantitative vegetation cover estimates gave results that were similar to visual vigor scores.

4.2 Materials and Methods

4.2.1 Field Site and Cultivars Selected

The field trials were conducted at the Barenbrug Turfgrass Research Station in Wolfheze, The Netherlands (52°00' N, 5°47'E). The first field trial was sown on July 13th, 2018, and the replication of the experiment was sown 22nd of August 2019, on an adjacent field. Both experiments were conducted for 84 days each. Soil in the upper 15 cm was loamy sand (79% sand, 12% silt, 3% clay) with a pH of five and an organic matter content of 6%. Each plot measured 1.5 m x 1.5 m. On the first day of each field trial, 30 plots were sown with an equal number of seeds (20150 per m^{-2}) of the following fine and tall fescue cultivars: Musica (Chewings fescue), Mentor (hard fescue), Samanta and Barpearl (slender creeping red fescue), Barisse (strong creeping red fescue), and Melyane (tall fescue). We originally planned to include varieties that were also investigated by Bertin et al. (Bertin et al., 2009). The authors of this study observed the visual weed suppression ability of fine fescues in Ithaca, NY from 1998 to 2002. Unfortunately, none of these cultivars are commercially available in Europe. Therefore, we selected cultivars from the British Society of Plant Breeders list (BSPB, 2017). Sowing rates were based on guidelines published by Beard (1973) (Table 4.1).

Table 4.1. Sowing rates (g m^{-2}) and number of seeds (seeds m-^{2}) of fine (*Festuca* L. spp.), tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.) cultivars, and weed species used in the study.

Cultivar	Species	Common name	g m^{-2}
Mentor	*F. brevipila*	Hard	21.8
Musica	*F. rubra commutata*	Chewings	17.3
Barpearl	*F. rubra littoralis*	Slender creeping	22.9
Samanta	*F. rubra littoralis*	Slender creeping	19.5
Barisse	*F. rubra rubra*	Strong creeping	28.2
Melyane	*Schedonorus arundinaceus*	Tall	47.8
-	*Achillea. millefolium* L.	Yarrow	0.9
-	*Bellis perennis* L.	Daisy	1.0
-	*Trifolium repens* L.	Clover	4.9

The seedbed was prepared by harrowing, removing stones by hand, raking and rolling. Before sowing, the soil was loosened again through raking. Subsequently, plots were seeded by evenly spreading seed by hand in two directions perpendicular to one another. After depositing the seeds, the seedbed was carefully raked to cover the seeds with soil, and then irrigated. The plots were then covered with a thin fleece to retain moisture in the soil and to promote seed germination.

Fourteen days after seeding of the grasses (DAS), all plots except grass controls were oversown with one of three weed species, namely clover, daisy, and yarrow, or with a mixture of all three species that included each weed at one-third of the full rate (hereafter referred to as 'weed treatments'). Weed seeding rates are listed in Table 4.1. The 14 day delay in seeding of the weeds was chosen to allow time for allelopathic chemicals produced by donor plants (grasses) to be released into the soil medium and potentially have a growth inhibitory effect on receiver plants (weeds) (Lalljee and Facknath, 2000; Bertin et al., 2003a, 2007).

Twenty-one days after seeding of the grasses (DAS), the area was mowed at a height of 20 mm, using a Jacobson TR3 reel mower (TR3, Jacobson, Racine, WI, USA) without box attachments to return clippings. Subsequently mowing was applied twice per week at 15 mm. At 28 DAS, 42 DAS, 56 DAS, and 70 DAS, granular fertilizer (NPK 12-10-18 Arm, Eurosolids, Westmaas, The Netherlands) was applied at a rate of 200 kg ha^{-1} $year^{-1}$, which amounted to 2.4 g m^{-2} $year^{-1}$ of nitrogen.

4.2.2 Data Collection

Digital image analysis was used to determine percent green vegetation cover, which includes both grasses and weeds cover of each plot (referred to as vegetation cover) (Karcher and Richardson, 2003, 2013) at the end of the research period (84 DAS). A picture covering an area of 0.9 by 1.1 m was taken of the center of each plot with a Canon PowerShot SX 200 (Canon Inc., Tokyo, Japan) set at ISO 200, Aperture 2.6 and shutter speed 1/60 s. The camera had a distance to the ground of 60 cm and was housed in an enclosed box fitted with four halogen light bulbs designed to provide uniform light coverage (Ikemura, 2003). The images were subsequently processed with the software Turf Analyzer (Turf Analyzer, 2018), which applies a green pixel recognition algorithm to calculate the percentage of vegetation cover.

Plots were also rated for visual quality on a scale of one to nine (Krans and Morris, 2007; Leinauer et al., 2014) on 84 DAS. Visual quality is a score that includes density, color, homogeneity, and uniformity, with one representing a poorly established sward with many weed species being present and nine representing a dense canopy with a monostand of sown turfgrass species free of weeds (Leinauer et al., 2014; Morris, 2020).

Scores for turfgrass vigor were collected on 14, 39, and 84 DAS and weed cover were recorded on 26, 54 and 84 DAS. Vigor was recorded visually from zero to nine (Morris, 2020) with zero denoting no grasses, one representing a turf sward that just germinated and nine indicating a perfectly established, dense sward. Hence vigor can be described as a combined score of germination speed, development of biomass and turf cover (Morris, 2020). On the first two sampling dates, turfgrass scores and weed cover measurements were not collected on the same day because initial seeding of grasses and weeds was staggered. Plots were first seeded with grass, then overseeded with weeds two weeks later. Thus, the first weed cover data were collected 12 days after first turfgrass vigor scores (26 DAS vs. 14 DAS) to allow weeds to grow sufficiently to accurately estimate weed cover. The second set of turfgrass vigor data were collected 35 days after the first set, and the second set of weed coverage data were collected 32 days after the first set. The third and last set of data all were collected on the same day, 84 DAS. Number of weeds were determined by line intersect analysis (LIA) placing a 1 m x 1 m frame with 100 intersections, each 10 cm apart, on each plot and counting presence or absence of weed species under each intersection. Presence of a weed species under an intersection was recorded as '1%' weed cover (Krans and Morris, 2007; Hoyle et al., 2013).

4.2.3 Environmental Conditions

During the 2018 experiment, air temperatures averaged 17.8°C and rainfall was recorded on 24 days (total rainfall 205 mm). In 2019, temperatures averaged 12.9 °C, and rainfall was recorded on 50 days (total rainfall 322 mm) (see table 4.2). Weather data were recorded by a weather station, which was located onsite.

Table 4.2. Monthly average air temperatures (°C) and precipitation (mm) at Barenbrug research station, Wolfheze, The Netherlands.

	2018				2019			
Climate Parameters	Jul.	Aug.	Sept.	Oct.	Aug.	Sept.	Oct.	Nov.
Air temperature (°C)								
Minimum	13.7	12.3	8.7	6.0	12.6	9.4	7.6	3.7
Maximum	31.6	26.0	21.7	16.8	30.8	20.5	15.5	10.8
Average	22.7	18.8	14.7	10.8	20.8	14.3	11.4	7.1
Precipitation (mm)								
Average	0.3	4.2	2.2	0.8	1.9	3.6	4.2	5.1

4.2.4 Statistical Analysis

The general experimental layout of the plots was an Extended Factorial Design which consisted of two treatment factors, one with seven grasses and another with five weeds. This type of designs are also known as Augmented Factorial (Lentner and Bishop, 1993). Plots were arranged as a randomized complete block (RCB) with each treatment replicated four times. The treatment design was incomplete because it did not include a grass control x weed control plot. Therefore, we combined both treatment factors (grass and weeds) to one treatment factor with 34 levels, as in Marini (Marini, 2003), and applied a pairwise comparison to determine significant differences between the treatment combinations. Calculations of weed cover are based on counts and data were analyzed using a Negative Binomial distribution. Vigor and quality data were analyzed based on a normal distribution. Since vigor was only recorded for grass species, data of plots sown with weeds only (i.e., weed controls) were removed from the vigor analysis. Initial statistical analyses revealed a significant DAS main effect, and DAS was also observed in each relevant interaction term that was shown to be significant. Consequently, ANOVA was used to analyze weed cover and vigor separately for each DAS. Weed cover, grass vigor, and visual quality data were analyzed using PROC GLIMMIX in SAS statistical software version 9.4 (SAS Institute, Cary, NC). The level of significance was set equal to 5%. The SIMULATE method was selected to control for multiplicity. To explore the relationship between visual quality and grass vigor and weed cover, and between vegetation cover and grass vigor and weed cover, Pearson's correlations among these output values collected 84 DAS were computed and coefficient of determination values (r) were reported.

4.3 Results

4.3.1 Grass Vigor

In 2018, vigor scores 14 DAS ranged from 1.0 (Samanta in combination with all four tested weeds and Musica in combination with daisy and yarrow) to 2.8 for Mentor in combination with clover. Mentor was consistently among the cultivars that rated highest for vigor (Table 4.3). Results were different in 2019, when Melyane consistently placed in the group with the highest vigor ratings, indicating an early, fast establishment when compared to other cultivars (Table 4.3). On DAS 39 in 2018, vigor scores again ranged from 1.0 (cultivar Samanta in weed control plots) to 3.5 for Mentor in combination with clover and Melyane in combination with daisy (Table 4.3). Vigor scores in 2019, 39 DAS only differed between Musica with yarrow and Melyane with clover, for which ratings of 5.5 and 3.9, respectively, were recorded (Table 4.4). In 2018, vigor differences among cultivars were no longer discernable at the end of the research period on DAS 84 (Table 4.3). Although at 84 DAS, in 2019, Barpearl and Musica placed in the group with the highest vigor scores for all weed treatments, there was extensive overlap with other cultivars and no clear trends emerged (Table 4.4).

4.3.2 Weed Cover

There were no differences in weed cover on 14 DAS in 2018. Lowest weed cover in 2019 was recorded on control plots seeded with Mentor, and there were no differences among any other grass x weed combinations (Table 4.4). On 54 and 84 DAS, grass control plots generally exhibited the lowest weed coverage (Table 4.4) and weed control plots had the highest percent weed cover. However, coverage did not separate clearly and consistently among the different grass by weed combinations (Table 4.4). In 2018, 84 DAS, plots sown with the cultivar Samanta placed in the group with the highest weed cover regardless of weed treatment, however no clear trends emerged because of significant overlap with other grasses (Table 4.4). Aside from weed cover on control plots, weed cover in 2019 was greatest on Melyane plots, but again no clear trend emerged among the other treatments.

Table 4.3. Grass vigor scores of six fine (*Festuca* L. spp.) and tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.) cultivars sown in 2018 and 2019 14, 39, 84 days after seeding (DAS) with white clover (*Trifolium repens* L.), daisy (*Bellis perennis* L.), yarrow (*Achillea millefolium* L.), a mixture of all three weed species or without weeds (control). Grass vigor describes a combined score of germination speed, development of biomass and turf cover, and ranges from 0 to 9 with 0 = no germination and 9 = fully established, dense turf sward.

Weed	Species	Cultivar	DAS 14		DAS 39		DAS 84	
			2018	2019	2018	2019	2018	2019
Clover	*F. rubra l.*	Barpearl	1.5ABC‡	1.6E	1.9BCDE	4.9AB	7.0A	6.5AB
	F. rubra l.	Samanta	1.0C	1.3E	1.4DE	4.4AB	6.8A	6.3ABC
	F. rubra c.	Musica	1.3BC	2.0BCDE	2.6ABCD	5.3AB	7.0A	6.0ABCD
	F. rubra r.	Barisse	1.5ABC	1.3E	3.0ABC	4.4AB	6.9A	5.9ABCDE
	F. brev.	Mentor	2.8A	1.3E	3.5A	4.0AB	6.9A	5.5ABCDE
	Schedon. a.	Melyane	1.8ABC	3.3AB	2.8ABCD	3.9B	6.6A	4.5DE
Daisy	*F. rubra c.*	Musica	1.0C	2.0BCDE	1.9BCDE	5.1AB	6.6A	6.8A
	F. rubra l.	Barpearl	1.5ABC	1.4E	2.4ABCDE	4.5AB	6.8A	6.6A
	F. rubra l.	Samanta	1.0C	1.5E	1.5CDE	4.4AB	7.0A	6.3ABC
	F. rubra r.	Barisse	1.8ABC	1.4E	2.8ABCD	4.6AB	6.6A	5.8ABCDE
	F. brev.	Mentor	2.0ABC	1.1E	2.6ABCD	4.0AB	6.6A	5.5ABCDE
	Schedon. a.	Melyane	2.5AB	3.1ABC	3.5A	4.0AB	6.4A	4.4E
Yarrow	*F. rubra l.*	Barpearl	1.3BC	1.8DE	2.0ABCDE	4.8AB	7.0A	6.9A
	F. rubra c.	Musica	1.0C	2.1ABCDE	1.9BCDE	5.5A	7.0A	6.8A
	F. rubra r.	Barisse	1.3BC	1.1E	3.3AB	4.5AB	6.6A	6.1ABC
	F. rubra l.	Samanta	1.0C	1.3E	1.4DE	4.6AB	5.8A	6.1ABC
	F. brev.	Mentor	2.5AB	1.4E	3.3AB	4.0AB	7.1A	5.8ABCDE
	Schedon. a.	Melyane	2.0ABC	3.4A	2.6ABCD	4.3AB	6.4A	5.0BCDE
Mixture	*F. rubra l.*	Samanta	1.0C	1.3E	1.6CDE	4.4AB	6.8A	6.5AB
	F. rubra c.	Musica	1.3BC	1.5E	2.5ABCDE	4.8AB	6.0A	6.4ABC
	F. rubra l.	Barpearl	1.3BC	1.4E	2.1ABCDE	5.1AB	6.9A	6.3ABC
	F. rubra r.	Barisse	1.8ABC	1.4E	2.6ABCD	4.8AB	6.8A	6.0ABCD
	F. brev.	Mentor	2.0ABC	1.0E	2.6ABCD	4.5AB	7.1A	5.8ABCDE
	Schedon. a.	Melyane	2.5AB	3.4A	3.4AB	4.1AB	6.5A	5.0BCDE
Control	*F. rubra c.*	Musica	1.0C	1.9CDE	2.1ABCDE	4.9AB	6.8A	6.9A
	F. rubra l.	Barpearl	1.0C	1.6E	2.1ABCDE	4.6AB	6.5A	6.8A
	F. rubra l.	Samanta	1.0C	1.3E	1.0E	4.6AB	6.6A	6.4ABC
	F. rubra r.	Barisse	1.3BC	1.3E	2.5ABCDE	4.8AB	6.9A	5.9ABCDE
	F. brev.	Mentor	2.5AB	1.3E	3.0ABC	4.0AB	6.9A	5.6ABCDE
	Schedon. a.	Melyane	1.8ABC	3.0ABCD	2.8ABCD	4.0AB	6.4A	4.9CDE

‡Values in each column (i.e., separately for each year and DAS) followed by the same letter are not significantly different according to simulated adjustment (0.05).

Table 4.4. Percent weed cover (estimated means) of fine (*Festuca* L. spp.) and tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.) cultivars and in control plots (no grass) sown in 2018 and 2019 on 26, 54, 84 days after seeding (DAS). Fescue grasses were seeded with either white clover (*Trifolium repens* L.), daisy (*Bellis perennis* L.), yarrow (*Achillea millefolium* L.), a mixture of all three weed species or without weeds (Control). Treatments also included weed only (Control II) plots.

Weed	Species	Variety	DAS 26		DAS 54		DAS 84	
			2018	2019	2018	2019	2018	2019
Clover	*Schedon. a.*	Melyane	5.9A[‡]	17.1A	17.0DEFGH	26.7AB	38.4ABCDE	32.7ABCD
	Control II		6.5A	5.8AB	35.7ABCD	17.5ABCD	71.3ABC	25.8ABCDE
	F. rubra l.	Samanta	5.5A	3.9AB	22.2BCDEFG	15.4ABCD	53.3ABCD	23.6ABCDE
	F. brev.	Mentor	5.3A	3.6AB	9.6FGHIJ	17.1ABCD	26.7DEFG	21.7ABCDE
	F. rubra l.	Barpearl	3.5A	7.4AB	15.6DEFGH	14.5ABCD	31.9CDEF	21.2ABCDE
	F. rubra r.	Barisse	8.0A	7.9AB	15.6DEFGH	15.6ABCD	40.1ABCDE	20.8ABCDE
	F. rubra c.	Musica	3.9A	8.0AB	16.4DEFGH	14.0ABCD	28.4DEFG	18.0ABCDE
Daisy	*Schedon. a.*	Melyane	4.5A	16.7A	19.9CDEFG	28.0AB	40.9ABCDE	37.0AB
	Control II		3.2A	9.1AB	36.6ABCD	29.4A	79.3AB	38.6A
	F. brev.	Mentor	3.0A	11.7AB	15.1DEFGH	23.8ABC	27.0DEFG	32.5ABCDE
	F. rubra r.	Barisse	4.6A	6.6AB	10.4EFGHIJ	18.6ABCD	21.5EFG	26.5ABCDE
	F. rubra c.	Musica	6.4A	12.8AB	23.8BCDEF	18.7ABCD	41.7ABCDE	22.7ABCDE
	F. rubra l.	Samanta	8.4A	15.1A	31.2ABCDEF	18.7ABCD	50.7ABCDE	22.4ABCDE
	F. rubra l.	Barpearl	7.4A	11.5AB	16.6DEFGH	16.4ABCD	40.4ABCDE	20.8ABCDE
Yarrow	Control II		12A	17.8A	76.1A	28.6AB	82.6A	35.6ABC
	F. rubra r.	Barisse	6.2A	14.0A	31.9ABCDE	18.7ABCD	44.5ABCDE	26.1ABCDE
	Schedon. a.	Melyane	9.6A	12.9AB	26.3BCDEF	20.9ABC	34.7CDE	24.8ABCDE
	F. rubra c.	Musica	9.4A	15.5A	34.9ABCD	17.7ABCD	45.7ABCDE	23.1ABCDE
	F. rubra l.	Samanta	9.6A	14.1A	50.3ABC	14.9ABCD	56.7ABCD	19.6ABCDE
	F. brev.	Mentor	8.5A	8.5AB	34.2ABCD	9.4ABCD	51.6ABCD	17.3ABCDE
	F. rubra l.	Barpearl	7.4A	10.7AB	41.1ABCD	12.4ABCD	56.4ABCD	15.1BCDE
Mixture	Control II		5.6A	12.5AB	61.9AB	22.3ABC	78.4AB	32.7ABCD
	Schedon. a.	Melyane	3.4A	17.1A	13.5DEFGHI	23.2ABC	34.4CDE	30.1ABCDE
	F. rubra l.	Barpearl	6.0A	11.9AB	31.1ABCDEF	16.2ABCD	49.0ABCDE	25.8ABCDE
	F. rubra l.	Samanta	4.5A	13.9A	33.8ABCD	19ABCD	48.9ABCDE	25.3ABCDE
	F. brev.	Mentor	4.7A	14.9A	21.3CDEFG	20.9ABC	36.1BCDE	23.3ABCDE
	F. rubra c.	Musica	7.1A	10.4AB	19.7CDEFG	13.9ABCD	27.2DEFG	21.0ABCDE
	F. rubra r.	Barisse	5.4A	8.3AB	18.5CDEFG	11.5ABCD	37.7ABCDE	19.6ABCDE
Control	*F. rubra l.*	Barpearl	1.0A	3.6AB	6.3GHIJ	10.2ABCD	12.1FGH	16.1ABCDE
	Schedon. a.	Melyane	0.5A	7.2AB	2.0J	10.3ABCD	4.3H	15.5ABCDE
	F. rubra r.	Barisse	0.2A	5.2AB	1.5J	8.5BCD	2.8H	12.7DE
	F. rubra c.	Musica	0.2A	4.4AB	2.2J	7.0CD	5.5H	13.7CDE
	F. rubra l.	Samanta	0.5A	5.0AB	4.0HIJ	9.2ABCD	10.4GH	13.1DE
	F. brev.	Mentor	0.5A	0.5B	2.7IJ	4.7D	10.4GH	12.2E

[‡]Values in each column (i.e., separately for each year and DAS) followed by the same letter are not significantly different according to simulated adjustment (0.05).

4.3.3 Visual quality

Generally, visual quality of plots seeded with grasses and weeds was higher in 2019 compared to 2018. In 2019, ten grass x weed treatment combinations exhibited a visual quality of six or higher, whereas in 2018 only Musica in combination with the weed mixture reached

6.0 (Table 4.5). As expected, control plots (plots sown with grasses only) were among the plots with the highest average visual scores, except for Melyane in 2019. They all rated 6.0 or higher, which is considered an acceptable level of turfgrass quality. Only Mentor and Melyane reached 6.5 in 2018 and Barpearl, Musica, and Samanta without weeds rated 6.8 in 2019 (Table 4.5). However, visual quality of the grass-only plots did not exceed quality ratings of plots seeded with grasses plus weeds, and there was no clear trend indicating that grasses alone exhibited greater quality than grasses in combination with weeds.

Table 4.5. Visual quality of fine (*Festuca* L. spp.) and tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.) cultivars and in control plots (no grass) sown in 2018 and 2019, 84 days after seeding (DAS). Fescue grasses were seeded with either white clover (*Trifolium repens* L.), daisy (*Bellis perennis* L.), yarrow (*Achillea millefolium* L.), a mixture of all three weed species or without weeds (control). Treatments also included weed only (control II) plots.

Weed	Species	Variety	Quality	
			2018	2019
Clover	*F. rubra c.*	Musica	4.7ABCD‡	6.3AB
	F. rubra l.	Barpearl	5.7ABC	6.0AB
	F. rubra l.	Samanta	4.2BCDE	5.5AB
	F. rubra r.	Barisse	4.2BCDE	5.3ABC
	F. brev.	Mentor	4.5ABCDE	4.5BCD
	Schedon. a.	Melyane	4.2BCDE	3.0DE
	Control II		1.5F	1.0E
Daisy	*F. rubra c.*	Musica	5.0ABCD	6.3AB
	F. rubra r.	Barisse	5.2ABCD	5.5AB
	F. rubra l.	Barpearl	5.0ABCD	5.3ABC
	F. rubra l.	Samanta	4.5ABCDE	5.0ABCD
	F. brev.	Mentor	5.2ABCD	4.8ABCD
	Schedon. a.	Melyane	3.2DEF	3.3CD
	Control II		1.2F	1.0E
Mixture	*F. rubra c.*	Musica	6.0ABC	6.5AB
	F. rubra l.	Barpearl	5.0ABCD	6.5AB
	F. rubra l.	Samanta	4.7ABCD	6.0AB
	F. rubra r.	Barisse	5.0ABCD	5.8AB
	F. brev.	Mentor	5.2ABCD	5.3ABC
	Schedon. a.	Melyane	5.0ABCD	3.3CD
	Control II		1.2F	1.0E
Yarrow	*F. rubra l.*	Barpearl	4.7ABCD	6.8A
	F. rubra l.	Samanta	4.0CDE	6.7A
	F. rubra r.	Barisse	5.2ABCD	6.3AB
	F. rubra c.	Musica	4.5ABCDE	6.3AB
	F. brev.	Mentor	4.5ABCDE	5.3ABC
	Schedon. a.	Melyane	4.7ABCD	3.0DE
	Control II		2.5EF	1.0E
Control	*F. rubra c.*	Musica	6.5A	6.8A
	F. rubra l.	Barpearl	6.2AB	6.8A
	F. rubra l.	Samanta	6.0ABC	6.8A
	F. brev.	Mentor	6.5A	6.0AB
	F. rubra r.	Barisse	6.2AB	6.0AB
	Schedon. a.	Melyane	6.0ABC	3.3CD

‡Values in each column (separately for each year) followed by the same letter are not significantly different according to simulated adjustment (0.05).

4.3.4 Vegetation cover

Vegetation cover data were only used for correlation analyses and are not presented or discussed in detail. Vegetation cover at 84 DAS was generally higher in 2018, ranging from 65% to 97% compared to 20% to 90% in 2019 (Figure 4.1).

4.3.5 Correlations

All measured parameters were significantly correlated with one another, except for grass vigor with weed cover and visual quality in 2018 (Figure 4.1).

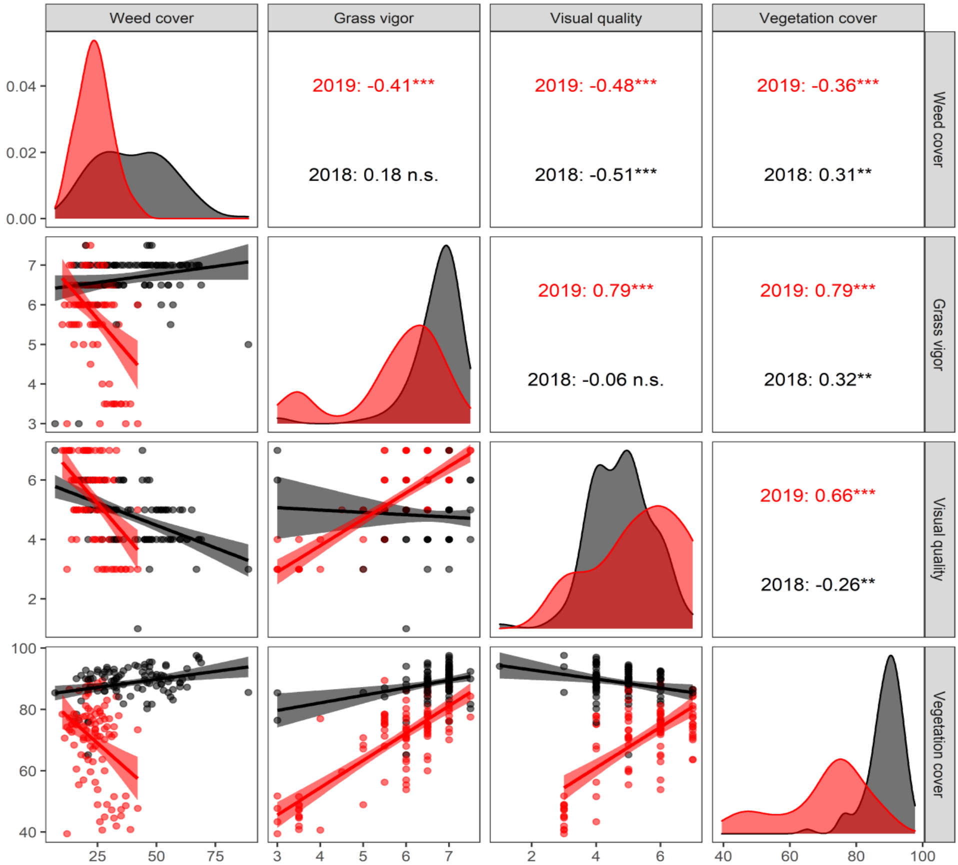

Figure 4.1. Pearson's coefficients of determination (r) (top right) between weed cover (measured by a 100-point quadrat, from 1-100%), grass vigor (visual score, from 0-9), visual quality (visual score, from 1-9), and vegetation cover (measured by digital image analysis using TurfAnalyzer software, from 1-100%). in 2018 (black) and 2019 (red), data were collected 84 days after seeding on plots sown with one of six grasses, one of four weed treatments and replicated four times. Line charts (bottom left) indicate linear regression line and data distribution between output variables. Graphs (diagonal) depict data density of variables. **,*** Significant at the 0.01, and 0.001 probability level, respectively. n.s Not significant at the 0.05 probability level.

When data for both years were compared, the correlation coefficient was highest between turfgrass vigor and vegetation cover (0.76) and lowest between turfgrass vigor and weed cover (0.21) (data not shown). Turfgrass vigor contributed strongly to vegetation cover (r= 0.76) but only moderately to visual quality (r= 0.36). An increase in weed cover resulted in a lower visual score, as indicated by the negative association (-0.48).

When correlations were examined separately for each year, visual quality was moderately negatively correlated with weed cover in both years, reaching -0.48 in 2018, and -0.51 in 2019 (Figure 4.1). Grass vigor was strongly positively correlated with vegetation cover in 2019 (0.79) but only weakly in 2018 (0.32). The relationship between visual quality and vegetation cover showed a moderate positive correlation in 2019 (0.66) and a weak negative correlation (-0.26) in 2018. A significant correlation between grass vigor and weed cover (-0.41) and grass vigor and visual quality (0.79) was only observed in 2019.

4.4 Discussion

The prime objective of our study was to assess the capacity of fine and tall fescues to interfere with growth of several common turf weeds. Our investigation revealed that percent weed cover was generally higher in 2018 than in 2019, which we attributed to higher overall temperatures in 2018. Maximum daily temperatures were higher in 2018 (25.3°C) compared to 2019 (18.4°C), most likely contributing to the more vigorous weed growth observed in 2018. Optimal temperatures for clover seed germination have been determined to range between 10.9°C and 17.2°C (Baxter et al., 2019). This might explain the difference in weed cover between the two years, as average daily temperatures were within this optimal range on 74 days in 2018 compared to only 57 days in 2019. Maximum germination rates (82%) for yarrow under frequent irrigation rates have been reported between 22°C to 29°C, and 25°C was reported to be an optimal temperature for germination of daisy (Robocker, 1977; Pêgo et al., 2012). Therefore, we conclude that regardless of weed treatment, germination of weed seed was favored in 2018 due to higher average temperatures, resulting in greater weed cover. However, based on our data we were not able to determine if any of the weed species tested were more or less susceptible to growth interference by fescue.

Fescue cultivars did not differ in their capabilities to interfere with growth of weeds during establishment. Our results do not support the few existing studies that suggest that both Chewings fescue (= Musica) and strong creeping red fescue (= Barisse) naturally interfere with growth of mature broadleaf weed species (Horgan et al., 2007; Bertin et al., 2009; Braun et al., 2020) in part because both species produce detectable amounts of the allelopathic non-protein amino acid m-tyrosine, which contributes to growth interference of other receiver plants.

To examine potential drivers of growth interference, we collected vigor data, based on the assumption that a more rapidly developing sward is likely to outcompete neighboring weed species (Busey, 2003). Shortly after germination (14 DAS), differences in vigor between cultivars and year were minor, except for the tall fescue cultivar Melyane, which showed higher vigor (greater than three) compared to other cultivars (vigor scores of two or lower for most cultivar x weed combinations) in 2019. However, the competitive advantages conferred by early vigor in Melyane were short lived, with several cultivar x weed combinations reaching similar vigor at 39 DAS in both years. Some authors have reported that Chewings- and strong creeping red fescue develop vigor more rapidly than hard fescue during establishment (Bertin et al., 2009; Braun et al., 2020), which we could not confirm based on our data. On the final day of the experiment in 2018, all cultivars established with the same degree of vigor, whereas in 2019 we observed treatment differences and Barpearl and Musica placed in the group with the highest vigor scores for all weed treatments. Tall fescue varieties are known to lose turfgrass cover under low mowing regimes (Beard, 1973; Moore and Christians, 1989), which we observed in both years after mowing was initiated. Tall fescue varieties are used for athletic fields in European transition zones (Pornaro et al., 2016), however we identified Melyane as an unsuitable cultivar for natural weed suppression in turf mowed at 15 mm or less twice a week in cool-season climates such as the Netherlands. We came to this conclusion based on visual quality data, not on weed cover data.

Even though visual quality in 2018 did not vary among cultivars within the different weeds, we observed that in 2019 Melyane performed poorly, with scores never exceeding 3.3. Most other cultivars scored greater than six in 2019, with Barpearl and Musica consistently scoring above six in all weeds. Nonetheless, upon examination of visual scores of fescues subjected to different weed treatments, we were unable to determine if any weed treatment resulted in particularly good or bad scores, as all weed control plots scored lower than the cultivar x weed plots.

In general, annual differences observed between data collected from both years of this study can be attributed to different weather conditions. In 2018, 19 days out of 84 reached a maximum temperature of greater than 30°C, whereas the next year only seven days of the experimental period reached temperatures above 30°C. Even though maximum daily temperatures exceeding 30°C were first recorded late into both years of the study (day 59 in 2018 and day 79 in 2019), the higher mean temperatures in 2018 probably promoted better grass establishment and consequently higher grass vigor. In 2018, irrigating regularly was necessary because of low precipitation rates in November, whereas in 2019 irrigation was only required in the first two weeks, as precipitation was sufficient to ensure good establishment.

Annual differences were also reflected in the correlation analysis. Across both years, we can state with confidence that visual quality decreased with an increase in weed cover. This was expected, as weed cover is a factor that influences visual quality (National Turfgrass Evaluation Program, 2020). We also found that grass vigor was significantly correlated with vegetation cover, strongly in 2019 and to a lesser degree in 2018. Vegetation cover describes the surface area covered by all green vegetation, with no distinction between weeds and grasses. The stronger association between vigor and cover in 2019 is the result of greater differences in the extent of establishment among grasses, reflected by data points distributed over a wider range of cover, from low to high. In 2018, all grasses established equally well, and data points were concentrated at the high cover area (Figure 4.1).

Visual quality was positively and significantly associated with vegetation cover and grass vigor in 2019, but not in 2018. Similar to the observed association between grass vigor and vegetation cover, differences in vigor among varieties were greater in 2019 because grasses established differently, which resulted in a wider spread of data points and a stronger association. Generally, we found that a more vigorous growing turf resulted in less weeds. However, our experiment was not designed to examine in detail the underlying mechanisms of growth interference.

4.5 Conclusion

Differences in mean temperatures and precipitation between the two years of the study resulted in differences in growth of grasses and weeds, as well as in the extent of weed interference of *Festuca* cultivars. Nonetheless, cultivars Musica (Chewings fescue) and Barpearl (slender creeping red fescue) were the least affected by weed growth during both years resulting in acceptable aesthetic quality of these turf stands after establishment. Both grasses appear to be interesting candidates for further investigations into the mechanisms responsible for growth interference of broadleaf weeds. The three weed species selected for this study were all similarly affected by the grasses used in the study. Tall fescue Melyane appeared to be an unsuitable turfgrass for areas destined for maintenance without herbicides, because of the low visual quality and aesthetic appearance after mowing at a height of 15 mm or less. Further research needs to be conducted to better understand the mechanisms of growth interference. Plant and root morphology, as well as allelopathic exudates are all traits that contribute to competition mechanisms, but extent and efficacy of these mechanisms not just in establishing but also in mature turf needs further investigation.

4.6 Acknowledgements

We are grateful for the contribution of Barenbrug Holland B.V. in Nijmegen, Netherlands. Dr Rossana Sallenave's help with the manuscript is also greatly appreciated. Financial support of this report was provided by the Turfgrass University Research Foundation (TuRF), Nederlandse Golf Federatie (NGF), Nederlands Vereniging van Golfaccommodaties (NVG), and Nederlands Greenkeepers Associatie (NGA). The authors are also grateful for the constructive and insightful comments provided by the anonymous reviewers; they greatly improved the manuscript.

CHAPTER 5

Detection and quantification of broadleaf weeds in turfgrass using close-range multispectral imagery with pixel- and object-based classification

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This chapter has been published as Hahn *et al*., 2021. International Journal of Remote Sensing 42(21): 8035-8055.

Abstract

The current practice used to evaluate broadleaf weed cover in turfgrass is visual assessment, which is time consuming and often leads to inconsistencies among evaluators. In this study, we investigated the effectiveness of constructing Random Forest models (RF), either pixel-, object-based (OBIA) or a combination of both to detect and quantify broadleaf weed cover. High resolution multispectral images were captured of 136 turfgrass plots, seeded with five species of *Festuca* L. and overseeded with either clover (*Trifolium repens* L.), daisy (*Bellis perennis* L.), yarrow (*Achillea millefolium* L.), or a mixture of all three weeds. Ground measurements of vegetation cover and bare soil were taken with a point quadrat and digital image analysis. Weeds were detected with 99% accuracy by OBIA, followed by the combined approach (98%) and Pixel-based approach (93%). Accuracy at distinguishing among weed species was somewhat lower (89%, 81% and 90%, respectively), with yarrow contributing most to the decrease in accuracy. The predictions based on ground measurements were further compared to field measurements. For both soil and weed classification, models that used shape features (OBIA and combined) resulted in better agreement with field measurements compared to Pixel- based classifications. Our study suggests that broadleaf weed cover comprised of species such as clover and daisy can be accurately quantified with high resolution multispectral images; however, quantifying yarrow cover remains challenging.

Keywords: random forest classification, weed detection, point quadrat, digital multispectral imagery.

5.1 Introduction

The presence of weeds disrupts the playing quality and aesthetic appearance of turfgrass areas (Larsen et al., 2004; McCarthy and Murphy, 1994; McElroy and Martins, 2013). Since the development of selective herbicides such as 2,4-D (2,4-dichlorophenoxyacetic acid) (Marth and Mitchell, 1944), herbicides have become the main tool used by managers to control weeds in turfgrass (Dahl Jensen et al., 2017; Hatcher and Froud-Williams, 2017; Heap, 2014; McElroy et al., 2013).

The European Union actively promotes the use of alternative non-chemical products or techniques to control weeds (European Parliament, 2009) because of potential health risks associated with exposure to herbicides (Kim, Kabir, and Jahan, 2017), environmental concerns (Aktar, Sengupta, and Chowdhury, 2009) and the increasing risk of herbicide resistance due to overuse (De Prado and Franco, 2004). Turfgrass managers are encouraged to adopt integrated pest management (IPM) approaches to reduce the input of herbicides (Busey, 2003). However, a lack of established weed treatment thresholds, and the absence of time efficient, low-cost alternative control methods limit the ability of turfgrass managers to follow clear IPM protocols (Latimer et al., 1996). Management practices such as increasing mowing heights and nitrogen fertilization enhance the competitiveness of turfgrass against weeds (Voight, Fermanian, and Haley, 2001), but full weed suppression generally requires the use of herbicides (Busey, 2003).

Remote sensing tools used in precision agriculture could be designed to detect and treat localized high weed densities in turfgrass, thereby reducing the overall herbicide loads required to control weeds (Zhang and Kovacs, 2012). In addition to reducing herbicide use on turfgrass areas, automated weed detection systems could also help turfgrass breeders accurately assess the competitiveness of grasses against weeds. Currently, turfgrass breeders typically use visual scores to assess turfgrass quality or weed cover (Bunderson et al., 2009; Kaur et al., 2016; National Turfgrass Evaluation Program, 2020). However, visual scoring is subjective, time consuming, and can be inconsistent over time and amongst evaluators. As a result, the reproducibility of such data has been questioned (Horst et al., 1984; Leinauer et al., 2014; Trenholm et al., 1999). Regardless, in the absence of high throughput alternatives, breeders and turfgrass scientists still rely on visual assessments to quantify weed cover and turfgrass quality.

Digital image analysis has been adopted by some turfgrass scientists to quantify vegetation cover and turfgrass quality. High values obtained from dark green color index

(DGCI) analysis correlate strongly to high chlorophyll content and genetically desirable dark green color of cultivars (Karcher and Richardson, 2013). This technology can readily distinguish vegetation from soil but cannot discriminate between desirable plants and weeds.

In situ strategies to objectively separate vegetation cover includes the use of point-based reflectance data collected by hand-held spectroradiometers. This method has been successfully used to distinguish two grassy weed species, dallisgrass (*Paspalum dilatatum* Poir.) and southern crabgrass (*Digitaria ciliaris* (Retz.) Koeler), and two broadleaf weed species, namely virginia buttonweed (*Diodia virginiana* L.) and eclipta (*Eclipta prostrata* (L.) L.), from a variety of warm and cool season turfgrasses (Hutto et al., 2006). However, this procedure is labor intensive and requires expensive equipment to collect the hyperspectral data. An additional disadvantage is that spectroradiometry measurements are point observations, which cannot be used to map spatial distribution of weeds. Remotely sensed data may offer a solution to these limitations by providing an empirical, cost-effective and reliable source of data that could detect and distinguish weeds from turfgrass, and map their spatial distribution. In recent years, the development and application of novel algorithms to analyze remotely-sensed imagery in combination with increased computational power and ease of data acquisition via unmanned aerial vehicles, has led to considerable advances in the use of remote sensing techniques (Ma et al., 2015; Gómez, White, and Wulder, 2016; Mulla, 2013). Remote sensing is used in a large variety of applications at spatial scales ranging from individual plants to fields. Examples include the estimation of plant-specific parameters such as leaf area index, chlorophyll content, and canopy cover (Roosjen et al., 2018; Yang et al., 2017), and the assessment of characteristics such as ground cover, vegetation type, and drought stress, to name a few (Gómez, White, and Wulder 2016; Nijp et al., 2019; Olmstead et al., 2004).

Using remotely sensed imagery followed by color modelling, Tang et al. (2016) were able to identify weed-covered areas in crop rows of agricultural fields with 92% overall accuracy (OA). While we acknowledge that OA provides a limited perspective on classification accuracy, it can be used for approximate comparisons (Alberg et al., 2004). Huang et al. (2018) demonstrated how remote sensing tools could provide a time and labor-saving alternative to ground collected spectral reflectance data or digital image analysis for the assessment of weed density in agricultural settings. In agricultural studies, hyperspectral radiometry and multispectral aerial imagery have been successfully used to quantify johnsongrass (S*orghum halepense* (L.) Pers.), which is also a problematic turfgrass weed (Thorp and Tian, 2004). Yu et al. (2020) tested the performance of deep convolutional neural networks to detect a variety of grassy weeds, including crabgrass (*Digitaria* spp.), in a bermudagrass sward, yielding high overall precision (>93% of grassy weed species). However, detection performance was dependent on the algorithm used and decreased considerably with reduced abundances of weeds likely caused by pixel mixing resulting in classification error (Yu et al., 2020; Hsieh, Lee, and Chen, 2001).

Other approaches used to separate and map different types of vegetation include utilizing contextual information derived from object-based image analysis (OBIA), which may improve the accuracy of discerning among vegetation classes of interest beyond pixel-based reflectance patterns (Blaschke et al., 2014). One example was the detection of bermudagrass (*Cynodon dactylon* (L.) Pers.) in vineyards using multispectral aerial images and OBIA (Jiménez-Brenes et al., 2019). Given that spectral characteristics of weeds and grasses differ very little whereas their shapes and texture can vary greatly (Weis et al., 2009), OBIA shows potential for accurate detection of broadleaf weeds in turfgrass. One challenge of detecting broadleaf weeds in turfgrass is the small size of some species, depending on their growth stage.

Currently, no reliable, affordable methods based on digital imagery exist to detect, quantify and map broadleaf weed cover in turfgrass areas with a high degree of spatial resolution. Research is needed to assess the usefulness of OBIA or pixel-based classification in detecting and quantifying broadleaf weeds in closely mowed turfgrass settings wherein differences in shape and spectral characteristics among species are minute. The objective of our study was to investigate the effectiveness of OBIA- and pixel- based classification derived from multispectral imagery at distinguishing among broadleaf weeds, grasses and soil. To that end, we compared the performance of OBIA and pixel-based classification methods using high resolution imagery collected in a controlled field experiment that included five species of *Festuca* and three common European broadleaf weed species.

5.2 Materials and Methods

5.2.1 Field trial

To explore the potential of using remote sensing techniques to distinguish and map percentage cover of weeds, grass and soil, we collected remotely sensed imagery data from a field experiment conducted at the Barenbrug Turfgrass Research Station in Wolfheze, The Netherlands (52°00’N, 5°47'E). The soil consisted of 79% sand, 12% silt and 3% clay, 6.4%

organic matter and had a pH of five in the upper 15 cm of the soil profile. The study area was located within the controlled traffic region (CTR) of Deelen Airport in Arnhem, The Netherlands (52°03'N, 5°52'E). The experiment was initiated to investigate the competitiveness of fescue cultivars (sown on July 13, 2018), against three common turf weeds that were sown on July 27, 2018.

Treatments included six *Festuca* cultivars, namely Chewings fescue [*Festuca rubra* L. spp. *fallax* (Thuill.) Nyman 'Musica'], hard fescue (*Festuca brevipila* Tracey 'Mentor'), slender creeping red fescue [*Festuca rubra* L. ssp. *littoralis* (G. Mey.) Auquier 'Samanta'], strong creeping red fescue (*Festuca rubra* L. *rubra* 'Barpearl' and 'Barisse'), and tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons. 'Melyane']. With the exception of grass controls, all plots sown with grass were oversown with weed treatments including either clover, daisy, yarrow or a mixture of all three weed species. The experiment included six cultivars oversown with weed seeds, six grass controls (grass cultivars only) and four weed controls (weed seeds only). All treatments were replicated four times. The individual plots measured 1.5 x 1.5 m and were arranged in a randomized complete block design.

Fescue cultivars were sown at a density of 20,150 seeds per m^2, following guidelines by Beard (1973) and weeds were sown at a density of 6,200 seeds per m^2. Granular fertilizer (NPK 12-10-18, Arm, Eurosolids, Westmaas, The Netherlands) was applied to the plots 28, 42, 56 and 72 days after sowing (DAS) the grass seeds at a rate of 200 kg ha^{-1}. The field was rolled 21 DAS and mowed for the first time at 20 mm. From 28 DAS onwards, the field was mowed twice per week with a Jacobson TR3 reel mower (TR3, Jacobson, Racine, United States) at a cutting height of 15 mm, with clippings returned. We achieved uniform establishment of grasses and weeds 84 DAS. Before data collection dew and clippings were removed with a hand blower.

5.2.2 Data collection

Percent weed and vegetation cover of 136 experimental plots was measured 84 DAS. To determine the percentage of each plot covered by weeds (i.e. weed cover), a point quadrat frame was constructed with wires spaced 10 cm apart creating a mesh with 100 intersections (Laycock 1980). The frame was placed in the middle of each plot and presence or absence of weeds underneath each intersection was recorded on October 3, 2018. Similar methods were used by Gaussoin and Branham (1989) and Proctor et al. (2015). On the same day, total vegetation cover (i.e. weeds and grasses) of each plot was determined by photographing them using a lightbox. The custom-made lightbox was a 60 x 50 x 50 cm metal box with a hole on the top large enough to insert a digital camera. Four lamps (5000 k color and 450 lumen) were arranged inside the lightbox to produce consistent lighting conditions during image capturing, similar to methods used by (Karcher and Richardson, 2013).

Images were taken with a digital camera (Canon Power shot SX 200 IS, Canon, Tokyo, Japan). Manual settings used were ISO 200, Aperture 2.6 and shutter speed 1/60 s, which provided the highest image quality in combination with the lightbox. The images were processed using the software Turf Analyzer (Turf Analyzer, 2018), which applies a green pixel recognition algorithm to calculate the percentage of green vegetation in images. To determine non-vegetation cover (i.e. *bare soil*) for each plot, we subtracted the green pixels computed by Turf Analyzer from the total pixels.

Weed cover measurements obtained with the point quadrat method and bare soil measurements obtained from the lightbox images and the Turf Analyzer methods are referred to as 'observed data'. The observed data can be considered the quantitative industry standard, which are recorded on the ground. The following sections describe a new proposed methodology, which will be referred to as 'predicted data' using an airborne camera system to capture multispectral images and analysis by random forest models (RF classification) to determine weed cover and percentage bare soil.

Multispectral images of the field experiment were collected with a Parrot Sequoia+ camera (Parrot Sequoia+, Parrot, Paris, France). The camera collected images of 1280×960 pixels at four spectral bands: green (550 nm), red (660 nm), red edge (735 nm), and near infrared (NIR, 790 nm). In the field, prior to the image collection, calibration images were taken of a grey reference panel (Parrot Sequoia Calibration Target, Parrot, Paris, France) with known reflectance values (green: 18.4%, red: 19.7%, red edge: 22.7%, NIR: 27.6%) at the same spectral bands as the camera. To accurately geo-reference the images, 30 ground control points (GCPs) were placed in the field and their GPS coordinates measured with a Real Time Kinematic unit (HiPerV, Topcon, Tokyo, Japan), which has a horizontal and vertical accuracy of 5 mm + 0.5 ppm and 10 mm + 0.8 ppm, respectively.

The sequoia camera is designed to be used with an unmanned aerial vehicle (UAV). Because our study site was located in CTR of Deelen Airport, permission to fly a UAV was not obtained at the time of image acquisition. Therefore, the camera was dismounted from a set-up with a UAV and attached to a three-meter pole with the downwelling light sensor pointing upward to the sky and the camera towards the ground. Both sensor and camera were undisturbed by shade and were positioned parallel to the ground simulating the original UAV set-up. The pole itself was attached to a platform four-wheeled vehicle which was manually pushed across the research area to take images from 2.5 m above ground level (AGL), resulting in a ground sample distance (GSD) of 2.8 mm/pixel. Images were collected every two seconds with a forward and sideward overlap of approximately 95% and 80%, respectively, resulting in a uniform coverage of the study area. The images were taken under partially clouded conditions on October 3, at 10 am (CEST). A total of 807 images were collected from the experimental area. The internal GPS of the camera stored the coordinates from which each image was taken. After data collection, we began image processing following the workflow outlined in Figure 5.1, which will be explained in more detail in the following sections.

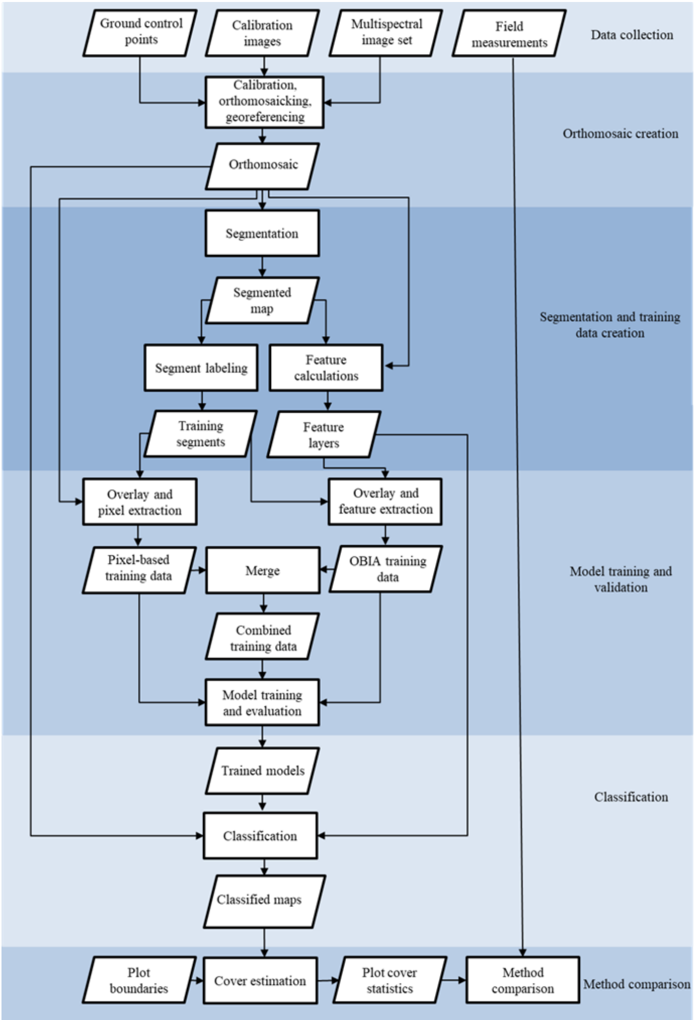

Figure 5.1 Project workflow from constructing object-based image analysis (OBIA), pixel based and combined classifications to classify vegetation cover. Rectangles indicate processes and parallelograms show products. The figure lists all steps from data collection (field measurements and multispectral image collection) to construction of the models and final comparisons between model data and ground measurements.

5.2.3 Orthomosaic creation

The multispectral images obtained were used to create an orthomosaic, using AgiSoft Metashape version 1.5.2 (AgiSoft LLC, St. Petersburg, Russia). An orthomosaic is a large image that is created by combining many georeferenced small images (Brown, 1992; Laganiere, 2000). First, pixel values of all four bands were calibrated using the measurement of the grey reference panel. The images were subsequently aligned at the *highest* accuracy setting using

GPS data of the images and the GCPs. A dense point cloud was built at *medium* quality-setting without depth filtering. From this, an orthomosaic containing the four spectral bands was constructed with a ground pixel size of 3 mm.

5.2.4 Orthomosaic segmentation

The orthomosaic image was segmented in GRASS GIS 7.4.4 (GRASS Development Team, 2017). Image segmentation groups adjacent pixels that are similar into segments, which are referred to as objects. The segmentation algorithm was driven by two parameters: 1) the minimal segment size, which is the minimum number of pixels that each segment can comprise and 2) a similarity parameter, which describes how similar pixels should be before they are assigned to a segment. For the segmentation process of the orthomosaic, we used a minimum segment size of 10 pixels and a similarity threshold of 0.025, respectively. After testing several combinations of these parameters, these values gave the best segmentation results. After segmentation of the orthomosaic, 1022 segments were manually labelled as grass (referred to as '*grass*'), no vegetation cover (referred to as '*soil*') or weed species (referred to as either '*clover*', or '*daisy*', or '*yarrow*') (Table 5.1).

Table 5.1. Number of annotated segments and pixels within each segment, separated by vegetation class. Segments were selected from an orthoimage of a field trial. Data from annotated segments were extracted to create the training data for vegetation classification.

Class	Segments	Pixels
Clover	295	6964
Daisy	196	4968
Grass	167	502572
Soil	227	39746
Yarrow	137	3975

5.2.5 Random forest models to classify vegetation

For our study we used RF classifications to sort vegetation, which are frequently used to categorize remotely sensed imagery, and other than the traditional Maximum Likelihood classification, do not rely on data distribution assumptions (Brodley and Friedl, 1997; Nitze, Schulthess, and Asche, 2012). Random forest models tend to classify weeds better than alternatives and have been used to successfully detect weeds, such as Chamomile (*Chamaemelum nobile* L.) and Thistle (*Cirsium arvense* L.), in aerial images of agricultural fields sown with oats (Gašparović et al. 2020). For RF classification, training data are randomly selected, followed by a decision tree procedure to make predictions (Belgiu and Drăgu, 2016; Breiman, 2001). Our RF classifications were implemented in the R package 'ranger' (Wright and Ziegler, 2017).

Training data for RF classifications were constructed by extracting shape, texture, and spectral features from the labelled segments within the orthomosaic (Table 5.2). The training data were used to construct three types of RF classifications to classify vegetation including (1) a pixel-based classification (referred to as 'Pixel classification'), (2) an 'OBIA' classification and (3) a combination of OBIA and Pixel-based classifications (referred to as 'combined classification'). We use 'RF classifications' throughout the manuscript as a hypernym for the Pixel-, OBIA- and combined classifications.

Table 5.2. Parameters and features used to construct the object based (OBIA), Pixel based, and combined classifaction. Training data for the RF (random forest) classifications were generated from labelled segments of an orthoimage of a field trial.

Parameter	Feature	Description
Shape	Area	Area of each segment
	Compactness	Compactness of each segment
	Fractal dimension	Statistical index, that provides a ratio how segment boundaries change with scale (Mandelbrot, 1982)
	Length	Length of each segment
Texture	Max	Maximum pixel value within each segment per band
	Mean of entropy	Mean entropy of pixels within each segment per band (Haralick, Dinstein, and Shanmugam, 1973)
	Mean	Mean pixel value within each segment per band
	Mean SV[a]	Mean sum of variance of pixels within each segment per band (Haralick, Dinstein, and Shanmugam, 1973)
	Min	Minimum pixel value of pixels within each segment per band
	SD[b] of entropy	Standard deviation of entropy within each segment per band (Haralick, Dinstein, and Shanmugam, 1973)
	SD	Standard deviation of pixels value within each segment per band
	SD of the sum of variance	Standard deviation of sum of variance within each segment per band (Haralick, Dinstein, and Shanmugam, 1973)
Spectral features	green, red, red-edge, NIR	All individual pixel values within each segment per band

For the Pixel-based classifications, pixel values of four spectral bands (i.e. green, red, red-edge and NIR of all pixels) within each segment were extracted. Neither textural nor shape features were used. For the object-based classifications, shape and texture features were calculated within each segment. This resulted in a total of 36 features (eight texture features x four bands + four shape features) for each segment. In the object-based classifications, average spectral characteristics were calculated for each segment. Lastly, the combined classifications were developed using the same 36 shape and textural features as for the OBIA classifications and the spectral information of the four bands of each pixel (following the Pixel classifications) within each segment. Therefore, in the combined classifications, all pixels within a segment had identical object features, but had different spectral characteristics.

5.2.6 Random forest model classification training and validation

For each of the three RF classification methods, we developed two further models (referred to as either '*5-class model'* or '*3-class model'*). The *5-class models* were used to categorize the percentage of the area covered by *clover, daisy, grass, soil* and *yarrow*. For the *3-class models* we used the sum of *clover*, *daisy*, and *yarrow* to create a simplified '*weed*'-class.

To quantify the balanced predictive accuracy of each trained RF (i.e. the accuracy is calculated independently for each class as the fraction of cases correctly classified, and these individual accuracy values are then averaged across all classes), a repeated five-fold cross validation scheme was used for a total of 15 evaluations for each type of classification. Five-fold cross validation requires that for each evaluation, the dataset is randomly split into five subsets (Hastie, Tibshirani, and Friedman, 2009). Four of the data subsets (i.e. 80% of the data) are used to train the algorithm and the remaining subset (20% of the data) is used to test predictions by the RF classifications. In the case of the combined classifications, all pixels that belonged to the same segment were sampled as indivisible units to avoid having pixels from the same segment in training and testing datasets (which would violate the principle of independence of the testing dataset, since all pixels within a segment share the same segment information). In all cases, the random sampling was stratified across classes to ensure the same relative proportions of classes as in the total dataset (i.e. the proportions of each class were maintained in each sample as in the total dataset) (Hastie, Tibshirani, and Friedman, 2009). As the number of annotations per class differed (Table 5.1), each class was weighted during training by the inverse of the total number of annotations in that class, to avoid the negative effects of class imbalance. Confusion matrices were constructed to show the producer-, user-, overall accuracy (OA) of the RF classification predictions (Stehman 1997). Additionally, average accuracy (AA) and the kappa coefficient (K) were calculated.

5.2.7 Comparison of field- and random forest model classifications

Plot statistics were obtained by first using the trained RF classifications to categorize each segment of the whole orthomosaic. We then drew a polygon around each experimental plot, labeled the plot number and extracted data for each plot. Total weed cover and bare soil quantified by observed field measurements (i.e. point quadrat method for *weed, clover, daisy* and *yarrow* estimations and lightbox/ Turf Analyzer method for *soil* estimation), were compared with values obtained using the predictions from all RF classifications (i.e. OBIA, Pixel and combined classifications for each *3-class model* and *5-class model*).

In order to evaluate the accuracy of the predictions for weed cover and bare soil we numerically evaluated the agreement between the observed and predicted values. Every scatterplot between the observed and predicted values suggested an exponential relationship. Thus, a logarithmic transformation was used to linearize the trend; as a result, a simple linear regression model was utilized to evaluate the log-transformed variables agreement. The AIC (Akaike Information Criterion) and MAE (Mean Absolute Error) were computed as metrics to summarize model fit and used to determine the best model to explain agreement between observed and predicted data (Akaike, 1974; Willmott and Matsuura, 2005).

5.3 Results

5.3.1 Data extraction per experimental unit

For each of the 136 experimental plots, we computed vegetation cover plot statistics based on the RF classification predictions following the example shown in Figure 5.2. The plot shown in Figure 5.2 was sown with Chewings fescue ('Musica') and clover. We calculated the surface area covered by each vegetation class.

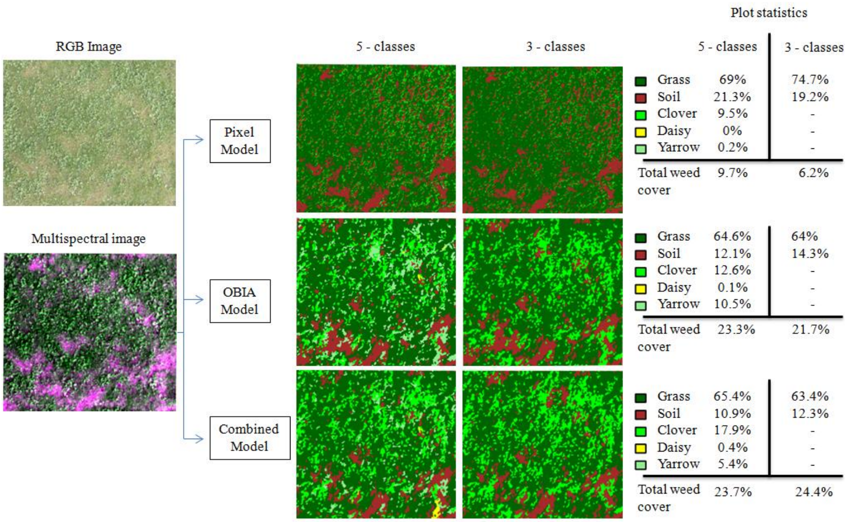

Figure 5.2. Visualization of an object- based- (OBIA), pixel- based and combined RF (random forest) classification approach to classify vegetation cover of field plots consisting of *Festuca* cultivars and broadleaf turfgrass weeds. Figures show classification differences between either grass, soil, and weed (*3-class model*) or between clover, daisy, yarrow, grass, and soil (*5-class model*) and multispectral aerial images from the vegetation cover. Images were derived from one experimental plot established with Chewings fescue ('Musica') and clover.

5.3.2 Pixel classification accuracy

The Pixel classification using the *3-class model* resulted in the lowest accuracies of any of the *3-class model* RF classifications, but was still able to correctly categorize grass, soil and weed cover with 90 to 95% OA (Table 5.3).

Table 5.3. Accuracy assessment of object-based models (OBIA), Pixel and combined RF (random forest) classifications to separate the percentage of area covered by different vegetation classes of a field trial. Results are grouped by two subset models with *3-class model* (grass, soil, and weed) and *5-class model* (clover, daisy, grass, soil, and yarrow) for each model.

		Percentages (%)		
		OBIA	Pixel	combined
	Grass	98 (± 2)	93 (± 0)	98 (± 3)
3-class model	Soil	99 (± 1)	95 (± 0)	98 (± 3)
	Weed	99 (± 1)	90 (± 1)	98 (± 3)
	Clover	82 (± 2)	77 (± 1)	92 (± 2)
	Daisy	88 (± 3)	77 (± 1)	98 (± 2)
5-class model	Grass	99 (± 1)	93 (± 0)	97 (± 3)
	Soil	100 (± 1)	96 (± 0)	86 (± 6)
	Yarrow	77 (± 5)	60 (± 1)	78 (± 6)

The worst performing model (K= 83 and AA= 81%), based on 15 validation iterations, highlighted the difficulty in correctly classifying the *weed* class due to frequent confusion with *grass* (i.e. 19.7%, Table 5.4).

Table 5.4. Confusion matrix of *3-class models* (grass, soil, and weed), from: object-based image analysis (OBIA) classification, Pixel based classification (Pixel) and a combination of both classifications (combined). Training data for the model was obtained from a segmented orthoimage of a field trial to investigate the competitiveness of *Festuca* cultivars with broadleaf turfgrass weeds.

		Grass	Soil	Weed	Sum	User accuracy
OBIA	Grass	33	0	0	33	100%
	Soil	0	45	0	45	100%
	Weed	1	0	125	126	99.2%
	Sum	34	45	125	204	
	Producer accuracy	97.1%	100%	100%		
	Overall accuracy					99.4%
	Average accuracy					45.4%
	Kappa x 100					99
Pixel	Grass	98331	1270	914	100515	97.8%
	Soil	696	7248	6	7950	91.2%
	Weed	628	9	2544	3181	80%
	Sum	99655	8527	3464	111646	
	Producer accuracy	98.7%	85%	73.4%		
	Overall accuracy					87.7%
	Average accuracy					81.0%
	Kappa x 100					83
Combined	Grass	56865	0	24	56889	100%
	Soil	1408	11332	0	12740	88.9%
	Weed	0	0	3265	3265	100%
	Sum	58273	11332	3289	72894	
	Producer accuracy	97.6%	100%	99.3%	97.6%	
	Overall accuracy					97.6%
	Average accuracy					65.3%
	Kappa x 100					94

We present outcomes of the worst performing model in a confusion matrix to highlight where misclassification occurred; this information would not be evident by simply presenting average classification accuracies of all validation iterations.

The *5-class model* highlighted that *yarrow* was particularly difficult to detect, with a low accuracy of 60% (Table 5.3). The confusion matrix with the worst performing model of the Pixel classification (K= 77 and AA= 80.5%) using the *5-class model* highlighted that the misclassification of plants belonging in the *weed* class was primarily attributed to the difficulties in classifying *yarrow*. *Yarrow* was more frequently misclassified as *clover* or *grass* than it was correctly categorized, resulting in low producer and user accuracies of 19.2% and 18.4%, respectively. Additionally, *daisy* was misclassified as *clover* 27.5% of the time and *clover* was misclassified as *grass* 19.4% of the time (Table B1).

5.3.3 OBIA classification accuracy

The accuracy assessment of the OBIA classification using the *3-class model* after 15 evaluation runs resulted in an average accuracy of 98% for *grass*, 99% for *soil*, and 99% for *weed* (Table 5.3). The confusion matrix with the lowest OA (K= 99 and AA= 45.5%) of the 15 evaluations, showed that *weed* was confused with *grass* on one occasion, resulting in an OA of 99.4% (Table 5.4).

Further separating the *weed* class into species (*clover*, *daisy*, and *yarrow*) by the *5-class model* decreased the OA of detecting weeds to 82.3% (± 3.3) (Table 5.3), due to the difficulty in distinguishing among weeds. The confusion matrix of the evaluation with the lowest OA (K= 84 and AA= 21.5%) showed that yarrow was the most difficult weed to classify. *Yarrow* was frequently (33.3%) misclassified as *clover* (Table B1). Nevertheless, using the *5-class model* resulted in similar accuracy of classification of the *soil* and *grass* classes.

5.3.4 Combined classification accuracy

The combined classification with the *3-class model* achieved an OA of 98% (Table 5.3). W*eed* and *grass* were detected with 100% user accuracy, while *grass* was confused with *soil* 11.6% of the time in the worst performing confusion matrix (K= 94 and AA= 65.3%) (Table 5.4).

Separating the *weed* class into *clover*, *daisy* and *yarrow* resulted in a drop of accuracy, with *yarrow* contributing most to any misclassification. Overall classification accuracy for *yarrow* was 78%, and *daisy* was correctly classified with 98% accuracy (Table 5.3). In the worst performing confusion matrix, *yarrow* was mainly misclassified as *clover* by 32.2% of the time. Furthermore, *daisy* was frequently misclassified as *yarrow* 35.6% of the time (Table B1).

5.3.5 Feature importance of random forest model classifications

Pixel values for each vegetation type indicated that *soil* in particular showed lower values in the NIR and red-edge spectra (Figure B1). Within all bands, *clover* had similar pixel values as *daisy*, with overall differences most prominent in the red band. *Grass* showed unique pixel values in the NIR and red-edge band, being lower than any of the *weeds* and higher than soil. In the red band, *grass* showed the lowest mean pixel values of all classes. Pixel values of *yarrow* were similar to those of *daisy* and *clover* in particular in all bands.

For the Pixel classification using the *3-class model*, the green band was most important to detect features closely followed by the red band with 17% less relative importance (data not shown). Red edge and NIR were the least important features with 72.9% and 77% less relative importance compared to the green band. For the *5-class model*, Pixel classification differences were more prominent with the red band being most important closely followed by the green band (2% less relative importance) and 86% for red edge and NIR (data not shown).

For both the *3-class* and *5-class models* OBIA classifications, mean green pixel values for each segment contributed most to the classification of vegetation type (Figure 5.3 & Figure 5.4). In general, the mean pixel values of each band were among the seven most important features for both models. Furthermore, the sum of variance (SV) of pixels values proved to be an important feature, particularly in the green band of the *5-class model*. Also, the maximum NIR feature was an important feature to classify vegetation in both modes. The OBIA-specific shape parameters scored low in importance, and for the *5-class model*, all of the shape parameters were the least important features.

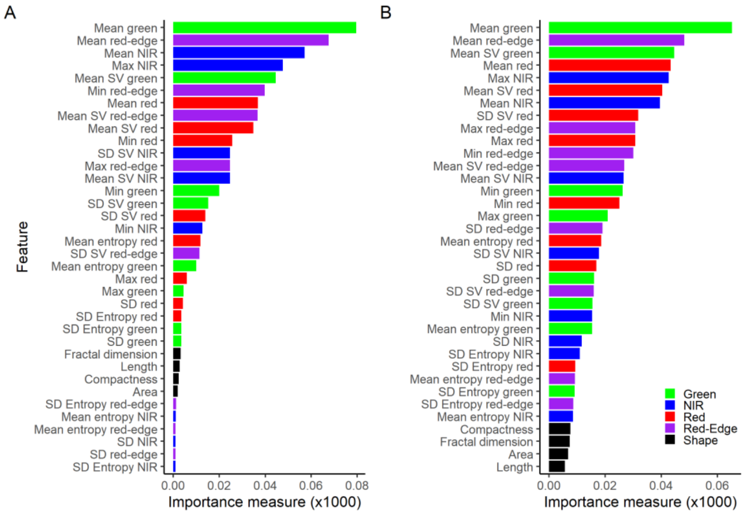

Figure 5.3. Feature importance charts of (A) a *3-class model* (grass, soil, and weed), from object-based image analysis (OBIA) classification and (B) from *5-class model* (clover, daisy, grass, soil, and yarrow) OBIA classification to quantify vegetation cover in a *Festuca* cultivars and broadleaf weeds field trial. Importance measure is a dimensionless/ relative measurement. Colors indicate the spectral bands green, blue (NIR), red, red-edge (purple) and object- based shape parameters (black). NIR, near infrared; SV, Sum of variance; SD, Standard deviation.

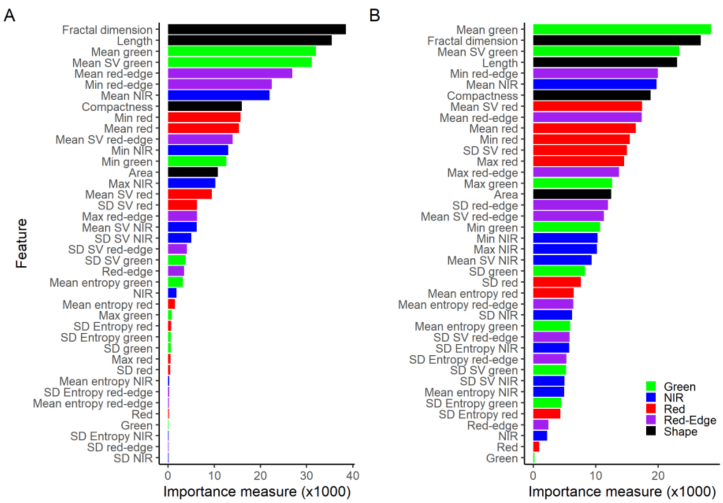

Figure 5.4. Feature importance charts of (A) a *3-class model* (grass, soil, and weed), from combined- image analysis classification (OBIA shape parameters and spectral features of Pixels) and (B) *5-class model* (clover, daisy, grass, soil, and yarrow) from combined- image analysis classification to quantify vegetation cover in a *Festuca* cultivars and broadleaf weeds field trial. Importance measure is a unit less/ relative measurement. Colors indicate the spectral bands green, blue (NIR), red, red-edge (purple) and object- based shape parameters (black). NIR, near infrared; SV, Sum of variance; SD, Standard deviation.

For the *3-class model* combined classification, shape parameters such as fractal dimension and length proved to be the most important features (Figure 5.4). This was followed by the mean pixel values for green, SV for green and red edge. Eight features were of no relative importance for classifying vegetation. For the *5-class model* combined classification, there was less of a difference in importance among the features, with only one feature (the green band) not contributing to the classification of vegetation.

5.3.6 Comparison of observed and predicted data

The least agreement between observed and predicted data (high AIC and MAE) used to estimate weed cover were reported in plots sown with grass only (Grass controls) for all RF classifications (Table 5.5). In plots sown with weed treatments, both OBIA and the combined classifications showed better agreement with observed data compared to the Pixel classifications for both *3-class* and *5-class models*. Differences between OBIA and combined classifications were marginal except for the *3-class model*s' detection of weed cover in mixtures. In mixtures, OBIA classification using the *3-class model* performed worse (AIC= 49.4) than the *5-class model* (AIC= 28.10). The MAE for OBIA and the combined classifications also showed that both classification methods performed equally in detecting weed cover, with no clear difference between *3-class* and *5-class models*.

Table 5.5. Comparison of remotely sensed total weed cover estimates with field observations using Akaike information criterion (AIC) and Mean absolute error (MAE) on log-transformed values. Methods to estimate weed cover included ground measurements with a 100-point quadrat and analysis of aerial multispectral images using object-based image classifications (OBIA), Pixel based classifications (Pixel) and a combination of both classifications (combined), for *3-class model* (grass, soil, and weed) and *5-class model* (clover, daisy, grass, soil, and yarrow).

		3-class model			*5-class model*		
	Sowed treatment	Pixel	OBIA	Combined	Pixel	OBIA	Combined
AIC*	Clover	42.7	27.5	26.2	42.4	27.5	26.6
	Daisy	48.6	34.1	34.7	47.5	34.6	34.1
	Yarrow	42.8	37.6	37.1	42.1	37.4	37.4
	Mixture	54.5	26.4	28.8	52.9	28.1	29.9
	Grass c.*	52.2	49.4	50.1	52.1	50.2	50.0
MAE*	Clover	0.37	0.27	0.26	0.36	0.27	0.26
	Daisy	0.39	0.29	0.29	0.38	0.29	0.29
	Yarrow	0.32	0.29	0.29	0.32	0.29	0.29
	Mixture	0.43	0.25	0.26	0.42	0.26	0.27
	Grass c.*	0.53	0.47	0.49	0.52	0.48	0.49

* MAE= Mean Absolute Error; AIC= Akaike Information Criterion; Grass c.= Grass control.

For *soil* estimations, we generally observed better agreement between methods, with overall lower AIC and MAE compared to estimations of weed cover for all models (Table 5.6). However, the Pixel classifications again performed worse than the OBIA and combined classifications, except in plots sown with yarrow, where there was similar agreement between all RF classifications and observed data (AIC between 31.1 and 33.1). The MAE between predicted and observed data in plots sown with yarrow were the same (0.26- 0.27). Overall, combined classification using the *5-class model* scored the lowest AIC scores (162.2), closely followed by combined classification using the *3-class model* and OBIA classification using the *5-class model* (164.1 and 164.2 respectively). The MAE for combined and OBIA classification using the *3-class* and *5-class models* were similar.

Table 5.6. Akaike information criterion (AIC) and mean absolute error (MAE) for log transformed values of two methods for estimating bare soil in between vegetation cover using aerial image analysis of a field trial. Ground measurements included, single picture analysis of 136 plots captured with a RGB camera and subsequent analysis with Turf Analyzer software. Aerial multispectral images were analyzed using object-based classifications, Pixel-based classifications (Pixel) and combined classifications (combined) for a *3-class* model (grass, soil, and weed) and a *5-class model* (clover, daisy, grass, soil, and yarrow). Data was log transformed.

		3-class model			*5-class model*		
	Sowed treatment	Pixel	OBIA	Combined	Pixel	OBIA	Combined
AIC*	Clover	45.6	35.7	32.3	45.2	34.5	31.7
	Daisy	62.5	47.6	45.8	62.6	42.6	44.1
	Yarrow	33.1	33.0	31.9	32.4	32.8	31.1
	Mixture	43.0	29.4	32.1	41.7	29.5	32.6
	Grass c.*	31.9	26.3	22.0	30.1	24.8	22.7
MAE*	Clover	0.33	0.30	0.26	0.33	0.29	0.26
	Daisy	0.49	0.40	0.38	0.49	0.36	0.36
	Yarrow	0.26	0.26	0.27	0.26	0.26	0.27
	Mixture	0.35	0.27	0.28	0.34	0.26	0.28
	Grass c.*	0.31	0.26	0.23	0.30	0.25	0.23

* MAE= Mean Absolute Error; AIC= Akaike Information Criterion; Grass c.= Grass control.

5.4. Discussion

Using our RF classifications, we were able to successfully distinguish green vegetation cover (grass and weeds) from non-vegetation cover (bare soil). The OBIA classification using the *5-class model* was able to classify soil with 100% accuracy due to the high reflectance of green vegetation in the NIR portion of the electromagnetic spectrum in comparison to the lower reflectance of soil. Digital analysis of the green fraction of pixels has been used by other researchers to measure cover and quality of turfgrasses (Karcher and Richardson, 2013).

However, the methodology used in their study was not able to distinguish between weeds and grasses because both classes have similar reflectance values in the green portion of the electromagnetic spectrum. Identifying plant species from within a landscape of green vegetation is generally more complex and challenging (Lamb and Brown, 2001). We encountered these challenges with regard to *clover*, which had similar pixel values at all measured bands, compared to other weeds (Figure B1). As reported by Casapia et al. (2020), we found that the mean green pixel values within segments were an important feature for OBIA classifications (Figure 5.4). The average spectral reflectance within segments was used, whereas the shape parameters of the objects did not appear to be important features. The OBIA classifications performed much better than the Pixel model, indicating that even less important shape parameters led to better classification accuracy (Tables 5.4 & 5.6). For the combined classification, all individual pixels within segments were classified. In that case, we observed that the shape parameters such as fractal dimension and length were the most important features, particularly for the *3-class model*. However, the shift in relative importance of shape parameters in OBIA classifications compared to the combined classifications, did not significantly alter the predictive accuracy of the models. Both models showed high accuracy when using the *3-class model* (98-99%), and while the OBIA *5-class model* was superior at detecting *grass* and *soil,* the combined model scored higher accuracies for detecting *clover* and *daisy* (10% more accurate for both classes). Hence, if the overall goal is to detect general weed cover, the OBIA classification approach is recommended.

When comparing cover estimations based on industry-standard in-field measurements (observed) and the RF classifications (predicted), we found that agreement between observed and predicted estimates of weed cover was particularly good in plots sown with daisy and mixtures for both OBIA and combined classifications (Table 5.5). This could be due to the fact that daisy growth flat on the surface with sharp leaf edges. Compared to the other tested weed species daisy clearly stood out. A weed species that can be easily separated visually is also easier to detect in an image analysis approach. The highest discrepancy between observed and predicted estimates for both OBIA and the combined classifications occurred in plots sown without weeds (grass controls). For estimates of *soil* cover we found the opposite to be true, with the best agreement between observed and predicted values found in plots sown with grass only and the highest discrepancy found in plots sown with daisy, for OBIA and combined classifications. Some of the weed species such as yarrow do not have a solid leaf blade but a feather type of blade. Because of the segmentation process an object like a yarrow leaf could potentially be classified as soil if the reflectance of the soil through the yarrow leaf blade overrides the reflectance of the yarrow leaf blade itself. Grasses such as *Festuca* spp. have an upright growth habit and because the camera was pointed vertically at the plot, it is likely that in grass control plots the clear edges between soil and grass patches were visible, leading to high classification accuracy of soil in grass control plots.

Using remote sensing to detect weeds in regularly mowed turfgrass is generally challenging because of the small size of individual weed plants, which requires high resolution imagery. In our study, we found *yarrow* to be a particularly problematic weed to detect, which was most likely due to its similarity in leaf shape and spectral features to grasses (Fig. B1). Compared to natural grasslands, where vegetation cover and characteristics show strong seasonality (Zillmann et al., 2014), cover of turfgrass is much less dynamic due to the intense management regimes. This consistent and uniform cover is better suited to classification models such as those developed in this study and result in improved classification accuracy. Due to the mowing regime of turfgrass, we expect spectral and shape characteristics to be more seasonally uniform and suggest that our results could be applicable throughout the entire season. More research is needed to confirm this suggestion.

In this study we focused on three weed species that commonly occur on golf courses, however there are many more. Future research efforts should focus on examining the influence of different weed species on the predictive accuracy of image analysis approaches. For the purposes of identifying weed cover, our results are highly encouraging, given the high accuracy (98-99%) of the OBIA classification that grouped all weeds into a single class (*3-class model*). Apparently, the three contrasting weed species used in this study share common characteristics that can be successfully captured by our method and are considerably different from grass characteristics. The spectral reflectance of all features used to construct the models (Fig. B1) suggests that main differences between weeds and grass are in mean red entropy, shape parameters and mean green. Accordingly, we anticipate that our method may also be successful in identifying weed species that have a growth form similar to clover, daisy, and to a lesser degree, yarrow. To what extent our results can be generalized to other weed species is an open question that is of interest for future research.

In conclusion, our research demonstrated that OBIA classification is a useful tool for weed detection, especially when compared to the currently employed time consuming in-field measurement with a point quadrat and lightbox. Our study showed that using texture features

(OBIA- and combined classifications), the *3-class models* classified soil, weed, and grass with 98-99% accuracy, and the *5-class models* discriminated between soil, grass, and the three weed cover types with 81-80% accuracy. Agreement between predicted estimates of vegetation type and bare soil and observed estimates obtained using point quadrat and Turf analyzer methods varied depending on the grass/weed seeding treatment.

5.6. Acknowledgments

First and second author have contributed equally to this report. We especially thank Dr Rossana Sallenava for her help with the manuscript. Financial support of this report was provided by the Turfgrass University Research Foundation (TuRF), Nederlandse Golf Federatie (NGF), Nederlands Vereniging van Golfaccommodaties (NVG), and Nederlands Greenkeepers Associatie (NGA). The authors are also grateful for the constructive and insightful comments provided by the anonymous reviewers; they greatly improved the manuscript.

CHAPTER 6

General discussion

6.1 Current European policy regarding herbicide use in turfgrass management

There is a growing opposition in Europe to the use of herbicides to control broadleaf weeds in turfgrass management due to concerns about herbicide resistance and human health impacts associated with direct contact during and after product application. This has led to a shift towards integrated pest management (IPM) strategies in Europe, with herbicides being identified as the last resort for broadleaf weed control (European Parliament, 2009). In the US, "alternative control strategies" seems to be focused on finding new modes of action (MOAs), rather than focusing on strategies to eliminate herbicide use entirely (Brosnan et al., 2020). The entire process of developing a new herbicide, from research into new MOAs to development of the final product costs about 241 million Euros and does not guarantee efficacy against weeds that have already developed resistance to other herbicides (Brosnan et al., 2020). Resistant broadleaf weeds can develop non-target site resistance mechanisms, physically hindering the active ingredients from reaching the target sites (Ghanizadeh and Harrington, 2017; Brosnan et al., 2020). Turfgrass areas can be managed for some time without herbicides, but Dahl-Jensen et al. (2014) found that weed densities severely increased after a period of around three years in Scandinavian golf courses. Weed management without herbicides will become more challenging, which requires re-examination of the problem and existing control strategies.

6.2 A holistic approach to control weeds in the absence of herbicides

The use of herbicides can be seen as an effective and efficient curative tool for turfgrass managers to selectively control broadleaf weeds, such as white clover (*Trifoloum repens* L.) or broadleaf plantain (*Plantago major* L.). When broadleaf weeds establish, a selective herbicide application can provide quick control (Busey, 2003; Brewer et al., 2017). If herbicides are removed from the toolbox of practitioners, much more diverse strategies, with an emphasis on prevention, are required to maintain a dense turf and limit the niches for weed invasion. Broadleaf weed control in the absence of herbicides is likely to be most effective when a variety of measures are implemented to interrupt the cycle of broadleaf weed establishment from seed germination, to colonization and vegetative propagation (Busey, 2003; Abu-Dieyeh and Watson, 2007; Marble et al., 2015). Before seeding a new turfgrass area, agricultural methods, including flaming or steaming, could be employed to kill broadleaf weed seeds in the natural seedbank (Bond and Grundy, 2001; Hoyle et al., 2012). However, such methods are applied to the surface and weed seeds are often located deeper in the soil profile. Therefore damaging a buried seed to the point where it can no longer germinate, is challenging (Turner et al., 2012).

In newly seeded areas broadleaf weed establishment is also hindered when the ecological niche is rapidly invaded by desirable turfgrasses (Beard, 1973; Watschke and Engel, 1994; Larsen et al., 2004). For this reason, it is important to select a grass species or grass species composition, which are well adapted to the local climate to ensure it establishes rapidly and is well suited to outcompete weeds. For this Doctoral research project, we focused mainly on *Festuca* spp., a turfgrass species that has many attributes that make it attractive to practitioners. *Festcua* spp. are well adapted to moderate climates and free-draining- low nutrient soil environments and are often described as the most sustainable turf species because of their low requirements for water, fertilizers and pesticides (Peters and Mohammed Zam, 1981; Watkins et al., 2010; Rice, 2012). Sustainable turfgrass species selection is an important aspect for turfgrass managers because they face other management pressures, such as water and nutrient restrictions, in addition to restrictions on herbicide use. *Festuca* species were also identified for their growth interference potential on common broadleaf weeds (Bertin 2003a; 2009). However, it should be pointed out that in locations where the soil composition, drainage rates, climate and so forth are far from ideal to establish *Festuca* grasses, species selection based solely on low input criteria could lead to abiotic and biotic stress that result in exposure of soil and niches to weed invasion. A mixture of a variety of turfgrass species and cultivars with high leaf area index might be more appropriate in such circumstances, to maintain a vigorous turfgrass sward that outcompetes broadleaf weeds (McKernan et al., 2001; Busey, 2003).

In established turf, management practices should be adjusted to provide a competitive sward which in turn increases competitiveness of desirable turfgrass species with unwanted broadleaf weed species (Busey, 2003). Generally speaking, any management practice can be adjusted in some way to improve competitiveness against weeds. For example, increasing mowing heights reduces annual weed densities such as crabgrass (*Digitaria* spp.) over time (Voigt et al. 2001; Dernoeden et al., 1998). The effect of different mowing heights on broadleaf weeds is poorly understood. Also, research on mowing heights focusses on large differences of a few centimeters, whereas turf managers need to know if changing the mowing height within an acceptable range (often a few mm) makes a difference. Such differences for golf fairways are for example a minimum of 8 mm and a maximum of 25 mm to maintain usability. However, it is unclear if such small changes affect development and growth of weeds. Turf managers can mow turfgrass areas with box attachments in front of the mowing units or without box attachments, which returns the clippings to the turf grass sward. Exudates from shoots of some species are known to have allelopathic potential (Akbari et al., 2015; Bertin et al, 2003a), but the effect of leaving clippings on the surface as weed control mechanism has not been investigated. Clippings contain nutrients which are released to the soil after decomposition (Knot et al., 2017; Qian et al., 2003). Increasing the nitrogen inputs, through leaving clippings on the surface or increasing fertilization, is known to change the competitive ability of grasses to compete against broadleaf weeds (Silvertown 1987, Silvertown et al., 2006). Adjusting fertilization rates to provide competitive turfgrass swards should be considered an important management practice when herbicides cannot be used for weed control. Turf managers face environmental pressures and a development on “how low can we go?” has been observed in Europe in recent years. Reassessing fertilization regimes when herbicides can not be used, are likely to be one of the most important considerations.

Adjusting management practices is vital to establish a competitive sward, but in situations where weeds have already established, managers also need direct control options. To that end, research on biological herbicides continue to progress, with some promising results emerging over the past couple of years. Biological herbicides with selective mechanisms are formulated with microorganisms (fungal pathogens or bacteria) or plant extracts such as allelochemicals (de Souza Barros et al., 2021). Control of broadleaf weeds is dependent on environmental conditions, soil type, organic matter content and other factors (Bailey et al., 2011, 2013). Bioherbicides are highly selective and the risk of weeds developing resistance to them is low (de Souza Barros et al., 2021). However, to date, only 20 bioherbicides have been registered and on top of that many of the commercially available products are no longer sold (de Souza Barros et al., 2021). In practice the biggest challenge for bioherbicides is achieving successful infection of the plant with the microorganism, which starts with the correct transport and storage of the living organisms, using the right spraying equipment with adequate droplet size for product application, and spraying during adequate environmental conditions to achieve sufficient fungal infections of the target organism (Auld et al., 2003; de Souza Barros et al., 2021). All these challenges and limitations are reasons why more research is needed to increase biological herbicide efficacy and for the time being, relying on development of these products alone is not a sensible strategy.

The examples mentioned reflect a small selection of possible management adjustments and developments, however highlights that in the absence of herbicides a holistic concept is required to prevent turf loss, which in turn can lead to rapid weed invasion. Apart from turfgrass management considerations and alternative products to herbicides, the genetic component of turfgrasses can be considered in search towards alternative control strategies for broadleaf weeds in turfgrass. It is this genetic component, more specifically growth interference potential, that we focused on in the research chapters.

6.3. Growth interference potential of *Festuca* species with weeds

The general objective was to conduct controlled growth chamber screenings to identify *Festuca* species or cultivars with allelopathic potential with broadleaf weeds (clover, daisy and yarrow) and to evaluate the effectiveness of this technique as a preliminary screening method by comparing results to a subsequent field trial. This was to some extent previously achieved by Bertin et al (2003a), who grew donor (*Festuca*) and receiver plants on agar to observe the root inhibitory effect of *Festuca* on weeds. We were particularly interested to select an experimental design that reduced the competition for resources as much as possible and highlighted the allelopathic effect more prominently, which is an adequate experimental design to preliminarily screen for allelopathic plants species (Duke, 2015). Allelopathy of *Festuca* species in laboratory screenings has proven to be an important mechanism in weed growth interference (Bertin et al 2003a), however to what extend this growth interfering mechanism contributes to weed growth interference with other resource competition mechanism for light, nutrient, water and space is fairly unknown (Busey, 2003; Rice, 2012; Swanton et al., 2015).

For the growth chamber we adopted the experimental design from Bertin et al (2003a) but used a much larger amount of *Festuca* seeds (60 instead of 30) and allowed *Festuca* seeds to germinate for 13 days rather than seven to allow production of more allelopathic compounds. We also did not include known indicator species such as curly cress (*Lactuca sativa* L.) or lettuce (*Lepidium sativum* L.) but rather a variety of broadleaf weeds species found in turfgrass such as clover, daisy, and yarrow. Growth interference in our climate chamber study was assessed by measuring the root length of weeds, by recording the number of germinated seeds over time (mean germination period= MGP) and the final number of germinated seeds (full germination percentage).

In our growth chamber study, we found that neither *Festuca* species nor cultivars influenced measured germination characteristics (FGP or MGP) of weeds. No published study has shown that the naturally produced allelopathic compounds of *Festuca* species, when grown for a period of time before introducing weed seed, are strong enough to influence weed seed germination. It is known that allelopathic compounds are produced slowly and in small quantities over time (Duke, 2015), therefore future research should focus on identifying time periods required for *Festuca* species to build up a sufficient amount of exudates in the growing medium to have a growth interfering effect on weed germination. When we examined growth interference of *Festuca* species on weed root length, we observed that clover root length differed between species and a growth interfering effect on yarrow roots that did not differ between species. Daisy entries were removed from the analysis, as all species appeared photobleached and roots were too short to be measured. These results showed that screening for allelopathy is species x species specific. For weed species this implies that clover species are more appropriate as indicator species for allelopathic screenings, because *Festuca* species effects were observed, and a wider range of cultivar differences were detected. We also observed larger differences for cultivars than for species. Therefore, the results also implied that any cultivar regardless of *Festuca* species can have high allelopathic potential, which complicates a preliminary screening process for allelopathic species, because cultivars from all *Festuca* species need to be considered as interesting candidate species.

In the subsequent field trial, we observed large differences by year with no significant difference among *Festuca* species for weed cover. Weed establishment in field trials was significantly dependent on weather conditions. As we did not observe any differences in weed cover, we could not compare the results from the growth chamber to the field trial. We suggest for future research to include equal number of entries for growth chamber screenings and field trials. Equal number of entries allows for establishing a ranking for weed suppressiveness which could be comparable to one another. Also, our growth chamber experiment was designed to reduce resource competition but not fully exclude it. A set of different experimental designs would be useful to separately screen for resource competition mechanism. For each of the separate experiments, a ranking of the weed suppressiveness potential of *Festuca* cultivars could be compared to the results observed in field trials. This would for one help to determine which aspects of competition contribute most to growth interference potential of *Festuca* species with weeds and two to help find an experimental set-up, which ranks cultivar suppressiveness similar to field observations. To screen explicitly for *Festuca* allelopathy competition one experimental set-up could be designed to separate *Festuca* and indicator weeds on two separate sides of agar, physically hindering competition for space but allowing diffusion of exudates through the growing medium to explicitly study the effect of allelopathic compounds interfering with receiver plants (Duke, 2015). It might be that our experimental design is already useful for preliminary screening of *Festuca* species, but we were unable to conclusively examine this because result were not comparable to the outcomes of the field trial.

This leads to the question how difference in *Festuca* growth interference in field studies could be better observed in the future. In our design, we used a high seeding rate of weeds seeds (ratio 3:1) to homogenously establish weed cover, which is important to observe differences in weed suppressiveness of *Festuca* species. In future research, it might be useful to not overseed with weeds, but simply let weeds establish from the natural seedbank, which reflects a more realistic scenario encountered by practitioners. The risk of failure of such an experimental set-up is high, therefore careful examination of weed seed abundance in the natural seedbank should be a preliminary step before conducting field experiments. Also, such a set-up does not ensure homogenous weed seed establishment, because in some areas high abundant of weed seeds might be presence whereas in other areas no seeds might be present. Therefore, a multi-location experiment would be useful to increase the number of experimental observations and to increase the chance of collecting meaningful results. Also increasing the replications per year from two (our study) to three would be helpful to increase the chance of collecting data of field trials that were exposed to similar environmental conditions. The major limitation of our field trial, was the amount of cultivar entries (six), because of the time it requires to measure weed cover objectively. Therefore, to advance growth interference studies in turfgrass with weeds, we included a research chapter to objectively record weed cover by using aerial multispectral images and subsequent analysis of weed cover by digital image analysis and machine learning approaches.

6.4 Limitations in turfgrass field trials investigating effects on weeds

In our research we used the point quadrat method (weeds are counted manually) to quantitatively assess weed cover. It is a time-consuming method and therefore limited the number of cultivar entries in our experiments. Therefore, we investigated the use of aerial multispectral digital images and data processing, training and classification with random forest models (RF), which are machine learning algorithms that initially use multiple decision trees to independently classify the type of vegetation based on supervised learning. Our RF classifications appear to be a simple way to distinguish vegetation cover from non-vegetation and could therefore potentially replace visual vigor scores that require special training. We observed that broadleaf weeds were best detected by extracting average pixel values from object-based shaped features to train the RF classifications. Further investigations are needed to determine if using high resolution images in the red, green and blue band (RGB) would provide similar results to using multispectral images. Using conventional cameras (RGB) for data collection, would considerably reduce the cost of this method, which would increase the likelihood of it being adopted by practitioners (Ashapure et al., 2019; Marcial-Pablo et al., 2019). Adaptability to practitioners would also be improved by keeping the processing time and memory space to a minimum, which will require further research into the optimal image resolution for broadleaf weed detection in close mown turf. Recently, deep learning algorithms were used to detect a variety of broadleaf weeds such as white clover, chickweed [*Stellaria media* (L.) Vill.] and dandelion (*Taraxacum officinale* F.H. Wigg) in dormant bermudagrass [*Cynodon dactylon* (L.)] (Yu et al., 2019). The use of neural networks is a promising development that could ideally lead to building a large publicly accessible data set, because these networks do not require manual extraction of features, unlike machine learning approaches (Shah et al., 2021). In recent publications, convolutional neural networks were used to detect weeds for GPS removal practices in agricultural settings (Sudars et al., 2020). Such networks could also be employed for precision spot removal of broadleaf weeds in turfgrass areas.

6.5. Redefining acceptable threshold levels for weed control

Access to herbicides allowed turfgrass managers to strive for an ideal target of establishing weed-free turfgrass areas. Restricting the use of herbicides greatly limits turfgrass managers in their ability to control weeds. Therefore, it needs to be examined if turfgrass areas really need to be kept weed-free, or whether some weeds can simply be tolerated without any negative effects on its quality. Relevant questions would then become how much weed cover and which species can be tolerated related to the intended use of the turfgrass area. In the turfgrass literature, broadleaf weeds are simply seen as unwanted plant species, as they interfere with the usability of the desired turfgrass area, are seen as aesthetically unattractive, and reduce playing quality of sports turf (Larsen et al., 2004; McElroy and Bhowmik, 2015; Yu et al., 2019). In agriculture, action threshold levels for weeds are defined, and clearly indicate at which percentage of weed invasion control measures should be taken to prevent crop yield and financial losses (Munier-Jolain et al., 2002). For turfgrass areas a clear definition of how usability or playing quality is affected by broadleaf weeds is absent. Playing quality of turfgrasses is generally only characterized in terms of quantitative measurements such as smoothness, firmness, and uniformity (Dahl-Jensen et al., 2014). The impact of broadleaf weeds on these characteristics has not been described in the literature, however it can be argued that in many situations the negative effects are minimal or trivial. For example, on golf fairways, which make up the majority of the surface area of golf courses, neither the bounce of a golf ball is negatively affected when it hits a broadleaf weed patch, nor the ball lie is negatively impacted when it comes to rest on a weed patch. This is because most broadleaf weeds, such as clover (*Trifolium repens* L.) or daisy (*Bellis perennis* L.), lie flat on the surface and do not significantly absorb the impact, divert the direction after impact, or cover a golf ball when it comes to rest on a weed patch. Certainly, some areas should be free of broadleaf weeds, such as bowling greens or golf greens, otherwise ball roll is affected, because of the different growth habit of weeds compared with fine turfgrasses. However, such areas are usually small in size, and maintained intensively by regular close mowing and fertilization, which leads to a closed canopy with grass dominance and almost no natural niches for broadleaf weed establishment. For other turfgrass areas such as public parks and residential gardens, defining threshold levels to control weeds becomes even more difficult. Certainly, weed-free lawns are aesthetically pleasing and humans assign a higher quality to such lawns compared with a lawn with a certain level of broadleaf weed cover (Cheng et al., 2008). However, there is no doubt that cultural and societal norms influence what level of broadleaf weed invasion is perceived as problematic and these societal influences have contributed to the notion that a lawn needs to be weed-free. These cultural influences and perceptions vary from one country to another. In Sweden for example, lawns are valued for physical activity but users want diverse lawns that stimulate all the senses (Ignatieva et al., 2017). In China, biodiverse lawns are accepted in areas which focus on ecological management, but broadleaf weeds are accepted to a lesser extent in urban areas (Yang et al., 2019). Our research has shown that weed establishment, particularly in newly seeded areas is highly dependent on environmental conditions, therefore the perception that a perfect lawn is 100% weed free is no longer a realistic target, when herbicides can not be used for control. In my perception, future research should not only focus on finding alternative control strategies but should also investigate in which situations some degree of weed cover is acceptable.

6.6 Concluding remarks

Weed control in the absence of herbicides is a complex subject that requires a holistic management approach. Key to this approach is the selection of a grass species based on site-specific considerations to maintain a closed turf canopy all-year round and thereby minimize niches that weeds can exploit to establish. To what extend weed control without herbicides can be achieved is questionable. Turf managers not only face herbicide restrictions but also fungicide and insecticide restrictions. The outbreak of turf diseases for instance is dependent on environmental conditions, and susceptibility of the host plant. Turfgrass areas dominated by susceptible turfgrass species such as *Poa annua*, are at great risk to be severely damaged by disease outbreaks when environmental conditions are suitable, which leads to opening in the turf and subsequently weed invasion. Another concern is the environmental pressure turf manager face. Water is becoming a scarce resource in the future, with 95% of fresh produce at risk from climate change (Kelly, 2014). In the event of water scarcity, it is likely that water use will be strictly restricted for the turfgrass sector. Drought damage severely damages the turf, which leads to niches for weed invasion. When access to herbicides is restricted in the future, turfgrass managers face a constant battle to not lose turf and weed invasion will be a secondary effect. Another consideration is how to remove weeds once they established. Post-emergent weed removal is currently difficult and time-consuming, requiring non-selective spot treatments. Recent advancements in technology might lead to autonomous weeding robotic systems that would replace the need for time-consuming manual control. Once autonomous weeding systems are developed the problem of removing existing weeds is likely to be solved. In that case, the challenge for turf managers remains how to prevent turf loss and provide consistently performing turfgrass areas all year round.

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Appendix A

Table A1. Mean germination period (MPG) of 27 *Festuca* cultivars, grown in plastic boxes filled with water agar and placed in a growth chamber. Germination of each seed was counted every four days and MGP was calculated by separating each fourday interval into periods (1-4 days = Period 1; 5-8 days = Period 2; 9-12 days = Period 3). Smaller MPG numbers means seeds germinated earlier on.

Cultivar	Species	Mean	LS-means		
FRR	Mellori	2.10	A		
FRT	Baroyal	2.09	A		
FRT	Libano	2.06	A		
FRT	Samanta	1.97	A	B	
FRR	Staybo	1.95	A	B	
FRC	Barileneus	1.94	A	B	
FA	Barcesar	1.92	A	B	
FRT	Barcrown	1.92	A	B	
FA	Melyane	1.90	A	B	
FRR	Barisse	1.86	A	B	
FA	Regenerate	1.85	A	B	C
FRT	Charlotte	1.82	A	B	C
FRC	Annalena	1.81	A	B	C
FRT	Barpearl	1.78	A	B	C
FRC	Dancing	1.75	A	B	C
FRR	Relevant	1.73	A	B	C
FRR	Barjessica	1.73	A	B	C
FRC	Siskin	1.72	A	B	C
FRA	Mentor	1.66	A	B	C
FRT	Cathrine	1.65	A	B	C
FRR	Rossinante	1.60	A	B	C
FRA	Dumas 1	1.60	A	B	C
FRT	Nigella	1.59	A	B	C
FRC	Melitta	1.53		B	C
FRR	Livison	1.52		B	C
FRC	Ramona	1.49		B	C
FRR	Sergei	1.38			C

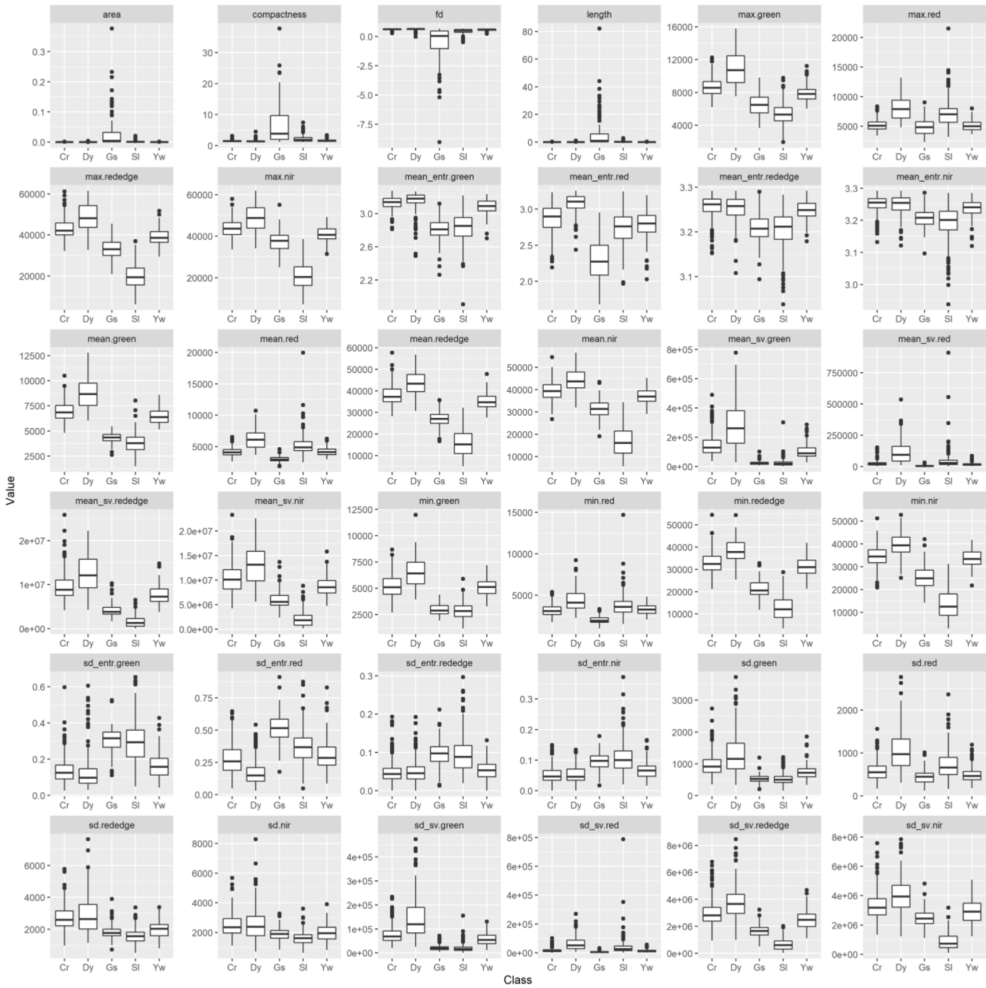
‡Values in each column followed by the same letter are not significantly different according to simulated adjustment (0.05).

Appendix B

Table B1. Confusion matrix of a *5-class model* (clover, daisy, grass, soil, and yarrow) from object-based image analysis (OBIA) classification, Pixel based classification (Pixel) and a combination of both classifications (combined). Training data for the model was obtained from a segmented ortho-image of a field trial (Barenbrug Turfgrass Research Station, Wolfheze, The Netherlands) to investigate the competitiveness of *Festuca* cultivars with broadleaf turfgrass weeds.

		Clover	Daisy	Grass	Soil	Yarrow	Sum	User accuracy
OBIA	Clover	48	4	0	0	7	59	81.4%
	Daisy	2	37	0	0	1	40	92.5%
	Grass	0	0	31	1	1	33	93.9%
	Soil	0	0	0	45	0	45	100%
	Yarrow	9	1	0	0	17	27	63%
	Sum	59	42	31	46	26	204	
	Producer accuracy	81.4%	88.1%	100%	97.8%	65.4%		
	Overall accuracy							86.3%
	Average accuracy							21.5%
	Kappa x 100							84
Pixel	Clover	777	174	270	3	168	1392	55.8%
	Daisy	273	545	85	0	91	994	54.8%
	Grass	445	111	97892	1712	354	100514	97.4%
	Soil	1	1	555	7391	1	7949	93.0%
	Yarrow	235	107	303	4	146	795	18.4%
	Sum	1731	938	99105	9110	760	111644	
	Producer accuracy	44.9%	58.1%	98.8%	81.1%	19.2%		
	Overall accuracy						62.1%	
	Average accuracy						80.5%	
	Kappa x 100						77	
Combined	Clover	1167	145	0	0	51	1363	85.6%
	Daisy	92	762	0	0	472	1326	57.5%
	Grass	0	0	114069	13	4	114086	100.0%
	Soil	0	0	549	11180	0	11729	95.3%
	Yarrow	283	58	0	162	375	878	42.7%
	Sum	1542	965	114618	11355	902	129382	
	Producer accuracy	75.7%	79.0%	99.5%	98.5%	41.6%		
	Overall accuracy							77.5%
	Average accuracy							78.9%
	Kappa x 100							93

Figure B1. Spectral reflectance of all features used to construct the object based (OBIA), Pixel based, and combined classifications. Training data for the classifications were generated from labelled segments (Cr = clover, Dy = daisy, Gs = grass, SI= soil and Yw= yarrow) of an orthoimage generated with data collected in a field trial.



Summary

In this thesis we examined building blocks towards non-chemical weed control strategies in turfgrass. Public health and environmental concerns associated with herbicide misuse led to herbicide restrictions in the recent past. To maintain usability of turfgrass areas with a potential scenario of further herbicide restrictions or complete bans, alternative management strategies need to be explored. In addition, turfgrass managers experience pressure to reduce water and fertilizer use and therefore try to maintain a low input turfgrass species composition. Species within the genus *Festuca* are considered low input species for which allelopathic potential against broadleaf weeds was also identified. Based on the existing body of knowledge, we used *Festuca* species for our experiments. The genus *Festuca* includes the fine Fescue complex with the red fescues (*Festuca rubra*) and the sheep fescues (*Festuca ovina*). From the *Festuca rubra* sub species we selected cultivars from slender creeping red fescue [*F. rubra* L. ssp. *littoralis* (G.Mey.) Auquier], strong creeping red fescue (*F. rubra L. ssp. rubra Gaudin)* and Chewings fescue [*F. rubra L.* ssp. *fallax* (Thuill.) Nyman]. From the *Festuca ovina* sub species we included hard fescue (*F. brevipila Tracey)* cultivars. Additionally, we used tall fescues [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.]. The objectives of our studies were to give a perspective on the existing literature about non-chemical weed control strategies, to screen *Festuca* species and cultivars for their growth interfering potential against common European broadleaf weeds including clover (*Trifolium repens* L.), daisy (*Bellis Perennis* L.) and yarrow (*Achillea millefolium* L.) and to investigate a different image analysis approach to improve data collection in turfgrass weed research.

In **Chapter 2** a perspective about broadleaf weed control without herbicides is given. I propose that threshold levels need to be defined to determine when control of broadleaf weeds is necessary. Also, published reports suggest that application of nitrogen (N) increases competitiveness of desirable turfgrass species against weeds, which also requires defining threshold levels to find a balance between how much N is needed to cause a weed suppressing effect and how much N is sustainable. Species selection to provide a competitive sward against abiotic and biotic stress is crucial to maintain dense turfgrass cover and avoid the development of niches for weed invasion. Another species selection criterion can be using the allelopathic potential of desirable grass species against broadleaf weed species. Moreover, established broadleaf weeds can be controlled with spot treatments of hot water, acetic acid or other means, such as mechanical removal by robotic, autonomous systems. Such systems are already

deployed in agricultural settings but have not been investigated for the use on turfgrass.

In **Chapter 3** we screened 27 *Festuca* cultivars from three sub species (red fescues, hard fescues and tall fescues) for their growth interfering potential against clover (*Trifolium repens* L.), daisy (*Bellis Perennis* L.) and yarrow (*Achillea millefolium* L.), which are common broadleaf weed species found in cool season turfgrass areas. The objective was to investigate if *Festuca* species and cultivars varied in their ability to affect germination and root length of daisy, clover, and yarrow. Also, we investigated growth interference sensitivity between weed species. The designed experiment was based on Bertin et al (2003a). We used plastic containers, filled with water agar (no nutrients) and placed 60 grass seeds in a matrix on the agar, which were allowed to germinate for 13 days in a growth chamber before 20 weed seeds were place in the middle of the *Festuca* seeds. Full germination percentage (FGP) and mean germination period (MGP) of weeds were not affected by presence of *Festuca* species. *Festuca* growth interference on weed roots was strongest on daisy weeds, which we had to remove entirely from the experimental analysis, because plants rapidly appeared photobleached and died shortly after germination. On average, root length of yarrow (reduction 79%) was more strongly affected by presence of *Festuca* species than that of clover (reduction 56%). Significant difference in root length reduction due to *Festuca* species were not observed for yarrow, whereas for clover root reduction compared to controls differed between 44.5% for FRT and 71.5% for FA. Clover could therefore be considered a more distinctive indicator species for *Festuca* interference with broadleaf weeds. Significant cultivar differences within species were observed for root length reduction of both yarrow and clover, indicating the presence of variability of this trait within species. Significant negative association between *Festuca* biomass and root length of clover (-0.264***) and yarrow (-0.181***) were observed. Examining cultivars, Relevant (FRR), Ramona (FRC), Rossinante (FRR) and Regenerate (FA) were identified as potential candidates for future investigations into growth interference studies as they reduced root length of clover to less than 2.6 cm, representing a 76.1% difference to controls. For yarrow, cultivars Melitta (FRC), Melyane (FA) and Barcesar (FA), which reduced yarrow root length to less than 2 cm (87.4% less than control), showed to be interesting cultivars for further investigations.

For the research project described in **chapter 4,** we selected six *Festuca* cultivars that were used in the experiment highlighted in chapter 3, with each of the species, namely Chewings fescues, slender creeping red fescue, strong creeping red fescue, hard fescue and tall fescue being represented. Individual plots measuring 1.5 m x 1.5 m were sown with *Festuca* species in a field trial. Fourteen days later, plots were oversown with weeds (clover, daisy, yarrow and a mixture of all three). Plots with no grasses (weeds only) were included as controls. We recorded vigor, i.e the speed of development of grasses into a mature sward, weed cover, and visual quality. The objective was to identify cultivars that interfered with weed growth (determined by weed cover in each plot) and to investigate if speed of *Festuca* development was associated with weed cover. Results differed between 2018 and the replication of the experiment in 2019 due to weather conditions. At the end of the experiment at 84 days after sowing grasses (DAS) we found Musica (Chewings fescue) and Barpearl (slender creeping red fescue) to be interesting candidate cultivars for natural weed suppression, as they interfered with weed growth in both years. However, other cultivars exhibited similar traits during one of the two years but not consistently during both years. The tall fescue cultivar Melyane seemed a promising cultivar, with good early establishment, but vigor declined rapidly after the first mowing which ultimately led to unacceptable visual scores. An inverse significant relationship between visual quality and weed cover was shown for both years (-.51*** in 2018 and -.48*** in 2019). Such a result was expected as uniformity of the turfgrass stand is an important parameter for visual quality of turfgrass swards and the experiment was designed to establish weeds which reduce the quality of a stand.

In Chapter 5 we used the field trial of Chapter 4 to take aerial multispectral images of the 136 experimental plots. Images were overlaid and an overview image (orthoimage) of the experiment was constructed. We used a segmentation algorithm to draw boarders around objects with similar properties. For some segments we labelled the vegetation composition and trained random forest algorithms to recognize species for the entire segmented orthoimage. We then drew a polygon around each of the 136 experimental plots and extracted spectral reflectance information and shape features. Three random forest models were trained to use (1) only spectral reflectance values from all pixels within a segment (Pixel classification), (2) only the average pixel values from one segment and shape features (Object-based classification), and (3) all pixel values from each segment in addition to the shape features (Combined classification). Vegetation was classified as either grass, weeds, and bare soil (*3-classes*) or grass, weeds, bare soil, clover, daisy and yarrow (*5-classes*). The weed class was detected with 99% accuracy with the Object-based classification. When weeds were further separated into clover, daisy and yarrow accuracy decreased to 82% for the Object-based classification whereas the combined classification reached 89% accuracy. When field measurements of weed cover, using point quadrat counts, were compared with the classification outputs, we found highest agreement for the object based and combined model approach.

In Chapter 6 we reemphasized the need to develop management strategies for weed control without herbicides. In this thesis we attempted to show how difficult it is to find alternative strategies and we therefore concluded that the definition of weeds needs to be revised. Perception of what a weed is, and acceptable threshold levels need to be examined in more detail. We summarized management practices discussed in the perspective paper that can be used to interrupt the weed seed cycle starting from the soil seed bank, to germination, establishment and finally removal of existing weeds. We highlighted the importance of selecting suitable grass species adapted to the local climate and the purpose of the turfgrass area. While our thesis focused on *Festuca* species, due to its allelopathic potential with weeds and low input requirements of water and fertilizer, it was emphasized that those species might not be ideal for situations where the soil or climate does not allow ideal establishment. Lastly, building on our weed detection approach in Chapter 5 I recognized that neural network gain ever increasing momentum in automated detection using deep learning approaches, which is a promising step, not just for furthering turfgrass research, but also in the development of automated weeding systems in general.

Acknowledgements

I want to first thank Paul Struik who acted in a co-supervising role throughout my thesis. I learned a lot from you how to precisely ask questions and how to efficiently reply to emails. I am not sure how you manage your workload, and I was always impressed with how quickly and precise you manage to reply to all my E-mails and personal requests. Thank you Lammert Bastiaans, for your support and guidance throughout this project. I was particularly impressed how polite you always remain and how much time, effort and thinking you invest in your communication. I particularly learned from you how to structure and organize my thinking. Thank you to my main supervisor Bernd Leinauer particularly for guiding me through the scientific writing and publishing part.

I would like to thank my father, who tragically and unexpectedly passed away during my time at Wageningen University. He taught me everything I know, particularly on a human level to be kind, generous, caring, and to smile and enjoy life every day. I would like to thank my family for always supporting me.

I would like to thank Kyle Callahan, who was my boss at Victoria National Golfclub for pushing me to my limits every single day I worked for him. Kyle taught me how much I am able to work, to love what I do and to produce a product every day to my best ability. After leaving Victoria National Golfclub, pursuing my master's degree at Imperial College felt like a vacation.

Thanks to the Dutch Golf Federation and all the other associations for financing my PhD and helping me to network. Thanks to Barenbrug for hosting our field experiments and helping with maintenance work.

List of publications

Hahn, D., Sallenave, R., Pornaro, C., & Leinauer, B. (2020). Managing cool-season turfgrass without herbicides: Optimizing maintenance practices to control weeds. *Crop Science*, 60(5): 2204-2220.

Hahn, D., Roosjen, P., Morales, A., Nijp, J., Beck, L., Velasco-Cruz, C. & Leinauer, B. (2021). Detection and quantification of broadleaf weeds in turfgrass using close-range multispectral imagery with pixel- and object-based classification. *International Journal of Remote Sensing*, 42(21): 8035-8055.

Hahn, D., Morales, A., Velasco-Cruz, C. & Leinauer, B. (2021). Assessing competitiveness of Fine Fescues (*Festuca* L. spp.) and Tall Fescue (*Schedonorus arundinaceous* (Schreb.) Dumort) established with white clover (*Trifolium repens* L., WC), daisy (*Bellis perennis* L.) and yarrow (*Achillea millefolium* L.). *Agronomy*, 11(11): 2226. https://doi.org/10.3390/agronomy11112226

Conference papers

Hahn, D. & VanLeeuwen, D. (2019). *Festuca* spp. interfere with germination of Trifolium repens L., Bellis perennis L., and Achillea millefolium L. in controlled environments. *ASA, CSSA and SSSA International Annual Meeting*, San Antonio, USA, November 2019.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)
Herbicide-free turfgrass management: Optimizing maintenance practices to control weeds (2018)

Writing of project proposal (4.5 ECTS)
Non-chemical weed control of turfgrass areas (2018)

Post-graduate courses (0.5 ECTS)
Introduction to R for statistical analysis; PE&RC (2019)

Laboratory training and working visits (2 ECTS)
Soil chemistry training, Mehlich 3 and KCL analysis (2019)

Invited review of (unpublished) journal manuscript (1 ECTS)
Agronomy Journal: Effect of soil water depletion rates, nitrogen and fungicide treatments on turfgrass quality (2019)

Deficiency, Refresh, Brush-up courses (3 ECTS)
Multiple turfnet webinars on: https:www.turfnet.com

Competence strengthening / skills courses (xxx ECTS)
Data management I,II,III (2019)

Understanding difference research perspectives (ethics in science)

PE&RC Annual meetings, seminars and the PE&RC weekend (0.6 ECTS)
PE&RC Day Utrecht: Exploring Sustainability: Now and for the future (2019)

Discussion groups / local seminars / other scientific meetings (xxx ECTS)
Multiple seminars at the Turf Industry Show San Antonio: Microbiology in turfgrass, use of UAV's in precision management, fertilizer development (2018)
ETS field days Padua, Bruno and Manchester (2018 & 2019)
Multiple seminars at the ASA meeting in San Antonio (2019)

International symposia, workshops and conferences (5.8 ECTS)
DTRF conference (2018, 2019, 2020)
German Greenkeeper association meeting Potsdam (2017)
ASA meeting San Antonio (2019)

Supervision of MSc students (3 ECTS)
Konstantinos Orolidis: Pixel based analysis of multispectral images for measuring weed density in turfgrass

About the author

Daniel Hahn was born on January 17th, 1989, in Starnberg, Germany. After finishing high school, he turned playing Golf professional and played in various international tournaments. In 2011, he decided to pursue a bachelor in Turfgrass Science and Management at Myerscough College/ UCLAN University, Preston, United Kingdom. After graduating, he worked as a greenkeeper on top golf courses around the world, including Gc Valley Munich and The Australian Golfclub before becoming an assistant superintendent in training at Victoria National Golfclub, Indiana, USA. Driven by an ambition to reduce the input of pesticides used to maintain top golf courses, he decided to pursue a M.Sc. in Applied Ecology at Imperial College London, United Kingdom. His master thesis was conducted in conjunction with DLF Seeds and Science in Store Heddinge, Denmark. In 2017, the Turfgrass University Research Foundation (TURF) raised money from several Dutch golf federations to finance a PhD position, with the goal to find non-chemical control strategies for weed management in turfgrass. Since 2017, Daniel is a PhD candidate at Wageningen University until September 1st, 2021, at which point he will become a self-employed golf course agronomist. The defense for this thesis is schedule for December 2021.

Cover design Firsova Kateryna

Layout Daniel Hahn

Printing GVO drukkers & vormgevers B.V., Ede, the Netherlands