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Pest Interactions in Agronomic Systems

Festuca sp. interfere with germination and early growth of three weeds

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Abstract

Herbicide restrictions require alternative strategies for turfgrass weed control. This growth chamber study investigated the interference of 27 *Festuca* cultivars selected from five *Festuca* species using white clover (*Trifolium repens* L.), lawn daisy (*Bellis perennis* L.), and yarrow (*Achillea millefolium* L.) as indicator species. At 13 days after sowing (DAS), 20 weed seeds were placed in between 60 grass seeds. Lawn daisy was highly sensitive to the presence of all grasses, and results are not presented. *Festuca* species or individual cultivars did not affect the germination percentage and mean germination period of white clover and yarrow. The presence of tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.]) species reduced white clover root length by 71.6% and slender creeping red fescue [*Festuca rubra* L. ssp. *littoralis* (G.Mey.) Auquier] by 44.5% at 30 DAS. Within cultivars, reductions of white clover roots ranged from 81.7% (Regenerate) to 24.8% (Cathrine). Root length for yarrow was reduced by an average of 75% with no difference among *Festuca* species. Cultivar effects ranged from 91.8% for Barcesar to 62.9% for Samanta. For both white clover and yarrow, negative correlations were determined between *Festuca* biomass and the root length of both weeds: −0.241*** (white clover) and yarrow −0.168* (yarrow). Such a relationship suggests that part of the inhibiting effect can be directly attributed to *Festuca* biomass. We conclude that differences in interference potential between cultivars within species are as important as differences between species. White clover appeared to be the most discriminative species for growth interference studies with *Festuca*.

1 | INTRODUCTION

Increasingly strict bans on herbicide use in amenity turf require alternative weed control strategies to provide esthetic 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

Abbreviations: DAS, days after sowing; F., *Festuca*; FA, tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort]; FGP, final germination percentage; FRA, hard fescue (*F. brevipila* Tracey); FRC, Chewings fescue [*F. rubra* L. ssp. (*F. brevipila* Tracey) (Thuill.) Nyman]; FRR, strong creeping red fescue (*F. rubra* L. ssp. *rubra* Gaudin); FRT, slender creeping red fescue [*F. rubra* L. ssp. littoralis (G.Mey.) Auquier]; MGP, mean germination period.

turfgrass canopy rapidly after sowing, thereby reducing the rate of weed seedling emergence (Islam & Kato-Noguchi, 2016; Masin & Macolino, 2016). After germination, desirable turfgrass species must be strong competitors for light, nutrients, water, and space to maintain dominance over weed species (Holt, 1995; Snaydon & Howe, 1986). Such dominance is achieved by plant species that are best adapted to efficiently exploit limited resources and space (Begon et al., 2007; Weigelt & Jolliffe, 2003).

Among turfgrass species, a specific group within the genus *Festuca*, referred to as fine-leaved fescues, appears to perform well under the preferred low-external input conditions, of which the restrictions in herbicide use are an example. The fine-leaved fescue grasses consist of two complexes or aggregates, *Festuca rubra* L. and *Festuca ovina* L. which are further divided into subspecies (Braun et al., 2020). Generally, fine fescues tolerate moderate shade and acidic soils (Bonos et al., 2006), are drought resistant (Fry & Huang, 2004), and require low inputs of water and nutrients (Dernoeden et al., 1994). Among the *F. rubra* complex, 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). For a detailed description on the fine fescue taxa, please see Braun et al. (2020).

The weed-suppressing ability of fescues was demonstrated in field experiments with 78 fine fescue cultivars from 6 species (Bertin et al., 2009). Particularly, cultivars from the red fescue complex (*F. 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 & Del Moral, 1997; Rice, 2012). Plant–plant interference effects caused by the release of allelopathic chemicals are either direct or secondary, through the microbial decomposition of plant materials (Inderjit & 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 & 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 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-tyrosine as the compound that interfered with the growth of the indicator species (Bertin et al., 2007).

Core Ideas

- Pesticide restrictions require alternative strategies for turfgrass weed control.
- Daisy (*Bellis perennis* L.) was highly sensitive to the presence of 27 cultivars from five *Festuca* species.
- *Festuca* cultivars did not affect the germination percentage and mean germination period of clover and yarrow.
- Root length for yarrow was reduced by an average of 75% with no difference between *Festuca* species.
- Root length of white clover was reduced in the presence of *Festuca* cultivars.

An alternative approach is to investigate the interference between donor and receiver plants 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* L.), and curly cress, on 0.8% water agar (80 mL) in plastic containers (Bertin et al., 2003). Root and shoot lengths of receiver plants were used as indicators of the allelopathic potential of the donor plant. In the presence of all investigated fescue cultivars, the mean root length of crabgrass and cress was 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 the root length of curly cress by 58% compared to the weed-only control (Bertin et al., 2003).

Previous research indicated that some varieties of *Festuca* species inhibit the growth of weed species to varying degrees. However, information is lacking on the ability of *Festuca* species in their growth interference potential against broadleaf weed species during germination and early growth stages. The objective of this study was to study differences in germination between *Festuca* species and varieties. Subsequently, we investigated the variability between and within *Festuca* species on weed seed germination percentage, germination speed, and root length. For this, we included three of the most problematic weeds in European turfgrass areas: white clover (*Trifolium repens* L.), lawn daisy (*Bellis perennis* L.), and yarrow (*Achillea millefolium* L.).

2 | MATERIALS AND METHODS

2.1 | Plant material

A total of 30 fescue (*Festuca* spp.) cultivars were selected from three subspecies, 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 leftover seeds from the first experiment were used. These seeds had been stored in permanent darkness at room temperature (20˚C).

Weed species included *T. repens* L. (white clover), *B. perennis* L. (lawn daisy), and *A. 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., 2003).

2.2 | Growth medium

In both experiments, purified agar with a working strength of 1%, moisture loss on drying less than 7.5%, and 'very low mineral' content (Oxoid purified Agar, Thermo Fisher Scientific) was mixed with Milli *Q* water (Milli-Q, Merck, Darmstadt, Germany) to produce 0.5% water agar. The mixture was then autoclaved at 120˚C for 2 h. Three hundred ninety-six plant tissue containers (Sterivent High Container, 107 by 94 by 96 mm^3, Duchefa Biochemie, Haarlem, the Netherlands) were prepared with 100 mL of water agar each and cooled at room temperature for 1 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.

2.3 | Experimental setup

Three hundred sixty containers (one for each of the 30 cultivars × three weed species × four replicates) were each seeded with 60 grass seeds by using an 8 × 8 matrix sowing template. No seeds were placed at any of the four corners of the matrix. We decided on 60 seeds per container based on recently published literature that reported the use of 50 seeds (Giolo et al., 2019; Goatley et al., 2017). Distance between individual grass seeds was 1.2 cm in all directions. Thirteen days after sowing of grass seeds (DAS), 20 weed seeds were placed randomly but always exactly in the middle of 4 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 7 DAS 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 that 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 × three containers × four replications = 36 control containers).

2.4 | Climate chamber

The climate chamber was set at 16/8 h 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 emitted from fluorescent tubes (Philips TL-D 58 W/840 Reflex, Philips, Amsterdam, the Netherlands). Inside the climate chamber, two 1- by 1-m tables were placed against opposite facing walls, with a walking lane of 80 cm in between. Containers were placed on the tables and their positions on the tables were randomly rearranged every 4 days to account for potential microclimatic differences within the growth chamber and to avoid edge effects. Settings of the second experiment were identical to those of the first experiment.

2.5 | Measurements

Germination of grasses and weeds was recorded every 4 DAS. 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 of the grasses, grass and weed seedlings were removed from the water agar and dried with paper towel. Total biomass of grasses within a container (g), root length (cm), and shoot length (cm) of individual weed plants were recorded. For root length, the total length of all roots, including the length of secondary roots, was summed up.

Mean germination time was calculated following Orchard (1977) using four consecutive time periods of 4 days (referred to as 1–4), for which germination was counted every fourth day. The result was expressed as mean germination period (MGP), indicating the average time period for which germination occurred:

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

where $n1$ is the total number of seeds germinated in period one (1–4 DAS), $n2$ is the total number of seeds germinated in period two (5–8 DAS), $n3$ is the total number of seeds germinated in period three (9–12 DAS), $n4$ is the total number of seeds germinated in period five (12–16 DAS), and N is the 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).

2.6 | Data analysis

Analyses for all *Festuca* and weed variables were conducted with experiment and interactions with experiment as random effects. Cultivars were treated as nested within *Festuca* species. *Festuca* variables included weed as a fixed factor and adjusted the weed main effect test as recommended in VanLeeuwen et al. (2013). 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 among species and cultivars within species when differences were detected. 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 I were computed, and p-values are reported. For Yarrow, one replicate of cultivars Dumas 1 and Charlotte exhibited unexplainable long root growth, which was noticeably different from all other entries. The same was true for one replicate of Dumas 1 and Mentor with white clover. The studentized marginal residuals had magnitudes of greater than four. Consequently, these entries were identified as true outliers and removed. Nontheless, we present the results with outliers included for white clover and yarrow root length in the supplemental material. Analyses were performed using SAS version 9.4 software with updated SAS/STAT 14.3 (SAS Institute, 2017), and significance was defined at $p \leq 0.05$.

TABLE 1 p and F-values and degrees of freedom from analysis of variance to test the effects of weed species (Weed), *Festuca* species (Species), *Festuca* cultivars within species and their interactions on *Festuca* germination percentage at 30 days after sowing, as obtained from a replicated growth chamber experiment.

Effect	ndf	ddf	F value	p Value
Weed	1	27.14	0.78	0.3836
Species	4	25.86	5.73	0.0019
Species × weed	4	27.20	0.70	0.5958
Cultivar (species)	22	25.84	6.26	<0.001
Cultivar × weed (species)	22	27.03	1.09	0.4084

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 lawn daisy were removed, because in the presence of *Festuca* species, lawn daisy plants appeared photobleached, and it was not possible to record root length.

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 1). The type of weed species did not influence the FGP of species or cultivars.

The FGP of species ranged from 93.2% (Standard Error [SE] ± 1.7%) for hard fescue to 86.4% (SE ± 0.8%) for slender creeping red fescue (Figure 1). Final germination percentage of strong creeping red fescue (FRR), FRA, and Chewings fescue (FRC) was greater compared to that of FRT, whereas FGP of tall fescue (FA) did not differ from any of the other species.

Full germination percentage among cultivars varied from 96.8% (SE ± 2.3%) (Barcrown, FRT) to 71.1% (SE ± 2.4%) (Samanta, FRT) (Figure 1). Within all species, except for FRA, which was only represented by two cultivars, the FGP of cultivars within species varied.

MGP did not differ among species ($p = 0.1661$) and ranged from 1.6 (SE ± 0.1) for FRA to 1.9 (SE ± 0.1) for FA (data

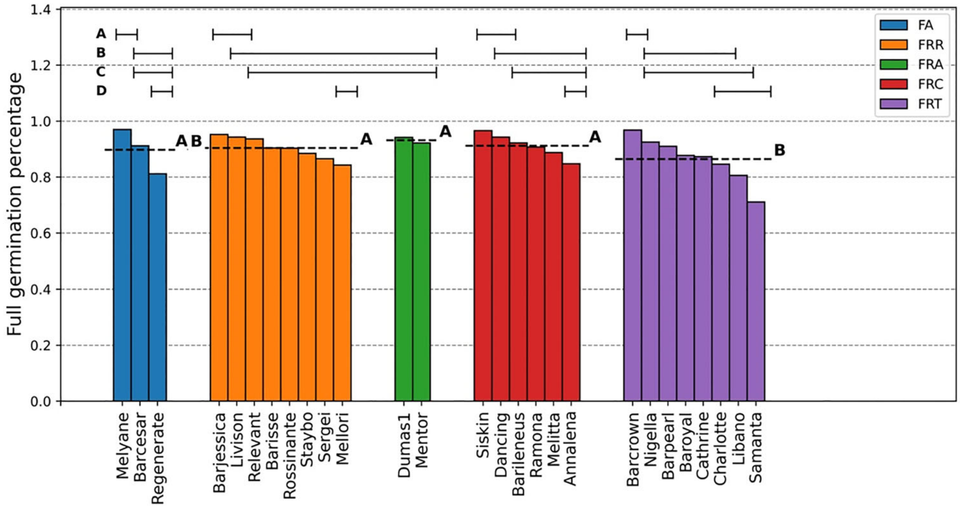

FIGURE 1 Final germination percentage (FGP) of 27 *Festuca* cultivars grown on water agar at 30 days after sowing, grouped according to species (FA, tall fescue; FRR, strong creeping red fescue; FRA, hard fescue; FRC, Chewings fescue; FRT, slender creeping red fescue). Dotted horizontal lines indicate species averages, and solid lines indicate shared cultivar mean separation letters. Means sharing the same letter are not significantly different from one another at the 0.05 probability level according to Tukey's honestly significant difference test.

not shown). Significant differences were observed among individual cultivars (Table 2).

FRR cultivars Livison and Sergei and FRC cultivars Melitta and Ramona had an MGP close to 1.5 or below and germinated faster than Mellori (FRR) and Baroyal and Libano (both FRT), which had an MGP just above 2 (SE for individual cultivars ± 0.1).

Biomass data for *Festuca* species and cultivars at 30 DAS was based on observations in containers, including weeds (yarrow and white clover), which were introduced at 13 DAS. Although weed species did not impact *Festuca* biomass ($p = 0.87$), the *Festuca* species × weed species interaction had a p value of 0.07 (Table 3). Species and cultivars within species differed in the amount of developed biomass after 30 DAS (Table 3 and Figure 2).

Average biomass weight of FA (1022.3 [SE ± 29.6] mg) was higher compared to all other species. Even though FRR was also higher than the other three species, it produced less than 50% of the biomass of FA (488.8 [SE ± 28.5] mg). The other three species, FRA (435.8 [SE ± 30.6] mg), FRC (409.2 [SE ± 28.7] mg), and FRT (404.4 [SE ± 28.5] mg), had similar biomass (Figure 3).

Differences in biomass among cultivars within species were observed for FRR, FRC, and FRT, which were also the species with most cultivars. Within FRR, the cultivar Relevant (587.5 [SE ± 33.1] mg) developed most biomass, which was 33.9% more compared to the FRR cultivar with the lowest biomass (Sergei, 388.1 [SE ± 33.1] mg). Within FRC and FRT, the difference among the cultivars with the highest and lowest biomass was 24.4% and 25.1%, respectively.

A correlation analysis was conducted to investigate the association between germination and biomass. A higher germination percentage of *Festuca* was associated with an earlier MGP and more biomass (Table 4).

3.2 | *Festuca* growth interference with white clover

White clover FGP in the presence of *Festuca* grasses was similar to that of the white clover control (FGP = 94.8% [SE ± 1.9%]) and ranged from 91.2% (SE ± 1.0%) in the presence of FRR to 89.0% (SE ± 1.6%) in the presence of FA (data not shown). Moreover, no cultivar differences were observed for white clover FGP and ranged from 94.4% (SE ± 2.8%) (Charlotte, FRT) to 86.9% (SE ± 2.8%) (Regenerate, FA). Similar to the white clover control, white clover in the presence of *Festuca* also scored an MGP of 1.0 (data not shown).

White clover root length was affected both by the presence of *Festuca* species ($p < 0.001$) and cultivars ($p < 0.001$). White clover root length in the presence of all species was shorter compared to the control. Negative impacts on white clover root length were strongest in the presence of FA, which reduced root length by 71.6% (3.1 [SE ± 0.2] cm) compared to the control (10.9 [SE ± 0.3] cm) (Figure 3). Root length of white clover seedlings germinating with either FRR, FRA, or FRC did not differ and averaged between 4.4 (SE ± 0.2) cm (FRC) and 4.6 (SE ± 0.3) cm (FRA). White clover root length was affected the least by FRT and reached 6.02 (SE ± 0.2) cm but was still reduced by 44.5% compared to the control.

For 20 out of 27 cultivars, white clover root length was more than 50% reduced compared to the control. Among the seven cultivars with the lowest growth-reducing effect on root

TABLE 2 Mean germination period (MGP) of 27 *Festuca* cultivars representing Chewings fescue [FRC, *Festuca rubra* L. ssp. Fallax (Thuill.) Nyman], slender creeping red fescue [FRT, *F. rubra* L. ssp. Littoralis (G.Mey.) Auquier], strong creeping red fescue FRR, *F. rubra* L. ssp. Rubra Gaudin), hard fescue (FRA, *Festuca brevipila* Tracey), and tall fescue (FA, *Schedonorus arundinaceus* (Schreb.) Dumort., nom. Cons.).

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

Note: Germination of each seed was examined every fourth day after sowing, and MGP was calculated by defining each 4-day interval as period (1–4 days = Period 1; 5–8 days = Period 2; 9–12 days = Period 3). Smaller values indicate earlier germination. Values in each column followed by the same letter are not significantly different at the 0.05 probability level according to Tukey's honestly significance difference test.

length, five were of FRT. Within the group of cultivars affecting white clover root length the most, four reduced white clover root length to below 3 cm: Relevant (2.6 [SE ± 0.4] cm, FRR), Ramona (2.3 [SE ± 0.4] cm, FRC), Rossinante (2.1 [SE ± 0.4] cm, FRR), and Regenerate (2.0 [SE ± 0.4] cm, FA). Significant differences between cultivars within species were present for FRR, FRC, and FRT. Only within the species that were represented by just two of three cultivars (FA and FRA) cultivar differences were not observed.

TABLE 3 *p* and *F*-values and degrees of freedom from analysis of variance to test the effects of weed species (Weed), *Festuca* species (Species), *Festuca* cultivars within species and their interactions on biomass of grasses at 30 days after sowing, as obtained from a replicated growth chamber experiment.

Effect	ndf	ddf	*F* value	*p* Value
Weed	1	1	0.05	0.8664
Species	4	370	729.69	<0.001
Species × weed	4	370	2.16	0.0731
Cultivar (species)	22	370	8.22	<0.001
Cultivar × weed (species)	22	370	0.54	0.9580

Shoot length of white clover control plants averaged 3.2 (SE ± 0.1) cm and differed from shoot length when *Festuca* species (2.3 cm on average) were present. Among species, only FA (2.1 SE ± 0.1) and FRT (2.5 SE ± 0.1) differed. Within species, cultivars differed for FA ($p < 0.01$), FRC ($p < 0.05$), and FRR ($p < 0.001$). Cultivar differences ranged from 2.74 (SE ± 0.2) cm for Barisse to 1.63 (SE ± 0.2) cm for Relevant. Differences were more pronounced for root length data; therefore, data for shoot length is not presented in more detail.

A correlation analysis showed that the biomass of *Festuca* had a significant negative association with the root length of white clover (Table 5), whereas FGP and MGP of white clover had no relationship with *Festuca* biomass.

3.3 | *Festuca* growth interference with yarrow

None of the *Festuca* species or cultivars had a significant effect on the FGP or MGP of yarrow. For FGP, species ranged from 97.3% (SE ± 1.3%) (FRA) to 96.3% (SE ± 1.1%) (FA), and cultivars ranged from 98.8% (SE ± 1.6%) (Libano, FRT) to 94.3% (SE ± 1.7%) (Samanta, FRT) (data not shown). Control treatments averaged 97.7% (SE ± 1.3%). MGP of all species was 1.1 (SE ± 0.04), and among the cultivars, MGP ranged from 1.2 (SE ± 0.04) for Regenerate (FA) to 1.0 (SE ± 0.04) 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 by at least 75% compared to the control (15.9 [SE ± 0.6] cm). No differences in yarrow root length among *Festuca* species were observed, with root length ranging from 3.8 (SE ± 0.5) cm (FRT) to 2.4 (SE ± 0.6) cm (FA) (Figure 4).

Differences were observed among individual *Festuca* cultivars, and all cultivars generated a significant reduction in yarrow root length. Yarrow root length was the shortest (5.9 [SE ± 0.6] cm) in the presence of FRT cultivar Samanta,

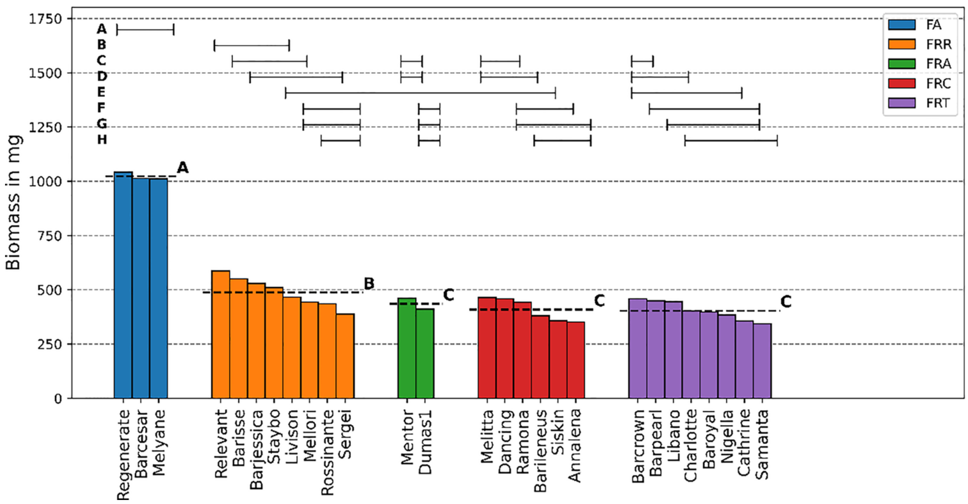

FIGURE 2 Biomass (mg/container) of 27 *Festuca* cultivars grown on water agar for 30 days in a controlled environment, grouped by species (FA, tall fescue; FRR, strong creeping red fescue; FRA, hard fescue, FRC, Chewings fescue; FRT, slender creeping red fescue). Data were averaged over weed species (white 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 at the 0.05 probability level according to Tukey's honestly significant difference test.

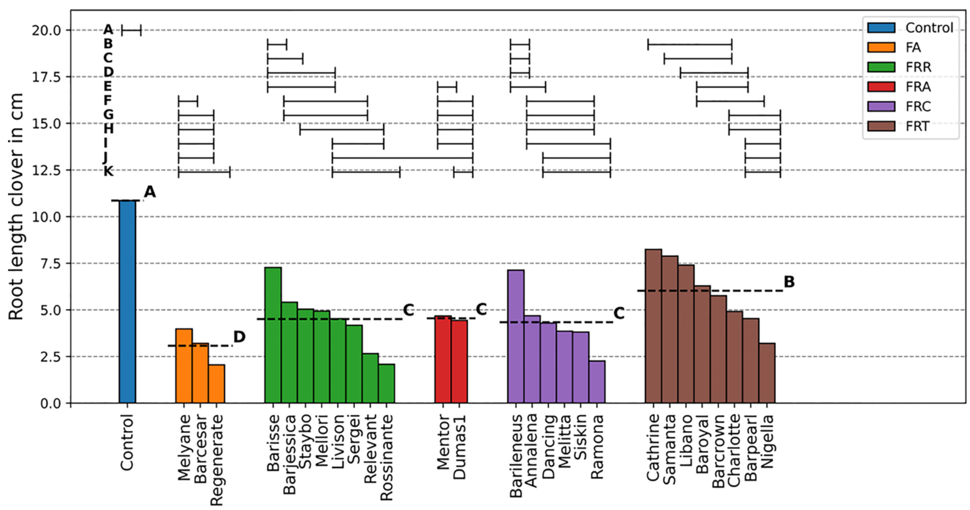

FIGURE 3 Root length of white clover (*Trifolium repens* L.), grown together with 27 *Festuca* cultivars or as stand-alone control on water agar for 30 days in a controlled environment, grouped by species (FA, tall fescue; FRR, strong creeping red fescue; FRA, hard fescue; FRC, Chewings fescue; FRT, 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 at the 0.05 probability level according to Tukey's honestly significant difference test. Two outliers were removed from the dataset as identified by means of studentized marginal residuals (magnitudes of greater than four).

yet still 63% shorter than the control. The reduction in root length of this cultivar was less than that of 14 cultivars that resulted in a yarrow root length of 3 cm or lower. The presence of Barcesar (FA) resulted in a yarrow root length of 1.4 (SE ± 0.6) cm, which was different from seven *Festuca* cultivars that had associated yarrow root lengths of 4.1 cm or longer.

Except for cultivars within FRA and FRR, there were significant differences in growth interference among cultivars within the other species. Tall fescue cultivar Regenerate had less of an effect on yarrow root growth (4.1 [SE ± 0.6] cm) compared to Barcesar (1.3 [SE ± 0.6] cm). Within FRC, the cultivar differences ranged from Barileneus with 4.8 (SE ± 0.6) cm yarrow root length to Melitta with 2.0

TABLE 4 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.

(SE ± 0.6) cm. Within FRT, yarrow root length ranged from 5.9 (SE ± 0.6) cm (in the presence of Samanta) to 2.0 (SE ± 0.6) cm (in the presence of Barpearl).

A correlation analysis was conducted to investigate the association of *Festuca* biomass on yarrow FGP, MGP, and root length (Table 5). *Festuca* biomass was negatively associated with MGP and weed root length, indicating that *Festuca* species with greater biomass production were associated with the advanced germination of yarrow and reduced yarrow root length.

Average shoot length of yarrow control did not differ from shoot length in the presence of *Festuca* species. Within species, differences were observed between cultivars except for the two cultivars within FRA ($p > 0.05$). Cultivars varied from 2.56 cm for Regenerate to 1.41 cm for Barcesar. The trends observed for shoot length were largely identical to those observed for root length, except that more pronounced differences were observed for root length. For this reason, data for shoot length is not presented in more detail.

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 (He et al., 2012; Inderjit & Del Moral, 1997; Scavo et al., 2018), but to date, designing an experiment that convincingly separates effects of each has proven to be difficult. As resource competition is prominent under field conditions, determining allelopathic potential is often attempted under laboratory conditions, with a minimal supply of nutrients. Because 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). Our experiment was conducted to study such a type of plant–plant interference.

Examining several different fine-leaved cultivars from different species allowed us to compare between and within species variability in interference potential. Differences in a number of cultivars among species are not ideal for such an analysis but resulted from our intention to focus on cultivars commonly used in athletic fields and golf course fairways. We observed that all *Festuca* species reduced weed root growth. As in previous experiments, shoot length was also measured as an indicator for growth interference.

The objective in this study was to examine the growth-interfering effects of *Festuca* on germination and early growth of common weed species and growth-interfering plant exudates are mainly produced at the ends of actively dividing fibrous roots as shown by Bertin et al. (2003). Our results are consistent with these findings and indicate that root growth is a better indicator for growth interference studies with turfgrasses. Therefore, the discussion focuses on root length data only. In the presence of *Festuca* species and compared to controls, the average root length of white clover was reduced by 56%, and yarrow root length was reduced by 79%. Data on lawn daisy was excluded from further analysis because the presence of *Festuca* species caused bleaching of lawn 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 between weed species. This was also observed by Bertin et al. (2003), who showed the root and shoot suppression of large crabgrass (*D. sanguinalis*) in the presence of *Festuca* species of up to 80% and only up to 58% for curly cress (*L. sativum* L.).

White clover root length was differentially impacted by the different turfgrass species, with FA having the greatest suppression and FRT having the least. Along with the differences among species, we also observed significant differences among cultivars within FRC, FRR, and FRT. We saw no overall differences among *Festuca* species for yarrow root length; however, significant differences were detected among cultivars within FA, FRC, and FRT. We conclude that cultivar differences in interference potential are more prominent than species differences within *Festuca*. This suggests that when selecting a *Festuca* with high interference potential, one is not necessarily restricted to a specific *Festuca* species, as presence and variability in interference potential can be found in several of the species.

Based on our findings, we suggest that white clover is a better indicator species to screen for the growth interference potential of *Festuca* species on weed characteristics such as root length. 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. Greater biomass production implies a stronger metabolic activity, and proportional to the metabolic activity an increased interference potential can be postulated. In fact, this hypothesis suggests that interference is not the result of specific compounds that are produced in higher quantity in one cultivar compared to another but results from more general compounds that are simply produced in a quantity proportional to growth rate. To investigate this premise, we

TABLE 5 Spearman's correlation investigating the degree of association between biomass of 27 *Festuca* cultivars and three white clover (*Trifolium repens* L.) and yarrow (*Achillea millefolium* L.) characteristics: full germination percentage (FGP), mean germination period (MGP), and root length.

	FGP		MGP		Root length	
Variables	Clover	Yarrow	Clover	Yarrow	Clover	Yarrow
Festuca biomass	n.s.[†]	n.s.	n.s.	−0.158*	−0.241***	−0.168**

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels. [†]ns, not significant.

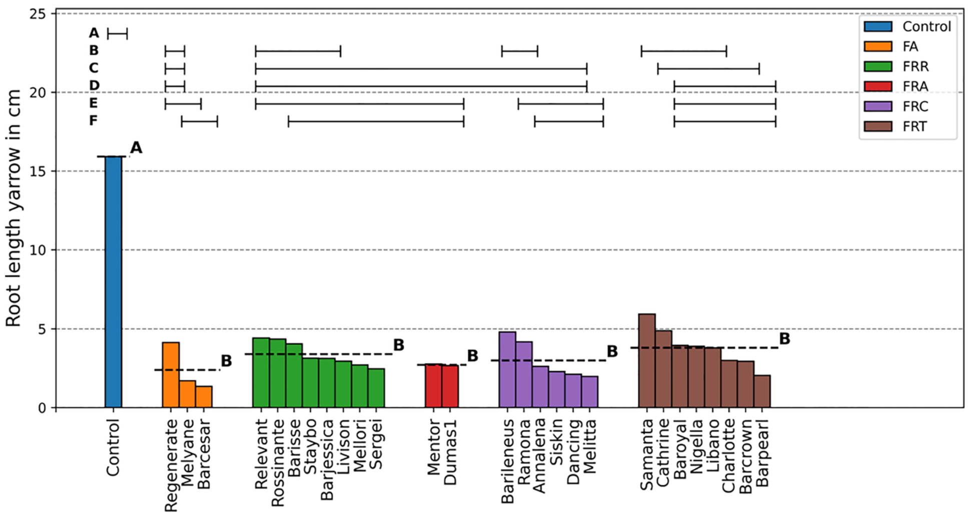

FIGURE 4 Root length of yarrow (*Achillea millefolium* L.), grown for 30 days on water agar in a controlled environment together with 27 *Festuca* cultivars or as a stand-alone control, grouped by species (FA, tall fescue; FRR, strong creeping red fescue; FRA, hard fescue; FRC, Chewings fescue; FRT, 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 different at the 0.05 probability level according to Tukey's honestly significant difference test. Two outliers were removed from the dataset as identified by means of studentized marginal residuals (magnitudes of greater than four).

determined the biomass of the different cultivars. Tall fescue (FA) produced the highest amount of biomass, about two times more than the next highest producing species (FRA) and approximately 2.5 times more than the other three species. Consequently, the strongest reduction in white clover root length was observed in the presence of FA. Interestingly, such a relation was not observed with yarrow, for which all Fescue species had an equal inhibitory effect on root length. The correlation analyses revealed an indirect relationship of *Festuca* biomass with white clover root length (−0.241***) and yarrow (−0.168*). Such a correlation indicates that Fescue species generally interfered with root growth of both white clover and yarrow, but to a greater degree with white clover and to a lesser with yarrow. However, the relatively low correlation coefficients further indicate that factors other than biomass contribute to such a relationship.

In addition to examining the effect of *Festuca* species and cultivars on weed root length, we investigated their influence on weed seed germination. We modified the experimental design of Bertin et al. (2003) and increased the establishment period of grasses from 7 to 13 days. Such an extended period would allow for the production and release of more allelopathic compounds. 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). Such a result could possibly indicate that *Festuca* species only influence weed growth processes but do not affect germination. The results might also be explained by the fact that, despite our modifications, the quantity of root exudates reaching the weed seeds was insufficient to influence development and growth. 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.). The 4-day observation period that was selected to quantify germination speed could also be considered insufficient to adequately assess weed interference. Our results show that all white clover seeds germinated within 4 days (MGP = 1), as did most of the yarrow seeds (MGP between 1 and 1.2). The evaluation interval should have been shorter, and reduced observation intervals are recommended for future studies (Supporting Information).

Nonetheless, to screen for the allelopathic potential of cultivars under controlled conditions independent from complex interactions that are faced in the field has several benefits. Proving allelopathy requires demonstration that plant exudates are produced by a donor plant, released into the environment (air or soil), absorbed by a receiver plant and ultimately cause a growth-interfering effect (Duke, 2015). Examining each individual step is possible in controlled conditions but immediately leads to 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 (i.e., root length reductions of more than 90%) may not be detected under field conditions. Are cultivar rankings for weed interference established under controlled climate conditions indicative of performance in the field? Published studies addressing this question are inconclusive. In a study on the weed suppressive ability of rice cultivars, Olofsdotter et al. (1999) found a weak correlation of root length reduction of barnyard grass [*Echinochloa crus-galli* (L.) Beauv.] between laboratory and field performance in rice. Contrary to Olofsdotter et al. (1999), Bertin et al. (2003) showed that the cultivar Rescue 911 (hard fescue) had only a minor effect on weed growth suppression of large crabgrass under controlled conditions but performed well in field trials (Bertin et al., 2003, 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 the growth interference potential of *Festuca* species with common turf weed species, and that this trait varies widely among cultivars within species.

AUTHOR CONTRIBUTIONS

Daniel Hahn: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; writing—original draft. **Bernhard Leinauer**: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; supervision; writing—review and editing. **Lammert Bastiaans**: Conceptualization; investigation; methodology; resources; supervision; validation; writing—review and editing. **Dawn M. VanLeeuwen**: Formal analysis; investigation; methodology; software; validation; writing—review and editing

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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