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TITOLO TESI

Germination, seedling growth and establishment of warm-season turfgrasses related to climate changes in the Mediterranean region

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Maurizio Giolo 29 settembre 2019)

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Summary

The Mediterranean region is located from the arid climate of North Africa up to the temperate and rainy climate of northern and central Europe. Climate simulations show a gradual increase in heating, drying and precipitation variability in this region. These effects will probably push warm-season turf species towards minor latitudes.

The consequences of climate change on turfgrass management have been investigated only recently. Germination and seedling growth have also been little studied for the response to climate change effects, although the establishment phase is central to any high-performing turfgrass. For warm-season grasses in the transition zone, a quick establishment increases winter survival and allows for earlier spring green-up and a more rapid recovery from winter injury.

In the Mediterranean region, warm-season grasses are commonly seeded in late spring when the temperatures are suitable for a rapid establishment. Warm-season species used extensively by the turf industry have fertile and sterile cultivars. Seeded cultivars are usually preferred over sterile cultivars because seeding is cheaper and easier to practice than vegetative propagation methods which include plugging, sprigging, and stolonizing.

The present study concerns two of the most used warm-season turf species in the Mediterranean area: Bermudagrass (*Cynodon* spp.) and seashore paspalum (*Paspalum vaginatum* Swartz) and one potential future new species i.e.buffalograss *[Buchloe dactyloides* (Nutt.) Engelm.].

The research activity scheduled consisted of five experiments conducted in the field and controlled environments (growth chamber and greenhouse) to investigate the effects of suboptimal temperatures and excess of water due to flooding on seed germination and seedling growth.

The main results were the following:

Effects of sub-optimal temperatures

The warm-season grasses tested germinated at a temperature equal or higher than 11.4 °C (base germination temperature) and early April is the optimal seeding time for establishing by seed warm-season turfgrasses in the Po-Venetian valley. From these results, we have concluded that the predicted warmer springs should lead to earlier seeding of warm-season grasses favouring turfgrass maturation before winter.

Effects of water excess

All three warm-season turf species under study tolerate flash waterlogging. However, bermudagrass is the one less affected by prolonged waterlogging (6-8 days). In general, warm-season grasses can be successfully used for establishing turfgrasses considering the future climate change predicting the increase of extreme precipitation events.

Riassunto

La regione mediterranea è ubicata fra il clima arido del Nord Africa ed il clima temperato e piovoso dell'Europa settentrionale e centrale. Le previsioni sui cambiamenti climatici nei prossimi anni presentano un graduale aumento delle temperature e delle condizioni di aridità, come pure l'aumento di eventi piovosi intensi e localizzati. Per effetto di questi cambiamenti climatici si prevede un crescente impiego di specie macroterme (C_4) a latitudini minori comprese quelle utilizzate per la formazione di tappeti erbosi.

Le conseguenze sulla gestione del tappeto erboso sono state studiate solo di recente ed inoltre mancano risultati scientifici riguardo gli effetti prodotti dai cambiamenti climatici sulla germinazione e sulle prime fasi di crescita delle plantule.

Nella regione mediterranea, le poacee macroterme vengono seminate in tarda primavera, quando le temperature consentono una pronta germinazione ed un rapido insediamento del tappeto erboso. Queste specie presentano sovente cultivar fertili e cultivar sterili che possono essere insediate unicamente per via vegetativa (*plugging* e stolonizzazione). Le cultivar da seme sono generalmente preferite alle cultivar sterili essendo la semina più economica e più facile da praticare di un insediamento per via vegetativa. Le fasi di insediamento del tappeto erboso seminato sono particolarmente importanti nella gestione dei tappeti erbosi in zone di transizione. Un rapido insediamento, infatti, consente alle specie macroterme di affrontare in condizioni ottimali i rigori invernali e riprendere rapidamente a vegetare in primavera. Il presente studio ha preso in esame due delle specie macroterme da tappeto erboso più utilizzate nell'area mediterranea: gramigna (*Cynodon* spp.) e paspalum vaginatum (*Paspalum vaginatum* Swartz) e una specie che potrebbe trovare possibilità di impiego nei prossimi anni e cioè buffalograss *[Buchloe dactyloides* (Nutt.) Engelm.].

L'attività di ricerca nell'ambito del dottorato prevedeva cinque esperimenti condotti in campo e in ambiente controllato (camera di crescita, serra), finalizzati allo studio degli effetti delle temperature sub-ottimali e dell'eccesso idrico, dovuto ad eventi piovosi intensi, sulla germinazione dei semi e sulla crescita delle plantule delle suddette specie macroterme.

I risultati più significativi sono di seguiti riportati:

<u>Effetti di temperature non ottimali</u>

È stato dimostrato che la germinazione delle specie macroterme testate avviene solamente quando la temperatura è uguale o superiore a 11,4 °C e che l'inizio di aprile può essere considerato il momento ottimale per la semina di tali specie nella pianura padano-veneta. L'incremento termico previsto nei prossimi anni dovrebbe consolidare tali risultati. Una semina ad inizio aprile, diversamente da quanto solitamente avviene (metà-maggio) consente di completare l'insediamento

entro maggio, così il tappeto erboso avrà a disposizione l'intera estate per maturare e affrontare nel migliore dei modi l'inverno.

Effetti dell'eccesso idrico

Le nostre ricerche hanno evidenziato la spiccata attitudine delle tre specie oggetto di studio a sopportare il ristagno idrico. In particolare, la gramigna è risultata la specie più tollerante all'eccesso idrico nel suolo durante l'insediamento, conservando la germinabilità ed una buona crescita delle plantule anche dopo 6-8 giorni di sommersione.

In generale, possiamo affermare che le specie macroterme da tappeto erboso possono essere di grande interesse per la realizzazione di tappeti erbosi nelle condizioni prospettate dai futuri scenari legati al cambiamento climatico.

Chapter 1

Introduction

Climate changes in the Mediterranean region: effects on turfgrass management

The Mediterranean region is located from the arid climate of North Africa up to the temperate and rainy climate of northern and central Europe (Giorgi and Lionello, 2008). Hot and dry summers and mild, wet winters characterize this region that roughly coincides with the transition zone for turfgrasses, and it is considered one of the most sensitive areas to climate change, global and regional climate simulations show a gradual increase in heating and drying. In particular, an increase in temperature and precipitation variability is expected (Moatti and Thiébault, 2018). Many pieces of evidence of changes underway in the main environmental parameters were found by the use of models, and a sharp increase in drought in the Mediterranean region with a decrease in average annual rainfalls was shown (Giorgi and Lionello, 2008; Olesen, 2011).

The decrease in average precipitation and the increase in unusual negative phenomena such as their intensity and the frequency of drought events are likely (Ciardini *et al.*, 2016; Moatti and Thiébault, 2018).

Human activities are considered responsible for climate change, which impact is estimated to be about 1.0 °C of the global warming above the pre-industrial level.

Climate change is destined to rise in the future, and it seems to reach 1.5 °C between 2030 and 2052, increasing at the current rate with a growth in the frequency of intense rainfall with relative floods and warmer winters (Carraro *et al.*, 2007; Intergovernmental Panel on Climate Change, 2018). Med-CORDEX (Coordinated Regional Climate Downscaling Experiment), an initiative of the scientific community that studies the weather prediction models, have elaborated some projections on future changes of climate in Italy (Desiato *et al.*, 2015, 2019).

Over a century, a steady increase in average temperature is expected between 2.5 °C and 4.4 °C. The escalation is caused both to the rise in the maximum daily temperatures and the minimum night temperatures. The variations provided by the models seem to affect the entire Italian territory even if the most significant increase in average temperature is expected in the summer season.

All models agree to indicate a reduction in the days with frost and an increase in tropical nights. Rainfall projections are much more uncertain than temperatures and indicate in the future, an increasing concentration of precipitation with more intense and less frequent events.

The effects of climate change affect plants by the duration of the leaf area and the photosynthetic efficiency controlled even with the closure of the stomata which can be strongly influenced by precipitation and the availability of soil water (Olesen and Bindi, 2002).

The C_4 plants, also known as warm-season plants, show better efficiency in the use of nitrogen compared with C_3 plants since they have a higher rate of photosynthesis per unit of nitrogen in the leaf (Gowik and Westhoff, 2011). Generally, the C_4 photosynthesis is an adaptation of the C_3 pathway which allows overcoming the limitations of the photorespiration by improving photosynthetic efficiency and minimizing the water loss during exposure of hot and dry conditions (Edwards and Walker, 1983; Gowik and Westhoff, 2011).

Looking ahead, the cool-season species (C3) in the Mediterranean region, which are less photosynthetically efficient, will be gradually more and more disadvantaged compared to C_4 species due to lack of water and temperature increase and especially during warmer months (Olesen and Bindi, 2002).

As expected for the USA (Hatfield, 2017) also in Europe the effects of global change will probably favour the plants that are better able to exploit the increase of CO_2 concentration favouring and pushing warm-season turf species towards minor latitudes (Olesen and Bindi, 2002).

Role and perspectives of warm-season turf species

Among utility functions of turfgrass, a relaxing environment that expresses naturalness and beauty is essential, especially in high-density urban areas. The climatic changes in progress that probably will continue in the coming decades lead us to reflect on the most suitable species to grow and develop in environmental situations different from the past. Many of the benefits of using turfgrass can help to mitigate the negative aspects associated with climate changes: protection of the soil from erosion, flood control, lowering runoff caused by rainfalls and mitigation of temperature (Beard and Green, 1994).

In the Mediterranean transition zone is possible the cultivation of both warm- and cool-season grasses, however, cool-season species are preferred over warm season-species even though the use of the latter has been encouraged over the last decade to reduce cultural inputs (Sever Mutlu *et al.*, 2011; Rimi *et al.*, 2011, 2013a; b). Warm-season grasses have optimum growth at temperatures between 27°C and 35°C that in transition zones this range corresponds to the period from late spring to late summer. Warm-season species go dormant in winter, and their leaves become yellow, the loss of colour during the winter months has been a significant impediment for more widespread use of these species. Conversely, cool-season species prefer cooler temperatures (18 - 24°C), have summer and winter dormancy but remain green during the cooler months (Romero and Dukes, 2016). Compared to cool-season species, warm-season species generally have a higher tolerance to drought, traffic, and fungal diseases (Puhalla et al., 1999) and use water more efficiently (Huang *et al.*, 2008; Romero and Dukes, 2016).

The difference in water use between C_3 and C_4 species mainly depends on differences in photosynthetic process and in leaf anatomy that produces higher efficiency of the C_4 species maintaining a high level of carbohydrates and growth capacity even when their stomates are partially closed. The C_4 photosynthetic pathway produces a four-carbon intermediate compound, the Oxaloacetate quickly converted to the C_4 acids malate or Asp, using the bundle sheath cells. This specialization produces a higher concentration of carbon, making C_4 organisms more suitable for warm habitats with low water availability (Gowik and Westhoff, 2011).

The general increase of temperatures account for a shift of both cool- and warm-season grasses towards Northward. However, the interactions between temperature and precipitations do not allow to predict the distribution precisely (Hatfield, 2017). The heat stress has a strong impact on the root system of plants, which is essential for extracting water and nutrients from the soil. Growth and root system development are affected by temperature variations and soil humidity levels (Waisel *et al*., 2002). The increase of temperatures reduces root growth and viability with a decrease in turfgrass quality (Huang *et al*., 1998). The highly stressed turf requires specific cultural practices influencing plant physiology. Heat damage to plants, due to high air or soil temperature, interrupts the water balance between shoots and roots, the transpiration of the leaves increases and the absorption of water from the roots could be inhibited. Furthermore, mowing height also produces significant effects on plant physiology: low mowing causes an increase in the respiratory rate of the entire plant (Huang, 2000). Light water distribution, in addition to regular irrigation, can be useful to cool turfgrass canopy to prevent excessive heat stress by evaporation. Although warm-season grasses show tolerance to heat stress, significant differences in their physiological response are documented, they also acquire thermotolerance due to exposure to high continuous temperatures (Pompeiano *et al.*, 2013). Different climate conditions in the Mediterranean region associated with a great variety of soils and the range in elevation do not allow to have grass best suitable for all situations (Goatley *et al.*, 2008). Warm-season grasses, however, offer a wide choice of cultivars to respond to the complex problems of turfgrasses in different cases. The main warm-season turf species include bermudagrass (*Cynodon* spp.), seashore paspalum (*Paspalum vaginatum* Swartz), bahiagrass (*Paspalum natatum* Flueggé), centipedegrass [*Eremochloa ophiuroides* (Munro) Hack], and buffalograss *[Buchloë dactyloides* (Nutt.) Engelm.] usually established by seed and zoysiagrass (*Zoysia* spp.), St. Augustinegrass [Stenotaphrum secundatum (Walter) Kuntze] generally vegetatively propagated (Christians, 2003).

Species studied and their behaviour in transition zones

The present study concerns two of the most suitable warm-season turfgrass species for the Mediterranean area: bermudagrass and seashore paspalum (Volterrani *et al*., 2001, 2008; De Luca *et al*., 2008), and buffalograss, a low-maintenance grass for light traffic widely used in the US that could also be successfully used also in Europe for low-maintenance turfgrasses. These species are characterized by the C_4 pathway, high tolerance to different types of stress, and an excellent recuperative potential due to vigorous stolons and rhizomes (Volterrani *et al*., 2012). Bermudagrass and seashore paspalum have been chosen because they are widely used in the Mediterranean transitional environment, and they can be propagated by seed. Bermuda grass is the most cultivated species for turfgrass, due to its adaptability and tolerance to numerous abiotic stresses. On the turf market are available fertile cultivars for seeding establishment and intraspecific hybrids obtained with the cross between *C. dactylon* var. *dactylon* and *C. transvaalensis*, which are sterile and should be propagated by sprigs, sod or plugging (Stier *et al.*, 2013).

There are numerous cultivars available for various uses: athletic fields, golf courses, recreational areas, and ornamental turf (Volterrani et al., 1997; Croce *et al.*, 2001, 2004).

Sports turfs require recommended varieties that often cannot be established by seed (Goatley *et al.*, 2008; Volterrani *et al.*, 2008).

The information on the different commercial cultivars of seashore paspalum and their performances in the Mediterranean transition area is lacking and largely not conclusive. Mediterranean transition zone includes at least two types of climates: Mediterranean hot summer climate (Csa) and Mediterranean warm/cool summer climate (Csb). In the latter adaptation may be difficult due to long thermal time and slow establishment (De Luca *et al., 2008; Giolo et al., 2019)*. Seed propagated and vegetatively propagated cultivars are available, usually by sprigs or sod cultivars (Stier *et al.*, 2013).

Buffalograss can be considered a new entry species for the Mediterranean region. Commercially are available vegetative types and seeded types, the new turf-type cultivars are dark green, short-growing and produce dense cover. Moreover, they have excellent resistance to weed invasion (Koski and Cox, 2014).

The presence of these three species in the European turfgrass market is quite different: bermudagrass is the best-known warm-season grass in Europe (Volterrani *et al.*, 1997, 2012; Rimi *et al.*, 2011) with several varieties registered in the Official Catalogs (OECD, 2019) seashore paspalum is also widely used in the Mediterranean countries of Europe which is particularly suitable for fine turfgrasses (Croce *et al*., 2001; De Luca *et al.*, 2008; Pornaro *et al.*, 2016). Buffalograss, instead, as previously stated, is well-known in the USA but not yet in Europe (Sever Mutlu *et al.*, 2011).

For some warm-season turf species, only vegetative material is available and sprigging, sodding or plugging are the only methods available for turfgrass establishment. For species with both seeded and vegetative cultivars, the latter often have higher aesthetic value than the seeded ones (Pornaro *et al*., 2019; Volterrani *et al.*, 2008). However, breeding programs have produced in the last twenty years for bermudagrass and other warm-season turf species high-quality fertile cultivars with morphological characteristics similar to those of vegetative cultivars (Richardson et al., 2003).

In the Mediterranean region, warm-season grasses are seeded in late spring when the temperatures are suitable for a rapid establishment. Seeded cultivars are usually preferred over sterile cultivars by turfgrass managers because seeding is cheaper and easier to practice compared to vegetative establishment methods, such as plugging, sprigging and stolonizing (Emmons, 2000).

Bermudagrass is the most popular warm-season grass for highly maintained areas in warm and transition zones of the USA and the Mediterranean Basin (Volterrani *et al.*, 1997, 2012). It owes its fortune both to its multiple adaptations to which an intense breeding activity and to the diversity of cultivars available on the seed market also contributed.

Bermudagrass is a name applied to several taxa of the genus *Cynodon* (L) Rich. which is a genus of Poaceae family and Chlorodoideae subfamily (Casler and Duncan, 2003). These grasses are ubiquitous and cosmopolitan between latitudes of 45° N and 45° S being able to grow in the warm-temperate, subtropical, and tropical climatic regions of the world. *Cynodon* species include common bermudagrass (*Cynodon dactylon var. dactylon*), sterile hybrid types (*Cynodon dactylon x C. transvaalensis*), and a natural sterile triploid hybrid (*Cynodon* x *magennisii*) (Beard *et al.*, 2014). Common bermudagrass is widely used for lawns with low or medium maintenance and to control soil erosion while hybrids are preferred for high-quality turfgrasses such as golf courses and sports fields (Emmons, 2000). Bermudagrass is a long-lived perennial grass with an excellent recuperative ability that produces a vigorous and dense turf; it is the most widely used warm-season turf species in Italy (Volterrani *et al.*, 1997; Rimi *et al.*, 2011). Only a few disadvantages are recognized to this grass: poor cold tolerance extended dormancy period, and poor shade tolerance (McCarty and Miller, 2002; Goatley *et al.*, 2008). In the Mediterranean region, bermudagrass maintains active growth for about six months between mid-spring to mid-fall. Dormancy begins with a decrease of growth rate when the soil temperature drops below the 10 °C followed by a gradual loss of the green colour (Goatley *et al.*, 2008; Rimi *et al.*, 2011; Schiavon *et al.*, 2016). The use of sterile bermudagrass hybrids is widespread. They usually have higher qualitative characteristics such as uniformity, density, colour, and close mowing tolerance than common bermudagrasses. Nevertheless, recently, the turfgrass industry has made available fertile cultivars with characteristics similar to sterile hybrids (Richardson *et al.*, 2003; Stier *et al.*, 2013; Macolino *et al., 2016).*

Seashore paspalum and bahiagrass (*Paspalum notatum* Flueggé) are two of the most used turf species of *Paspalum* genus, which originates from East-central South America (Beard, 1998). The genus *Paspalum* L. is one of the largest genera within the family Poaceae, it contains more than 400 species, but very few of them are suitable for turfgrass purpose. Seashore paspalum is a perennial grass characterized by several stress tolerances, including salt level (Casler and Duncan, 2003). This grass can be propagated by seed or vegetatively and it is considered a multi-purpose species as it is used for several purposes: golf courses, recreational area, park landscape, erosion control, dune stabilization, and also as a forage crop (Casler and Duncan, 2003; Stier *et al.*, 2013).

Buffalograss is a warm-season dioecious grass, native perennial shortgrass of genus Bouteloua belongs to the family of the Poaceae, subfamily Chloridoideae (USDA, Agricultural Research Service, National Plant Germplasm System, 2019), and it is codominant with blue grama (*Bouteloua gracilis*) over most of the shortgrass prairie. It is drought-, heat-, cold- and highly grazing-resistant (Howard, 1995). Buffalograss is characterized by grey-green colour and fine texture; it also has an excellent adaptation to semi-arid areas (Foy, 2006). Propagation usually is by seed (burs) and vegetatively by stolons (Beard *et al.*, 2014). In the transition zone, this species starts growing in April or May, and early fall goes into a dormant stage.

Aims and scientific hypothesis

Climate change mainly includes effects on temperature and precipitations; these effects will increasingly influence the choices of turf specialists as regards species selection and cultural practices (De Luca *et al.*, 2008). The consequences on turfgrass management were investigated only recently (Springer *et al.*, 2014), and no information is available on turfgrass establishment.

Most turfgrass problems met during the first or second growing season are often related to mistakes made before or during the establishment phase. Turf establishment is defined as the root and shoots growth following seed germination or vegetative planting needed to form a mature, relatively stable turfgrass (Beard *et al.*, 2014). However, germination is often considered part of the establishment phase, as evidenced by Bewley and Black (1994).

Turfgrass in the early stage of establishment is much more sensitive to damage than a mature stand. Germination is a primary component of seedling emergence which is governed mainly by soil temperature, water potential, and air quality (Forcella *et al.*, 2000). Shorten the establishment is a priority to have a satisfactory turfgrass. Chemical and physiological methods, such as wetting agent are available on the market (Lee, 2017). Germination is strongly related to the genetic basis, the minimum temperature (T_{BASE}) to which each species/cultivar can germinate and the thermal time (Tt, °C days) used to quantify germination and emergence are genetic characteristics. Also, the availability of water necessary for germination, measured as water potential, has a genetic basis. These fundamental variables are frequently included in models used for predicting germination, i.e. 'thermal time' and 'hydrothermal time' models (Bradford, 2002; Bewley *et al.*, 2013). Strictly speaking, germination ends with the protrusion of the radicle from the seed and does not include seedling growth (Bewley, 2013) which constitutes a subsequent phase towards the more or less complete establishment of the turfgrass.

Germination and seedling growth have been little studied in particular for the response to climate change effects. This thesis aims to investigate the effects of suboptimal temperatures and excessive water levels on seed germination and seedling growth of some warm-season species. In a temperate climate, the success of a newly formed warm-season turfgrass is primarily due to growth stage achieved by vegetation before cold stress (Munshaw G.C. *et al.*, 1998) to avoid winterkill or winter injury (Musser and Perkins, 1969) (Richardson *et al.*, 2004). Seeding warm-season grasses in late spring or early summer (June or July), as is usually done, does not guarantee a mature turfgrass before winter dormancy, while early seeding (April or May) results in higher winter survival (Richardson *et al.*, 2004).

The PhD research project consisted of five experiments conducted in the field and a controlled environment. Precisely three field experiments and two experiments under controlled environment conditions were performed to investigate and verify the following hypotheses:

I. *Seed germination and subsequent seedling growth of bermudagrasses under different water submersion periods.*

   Two experiments were set up in growth chamber and greenhouse comparing five bermudagrass cultivars: ‘Jackpot’, ‘La Paloma’, ‘Transcontinental’, ‘Yukon’ and ‘Sunbird’. Germination was tested in a growth chamber under the following conditions: 2, 4 and 6-days submersion with ‘seeds floating’; 2, 4 and 6-days ‘full submersion’ with seeds kept submerged using a plastic net; ‘no submersion’ (control).

   In the greenhouse, the cultivars were seeded in plastic trays with a mix of soil/sand to test seedling growth and numbers of yellow seedling leaves under four water submersion conditions (2, 4, 6 and 8 days) were measured.

II. The *Effects of sub-optimal temperatures on seed germination of three warm-season species: perspectives of cultivation in the transition zone.*

Seven turf-type cultivars of three warm-season species including five cultivars of bermudagrass: ‘Jackpot’, ‘La Paloma’, ‘Transcontinental’, ‘Yukon’, and ‘Riviera’, ‘SWI 2000’ buffalograss, and ‘Pure Dynasty’ seashore paspalum were tested in the germination chamber. Four temperature levels were applied consisting of two alternating temperatures each: 20/30 °C, as optimal temperature treatment according to ISTA methods (control), and 15/25, 10/20, 5/15 °C as suboptimal temperature treatments.

III. *Effect of simulated flash flooding events on six cultivars of three warm-season species at two seeding rates.*

The study explored the effects of waterlogging on turfgrass establishment comparing two seeding rates: recommended seeding rate (5.0 g m^{-2}) and reduced seeding rate (2.5 g m^{-2}), and two levels of waterlogging (with and without). Seedling emergence, dry seedling weight, cover and NDVI before and after the submersion were evaluated.

IV. *Influence of early seeding time on the establishment of bermudagrasses in the Venetian valley.*

The speed of establishment of four cultivars of bermudagrass (‘Transcontinental’, ‘Jackpot’ and ‘SR 9554’ and the ‘La Paloma’) was evaluated under three seeding dates with 30 days interval from late March (first date of seeding) to the beginning of May.

V. *Influence of T_{BASE} on calculating Degree Days (DD) of bermudagrass in a transition zone environment*. The effect of base temperature on Degree Days model was evaluated. For this purpose ten bermudagrass cultivars (‘Gobi’, ‘Sunbird’, ‘SR9554’, ‘Princess 77’, ‘Yukon’, ‘Riviera’, ‘Transcontinental’, ‘Casinò Royal’, ‘Savannah’, and ‘La Paloma’) were seeded at three different dates in spring (late March, late April, and late May). A T_{BASE} of 5°C (customarily used) was compared with a T_{BASE} of 15°C.

Abstracts of the five research chapters

The abstracts of the next five chapters are reported below for helping readers in understanding the outline of the study.

Chapter 2.

Effects of sub-optimal temperatures on seed germination of three warm-season turfgrasses with perspectives of cultivation in transition zone

Warm-season turfgrass species prevail in tropical and subtropical areas, but can also be grown in the transition zone. In this case, cold tolerance is a key aspect for germination and successful turfgrass establishment. The germination response to sub-optimal temperatures was investigated for bermudagrass (cvs ‘Jackpot’, ‘La Paloma’, ‘Transcontinental’, ‘Yukon’, ‘Riviera’), buffalograss (cv ‘SWI 2000’) and seashore paspalum (cv ‘Pure Dynasty’). Four temperature regimes were applied, i.e., 20/30 °C, 15/25 °C, 10/20 °C and 5/15 °C, with a 12:12 h (light:dark) photoperiod. Germination assays were performed twice, with six replicates (Petri dishes) per treatment in each experiment, fifty seeds per dish. The final germinated percentages at last inspection time (FGP) were obtained for each Petri dish and processed by using a generalized linear mixed model (binomial error and logit link). Germination curves were fitted to each Petri dish by using time-to-event methods, and germination rates (GR) for the 10th, 20th and 30th percentiles were derived and used to fit a linear thermal-time model. For all cultivars, FGP decreased with decreasing mean daily temperatures. Base temperatures (Tb) ranged between 11.4 °C and 17.0 °C, while the thermal time to obtain 30% germination ranged from 51.3 °C day for SWI 2000 to 144.0 °C day for Pure Dynasty. The estimated parameters were used to predict germination time in the field, considering the observed soil temperatures in Legnaro. The estimated date for the beginning of germination in the field would range from early April for SWI 2000 and Transcontinental to mid-May for Riviera. These results might be used as practical support for planning spring seeding, which is crucial for successful turfgrass establishment, especially without irrigation.

Chapter 3.

Germination and seedlings response of bermudagrasses under water submersion

Bermudagrass [*Cynodon dactylon* (L.) Pers.] at the mature stage is quite tolerant of submersion, but little information is available on the effects of submersion on seed germination and seedling development. Five cultivars (‘Jackpot’, ‘La Paloma’, ‘Transcontinental’, ‘Yukon’ and ‘Sunbird’) were compared in two experiments conducted at the Agricultural Experimental Farm of Padova University in Legnaro, Italy.

Germination percentage in petri dishes under ‘seed floating’ and ‘seed submerged’ conditions (0, 2, 4 and 6 days submersion) was measured. The cultivars tested displayed a different response to both submersion and floating treatments. ‘Jackpot’, ‘Sunbird’ and ‘Transcontinental’ were the most suitable under these conditions.

The same cultivars were also tested in the greenhouse where seedlings growth under 0, 2, 4, 6, and 8 days submersion was evaluated.

Results displayed a difference, limited to a short period, between floating and submerged seeds. Bermudagrass seedlings appeared to have short-term resilience to flooding, as long as it is not prolonged. All the cultivars showed good tolerance of submersion during germination, while submersion for 6 and 8 days resulted in a significant increase in the average proportion of yellow seedlings.

Chapter 4.

Influence of seeding time on bermudagrass (Cynodon spp.) establishment in the upper Mediterranean region

Studies showed that a fast and early establishment allowed bermudagrass to get a mature turf before winter to reduce cold injury and anticipate the spring green-up. We investigated the effect of early seeding on the speed of establishment of some bermudagrass cultivars in Venetian valley area. Three spring seeding times were compared (late March, late April and late May 2018 and 2019) and the time to reach 75% green coverage (full establishment) was evaluated. The study showed that the first period of seeding seeded bermudagrass (late March) is what allows faster coverage and thus guarantees an excellent establishment with a mature turf preventing winter injuries. The cultivars ‘La Paloma’, ‘SR9954’ and ‘Transcontinental’ were equally fast in reaching 75% cover, while ‘Jackpot’ needed more days.

Chapter 5.

Influence of waterlogging and seeding rate on the establishment of some warm-season turfgrass species

Debate on climate change seems to direct the attention towards warm-season grass species, and their adaptation to climatic projections in the Mediterranean region showing an increase of average temperature and precipitation intensity with increases in extreme weather events. Most studies about submersion do not focus on the first stages of the establishment, and, even less, take into consideration the seeding rate.

This study investigated the effect of simulated flash flooding events on emergence and seedling growth of bermudagrass, seashore paspalum and buffalograss cultivars at two seeding rates (5.0 g m^{-2}, and 2.5 g m^{-2}). Results indicated that a seeding rate of 5.0 g m^{-2} is needed to make the establishment faster. Moreover, all cultivars under study displayed high tolerance to flooding during seedling emergence and early establishment.

Chapter 6.

Germination and seedling emergence of bermudagrasses: a critical analysis of degree days formula

The germination of bermudagrass under different temperature regimes was widely investigated. The time of turfgrass establishment is often calculated in Degree-Days (DD). Differences in thermal requirement have been observed in studies conducted in different locations with the same species. These differences could be related to reference T_{BASE} used in DD calculation. The most used T_{BASE} for bermudagrass is 5°C although studies conducted in laboratory displayed a base temperature of about 15° C. In this study we investigated the impact of T_{BASE} 5°C and T_{BASE} 15°C on estimation of bermudagrass emergence prediction. Ten cultivars were seeded at three dates between 10 March and the end of April in 2013 and 2014. Based on our findings, a T_{BASE} of 15°C provides a more accurate estimation of emergence degree days than a T_{BASE} of 5°C.

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Chapter 2

Effects of sub-optimal temperatures on seed germination of three warm-season turfgrasses with perspectives of cultivation in transition zone

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Introduction

In arid regions, warm-season species are preferred over cool-season ones for lawns and other recreational landscapes due to their low water requirements [1,2]. Recently, because of global warming and the resulting climate change, the use of warm-season species for ornamental and sports turfs has also been encouraged in the Mediterranean countries of Europe. In these regions, which fall within the transition zone, cool-season species are the most common, but warm-season ones can successfully be used as, in addition to low water consumption, they have other environmental benefits: greater ability to compete with weeds, higher tolerance to biotic and abiotic agents, traffic and salinity [3,4].

Warm-season grasses are cold-sensitive, and in transition zones, they can suffer from the long winters resulting in a delay in spring green-up shortening the growing period. Proper cultural practices and the selection of cultivars adapted to the specific environment are essential to enhance the growing season length [5–10].

Moreover, warm-season grasses can be seeded or propagated vegetatively as sod, plugs or sprigs [11,12] but the establishment of turfgrass from seed is preferred over sodding or sprigging as it is cheaper [13]. However, for some species such as bermudagrass (Cynodon dactylon (L.) Pers.), St. Augustinegrass (Stenotaphrum secundatum (Walt.)

Kuntze), seashore paspalum (Paspalum vaginatum Swartz), and zoysiagrass (Zoysia spp.), the turfgrass industry developed vegetatively propagated cultivars that are widely used because of the high turf quality due to improved morphological uniformity [14]. Seeded-type cultivars can be a challenge to establish by seed in transition zones as they need higher temperatures to germinate and grow after emergence than cool-season species [15].

In transition zones of Europe, warm-season species are generally seeded in late spring when the temperature is sufficiently high for a rapid establishment [10,16]. Spring average monthly temperatures in European transition zones range roughly from 8–9 °C in March to 17–18 °C in May [17,18].

Warm-season grasses have cardinal germination temperatures shifted to higher levels in comparison with cool-season grasses, and tolerance to sub-optimal temperatures appears to be crucial for seeding them in transition zones.

Several studies highlighted the importance of an early seeding of bermudagrass for proper establishment [11,13,19,20], demonstrating that seeding in late spring or early summer (June or July) does not ensure proper turfgrass establishment before winter dormancy, while early spring seeding resulted in faster establishment and a reduction of the risk of winter injury [15,21]. There are a large number of bermudagrass cultivars, which give a range of adaptation to different environments [5–7,17]. Several studies [16,21,22] showed that vast differences in germination temperatures occur among bermudagrass cultivars. Giolo *et al.* [23], in a growth chamber experiment in which ten bermudagrass cultivars were tested, found an average base germination temperature of 15 °C corroborating field studies demonstrating that early spring seeding could be effectively used to successfully establish bermudagrass in transition zones [21]. Similar findings were also observed for ‘Sea Spray’ seashore paspalum [16], although Fontenot [24] found that this cultivar has a significantly higher mean germination time (MGT) when seeds are subjected to 20 °C, compared with 25 °C, 30 °C and 35 °C. Growing degree-day (GDD) units are often used to predict turfgrass establishment, although information on germination base temperature of warm-season grasses is limited. Unruh *et al.* [25] found a base temperature of 5 °C for vegetative bermudagrasses, later also widely used for germination of seeded-type cultivars. More recent studies indicated a base germination temperature for seeded-type cultivars close to 15 °C [23]. Knowledge of base temperature is fundamental to the choice of seeding dates for these species, but it has scarcely been studied. Cardinal temperatures are considered in thermal time (TT) models, developed to describe germination patterns [26]. The ordinal range of cardinal temperatures for seed germination includes a minimum or base temperature (Tb), optimum temperature (To) and maximum or ceiling temperature (Tc). Temperatures between Tb and To represent suboptimal temperatures. In TT models, the timing of germination can be described for suboptimal temperatures introducing the concept of thermal time (θT), which is considered constant for a given percentile (g) [27]. Differently from hydro time and hydrothermal time models, TT models do not account for water potential, which is assumed to be 0. This study aimed to reduce the lack of information related to base germination temperatures in warm-season species, which has been little studied [28]. A growth chamber experiment was conducted in 2018 to investigate the germination response to sub-optimal temperatures of some cultivars currently available in the European turfgrass markets of bermudagrass, one cultivar of seashore paspalum, and one cultivar of buffalograss (Buchloe dactyloydes (Nutt.) Engelm). Buffalograss is a warm-season species not yet widespread in Europe, but that may have a high potential for low-maintenance lawns and other non-trafficked areas [29,30].

Materials and Methods

Germination assay: experimental setting

The study was conducted in 2018 at the laboratory of CREA DC – Research Centre for Plant Protection and Certification of Lonigo (Vicenza, Italy). Seven turf-type cultivars of warm-season species were tested in a germination chamber under specific controlled conditions. They included five cultivars of bermudagrass: ’Jackpot’, ‘La Paloma’, ‘Transcontinental’, ‘Yukon’, and ‘Riviera’, ‘SWI 2000’ buffalograss, and ‘Pure Dynasty’ seashore paspalum. Four temperature regimes were applied consisting of two alternating temperatures each: 20/30 ◦C, as optimal temperature treatment according to ISTA methods [31], and 15/25 °C, 10/20 °C, 5/15 °C as suboptimal temperature treatments.

The sub-temperatures chosen for the experiment represent a possible temperature range that takes into account the temperatures in early Spring in the transition zone and studies and observations on the base temperature of warm-season turfgrass species [21,23]. For each treatment (i.e., each cultivar combined with each temperature treatment), six replicates (Petri dishes of 140 mm diameter) of fifty seeds were laid out in a randomized blocks design. All the seeds used, except for 'Jackpot', were unhulled, whereas for 'SWI 20000 buffalograss the bur was used, which includes 2–3 caryopses [32], and was considered as a seed unit. Before the test, seeds and burs were stored at a temperature of 5 °C and 50% relative humidity into a storage chamber for six months to break possible seed dormancy. The filter paper in Petri dishes was moistened with 6 mL solution 0.2% of KNO_3 [31] and then kept moist with deionized water. Petri dishes were incubated at 12:12 h (light:dark) photoperiod. Germinated seeds were counted five times a week for 20 days. Seed germination was assayed by monitoring primary root elongation.

A bur was considered germinated when at least one seed was germinated. Germinated seeds and burs were removed at the time of counting. The experiment was run twice using the same germination chambers. In total, we had 336 Petri dishes (2 runs × 6 blocks × 7 cultivars × 4 temperatures).

Soil temperature data

In order to predict germinations in field conditions (see later), soil temperatures were recorded by a weather station on the Experimental Farm of Padova University in Legnaro [33]. The area is a typical transition zone with sandy loam soil [6,7,23]. Seven-year temperatures (2008–2014) recorded by ground surface temperature sensors TSS of MTX srl were considered. Temperatures were recorded near to the soil surface, that is where the small seeds of these species are usually sown.

Data analyses

The final germinated proportions (FGPs) obtained for each Petri dish at the final inspection time were processed by using a Generalized Linear Mixed Model (binomial error and logit link), where the temperature and cultivar were included as fixed effects, while the run and block within the run were included as random effects. Back-transformed proportions were derived from model parameters. Standard errors on the link scale were derived by the Hessian and were back-transformed by using the delta-method [34].

Pairwise comparisons were performed by using the generalized hypothesis testing procedure devised by [27]. In order to evaluate the effect of temperature on germination rates, the time-course of germination was determined for each of the 336 treatment combinations (4 temperature levels × 7 cvs × 6 replicates × 2 runs). Model fitting was accomplished by using a time-to-event model, considering a Weibull distribution of germination times [35,36]. The cumulative probability function was:

$$g = d \exp\{-\exp[-b(\log(t) - \log(e))]\} \qquad (1)$$

where: g is the proportion of germinated seeds at time t, d is the higher asymptote, b is the shape parameter and e is the location parameter.

The fitted models were used to derive the germination rates (GRg) for the 10th, 20th and 30th percentiles (GR10, GR20 and GR30) for each Petri dish as the inverse of the germination times (t10, t20 and t30). The choice of using only up to GR30 instead of GR50, as would be usual, was made in agreement with Bewley *et al.* [26], based on the fact that, in our study, the FGP differed widely among cultivars and in some cases ('Yukon' and 'Pure Dynasty') was even lower than 50%.

The derived GRg values were used to fit the thermal-time model of Garcia-Huidobro [27]:

$$\begin{cases} \mathrm{GRg} = \dfrac{1}{\mathrm{tg}} = \dfrac{(\mathrm{T} - \mathrm{Tb})}{\theta_{\mathrm{T(g)}}} & \text{if T} > \text{Tb} \\ \qquad\quad 0 & \text{if T} \leq \text{Tb} \end{cases} \qquad (2)$$

where: GRg is the germination rate for the g percentile (i.e., the 10th, 20th and 30th percentile), T is the average daily temperature, Tb is the base temperature (in °C and common for all percentiles) and θT (g) is the thermal time for the germination of the g percentile (in °C d). By fitting this model, we could derive the main thermal-time parameters for all the species/cultivars. Nonlinear least squares were used to fit the model and the average daily temperature was used as explanatory variable [37]. A transform-both-sides technique was used to account for heteroscedasticity, with $\lambda = 0$ [38].

The estimated values for thermal time (θT (30)) and base temperature were used to predict the germination in field conditions, by using soil temperature data. The 10-day means for March, April and May (Table 1) were then calculated and used to determine germination degree days using the following equation [39,40]:

$$\text{Degree days} = \frac{(T_{MAX}+T_{MIN})}{2} - Tb \qquad (3)$$

where if $T_{MAX} < Tb$, then $T_{MAX} = Tb$, and if $T_{MIN} < Tb$, then $T_{MIN} = Tb$.

Table 1. Ten-day average of maximum, minimum, and mean daily temperatures (°C) in Legnaro (Padova, Italy) for the period 2008–2014, and averages and standard deviations over the seven years.

Temp.	Month	10-Day Interval	2008	2009	2010	2011	2012	2013	2014	Average	St. Dev.
max	March	1	10.0	10.0	6.9	9.5	12.8	11.7	12.4	10.5	2.03
mean			7.0	7.9	4.5	4.4	7.5	7.7	8.9	6.8	1.73
min			4.7	6.0	2.9	0.6	3.7	4.8	6.4	4.2	1.98
max		2	12.2	11.8	9.4	13.2	16.7	10.7	16.1	12.9	2.69
mean			8.9	8.3	5.6	10.1	10.0	7.5	10.4	8.7	1.72
min			6.2	5.3	2.7	7.5	5.1	5.0	6.3	5.4	1.49
max		3	11.1	11.2	14.0	15.6	18.3	9.4	17.4	13.9	3.41
mean			7.7	8.6	11.3	10.4	12.2	6.8	11.6	9.8	2.10
min			4.8	6.4	9.2	6.0	7.0	4.6	6.8	6.4	1.54
max	April	1	13.7	20.8	15.4	20.6	15.6	12.0	20.1	16.9	3.59
mean			10.1	14.4	11.2	14.5	12.3	9.4	14.6	12.4	2.20
min			7.0	10.4	7.9	9.6	9.3	7.4	10.1	8.8	1.36
max		2	15.2	23.7	16.9	21.0	14.3	18.4	20.3	18.5	3.36
mean			12.1	14.9	12.7	14.0	11.9	14.5	14.5	13.5	1.25
min			9.7	9.5	9.3	8.5	9.9	11.2	9.6	9.7	0.81
max		3	16.4	20.5	20.4	22.9	16.8	18.7	21.5	19.6	2.41
mean			13.5	13.7	16.1	16.5	13.4	15.3	16.8	15.0	1.49
min			11.3	9.5	12.7	11.7	10.7	12.1	13.3	11.6	1.27
max	May	1	18.2	25.4	20.4	26.0	24.4	23.1	22.9	22.9	2.78
mean			14.8	17.5	15.5	17.8	18.9	19.1	17.8	17.3	1.62
min			11.9	12.2	11.3	11.2	14.1	16.0	13.8	12.9	1.77
max		2	19.0	27.3	22.0	27.5	22.6	20.8	23.4	23.2	3.18
mean			16.0	20.5	16.8	20.2	17.4	17.4	18.0	18.0	1.69
min			13.2	15.8	13.1	14.0	12.6	14.4	13.7	13.8	1.06
max		3	24.2	29.1	26.8	31.0	24.4	20.3	25.8	25.9	3.50
mean			19.5	22.0	19.7	23.1	20.2	16.5	20.7	20.2	2.09
min			15.8	16.7	14.2	16.6	16.8	13.3	16.7	15.7	1.42

Results

The FGP (Table 2) varied with cultivar and temperature regime: Wald chi-square statistics showed that all the effects were significant (chi-square values equal to 1002.16, 244.37 and 355.69, respectively for cultivar, temperature and 'cultivar ×x temperature' interaction. The degrees of freedom were, respectively, 6, 3 and 18, corresponding to p-levels, always lower than 2.2 × 10−16).

Table 2. Germination percentage (FGP) at the final inspection time of five cultivars of warm-season turfgrass species: bermudagrass (*Cynodon dactylon* (L.) *Pers.*); buffalograss (*Buchloe dactyloides* (Nutt.) Engelm), and seashore paspalum (*Paspalum vaginatum* Swartz), subjected to four temperature regimes (5/15 °C, 10/20 °C, 15/25 °C, 20/30 °C) (The values represent the means of two experiment runs and six replications within each run; standard errors are in parentheses.)

Species	**Cultivar**	**Temperature Regime**			
		5/15 °C	**10/20 °C**	**15/25 °C**	**20/30 °C**
		FGP (%)			
bermudagrass	Jackpot	0.0 (0.0)	10.2 (1.8)	69.5 (3.5)	75.5 (3.1)
bermudagrass	La Paloma	0.3 (0.3)	20.5 (2.8)	71.7 (3.4)	86.8 (2.2)
bermudagrass	Riviera	0.0 (0.0)	1.1 (0.5)	33.0 (3.6)	70.1 (3.5)
bermudagrass	Transcontinental	0.0 (0.0)	48.2 (4.0)	80.4 (2.8)	91.6 (1.7)
bermudagrass	Yukon	0.2 (0.2)	2.9 (0.9)	43.4 (4.0)	49.0 (4.0)
buffalograss	SWI 2000	8.7 (1.6)	38.1 (3.8)	63.5 (3.8)	69.1 (3.5)
seashore paspalum	Pure Dynasty	0.2 (0.2)	0.0 (0.0)	33.3 (3.7)	47.8 (4.0)

In all cultivars, as expected, the highest FGP was achieved at the highest temperature regime (20/30 °C) [31]. In four cultivars of bermudagrass ('Jackpot', 'La Paloma', 'Riviera', 'Transcontinental') FGP was close to or higher than 70. For all the cultivars tested, the FGP decreased with decreasing temperature. At 10/20 °C, FGP dropped to almost nil for 'Pure Dynasty', 'Riviera' and 'Yukon'; it ranged from 10% to 20% for 'La Paloma' and 'Jackpot', while it was between 20% and 40% for 'Transcontinental' and 'SWI 2000′. At 5/15 °C, only 'SWI 2000′ buffalograss maintained a little germination (FGP = 8.7%).

Considering the germination time-course, the fit of Weibull cumulative probability functions was always good, although, in some cases, no germinations were observed and, therefore, no time-to-event model could be fit. In other instances, the higher asymptote could not be estimated with precision and, therefore, it was constrained to one, considering that our aim was to achieve the best fit within the observed time-lapse. Figure 1 shows the observed data (as averages of two runs and six replicates per run) and the fitted models, as obtained by averaging model parameters across runs and replicates. The estimated parameters for the different cultivars and temperature regimes, as averages across runs and replicates, are reported as supplemental data (Table S1).

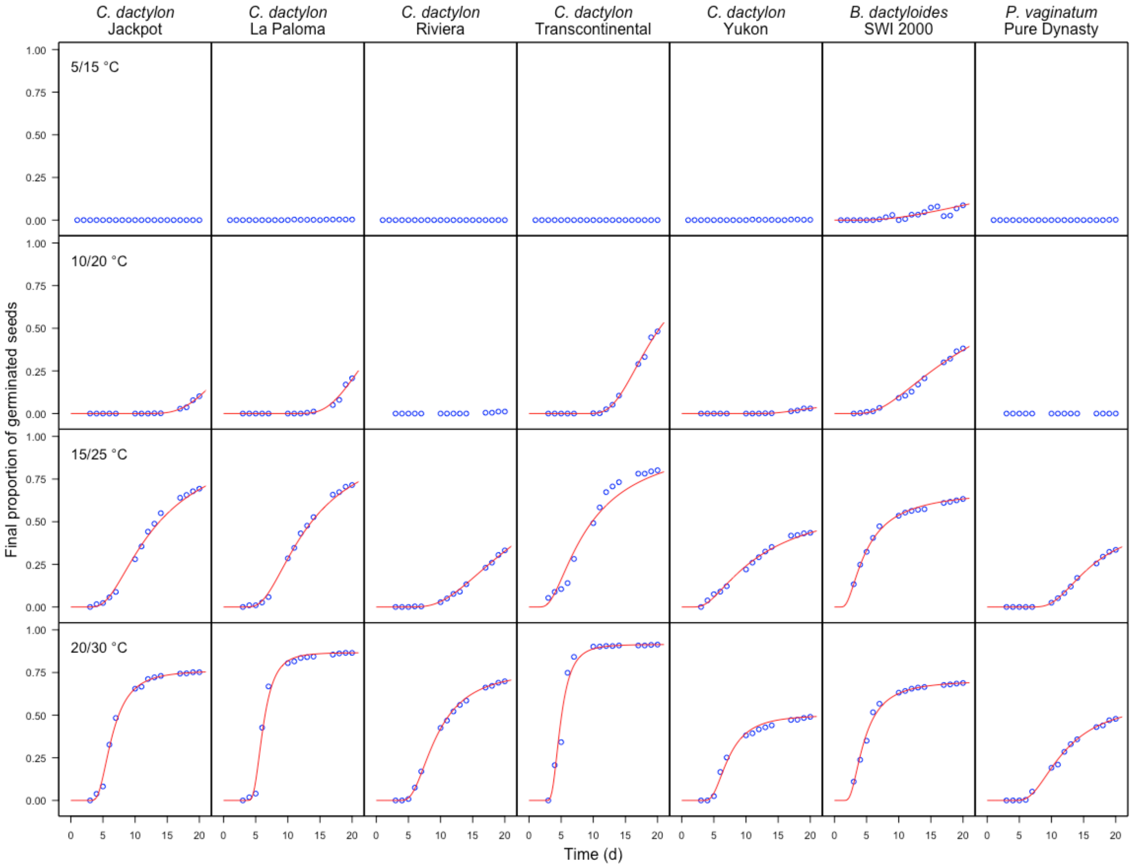

Figure 1. Time course of germination for seven cultivars of warm-season turfgrass species (Jackpot, La Paloma, Riviera, Transcontinental, Yukon; SWI 2000, Pure Dynasty; each variety is shown in a different column of the panel graph) subjected to four temperature regimes (5/15 °C, 10/20 °C, 15/25 °C, 20/30 °C; each temperature regime is shown in a different row of the panel graph). Symbols represent observed data and solid lines show the fitted models (Equation (1), together with the parameters in Table S1). The observed data represent the means of six blocks and two runs.

Fitted curves were used to derive the germination rates for the 10th, 20th, and 30th percentiles (GR10, GR20, GR30), which were used to parametrize Equation (2). The fit was always good; in agreement with Bradford [44], the three linear relationships of GR10, GR20 and GR30 merged at the intercept of the x-axis, confirming that Tb was equal for all seeds of the lot (Figure 2, Table 3) [44].

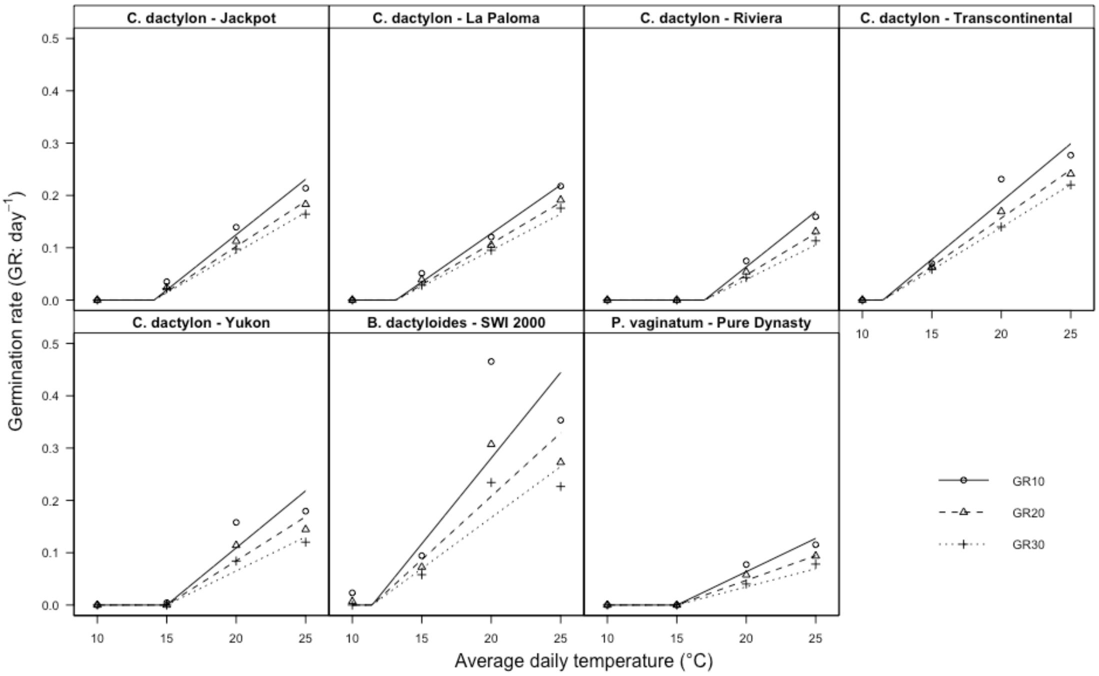

Figure 2. Germination rates of the 10th, 20th and 30th percentiles for seven cultivars of warm-season turfgrass species (Jackpot, La Paloma, Transcontinental, Yukon, SWI 2000, Pure Dynasty) subjected to four temperature regimes (20/30 °C, 15/25 °C, 10/20 °C, 5/15 °C, corresponding, respectively, to average daily temperatures of 25°C, 20°C, 15°C and 10°C) (Garcia-Huidobro *et al.*, 1982).

For all cultivars, except for ‘SWI 2000′, the fitted lines are very close (Figure 2) and, therefore, the thermal times required to reach 10, 20 and 30% germination (θT(10), θT(20), and θT(30)) were very similar (Table 3).

The five bermudagrass cultivars showed very different Tb varying from 11.5 for ‘Transcontinental’ to 17.0 °C for ‘Riviera’. The cultivar SWI 2000 also showed a very low Tb (11.4 °C), while that of ‘Pure Dynasty’ was intermediate (15.0 °C).

Based on the estimated values for thermal time ($\theta_{T(30)}$), base temperature (T_b) and considering the observed temperature levels (see Table 1), we calculated that, to reach 30% germination (Table 4), seeding time would range from early April for ‘SWI 2000′ and ‘Transcontinental’ to mid-May for ‘Riviera’ and ‘Pure Dynasty’.

Table 4. Cumulated degree days per 10-days for seven cultivars of warm-season turfgrass species in March, April and May. These values were calculated by using weather data and the estimated parameters in Table 3. The time intervals with degree days are those necessary to reach the thermal time for the 30th percentile ($\theta_{T(30)}$). The cultivars are listed by increasing Tb value.

Species	**Cultivar**	**Month and Decade**							
		March		**April**			**May**		
		2°	**3°**	**1°**	**2°**	**3°**	**1°**	**2°**	**3°**
		Cumulated degree days							
buffalograss	SWI 2000	7.5	20	47.5	83	125	190	261	355
bermudagrass	Transcontin.	7	19	46	81	122	186	256	349
bermudagrass	La Paloma			19	46	78.5	127.5	181.5	258.5
bermudagrass	Jackpot			14	36	63.5	107.5	153	220
bermudagrass	Yukon			9.5	27	50	89.5	130.5	188.5
seashore paspalum	Pure Dynasty			9.5	27	50	89.5	130.5	188.5
bermudagrass	Riviera				7.5	20.5	50	81	125.5

Discussion

In four cultivars of bermudagrass ('Jackpot', 'La Paloma', 'Riviera', 'Transcontinental'), FGP was close to or higher than 70%, which is the minimum threshold for the seed market of this species in the EU [45]. The low FGP of 'Yukon' (49%) was probably due to the low vigor of the seed lot. Low germination of 'Pure Dynasty' seashore paspalum corroborated previous results obtained by [46].

For 'SWI 2000′, the germination rates of the three percentiles were different; this result could be related to the fact that this species is dioecious and cultivars include many genotypes [47]. In agreement with Bradford [44], our result show that base temperature should be equal for all seeds of the lot (Figure 2, Table 3) [44], even though some exceptions to this rule can be found in literature [48].

The low Tb of 'Transcontinental' bermudagrass and 'SWI 2000′ buffalograss may allow early spring seedings, thus ensuring quick establishment reducing the risk of winter injuries [13,20]. In this regard, however, 'SWI 2000′ should be taken into consideration in virtue of the lower thermal time of the 30th percentile (51.3), both at 15/25 °C and 10/20 °C (Table 3, Figure 2). The Tb of 'Riviera' (17.0 °C) is in line with that observed in previous studies [22,23]. The wide range of Tb observed in the bermudagrass cultivars is of great interest for turf specialists who may select the most suitable cultivar for a specific location. Thirty varieties of bermudagrass are listed in the OECD catalogue [49], as a result of the considerable breeding activity on this species. Instead, buffalograss has only four varieties, and seashore paspalum has none currently listed.

In the transition zone, the recommended seeding period is between late spring and early summer [16]. Sub-optimal temperatures considered in this study generally occur in the earlier spring months (March and April).

The low germination temperature of ‘Transcontinental’ makes this cultivar very suitable for transition zones where early spring seeding is required [13,20]. Among the three species studied, buffalograss is the least known in Europe; however, our results demonstrated that ‘SWI 2000′ may have a good possibility to be successfully seeded in European transition zones under early spring conditions. Furthermore, ‘SWI 2000′, despite having a similar Tb to ‘Transcontinental’, showed a lower thermal time (Table 3, Figure 2). Instead, ‘Riviera’ bermudagrass and ‘Pure Dynasty’ seashore paspalum appear to be suitable only for late spring seeding. It is worth noting that ‘Pure Dynasty’ showed an intermediate Tb of 15 °C but a very high thermal time (144 °C) in comparison with ‘Yukon’ that showed the same Tb with a thermal time of 76.8 °C. Consequently, the turfgrass establishment with this species would be very slow and late, with related drawbacks in terms of irrigation requirements and weed competition and reduced cold tolerance during the first winter [9].

Conclusions

The results indicate that there is a wide variability among warm-season turfgrasses in the temperature requirements for germination. However, none of the species or cultivars was able to germinate below 11.4 °C and the earliest date for achieving 30% of seed germination in the Northern Italian plain is early April for the cultivars with the lowest base temperature, i.e., ‘SWI 2000′ buffalograss and ‘Transcontinental’ bermudagrass. This information is useful to identify the earliest seeding date in spring for each of the species and cultivars used in this experiment and to predict the time necessary for their germination. This approach might represent a practical tool for planning spring seeding, which is crucial for successful turfgrass establishment, especially without irrigation.

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Chapter 3

Germination and seedlings response of bermudagrasses under water submersion

An edited version of this chapter has been submitted to 'European Journal of Horticultural Science' (www.ishs.org/ejhs).

Introduction

The Mediterranean area mainly lies in the so-called transition zone, where it is possible to cultivate both warm- and cool-season grasses. The use of warm-season species has been encouraged over the last decade in the transition zones of Europe to reduce cultural inputs required to maintain turfgrasses (Sever Mutlu et al., 2011; Rimi et al., 2011, 2013a; b). In comparison to the more traditionally used cool-season species, warm-season species use water more efficiently (Casnoff, 1989; Huang et al., 2008) and have higher tolerance of drought, traffic and fungal diseases (Puhalla et al., 1999).

Current climate change projections for the Mediterranean area show an increase of extreme weather and climate events over the next years. Med-CORDEX (Coordinated Regional Climate Downscaling Experiment) has elaborated some projections on future climate changes in Italy (Desiato et al., 2015, 2019). Rainfall projections indicate an increasing concentration of more intense and less frequent events (Medri et al., 2013; IPCC, 2014; Zollo et al., 2016).

Climate change influences the choice of turfgrass species and management practices (De Luca et al., 2008) and warm-season species, and especially bermudagrass, are becoming popular in the transition zones of Europe (Macolino et al., 2016), however, only a few studies have investigated the effect of increased rainfall intensity on early-stage growth of warm-season turf species. Heavy rains may cause either flooding, which can cause partial or total plant submersion or waterlogging when plants are not submerged, but soil pores are saturated with water (Striker, 2012; Bailey-Serres et al., 2012). Waterlogging and, to a greater extent, submersion, can cause several visible symptoms on plants, especially when they are prolonged: reduced photosynthetic activity, abscission of leaves, chlorosis, lower growth of roots and stems (Batzli and Dawson, 1997). Flooding events can be extremely harmful to plants (Bailey-Serres et al., 2012), a decrease in oxygen diffusion rate (ODR), pH and soil redox potential, and an accumulation of toxic compounds are the most significant effects of rootzone pores saturation (Voesenek et al., 2006; Parent et al., 2008). The oxygen diffusion rate is a significant indicator of the availability of oxygen to plant roots that can restrict seedling emergence (Chesworth, 2008; Neira et al., 2015). Studies by Letey*et al.* ((1966) found that at ODR of 0.15 μg min^{-2} cm^{-1} roots growth of bermudagrass (*Cynodon* spp.) drastically reduced, even if this species showed more tolerance to compact soil than dallisgrass (*Paspalum dilatatum* Poir.) and bahiagrass (*Paspalum notatum* Flueggé). Flooding sharply limits CO_2 and light availabilities, which are essential for the photosynthesis process (Jackson *et al.*, 2005); light availability is also limited when heavy rains are associated with soil erosion because of soil particles muddy the water (Pedersen *et al.*, 2013). Several studies demonstrated that bermudagrass plants are quite tolerant of submersion, even for a long period. Wherley *et al.* (2011) studied the response to soil saturation over 72 days on plant development of two warm-season species: common centipedegrass [*Eremochloa ophiuroides* (Munro) Hack] and 'Tifway 419' hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. X *C. transvalensis* Burtt Davy]. Waterlogging reduced canopy cover by 30% in both species, but shoot biomass decreased in bermudagrass. Tan *et al.* (2010) demonstrated that bermudagrass plants could withstand prolonged and deep submersion through lowering their metabolism, increasing carbohydrate reserves and improving anti-oxidative activities of roots, which allow an adaptation to hypoxia and anoxia. Ashraf *et al.* (1991) experimented comparing the mechanism of waterlogging tolerance of kallar grass [*Leptochloa fusca (*L.) Kunth], a species known for its high tolerance of waterlogging and salinity, mangrove grass [*Aeluropus lagopoides* (L.) Trin.], and bermudagrass [*Cynodon dactylon* (L.) Pers.] under three water submersion conditions: no submersion (control), intermittent submersion (one-week submersion and then drained for one week), and six weeks continuous submersion. They found a significant reduction of chlorophyll content in all species, while bermudagrass showed less capacity of oxidising the rhizosphere region, concluding that bermudagrass can be evaluated as relatively sensitive to submersion in comparison with other species regarded as highly tolerant.

In a recent study, Ye *et al.* (2015) reported that physiological changes caused by submersion are more limited than those observed as a result of drought stress. Submersion causes growth inhibition as a consequence of a decrease in the metabolism of carbohydrate degradation and energy supply (Huang *et al*., 2019). The strategy adopted by bermudagrass seems to be oriented towards dormancy and delayed growth, which allows this species to be adaptive to a long-term submerged environment.

Although several studies investigated the effects of waterlogging on plant growth and survival, few of them examined the effects of waterlogging on seed germination and seedling growth. Oxygen at atmospheric levels is usually required for seed germination even; however, the response to oxygen varies strongly with species (Bewley *et al.*, 2013). Prolonged immersion progressively increases damage to cell membranes, which instead results in significant inhibition of seedling growth (Ye *et al*., 2015).

The first stage of the complex phenomenon of germination is imbibition. Germination of bermudagrass in water does not seem to be problematic because water potential (Ψ) is 0 Mpa and, according to the hydro time model, it facilitates seed imbibition (Bradford, 2002). Morinaga (1926) found that bermudagrass germinated better in bottles under 100 ml of distilled water than in Petri dishes with moistened filter paper.

The study aimed to reduce the information gap in the existing literature regarding the effects of excess of water during bermudagrass germination and early stage of growth. We investigated germination and subsequent seedling growth of five bermudagrass cultivars under different water floating/submersion periods.

Materials and Methods

Two experiments were carried out at the Agricultural Experimental Farm of Padova University in Legnaro, Italy (lat. 45°20’ N, long. 11°57’ E, elevation 8 m).

Laboratory experiment

In 2016, five seeded bermudagrass cultivars among the most widespread in the Italian turf market (Jackpot, La Paloma, Transcontinental, Yukon, and Sunbird) were tested in a growth chamber for seed germination under different water submersion conditions: 2, 4 and 6-days submersion with seeds floating, 2, 4 and 6-days full submersion with seeds kept submerged using a plastic net, and no submersion (control). Unhulled seeds were then submerged for different periods in plastic containers filled with deionized water, and then 100 seeds of each cultivar were placed on filter paper in 10 cm diameter Petri dishes. The filter paper was moistened with deionized water. No methods to increase germination, as indicated by the International Seed Testing Association, were used (ISTA, 2013). Seeds were tested in a KW incubator under alternating temperature 30/20 °C and 14/10 h (light/dark) photoperiod. Petri dishes were randomly placed in the incubator and replicated five times. The numbers of germinated seeds were counted three times a week for a period of 40 days from seeding, that it is about twice the period provided by the International Seed Testing Association, which is equal to 21 days (ISTA, 2013). Seeds were previously stored at a temperature of 5 °C for almost six months to interrupt possible dormancy. According to Munshaw seed vigour was not considered (Munshaw *et al*., 2014). A seed was included as germinated seed at the stage where it was possible to identify primary roots.

Greenhouse experiment

A greenhouse experiment was conducted during spring 2017 using the same bermudagrass cultivars and the same seed lots used for the growth chamber experiment. The seeds were placed in plastic trays with a mix of soil/sand to test seedling growth under different water submersion conditions: no submersion (control), 2, 4, 6 and 8-days submersion. Before the experiment, seeds were stored at a temperature of 5 °C for almost six months to interrupt dormancy. Greenhouse air temperatures were monitored with a digital datalogger 'Onset HOBO Pendant temp UA-001-64' and maintained between 11.1 and 32.1 °C with a mean of 22.4 °C. Each tray contained a 3 cm bed of expanded clay separated by a nonwoven fabric towel from a 3 cm layer of soil- sand mix (70% and 30% V/V). The soil was a sandy loam containing 60.8% sand, 28.9% silt 10.3% clay, with pH 8.3, organic matter 21%, C-to-N ratio of 12.2, Olsen P content of 4.2 mg kg^{-1} P, and exchangeable K content of 86 mg kg^{-1}, and it was previously oven-sterilized at 105 °C for 48 h. Two hundred uncoated seeds were sown in each tray on 17 March 2017 and covered with 1-2 mm of sand. Plastic trays were arranged in a randomized complete block design with four replicates. The top 3 cm of soil was previously moistened by automatic sprinkler irrigation and regularly irrigated until the beginning of the submersion, with the only exception being controlled trays that were irrigated for the entire experimental period. The beginning of the submersion was calculated empirically based on the progressive counts of the seedlings. The counts of seedlings number emerged allowed to produce experimental cumulative seedling emergence curves (Bewley *et al.*, 2013). The submersion thus coincided with seedlings average emergence of about 40%. At this experimentally calculated level, the emergence had peaked and was gradually slowing down, as shown in Figure 1.

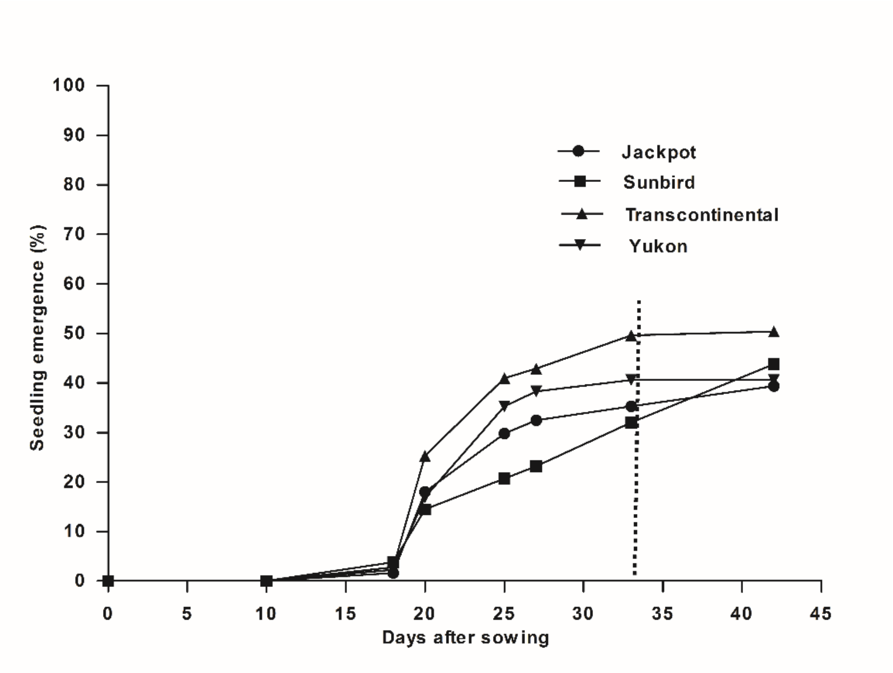

Figure 1. Greenhouse: experimental cumulative seedling emergence curves of four bermudagrass cultivars under no submersion (Control). The vertical dotted line indicates the last count (33 days after seeding) before the beginning of submersion.

This level represented 90% of maximum seedling emergence reached by the control after the submersion period.

Flooding treatment consisted of complete submersion of seedlings (1-2 cm of water above the soil surface). The comparison for each cultivar with the control was carried out by matching the end of the immersion of the four treatments (2, 4, 6, and 8 days) on the same day. The four submersions are then progressively occurred to 2 days away from one another. The number of growing seedlings was recorded weekly from April 4 to April 28, and yellow seedling leaves were also recorded on April 28.

Statistical analyses

The dataset of laboratory data (final cumulative germinated proportion and proportion of not germinated) was analysed using a Generalised Linear Model with binomial error and logit link; a dispersion parameter was added to account for overdispersion (quasi binomial model). Submersion treatment (different submersion types and durations), cultivar and the interaction ‘submersion x cultivar’ were added as fixed effects. The significance of fixed effects was tested using Wald F tests. Back transformed proportions were derived and are reported in table 1.

In the greenhouse experiment, the cultivar La Paloma showed low germination and was not included in the statistical analysis. Two variables were recorded after 2, 4, 6, and 8-

days submersion, i.e. the final cumulative proportion of seedlings surviving submersion and the proportion of yellow seedlings. Both variables were analysed using a Generalised Linear Model with binomial error and logit link; a dispersion parameter was added to account for overdispersion (quasi binomial model). Block, submersion, cultivar and the interaction 'submersion x cultivar' were added as fixed effects. The significance of fixed effects was tested using Wald F tests. Back transformed proportions were derived and are reported in tables 2 and 3.

Data analyses were performed using the R statistical environment (R Core Team, R 3.5.0, 2018) together with the package "emmeans" (Lenth *et al*., 2018).

Results and discussion

Laboratory experiment

The final cumulative germinated proportions (FGP) of the five cultivars tested in the laboratory are reported in Table 1. The FGP of the control resulted between 44.4 and 81.4%. In all the tested samples, a negligible presence of fungal hyphae on seeds was found. Except for 'La Paloma', the germination of the control samples was close to the minimum threshold for the seed market of this species in the EU that is 70% (The Council of the European Economic Community, 1966).

The analysis of variance showed a significant 'treatment x cultivar' interaction ($P = 2.042e^{-15}$). The five cultivars displayed a different response to both submersion and floating treatments, especially for the longest duration (6 days). Under no submersion 'La Paloma' revealed a higher cumulative germinated proportion in comparison with submersion and flooding treatments (Fig. 2). 'Yukon' also showed a decrease of cumulative germinated proportion after 4-6 days of continuous submersion and all floating treatments (Fig. 3).

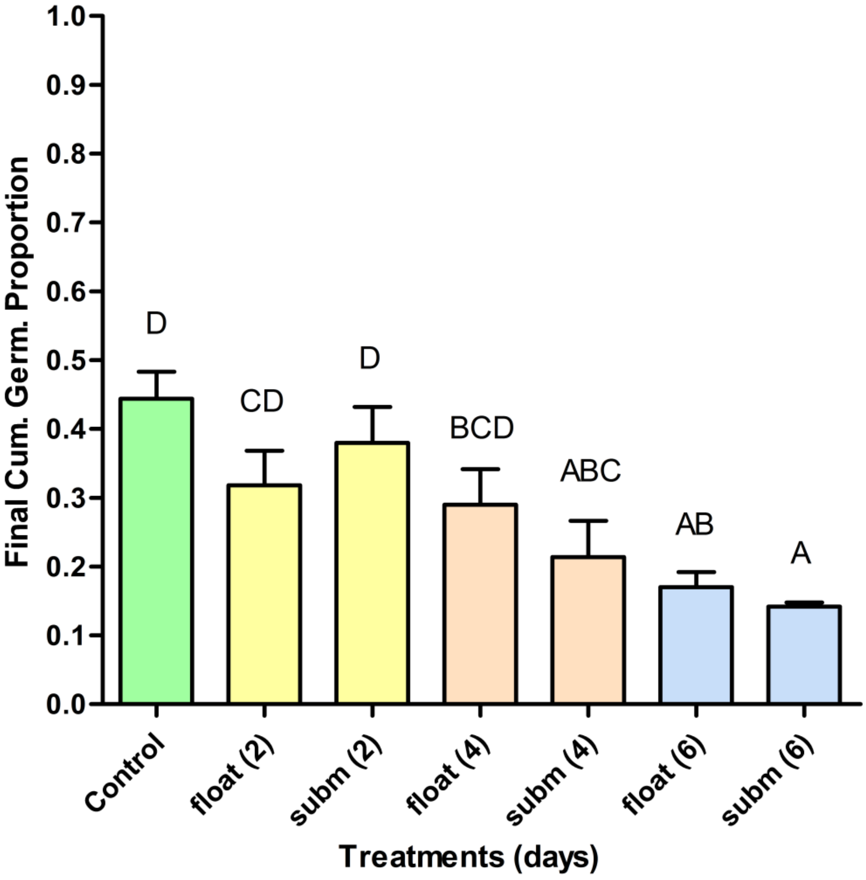

Figure 2. Laboratory: Final Cumulative Germination Proportion of 'La Paloma' bermudagrass at 0, 2, 4 and 6 days of floating and submersion. Vertical bars represent the standard error. Bars with a different letter are significantly different ($p<0.05$).

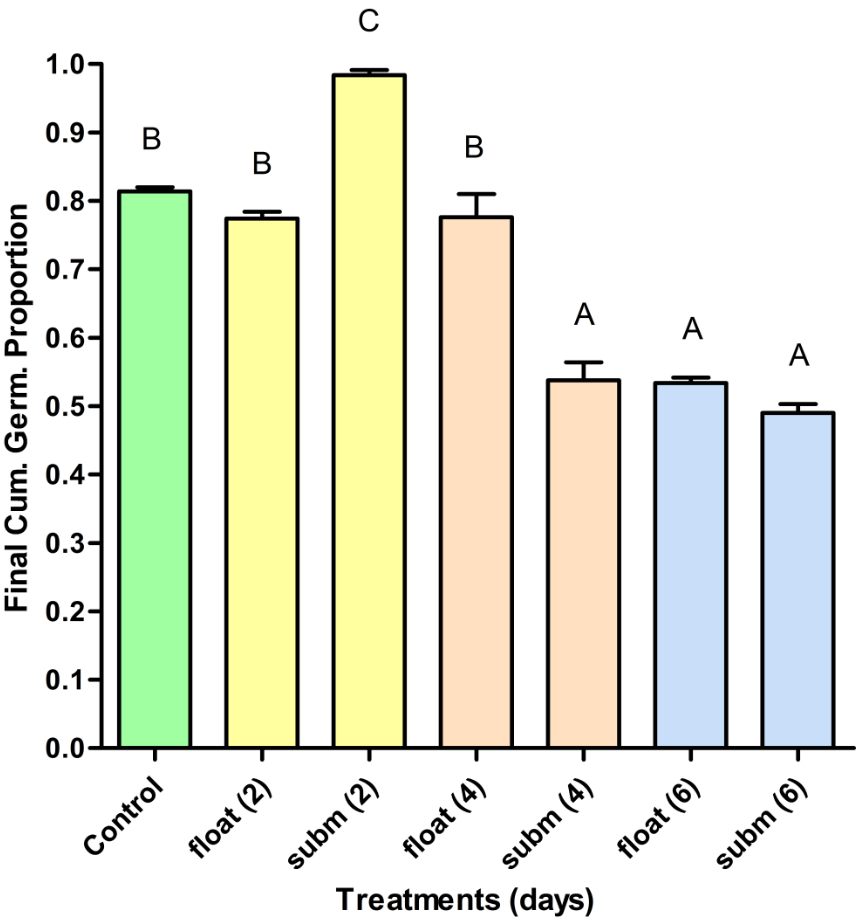

Figure 3. Laboratory: Final cumulative germination proportion of 'Yukon' bermudagrass at respectively 0, 2, 4 and 6 days of floating and submersion. Vertical bars represent the standard error. Bars with a different letter are significantly different ($p<0.05$).

The cultivars 'Jackpot', 'Sunbird' and 'Transcontinental', did not exhibit any significant difference among treatments (Table1), while 'La Paloma' and 'Yukon' showed a progressive decrease in final germination percentage with prolonged water submersion/floating conditions (2 days for submersion and 3-4 days for floating).

Table 1. Laboratory: final cumulative germinated proportion (prop.), standard error (SE) of five bermudagrass cultivars subjected to 0, 2, 4 and 6 days of floating or submersion (the experiment includes five replications).

	CTRL			Floating									Submersion								
	0			2			4			6			2			4			6		
	Prop.	SE		Prop.	SE		Prop.	SE		Prop.	SE		Prop.	SE		Prop.	SE		Prop.	SE	
Jackpot	0.726	0.037	A	0.660	0.039	A	0.758	0.036	A	0.686	0.039	A	0.734	0.037	A	0.800	0.033	A	0.714	0.038	A
La Paloma	0.444	0.041	D	0.318	0.039	CD	0.290	0.038	BCD	0.170	0.031	AB	0.380	0.040	D	0.214	0.034	ABC	0.142	0.029	A
Sunbird	0.612	0.040	A	0.608	0.041	A	0.694	0.038	A	0.666	0.039	A	0.630	0.040	A	0.738	0.037	A	0.646	0.040	A
Trans.	0.746	0.036	A	0.790	0.034	A	0.784	0.034	A	0.824	0.032	A	0.864	0.028	A	0.872	0.028	A	0.782	0.034	A
Yukon	0.814	0.032	B	0.774	0.035	B	0.776	0.035	B	0.530	0.041	A	0.984	0.010	C	0.544	0.041	A	0.488	0.042	A
Average	0.668	0.017		0.630	0.017		0.660	0.017		0.575	0.016		0.718	0.015		0.634	0.016		0.554	0.016	

Values in rows followed by different letters are significantly different, according to a familywise error rate ($P < 0.05$).

The difference in the final germination between floating and immersion (tab. 2 and 3) was probably due to the increase of seed weight by water imbibition under floating, allowing to complete the absorption phase.

Table 2 Greenhouse: final cumulative seedlings proportion (prop.) and standard error (SE) of four bermudagrass cultivars under four submersion treatments. (the experiment includes four replications).

	Control		Days of submersion									
Cvs	0		2		4		6		8		Average	
	Prop.	SE	Prop.	SE	Prop.	SE	Prop.	SE	Prop.	SE	Prop.	SE
Jackpot	0.394	0.064	0.456	0.065	0.481	0.066	0.397	0.092	0.394	0.064	0.424	0.032
Sunbird	0.409	0.074	0.314	0.061	0.292	0.070	0.299	0.071	0.489	0.095	0.360	0.034
Trans.	0.504	0.066	0.555	0.065	0.579	0.065	0.573	0.094	0.679	0.071	0.578	0.033
Yukon	0.406	0.065	0.573	0.065	0.480	0.077	0.529	0.133	0.553	0.093	0.508	0.040
Average	0.428	0.034	0.474	0.032	0.458	0.035	0.449	0.051	0.529	0.042	0.468	

Table 3. Greenhouse: the proportion of yellow seedlings and standard error (SE) of four bermudagrass cultivars after 6 and 8 days of submersion. (the experiment includes four replications).

	Days of submersion					
Cvs	4		8		Average	
	Prop.	SE	Prop.	SE	Prop.	SE
Jackpot	0.254	0.082	0.931	0.054	0.593	0.049
Sunbird	0.037	0.047	0.888	0.092	0.462	0.052
Trans.	0.410	0.115	0.949	0.043	0.680	0.065
Yukon	0.125	0.079	0.924	0.067	0.524	0.054
Average	0.207	0.045	0.923	0.037		

Differently, immersion is characterized by an initial phase of faster imbibition followed by a progressive decrease of oxygen availability which leads to lower germination *(*Bewley *et al.*, 2013). A significant difference between floating and submerged occurred only for a short initial period when oxygen availability was higher, and seed imbibition was in progress. These results are in agreement with Moriaga (Morinaga, 1926) who demonstrated in a laboratory study (in 'bottles') that bermudagrass is tolerant to submersion conditions and germination level can increase in comparison with germination in 'Petri dishes' (seeds tested for 42 days). As reported by Bewley *et al.* (2013), seed imbibition increases its fresh weight from 40 to 50%, and a wholly soaked seed can reach a percentage of moisture between 75 and 100%.

Greenhouse experiment

Results of analysis of deviance for the final cumulative seedlings proportion showed significant differences only for the effect of the cultivar (P = 0.00016), while the interaction ‘cultivar x treatment’ and the submersion effect were not significant (P = 0.67 and 0.44 respectively). The observed proportions are reported in Table 2. ‘Transcontinental’ displayed a significantly higher cumulative seedlings proportion (0.58) than ‘Jackpot’ (0.42) and ‘Sunbird’ (0.36), while ‘Sunbird’ (0.36) showed a cumulative seedlings proportion significantly lower than ‘Yukon’ (0.51) and ‘Transcontinental’ (0.58). Statistical analysis did not reveal significant differences among the five submersion treatments, suggesting that bermudagrass seedlings can easily withstand prolonged periods of waterlogging.

Unlike germination, the seedling phase did not show differences in behaviour among cultivars. By germination, bermudagrass shifts from a heterotrophic to the autotrophic organism, where leaflets and roots also play an essential role in response to submersion. Results obtained are in agreement with previous researches on bermudagrass, indicating that bermudagrass seedlings have a high tolerance to water submersion (Ashraf and Yasmin, 1991; Tan *et al.*, 2010; Springer *et al.*, 2014).

Regarding leaf yellowing (symptoms of hypoxia) results of the analysis of variance for flooding, treatments revealed a significant ‘treatment x cultivar’ interaction (p= $5.64e^{-07}$). No significant differences between 2 (T2) and 4 (T4) days submersion were observed, while water submersion for 6 (T4) and 8 days (T5) resulted in a significant increase in the number of yellow seedlings ($P = 1.5 \times 10^{-6}$), with no differences among cultivars (Table 3). Moreover, T5 showed a significant increase in the number of seedlings affected by leaf yellowing in comparison with T4. The period of 6 days of submersion indicates the beginning of visible suffering on bermudagrass seedlings, in agreement with previous studies reported bermudagrass moderately tolerant to flooding (Ashraf and Yasmin, 1991; Tan *et al.*, 2010). Studies carried out on sugarcane (*Saccharum officinarum* L.) that belongs to the same botanical family of bermudagrass, confirmed that submersion causes leaf yellowing (Gomathi *et al.*, 2015) as a consequence of depletion of chlorophyll content (Gomathi *et al.*, 2015). Another study conducted by Ye *et al.* (2015) reported that submersion negatively affects photosynthesis and redox-related pathways of bermudagrass.

Conclusion

Bermudagrass seeds and seedlings appeared to possess some short-term resilience to flood, as long as it is not prolonged.

The seeds of the five tested bermudagrass cultivars showed a moderate tolerance to floating/submersion during germination even though differences were found among cultivars. In particular, ‘Jackpot’, ‘Sunbird’, and ‘Transcontinental’ were the most tolerant. Future breeding programs should take into account the bermudagrass genetic variations for the selection of cultivars more tolerant of these stresses. Ultimately, six-eight days of full submersion represents the threshold for symptoms of hypoxia with yellowing of the seedlings basal leaves. We can, therefore, state that it is possible seeding bermudagrass without fear heavy rain events. However, it remains largely unknown to the seed/seedling environment interactions. Thus a field experiment could be necessary to deepen this aspect.

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Annex**:** Figures of the experiment in the greenhouse.

Figure 1. *Each tray contains a 3 cm bed of expanded clay separated by a nonwoven fabric towel.*

Figure 2. *Weighing of the soil-sand mix (70% and 30% V/V) for each tray.*

Figure 3. *Seeding in the plastic trays.*

Figure 4 Trays into the greenhouse with sprinkles for irrigation.

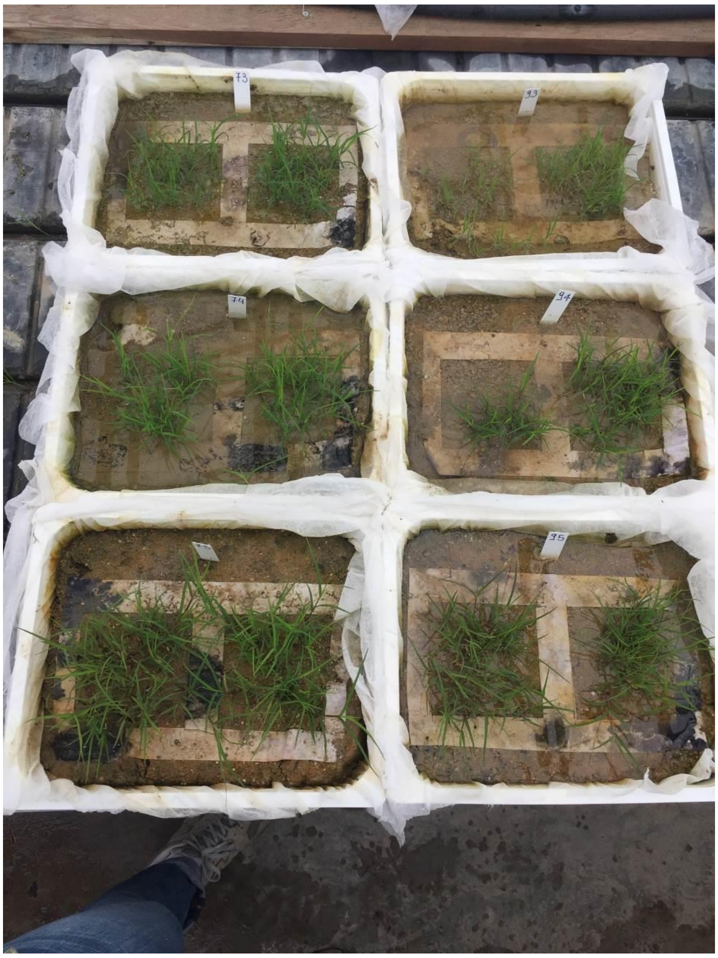

Figure 5. Trays during submersion period.

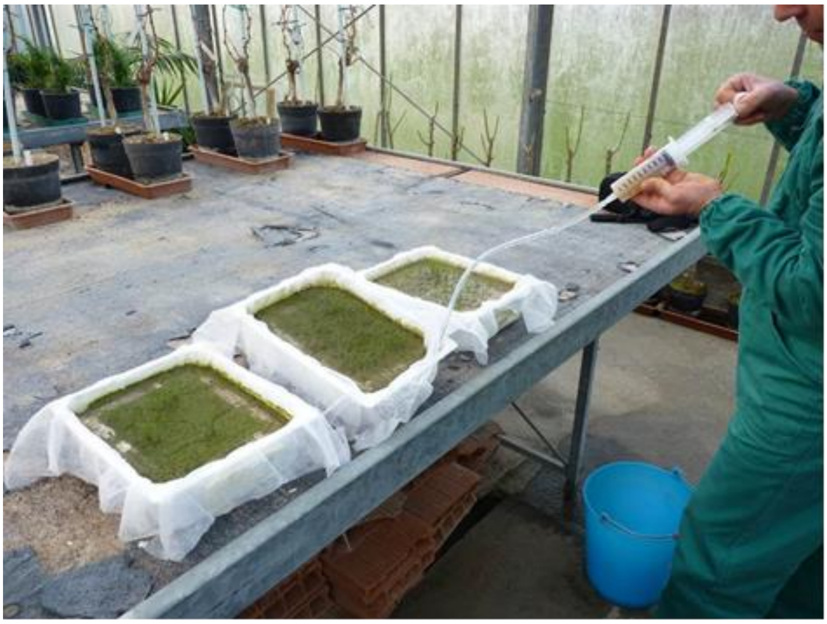

Figure 6*. Extraction of water in excess after submersion.*

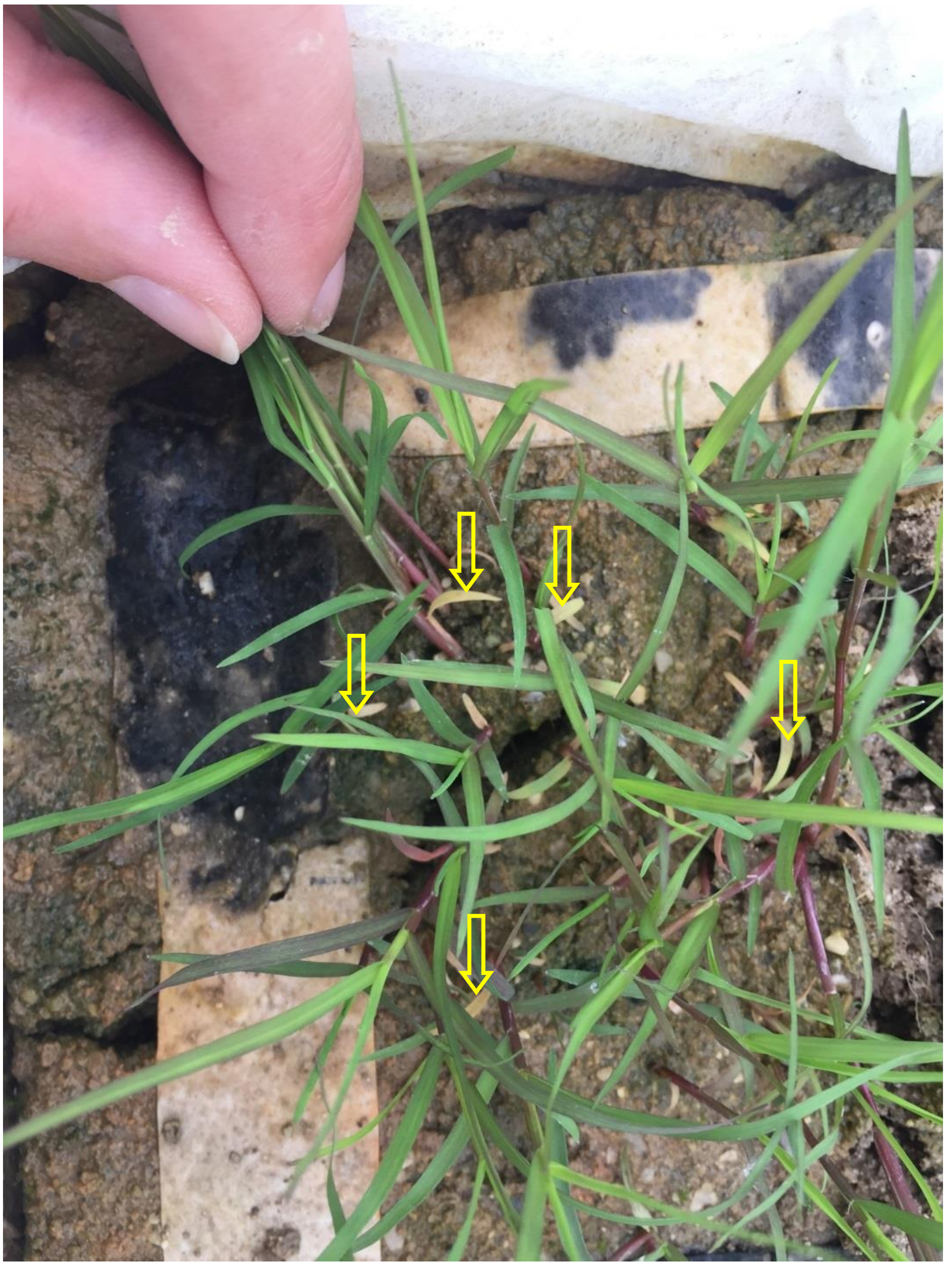

***Figure 7**. Evaluation of seedlings after submersion. The arrows indicate yellowed basal leaves.*

Chapter 4

Influence of seeding time on bermudagrass (*Cynodon* spp.) establishment in the upper Mediterranean region

Introduction

The Mediterranean region is included between the rainy climate of Central Europe and the arid area of North Africa (Giorgi and Lionello, 2008) and roughly coincides with the so-called transition zone (Minelli *et al.,* 2014). Warm-season turfgrasses are suitable for the Mediterranean region due to some advantages such as drought and heat stress tolerance (Magni *et al*., 2014). Until a few years ago, these species were not widely cultivated in Europe because of massive imports of cold season grasses from northern European Countries (Croce *et al.,* 2001). However, recently, due to the predicted increase of temperature and the decrease in water availability, they could be of great interest in this region (Hatfield, 2017). Warm-season turfgrasses include many species such as seashore paspalum (*Paspalum vaginatum* Swartz), zoysia (*Zoysia japonica* Steud.), kikuyu grass (*Pennisetum clandestinum* Hochst. ex Chiov.), but bermudagrass (*Cynodon* spp.) is dominant and it is the most widely-used species (Shaver *et al*., 2006; Magni *et al*., 2014) with a large number of cultivars available on the turfgrass market. In the OECD catalogue of 2019 are listed thirty cultivars of bermudagrass (OECD, 2019). In the transition zone, bermudagrasses are generally seeded in late spring or early summer, when the average daily temperatures are sufficiently high for germination (Pornaro *et al.,* 2016). Seeding is much cheaper than vegetative propagation and can be considered the best method for establishing low-maintenance turfgrasses. Hybrid bermudagrasses are sterile and must be propagated vegetatively, and are recognized as high-quality cultivars. Several improved seeded bermudagrass cultivars have been released since the early 1980s (Richardson *et al*., 2003, 2004, 2005). Nowadays the new seeded cultivars can be compared in turfgrass quality to the vegetative hybrids. They are increasingly popular even though one of the most debated aspects concerns the cold tolerance of new seeded cultivars during the first year of establishment (Richardson *et al.,* 2004). Due to this limitation in the upper transition-zone environment, seeded bermudagrass can suffer winter injuries, and the establishment phase is crucial to reduce the risk of winter injuries. Richardson *et al.* (2004) and Philley *et al.* (2009) shown that in transition zone early seeding lead to a faster establishment compared with later seeding, allowing a better cold tolerance during the first-year winter. T_{BASE} and thermal time of the different cultivars are aspects to be taken into consideration, and that can play a decisive role in the choice of the varieties to be used in early spring over most in the colder areas of the transition zone (Richardson *et al.*, 2004). This study aimed to study the effects of different seeding dates on the speed of establishment of some bermudagrass cultivars to determine the best seeding time for northern Italy.

Materials and methods

A field study was conducted from late March to early July 2018 at the Experimental Agricultural Farm of Padova University in Legnaro, northeastern Italy (45°20', 11°57'E, elevation 8 m) and it was repeated in 2019. The area is characterized by a humid subtropical climate with the annual minimum, average and maximum temperature respectively of 8.8°, 13.6°, 18.6° C and rainfall of 841 mm year^{-1} [Regional Agency for Environmental Protection of Veneto Region (ARPAV), 2019]. The soil at the site consisted of a clay loam (Soil Survey Staff, 1999) with 28.0% silt, 27.7% clay and 44.3% sand with pH of 8.17, 2.77% organic matter (Walkeley & Black method), N content of 0.14 mg kg^{-1}, 3.9 mg kg^{-1} of available phosphorus (P) (Olsen method), and 170.8 mg·kg^{-1} exchangeable potassium (K). From late March three seeding dates (S1, S2, and S3) with 30-day interval were tested (27 March, 26 April and 27 May during the 2018 and 22 March, 3 May, 4 June, during 2019). Four bermudagrass cultivars were compared: 'Transcontinental', 'Jackpot' and 'SR9554' and 'La Paloma'. The level of germination of each cultivar was preliminarily determined in the laboratory. The seeding rate was calculated as a number of seeds considering a Pure Live Seeds (PLS) rate in weight of about 2.5 gr m^{-2}. Plots were hand-seeded and irrigated daily with 5 mm of water using a sprinkler system. The experimental design was a split-plot with three replications with 'seeding dates' as plot and 'cultivars' as a subplot. A slow-release fertilizer (8-24-24) was applied at a rate of 50 N kg ha^{-1} before seeding. Additional nitrogen, at a rate of 60 N kg ha^{-1} was applied as urea (46-

0-0) 10 days after emergence. A metal frame (10 cm x 10 cm) was used to determine the number of seedlings per unit area.

At emergence (50% seedlings emerged) the number of seedlings enclosed in the frame was recorded, and five samples per plot were performed. Plots were 1.5 by 1.5 m with 0.5 m bare soil pathways arranged in a split-plot design with three replications with seeding date as main plot and cultivars ad subplot. The Degree Days (DD) from seeding to reach 75% green coverage were calculated. Percentage of the green cover was measured weekly from emergence using digital image analysis techniques (Richardson *et al.*, 2001) until the first decade of July. Three temperature sensors (thermocouples) connected to a data logger (CR10X; Campbell Scientific, Logan, UT), were installed at 2.5 cm soil depths to collect soil temperature. Soil temperature was recorded hourly and the average daily temperature from the first seeding date until the end of the study. Degree Days (DD) were calculated with daily average soil temperature at 2.5 cm depth using 15 °C as base temperature (Giolo *et al.* 2014). Digital image analysis was used to determine the speed of establishment (Richardson *et al.*, 2001). A sigmoidal association (Busey and Myers, 1979; Leinauer *et al.*, 2010) was identified to best describe the establishment (GraphPad Prism 5.1 for Windows; GraphPad Software, La Jolla, CA). According to Leinauer *et al.* (2009), establishment was considered satisfactory when 75% ground cover was reached. Sigmoidal models were used to calculate the number of days and Degree Days from seeding to reach 75% ground cover [Days From the S1 (DFS1), the Days From the Seeding Date (DFS), and Degrees Days (DD)].

The 3rd seeding date (S3) was not included in the statistical analysis because when S1 reached the reference coverage of 75% (beginning of June), the seedlings of S3 were not emerged yet.

Statistical analysis

Data were used to parametrise a Linear Mixed Model, where the ‘Year’, ‘Seeding Time’, ‘Cultivar’; and all the interactions therein were included as fixed effects, while the blocks within years and the main plots within blocks and years were included as random effects, to account for the clustering of observations and ensure the independence of model residuals. Normality and homoscedasticity of residuals were checked by using graphical analyses. The means for the significant interaction of the highest order were compared by using a multiple comparison testing procedure, accounting for multiplicity (Bretz *et al.*, 2011).

Results

The main effect of 'cultivar' was significant ($p< 0.001$) for all the parameters: DFS1, DFS, DD, while 'Seeding Date' was significantly ($p<0.01$) affected by DFS1 and DFS (Table 1).

Table.1. Results of analysis of variance testing the effects of days from the 1st seeding date (DFS1), days from seeding date (DFS) and Degree days (DD).

Factors	DFS1	DFS	DD
Year	NS[1]	NS	*
Seeding Date	**	**	NS
Cultivar	***	***	***
Year x Seeding Date	NS	*	NS
Year x Cultivar	NS	NS	NS
Seeding Date x Cultivar	NS	NS	NS
Year x Seeding Date x Cultivar	NS	NS	NS

NS[1] = not significant at the 0.05 level of probability
* Significant F-test at the 0.05 level of probability
** Significant F-test at the 0.01 level of probability
*** Significant F-test at the 0.001 level of probability

Interactions between DFS1, DFS, and DD were not significant for any parameter investigated while the interaction 'Year x Seeding Date' was significant for DFS. S1 reached the reference coverage of 75% faster than S2, but this result was limited to 2018 (Table 2).

Table 2. DFS1: comparison between 1st seeding date (S1) and 2nd seeding date (S2) for each year of experimentation.

Seeding date	**2018**		**2019**	
	emmean	**group**	**emmean**	**group**
S1	71.68	A	82.16	A
S2	93.49	B	92.98	A

Significant level: α=0.05

The ‘cultivar’ effect, for the same parameter, was also highly significant (Table 1). Multiple comparisons ($p<0.05$) displayed that three out four cultivars (‘Transcontinental’, ‘SR9554’, ‘La Paloma’) showed the same number of days (average of two years) to reach 75% ground cover, i.e. 80-86 days, while ‘Jackpot’ needed 92 days. The statistical analysis of DFS for separate years showed a significant difference between S1 and S2 in 2019 only.

Table 3. DFS: comparison between 1st seeding date (S1) and 2nd seeding date (S2) for each year of experimentation.

Seeding date	**2018**		**2019**	
	emmean	**group**	**emmean**	**group**
S1	71.68	A	82.16	B
S2	93.49	A	51.04	A

Significant level: α=0.05

The interaction 'Year x Seeding Date' (Table 1) was also significant ($p < 0.05$) highlighting the influence of different temperature trends in both 2018 and 2019.

Multiple comparisons test between the cultivars showed the same results for DFS1 and DFS parameters: 'Jackpot' needed more days to reach 75% coverage than all the other cultivars.

The ANOVA did not display any differences in DD between seeding times nor overall, nor considering the two years separately (data not shown). Degree Days displayed significant differences between years ($p<0.05$) and also among cultivars ($p< 0.001$). Multiple comparisons test among cultivars showed differences between the cultivars linked to their probable different thermal time (Giolo *et al.*, 2019): 'Jackpot' was significantly different from 'Transcontinental' and 'SR9554', but not from 'La Paloma'.

The number of seedlings per square meter estimated at 50% of emergence produced the following results in the three seeding dates (average of two years and three replications): S1 (684), S2 (1,017), S3 (648). In table 4 are reported the number of seedlings emerged per square meter of each cultivar.

Table. 4. General seeding dates (S1, S2, and S3); date of seeding in 2018 and 2019; date of 50% seedling emerged (average of three replications a); Days from seeding (DFS) to 50% seedling emerged (average of three replications), Degree days (DD) from seeding to 75% cover (average of three replications).

Year	Cultivars	Seeding dates	Date of seeding	DFS at 50% emergence	Seedlings n m^{-2} at 50% emergence	DD at 75% green coverage
2018	Jackpot	S1	27 March	37	333	402.94
	La Paloma			37	567	372.87
	SR9554			37	644	342.94
	Transcontinental			37	1,033	329.11
	Jackpot	S2	26 April	29	711	548.61
	La Paloma			29	1,400	523.33
	SR9554			29	1,533	354.15
	Transcontinental			29	1,450	353.97
	Jackpot	S3	27 May	21	667	Not available
	La Paloma			21	1,067	
	SR9554			21	1,067	
	Transcontinental			21	1,167	
2019	Jackpot	S1	22 March	49	396	367.43
	La Paloma			49	843	257.90
	SR9554			49	379	281.17
	Transcontinental			49	1,272	261.47
	Jackpot	S2	3 May	29	339	353.63
	La Paloma			28	1,006	244.10
	SR9554			29	158	267.37
	Transcontinental			28	1,539	247.67
	Jackpot	S3	4 June	34	156	Not available
	La Paloma			34	400	
	SR9554			34	129	
	Transcontinental			34	531	

DD = Degree Days

Discussion

The temperature increased during the three seeding dates in both years, although until mid-June the mean temperature in 2019 was lower than in 2018. In 2018, the average daily temperature was equal to or lower than the T_{BASE} of 15 °C (Giolo *et al.*, 2014) until mid-April while in 2019 non-optimal temperatures (<15°C) continued until mid-May (Figure 1).

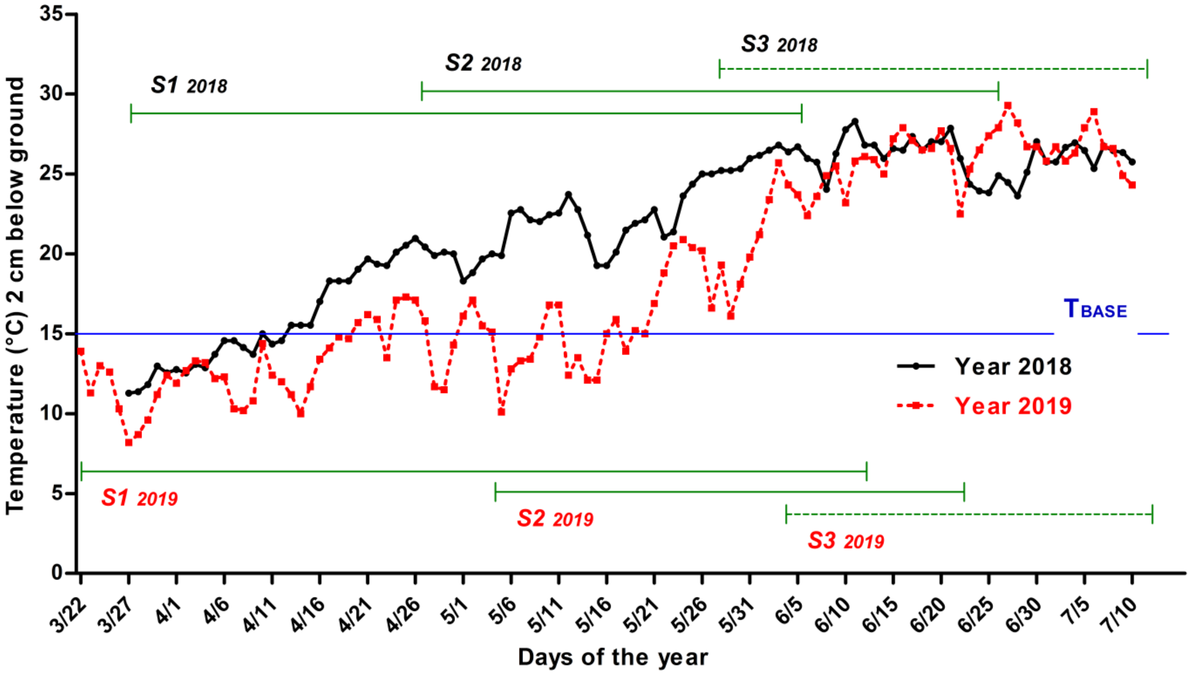

Figure 1. Daily ground temperatures (2 cm below ground) from April to July in 2018 and 2019. The blue line is the T_{BASE} of 15 °C; S1, S2, and S3 are the seeding times of each year. The green lines show the period from seeding time to reach 75% coverage; S3 is indicated by a dotted line as it should continue throughout July.

The significant effect of the parameter DFS1 shown in Figures 2 and 3 revealed that bermudagrass being tested in S1 were faster in reaching coverage of 75% compared to S2.

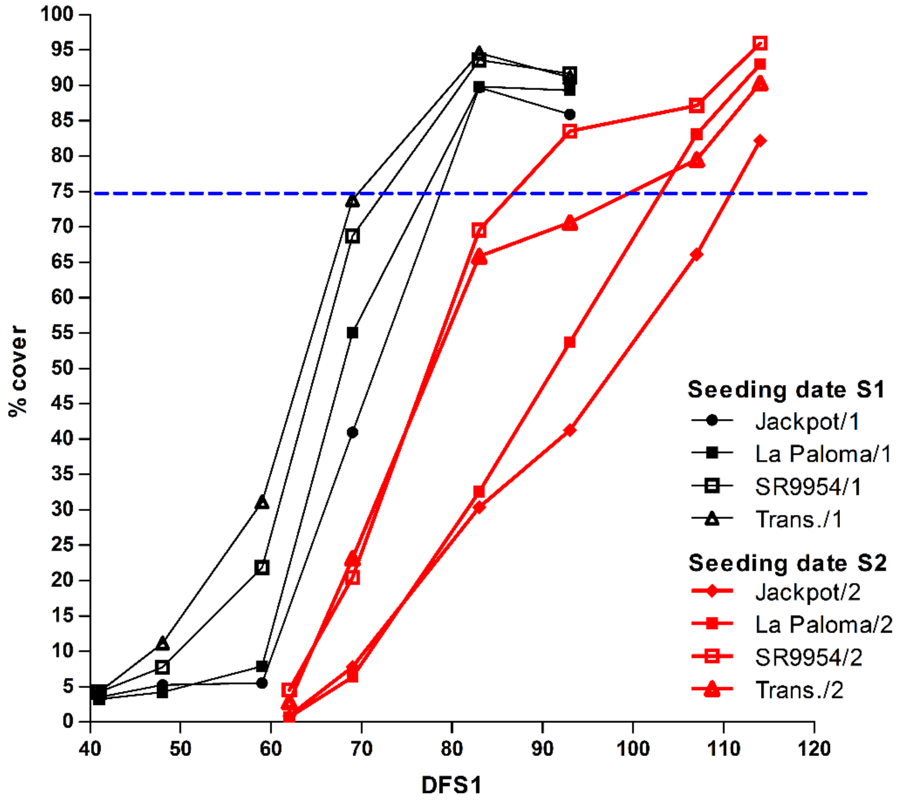

Figure 2. Percentage coverage reached by four bermudagrass cultivars from 1st seeding date (S1) and 2nd seeding date (S2) in 2018. The blue dotted line indicates the reference % cover level.

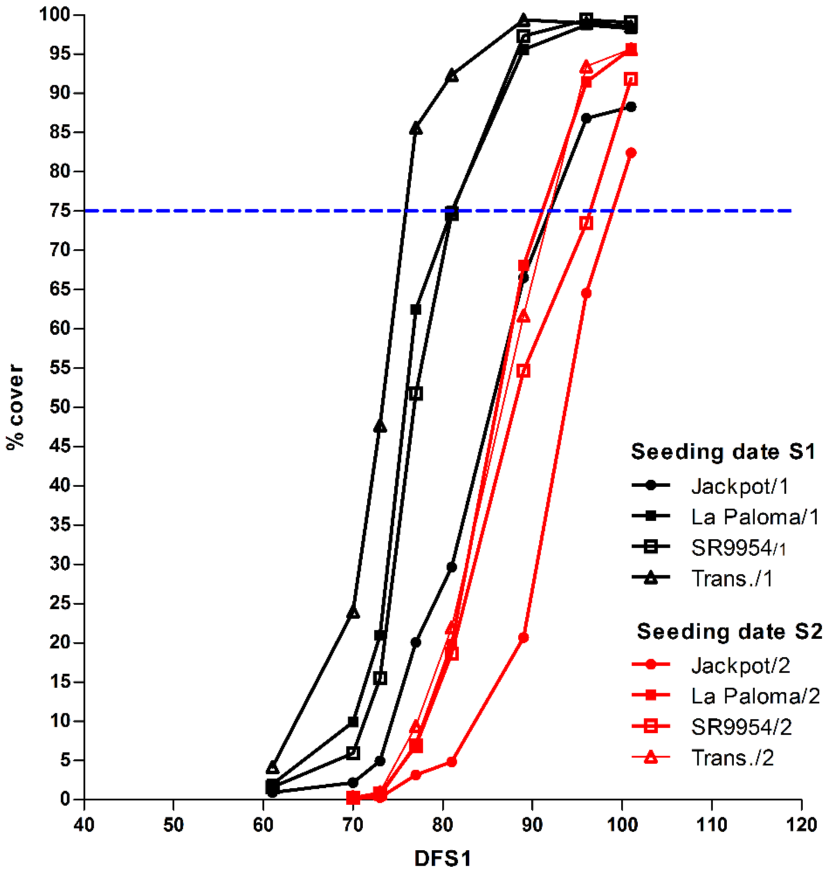

Figure 3. Percentage coverage reached by four bermudagrass cultivars from 1st seeding date (S1) and 2nd seeding date (S2) in 2019. The blue dotted line indicates the reference % cover level.

S3 reached 75% green cover at the end of July, much later than S1 and S2. For both 2018 and 2019, S1 allows cultivars to reach 75% coverage at the beginning of June. The 1st seeding date (end of March) guaranteed a faster establishment allowing the turfgrass to avoid winter injuries (figure 4).

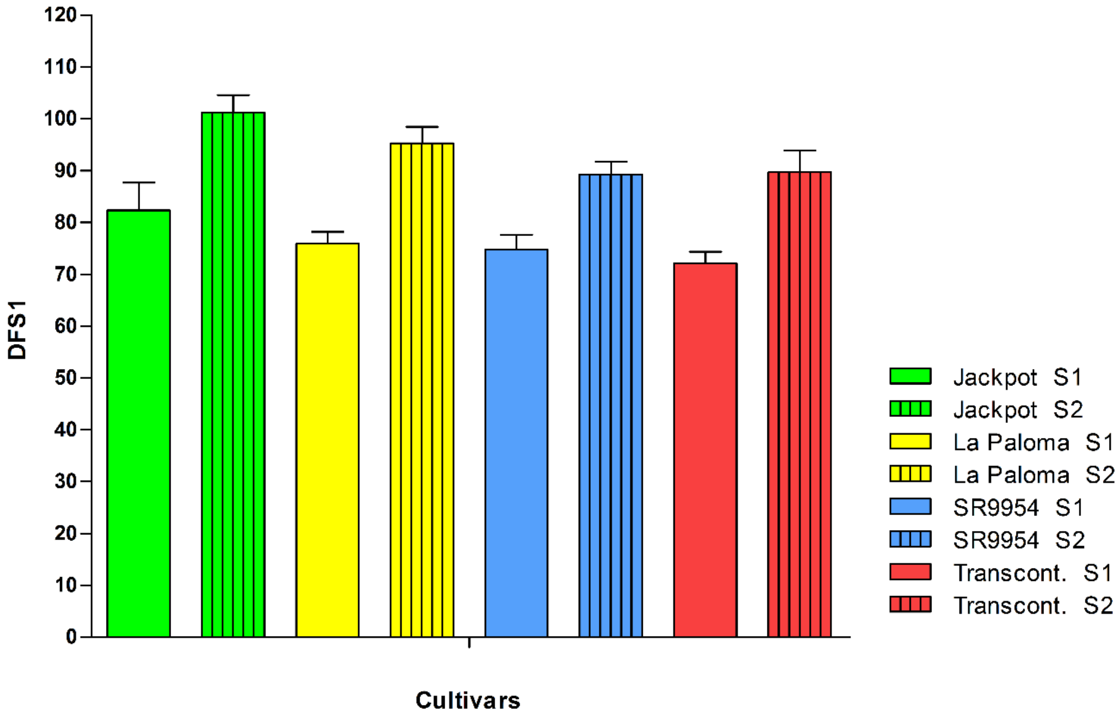

Figure 4. ‘Cultivar’ x ‘seeding date’ interaction: column bars represent Days from 1st seeding date (DFS1) to reach the 75% coverage (average of 2018 and 2019). S1 = 1st seeding date, S2 = 2nd seeding date.

These results are in line with those of Richardson *et al.* (2004), who reported that early spring seeding (April and May) guarantee a high recovery from cold winters and a more developed turf compared to late seeding. Results also agreed with Giolo *et al.* (2019), who demonstrated in a field study that the estimated date for germination of ‘Transcontinental’ and ‘La Paloma’ varied from early April to mid-April respectively. The 2nd seeding date takes fewer days to reach the coverage reference as it takes advantage of higher mean daily temperatures compared with S1. However, the higher temperatures which benefited S2 did not make it possible to recover the 30-day delay to S1. As shown in Figure 1, the second year of experimentation (2019) was affected by low temperature, mainly in the period following S1. The statistical analysis showed no significant differences between S1 and S2. ‘Jackpot’ had higher DD to reach the reference coverage than ‘Transcontinental’ and ‘SSR9554’ while no differences occurred between ‘Jackpot’ and ‘La Paloma’. This result is probably due to the similar thermal time for ‘Jackpot’ and ‘La Paloma’ according to Giolo *et al.* (2019) who found no differences in Θ_{T30} between the two cultivars. In 2019, poor and delayed germination was noted for S3 due to high temperatures occurred at that time. The calculation of DD based exclusively on temperature, without considering the water potential, has been slightly distorted and, in 2019, this approach led to a significant effect of ‘year’. The S3 characterized by high soil temperature resulted in reducing germination of small seeds such those of bermudagrass. Indeed, small seeds when seeded remain at or near the soil surface and they are negatively affected when the soil loses moisture (Evers and Parsons, 2009). This aspect is also confirmed by the average emergency values of S3, which were significantly lower than S2 for all the cultivars tested (Table 2).

Conclusions

The present study demonstrated that seeding bermudagrass in early spring allows a rapid and good establishment. Early spring seeding permits few seeds to germinate, but the high and vigorous seedling growth even at sub-optimal temperatures ensure a quick establishment. The cultivars ‘La Paloma’, ‘SR9954’ and ‘Transcontinental’ were equally fast in reaching 75% cover, while ‘Jackpot’ needed more days. In our experimental conditions, the optimum seeding time was late March. This finding further confirms the possibility of using seeded bermudagrass even in the upper Mediterranean transition zone, allowing an excellent establishment without running into winter injuries.

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Chapter 5

Influence of waterlogging and seeding rate on the establishment of warm-season turfgrass species

Introduction

The transition zone includes almost all the Mediterranean region (Geren *et al.*, 2009), where both cold- and warm-season grasses can be cultivated. Most of the warm season turf species are native to the tropical zone with optimum growing temperature between 21 and 35° C (Trlica, 2006) and are cold-sensitive, however, they can be successfully used the transition zone even if they are not entirely suitable (Geren *et al.,* 2009;) These are plant species with are low-water consumption as they use soil water more efficiently and have a lower ET coefficient rate than cool-season grasses (Romero and Dukes, 2016).

The increasing average temperature due to climate change seems to direct the attention towards warm-season grass species and their adaptation to new climatic projections that show an increase of average temperature in the Mediterranean region The predictions of mathematical models confirm that over the next century, the Mediterranean region will encounter drier general conditions (Stagge *et al.*, 2015).

The influence of climate change on the turfgrass is expected at different levels: temperature, precipitation intensity and carbon dioxide (Hatfield, 2017). Projections also include a considerable increase in extreme precipitation events and the risk of inland flooding. Trends in Mediterranean Region show heavy precipitation, especially during the spring season, which may impact on seed germination and seedling growth of warm-season grasses (Zong *et al*., 2015). A migration of warm-season turf species to areas that are becoming warmer due to climate change is also envisaged (Hatfield, 2017). No recent studies regarding submersion have been published, and the only available in literature concern cultivars propagated vegetatively and they not focus on the first stages of establishment. Heavy rain can often produce a flash flood because the duration of the event does not exceed six hours ((Knocke and Kolivras, 2007), and it is different from a regular flood.

Although an excess of humidity is harmful to crops, plant tolerance to flooding is variable (Pucciariello *et al.*, 2014). Plants which are sensitive to floods are severely damaged by 24 hours of anoxia, while flood-tolerant plants that can stand the lack of oxygen for a few days and wetland plants that have a unique adaptation and can resist long-term in anaerobic conditions such as rice (Taiz and Zeiger, 2010).

Roots need oxygen (O_2) for aerobic respiration and can find it from pores of the soil. If water occupies the space delimited by the pores, the diffusion of oxygen stops immediately, and only the portion of the ground closest to the surface remains partially oxygenated (Taiz and Zeiger, 2010).

Beard (1973) evaluated excellent submersion tolerance of buffalograss and bermudagrass. Insausti *et al.* (2001) showed that submersion in dallisgrass (*Paspalum dilatatum* Poir.) causes physiological changes with increasing root porosity and leaf aerenchyma.

Fry (1991) studied submersion tolerance of sodding turf and compared five warm-season grass species: zoysiagrass, common centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.], St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze], bahiagrass (*Paspalum notatum* Flueggé) and common bermudagrass submerged for 55 and 93 days. Results showed that all turfgrass species had high submersion tolerance, bermudagrass and bahiagrass showed the best tolerance performance, while centipedegrass exhibited the most inferior tolerance. Tan *et al.* (2010) studied the physiological responses of bermudagrass to water submergence showing that bermudagrass has a detoxification mechanism, lower metabolism and significative amounts of carbohydrate that allows long-term survival. On the contrary, Hansen *et al.* (2000) considered bermudagrass as not tolerant of waterlogging conditions.

Zhang *et al.* (2013) analysed germination and seedling growth by greenhouse experiments with four turfgrasses and found that buffalograss cv. Texoka was less tolerant than tall fescue cv Stonewall to both waterlogging a salinity. Zong *et al.* (2015) examined the growth and physiological mechanism of waterlogging tolerance on four warm-season turfgrasses, including seashore paspalum that showed a reduction in biomass under 30 days of waterlogging.

It is interesting to note that there is little information available regarding the seeding rate, even if it plays a crucial role in the establishment. Studies conducted on seeding rate are mainly based on different parameters (e.g. seeding rate, reference coverage) and therefore not conclusive. A recent study on seashore paspalum (cv Sea Spray) and bermudagrass (cvs Riviera and Sovereign) demonstrated no significant effect of seeding rate on the duration of the establishment phase (Poranro *et al.*, 2016). Differently, Leinauer *et al.* (2010) found for 'Panama' bermudagrass that applying the recommended seeding dose (10 g m^{-2}) the effect on coverage (50% level) was better compared to the reduced dose (5 g m^{-2}). Seeding rate is influenced mainly on the level of germination and environmental conditions. The number of germinated seeds that will produce seedlings is a minimal portion compared to those seeded (Pessarakli, 2007).

The objective of our study was to investigate the effect of simulated flash flooding events on three of the most used warm-season turf species in the transition zone (bermudagrass, seashore paspalum and buffalograss) at two seeding rate during the first stages of establishing which are crucial for future turfgrass performance. Furthermore, seedling emergence, seedling dry weight and percentage of seedling coverage were evaluated.

Materials and Methods

The experiment was set up at the Agricultural Experimental Farm of Padua University northeastern Italy (lat. 45°20'N, long. 11°57'E, elevation 8 m) on June 2017 and replicated in 2018.

The soil at the site was a clay loam (Soil Survey Staff, 1999) (44.3% sand, 28.0% silt, and 27.7% clay) with pH of 8.17, 2.77% organic matter (Walkeley & Black method), N content of 0.14 $mg \cdot kg^{-1}$, 3.9 $mg \cdot kg^{-1}$ of available phosphorus (P) (Olsen method), and 170.8 $mg \cdot kg^{-1}$ exchangeable potassium (K).

Four bermudagrass (*Cynodon spp.*) cultivars ('La Paloma', 'Transcontinental', 'SR 9554', 'Jackpot'), 'Pure Dynasty' seashore paspalum (*Paspalum vaginatum* Swartz) and 'SWI 2000' buffalograss [*Buchloe dactyloyde*s (Nutt.) Engelm] were compared from June to August. The area has a humid subtropical climate (Koppen, 1936)with an annual rainfall of 820 mm and means air temperature of 12.3 °C. Before seeding, a slow-release fertiliser (8N–24P–24K) was applied at a rate of 24 kg $ha^{-1}$of nitrogen. Plots were fertilised two weeks after emergence, beginning of July, with urea at 50 kg ha^{-1} of nitrogen. The cultivars were seeded on 19 June in 2017 and on 18 June in 2018. Two seeding/burs rates (pure live seed) were used: (2.5 $g.m^{-2}$) and (5.0 $g\cdot m^{-2}$) for bermudagrass cultivars, (5.0 $g\cdot m^{-2}$) and (10.0 g m^{-2}) for ‘Pure Dynasty’ seashore paspalum, and (10.0 $g\cdot m^{-2}$) and (20.0 g m^{-2}) of “burs” for ‘SW 2000’ buffalograss. However, for bermudagrass, the seeding rate was calculated as a number of seeds, for all the cultivars of the same species, the same number of Pure Live Seeds (PLS) was used. The seeding rate was therefore expressed as ‘number of seeds’ (Table1).

This study explored the effects of waterlogging on turfgrass establishment comparing two levels of waterlogging (with and without): 1) seedlings grew at field capacity and watered every other day until establishment, 2) seedlings grew under the same conditions with simulated flash flood events during a week period about four weeks after the seeding date. The experimental design was a strip-split-plot with three replications with irrigation as whole plot, species/cultivar as subplot and seeding rate as subplot (size 1.5 x 1.5 m).

A preliminary test was performed to determine the water infiltration rate of the soil using a double ring method (Burgy and Luthin, 1956; H. Gregory *et al.*, 2005). The test showed an infiltration rate of 30 mm/h. To determine how uniformly the water was applied by the sprinkler irrigation system, a catch can test was also conducted (Mecham, 2004; Bernd Leinauer, 2012). Based on water infiltration rates and run time of irrigation flooding treatment was calculated, and irrigation was carried out three times a week applying 150 mm of water for each application to simulate waterlogging conditions of an extreme rainfall event.

The percentage of germination of each cultivar was preliminarily determined in the laboratory.

The cultivars were seeded 19 June in the year 2017 and 18 June in the year 2018.

Table 1. Experiment plan: species, cultivars, irrigation level, seeding rate (PLS)

Species	Cultivars	Irrigation level			
		I		II	
		Seeding rate (PLS): n m^{-2} (g m^{-2})			
		1	2	1	2
Bermudagrass	Transcontinental Jackpot SR 9554 La Paloma	3,906 (~2.5 g m^{-2})	7,812 (~5 g m^{-2})	3,906 (~2.5 g m^{-2})	7,812 (~5 g m^{-2})
Seashore paspalum	Pure Dynasty	4,065 (~5 g m^{-2})	8,130 (~10 g m^{-2})	4,065 (~5 g m^{-2})	8,130 (~10 g m^{-2})
Buffalograss	SWI 2000	971 (~10 g m^{-2})	1,942 (20 g m^{-2})	971 (~10 g m^{-2})	1,942 (~20 g m$^{-2)}$

For bermudagrass, which included hulled cultivars (‘Transcontinental’, ‘Jackpot’,‘SR 9554’ and ‘La Paloma’), the number of seeds contained in 2.5 gr and 5.0 gr respectively from the cultivar SR 9554 was used as reference for the cultivars even if it can be roughly related to a weight. At emergence, 50% seedling emerged (visually assessed), the number of seedlings per unit area was determined using a metal frame (10 cm x 10 cm). Turf coverage was determined weekly from germination until full establishment using digital image analysis (Karcher and Richardson, 2003).

Soil volumetric water content (0-5 cm depth) was measured weekly before each submersion treatment using a portable time-domain reflectometer (Field scout 300, Spectrum Technologies, Plainfield, USA).

Normalised Difference Vegetation Index (NDVI) was measured weekly using chlorophyll meter Field Scout CM 1000 (Spectrum Technologies, Plainfield, USA). At the end of the experiment (August 10 and 11), two soil cores (0-8 cm depth) were collected in each plot and split into two depths: 0-3 and 3-8 cm and subsequently oven-dried to determine above-ground biomass and root dry weight.

Statistical analyses

Data were used to parameterise a Linear Mixed Model, where the 'Year', 'Irrigation Level', 'Seeding rate', 'Cultivar' and all the interactions therein were included as fixed effects, while the Irrigation Levels within year, the blocks within irrigation levels and years and the main plots within blocks, irrigation levels and years were included as random effects, to account for the clustering of observations and ensure independence of model residuals. Normality and homoscedasticity of residuals were checked by using graphical analyses. The means for the significant interaction of the highest order were compared by using a multiple comparison testing procedure, accounting for multiplicity (Bretz *et al*., 2011).

Results

EMERGENCE

As showed in Table 2, the analysis of variance displayed that the number of seedlings and cover percentage at 30 days from seeding was affected by ‘seeding rate’ unlike seedlings percentage. The ‘cultivar’ effect and the interaction ‘year x cultivar’ were significant for all the three parameters. The three-way interaction 'year x irrigation level x seeding rate' was also significant for a number of seedlings and seedlings percentage.

Table 2. Results of analysis of variance for the number of the parameters of seedlings, seedlings percentage, and cover percentage.

Factors	Number of seedlings	Seedings (%)	Cover (%)
Year	-[1]	-	-
Irrig. Level	-	-	-
Seeding rate	***	NS	**
Cultivar	***	***	***
Year x Irrig. Level	-	-	-
Year x Seeding rate	NS	NS	NS
Irrig. Level x Seeding rate	NS	NS	NS
Year x Cultivar	***	***	***
Irrig. Level x Cultivar	NS	NS	NS
SeedRate x Cultivar	NS	NS	NS
Year x Irrig. Level x Seeding rate	NS	NS	NS
Year x Irrig. Level x Cultivar	*	*	NS
Year x Seeding rate x Cultivar	NS	NS	NS
Irrig. Level x Seeding rate x Cultivar	NS	NS	NS
Year x Irrig. Level x Seeding rate x Cultivar	NS	NS	NS

-[1] = due to the experimental lay-out these effects have no correct denominator for the F test
NS[2] = not significant at the 0.05 level of probability
* Significant F-test at the 0.05 level of probability
** Significant F-test at the 0.01 level of probability
*** Significant F-test at the 0.001 level of probability

DRY WEIGHT

The ANOVA showed significant differences among 'cultivar' for the parameters aboveground dry weight (Aboveground DW) and root dry weight of zone 3-8 cm below of the soil surface (Roots 3-8) (Table 3). All the interactions were not significant. The analysis of variance of root dry weight of zone 0-3 cm (Roots 0-3) did not reveal a significant difference.

Table 3. Analysis of variance of parameters aboveground dry weight (Aboveground DW), roots dry weight at 0-3 (Roots 0-3) and 3-8 cm (Roots 3-8) depth.

Factors	**Aboveground DW**	**Roots 0-3**	**Roots 3-8**
Year	-[1]	-	-
Irrig. Level	-	-	-
Seeding rate	NS[2]	NS	NS
Cultivar	***	NS	*
Year x Irrig. Level	-	-	-
Year x Seeding rate	NS	NS	NS
Irrig. Level x Seeding rate	NS	NS	NS
Year x Cultivar	NS	NS	NS
Irrig. Level x Cultivar	NS	NS	NS
SeedRate x Cultivar	NS	NS	NS
Year x Irrig. Level x Seeding rate	NS	NS	NS
Year x Irrig. Level x Cultivar	NS	NS	NS
Year x Seeding rate x Cultivar	NS	NS	NS
Irrig. Level x Seeding rate x Cultivar	NS	NS	NS
Year x Irrig. Level x Seeding rate x Cultivar	NS	NS	NS

-[1] = due to the experimental lay-out these effects have no correct denominator for the F test

NS[2] = not significant at the 0.05 level of probability

* Significant F-test at the 0.05 level of probability

** Significant F-test at the 0.01 level of probability

*** Significant F-test at the 0.001 level of probability

Table 4. Results of analysis of variance for the parameters cover percentage (cover) before submersion and after submersion, the difference (b) – (a). NDVI (c) before submersion and after submersion (d), and difference (d) – (c).

Factors	Cover % before subm.(a)	Cover % after subm. (b)	Diff. cover % (b-a)	NDVI before subm.(c)	NDVI after subm.(d)	Diff. NDVI (d-c)
Year	-[1]	-	-	-	-	-
Irrig. Level	-	-	-	-	-	-
Seeding rate	**	NS	*	*	NS	NS
Cultivar	***	***	***	***	NS	**
Year x Irrig. Level	-	-	NS	-	-	-
Year x Seeding rate	NS[2]	NS	NS	NS	NS	NS
Irrig. Level x Seeding rate	NS	NS	NS	NS	NS	NS
Year x Cultivar	***	***	NS	NS	*	*
Irrig. Level x Cultivar	NS	NS	NS	NS	NS	NS
Seeding rate x Cultivar	NS	NS	NS	NS	NS	*
Year x Irrig. Level x Seeding rate	NS	NS	NS	NS	NS	NS
Year x Irrig. Level x Cultivar	NS	NS	NS	NS	NS	NS
Year x Seeding rate x Cultivar	NS	NS	NS	NS	NS	NS
Irrig. Level x Seeding rate x Cultivar	NS	NS	NS	NS	NS	*
Year x Irrig. Level x Seeding rate x Cultivar	NS	NS	NS	NS	NS	NS

-[1] = due to the experimental lay-out these effects have no correct denominator for the F test
NS[2]=not significant at the 0.05 level of probability
* Significant F-test at the 0.05 level of probability
** Significant F-test at the 0.01 level of probability
*** Significant F-test at the 0.001 level of probability

SUBMERSION

The cover percentage before submersion was significantly affected by ‘Seeding rate’, ‘cultivar’ and by the interaction ‘year x cultivar’. The cover percentage after submersion was only affected by the main factor, ‘seeding rate’ and the interaction ‘year x cultivar’ (Table 4).

Moreover, the difference between cover percentage before and after submersion (b-a) was significant for the effects of ‘cultivar’ and ‘seeding rate’.

The NDVI before submersion was significantly affected by ‘cultivar’ and ‘seeding rate’, while the NDVI after submersion displayed only a significant ‘year x cultivar’ interaction.

Furthermore, the difference between NDVI after and before submersion (d-c) showed a significant effect for ‘cultivar’ and ‘year x cultivar’, ‘seeding rate x cultivar’ and ‘Irrigation level x seeding rate x cultivar’ interactions.

Discussion

The average daily air temperature at 2 m (° C) in the two years of the experiment showed different trends. Comparing the two years of the test (2017, 2018), the average daily temperatures of 2018 have been much lower since mid-July (Figure 1).

Even the distribution of rainfall, although not accentuated, was different in the two years of the trial. Average daily rainfalls, concentrated in some days, were higher in 2018, starting from mid-July, with a peak of 40 mm of rain in an only day (Figure 2).

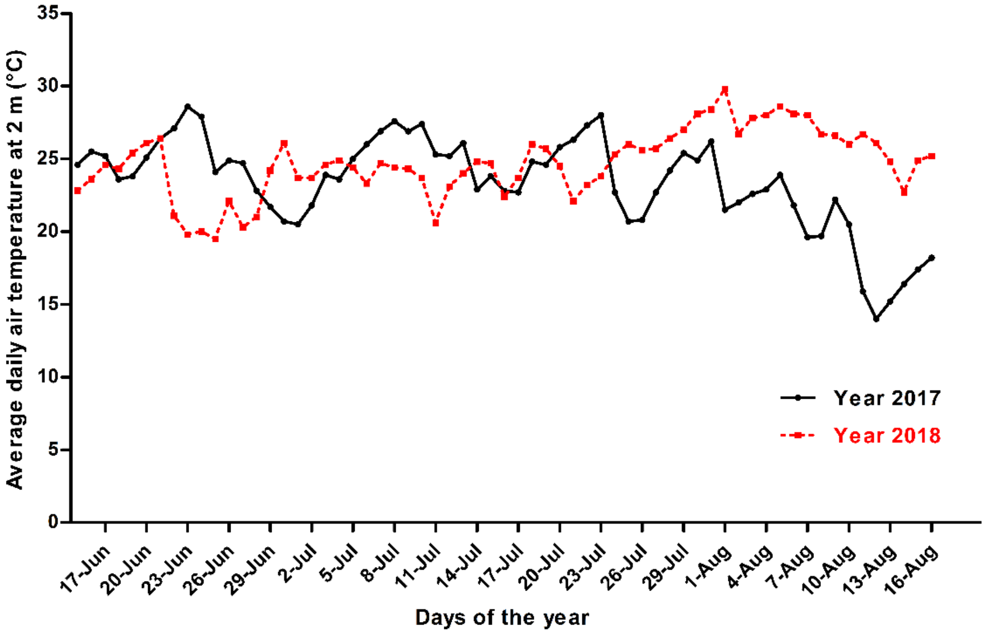

Figure 1 Average daily air temperature at 2 m (°C) from mid-June to mid-August in 2018 and 2019.

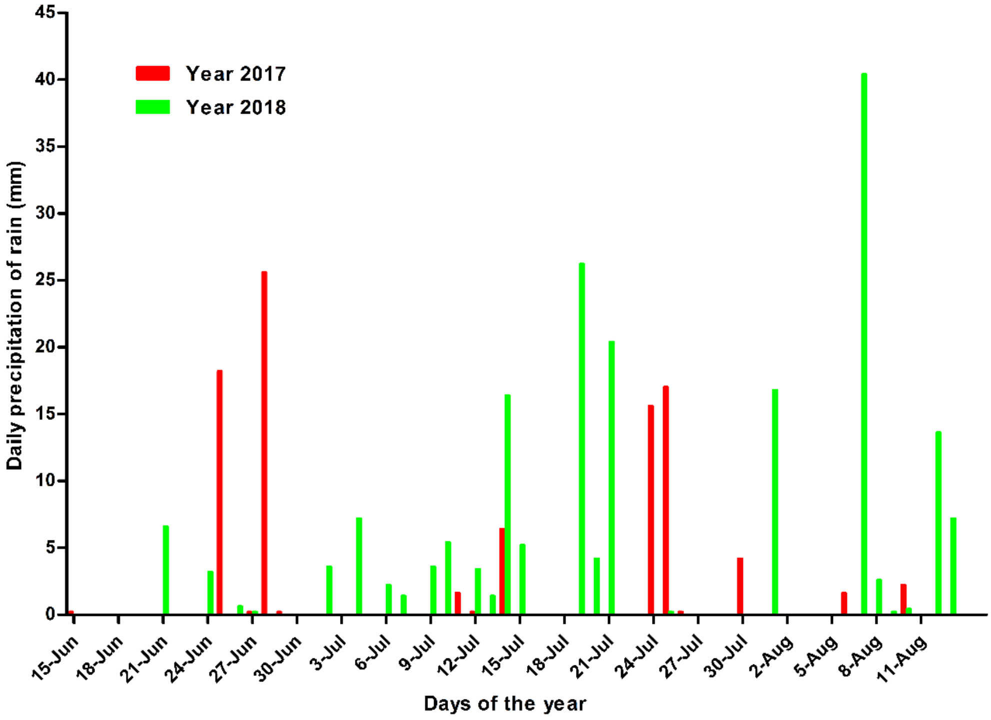

Figure 2. Daily precipitation of rain (mm) from mid-June to mid-August in 2018 and 2019.

EMERGENCE

Results highlight the influence of seeding rate on a number of seedlings per unit area, unlike what was found by Pornaro *et al.* (2016). The cover percentage at 30 days after seeding was also influenced by the seeding rate. Table 5 shows the uniformity of the emergency expressed as standard deviation (SD). The DS for each variety was calculated as the average of the replicates over two years. Table 5 also shows the average number of seedlings with the two seeding rate under test.

Table 5. Seedlings uniformity (SD) and Emergence 50% (number of seeds m^{-2}) for seeding rate 2.5 g m^{-2} (1) and seeding rate 5.0 g m^{-2} (2).

cultivar	**Seedlings uniformity: SD (average replications of 2 years)**		**Emergence 50% (number of seeds m^{-2})**	
	Seeding rate 1	**Seeding rate 2**	**Seeding rate 1**	**Seeding rate 2**
Jackpot	8.17	10.22	1,106	1,789
La Paloma	9.38	10.27	1,491	2,336
Pure Dynasty	5.31	6.49	593	1,015
SR 9554	8.91	10.69	1,485	2,066
SWI 2000	6.24	8.51	1,151	1,588
Transcontinental	10.80	13.00	1,868	3,142

The number of seedlings per unit area with a seeding rate of 5.0 g m^{-2} was much higher than that of seeding rate 2.5 g m^{-2}, and consequently, the percentage of seedlings emerged did not differ significantly. Our results are consistent with those of Leinauer *et al*. (2010) but are different from the results reported by Pornaro *et al.* (2016). These results indicate that the number of seedlings per unit area is relevant in affecting establishment speed.

The data of the parameter 'seedlings %' include only bermudagrass and seashore paspalum species. Buffalograss was established by burs, and it was not possible to calculate a percentage (a bur may contain from 2 to 5 seeds).

The significant 'year x cultivar' interaction can be explained by differences in the trend of daily temperatures in the two years of the experiment, already discussed above.

DRY WEIGHT

Significant differences among cultivars were found for root dry weight of zone 3-8 cm below of the soil surface (Table 3). Similarly to root also aboveground dry weight (aboveground DW) showed differences among cultivars (Table 3).

This result is consistent with that found by Dittmer (1973) who demonstrated that frequent mowing during its first growing season affected root and aboveground dry weight of bermudagrass but the root weight/aboveground weight ratio did not change.

The multiple comparisons between the cultivars of root dry weight of zone 3-8 cm below of the soil surface were not significant (data not shown).

In contrast to the previous one, the multiple comparisons of aboveground DW between the cultivars showed that Pure dynasty produced a significantly smaller quantity of dry weight (about half) than the other cultivars. The other cultivars were not significantly different from each other that were not significantly different from each other (data not shown).

SUBMERSION

Differences among cultivars occur for cover percentage and NDVI before and after the simulated submersion treatments with prolonged irrigations. The result concerning cover percentage is in agreement with Patton *et al.* (2008) who reported establishment differences among cultivars of bermudagrass. The speed of coverage is a genetic aspect and thermal time affect the establishment (Giolo *et al*., 2019). The results indicate that all the species and the cultivars under study are tolerant to flash floods in the early stages of the establishment. The comparison before (T1) and after (T2) simulated submersion treatments are shown in figures 3 and 4, where the two graphs highlighted how the cover continued over time without slowing down. This result is in line with those found by Giolo *et al.* in the greenhouse (oral communication).

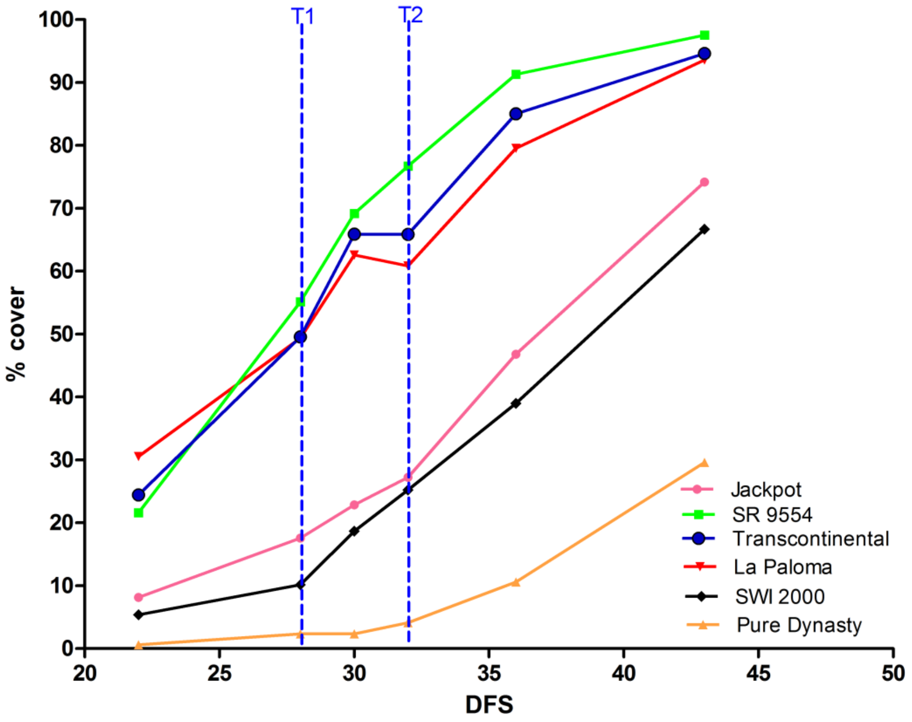

Figure 3. Mean percentage of ground cover at Days from seeding (DFS) for 7 cvs (trial, 2017). Vertical dotted lines show the beginning (T1) and the end (T2) of simulated submersion treatments**.**

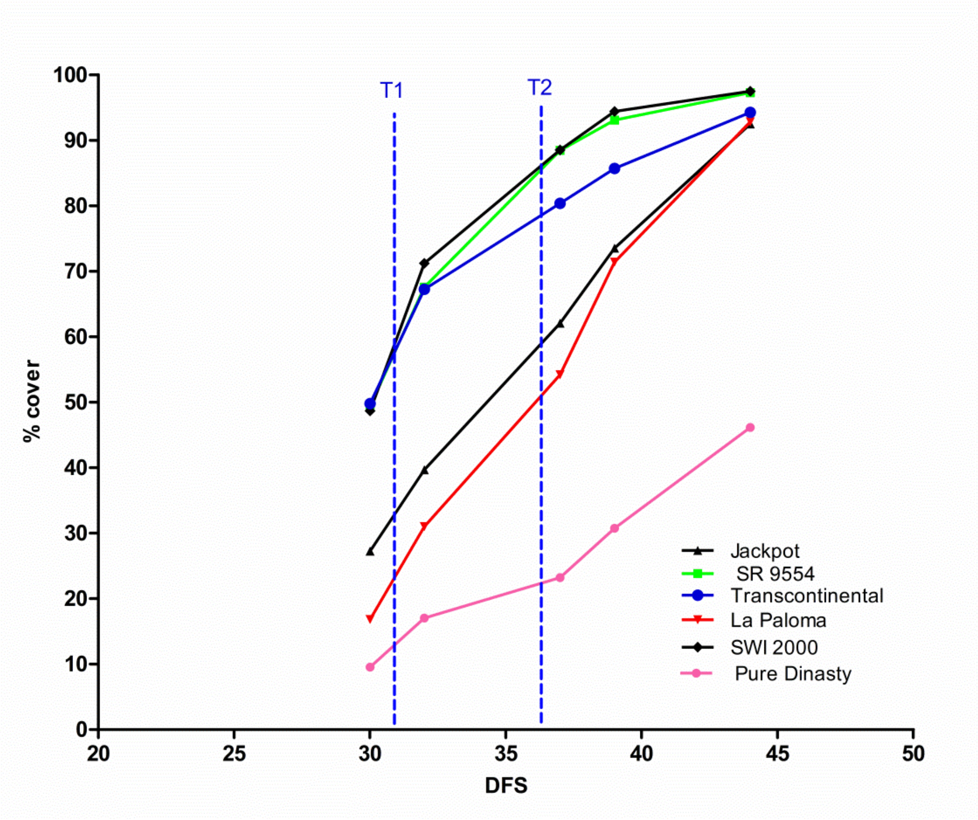

Figure 4. Mean percentage of ground cover at Days from seeding DFS for 7 cvs (trial, 2018). Vertical dotted lines show the beginning (T1) and the end (T2) of simulated submersion treatments.

Conclusion

The present study demonstrated that recommended seeding rate 5.0 g m^{-2} in comparison with reduced seeding rate 2.5 g m^{-2} produced a significantly higher number of seedlings per unit area and influenced positively the cover 30 days after seeding.

The results also demonstrated that the three species of warm-season turfgrasses and the cultivars included in the test are tolerant to flash floods in the early stages of the establishment when they are during seedling growth. These warm-season turfgrass species can withstand in the case of extreme rain events due to climate change.

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Annex: Figures of the trial 2017 -2018

Figure 1 Test with infiltrometer (Jun 2017)

Figure 2 Irrigation system calibration (2018).

Figure 3 Seedlings counting.

Figure 4 Seedlings counting at emergence 50% by metal frame (10 cm side).

Figure 5 Trial field after emergence (28 Jun 2018).

Figure 6 Trial plots: measuring of the moisture content of the soil by Field scout instrument (Aug 2017).

Figure 7 Plots *after the simulated submersion treatment (Aug 2018).*

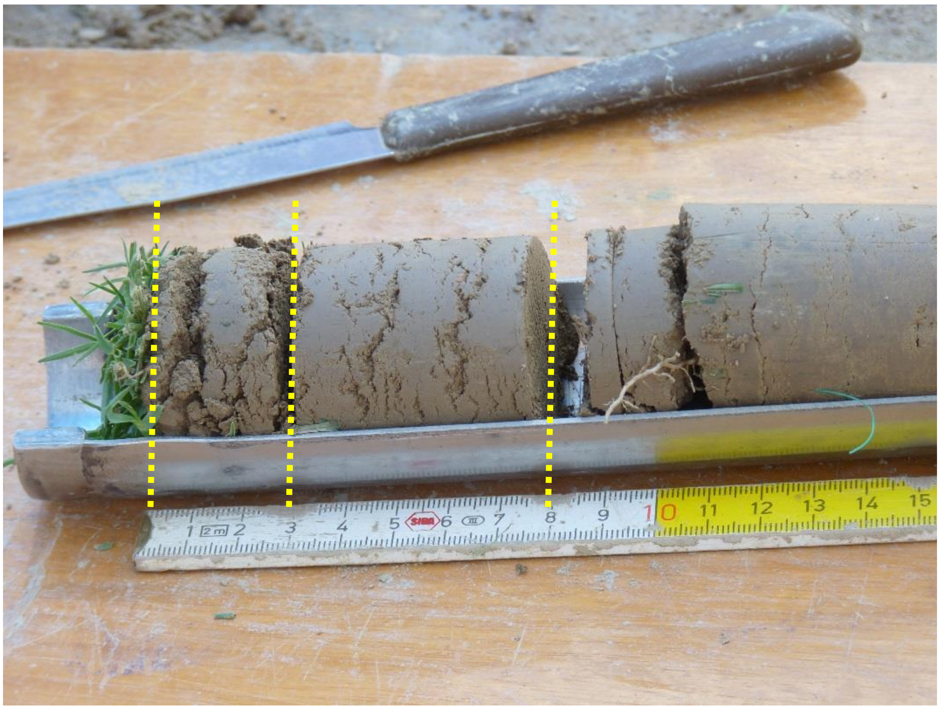

Figure 8 Soil carrot with three sub-units: vegetation at top-soil, root zone 0-3 cm depth, root zone 3-8 cm depth.

Chapter 6

Germination and seedling emergence of bermudagrasses: a critical analysis of Degree Days formula

Introduction

Bermudagrass [*Cynodon dactylon* (L.) Pers.] is a warm-season turf species widely used in Mediterranean regions of Europe of which most fall in the so-called transition zone (De Luca *et al.*, 2008; Sever Mutlu *et al.*, 2011; Rimi *et al.*, 2011, 2013a; b; Schiavon *et al.*, 2016). Some bermudagrass cultivars produce viable seed (seeded-type) while others are sterile hybrids derived from interspecific crosses between *Cynodon dactylon* and *C. transvalensis,* which must be propagated solely vegetatively (Jennings *et al.,* 2013). Hybrids have been for a long time improvement of the quality concerning, seeded cultivars, but hybrid turfgrasses require high costs for establishment and maintenance (Brosnan and Deputy, 2008). The quality of seeded cultivars has dramatically improved over the past fifteen years. Some of them are highly tolerant to cold temperatures and can be successfully used in transition zones where an early establishment is crucial to avoid cold damage during the first winter (Richardson *et al*., 2003, 2004; Patton *et al.*, 2008; Schiavon *et al.*, 2016). In the upper transition zone, an early seeding is recommended to anticipate the establishment which helping stolons and rhizomes formation to enhance winter hardiness (Patton *et al.*, 2008; Schiavon *et al*., 2012).

The main factors controlling seed germination are temperature, water potential, air quality, light quality and intensity. In temperate climates, the proper soil water at seeding time is generally guaranteed by precipitations, so germination and emergence are mainly influenced by temperatures (Forcella *et al*., 2000). Bermudagrass germination under different temperature regimes was investigated in several studies (Sandlin *et al*., 2006; Deaton and Williams, 2013a; Giolo *et al.*, 2014, 2019). Sandlin *et al.* (2006) documented no germination in chambers subjected to alternating temperatures of 15/5° C (day/night; photoperiod 12h). Also, Giolo *et al.* (2014) reported that several seeded bermudagrass cultivars successfully germinated at alternating temperatures of 20/10 °C (day/night; photoperiod 8/16h) corresponding to an average base temperature of 15 °C. These results are in agreement with Shaver (Shaver *et al*., 2006) who observed field germination of two bermudagrass cultivars (Princess 77 and Riviera) at lower temperatures than it had been previously found by others (Beard, 1973; McCarty, 2005). Moreover, recent research (Giolo *et al*., 2019) indicated that for the five cvs of bermudagrass studied (Jackpot, La Paloma, Riviera, Transcontinental and Yukon) the T_{BASE} ranged between 11.5 and 17 ° C, with an average slightly above 14 °C.

Germination and emergence affect turfgrass establishment and the time necessary to complete this phase is usually calculated in days from seeding to the achievement of coverage level between 75 and 100% (Patton *et al*., 2008; Leinauer *et al.*, 2009; Sever Mutlu *et al.*, 2011; Schiavon *et al.*, 2012; Pornaro *et al., 2016)*. Degree-Days (DD), a measure of heat accumulation in the growing environment, are used to determine the thermal requirement from seeding to the establishment of different species. The relationship between the average daily temperature and the time between seeding to the establishment is well described by a sigmoidal curve while the relationship between the same time and DD is linear (Todey and Taylor, 2019). The inherent simplification by using DD formula can partly explain why in different locations authors found (Bonhomme, 2000), for the same species, under no limit supply of water, different DD for establishment (Patton *et al.*, 2004; Schiavon *et al.*, 2012). These differences could be because germination is commonly considered part of the establishment while they are two separate stages with different thermal requirements. Due to widespread use of indexes such as ’percentage of emerged seedlings‘ or ‘cover percentage’ in agronomic practice, seedling emergence is often confused with germination, but it takes place when germination is already completed (Nonogaki *et al.*, 2010; Bewley *et al*., 2013).

Degree Days currently remain still widely used parameter to predict emergence and establishment despite some possible inaccuracies in the calculation (Bonhomme, 2000; Todey and Taylor, 2019). The algorithm for the calculation of DD (often called GDD) takes into consideration daily maximum (T_{MAX}), daily minimum (T_{MIN}) temperatures and the base temperature (T_{BASE}) of the species. Plant growth does not occur unless the temperature is above a minimum threshold value called $T_{BASE,}$ which is generally determined under laboratory conditions. The use of a too low T_{BASE} could lead to an overestimation of the amount of DD in periods when the average temperature on the soil is lower than the correct Tbase. The use of an incorrect T_{BASE} could be a problem for comparing DD obtained with different temperature trends or in different environments. The most used T_{BASE} for bermudagrass is 5°C, even though other T_{BASE} such as 18.3 °C or 10 °C have also been considered (Mittlesteadt, 2009; Deaton, 2012). The T_{BASE} of 5°C has been proposed by Unruh (Unruh *et al.*, 1996) who investigated 'vegetative' bermudagrass cultivars, but it has been widely used for 'seeded' cultivars too (Patton *et al.*, 2004, 2008; Sever Mutlu *et al.*, 2011; Schiavon *et al.*, 2012; Pornaro *et al.*, 2016). Significant differences have been observed in DD for the establishment of seeded bermudagrasses: Patton (Patton *et al.*, 2004), in Lafayette (Indiana), calculated 950 DD (T_{BASE} 5°C) for 'Mirage' while Schiavon (Schiavon *et al.*, 2012), in Las Cruces (New Mexico), noticed that over 2,000 DD (T_{BASE} 5°C) were required for 'Princess 77'. These differences could be related not only to genetic diversity among cultivars but also to the reference T_{BASE} used in DD calculation. The DD obtained in Lafayette, and Las Cruces should be similar even if they referred to seeding dates with very different average daily temperatures. They are very different probably because of the choice of a T_{BASE} of 5 ° C, which with early seeding and low average daily temperatures, can produce an overestimation of accumulated DD. A T_{BASE} at the soil surface of 15° C instead of 5 °C could be more appropriate for seeded bermudagrass cultivars as the T_{BASE} is related to seeds germination and not to propagules or plugs growth.

The bermudagrass emergence and establishment prediction are essential for the management of turfgrasses of this species. Unfortunately, the information available on T_{BASE}'s influence on DD calculation is also minimal. A 2-year study was conducted in northeastern Italy during spring 2013 and 2014 comparing the use of a temperature of 5°C and 15 °C for DD calculation. This study aimed to evaluate the inferences from the use of two different base temperatures in bermudagrass germination prediction based on the emergence response of ten cultivars sedded at different seeded dates.

Materials and Methods

The study was conducted at the Experimental Agricultural Farm of Padova University in Legnaro, Northeastern Italy (45°20′ N, 11°57′ E, elevation 8 m). The area has a humid subtropical climate with an annual mean temperature of 12.3° C and an annual rainfall of

823 mm. The soil at the site consisted of a sandy loam soil containing 63% sand, 31% silt, and 6% clay, with a pH of 8.2, 1.4% organic matter, C/N ratio of 15.3, 3.7 mg kg^{-1} available Olsen P, and 125 mg kg^{-1} exchangeable K (buffered $BaCl_2$ method). Ten cultivars of seeded bermudagrass, which included 'Gobi', 'Sunbird', 'SR9554', 'Princess 77', 'Yukon', 'Riviera', 'Transcontinental', 'Casinò Royal', 'Savannah', and 'La Paloma' were compared in this study. Plots measuring 1 m by 1m were seeded at a rate of 4 g m^{-2} except a 10 cm by 10 cm centre area on which exactly 100 seeds were placed by hand. A number of seeds matched the seeding rate which is the recommended rate by several authors (Munshaw *et al.*, 2001; Patton *et a*l., 2004; Macolino *et al*., 2010; Rimi *et al.*, 2011, 2013a). The experiment was carried out from March to June in 2013 and 2014. During each year there were three different seeding dates,13 March (D1), 3 April (D2), and 24 April (D3) in 2013 and 10 March (D1), 31 March (D2), and 22 April (D3) in 2014. Before seeding, seeds were stored at 5 °C for one month to break seed dormancy. After seeding, seeds were covered with a 2 mm layer of washed river sand and irrigated daily with 5 mm of water. The experimental design was a randomized complete block with three replications.

Soil temperatures were measured with a frequency of five minutes at a depth of 1 cm using thermocouples connected to a datalogger (CR10X; Campbell Scientific, Logan, UT) and T_{max} and T_{min} were selected from recorded values. The temperatures recorded during the experiment are reported in Figure 1.

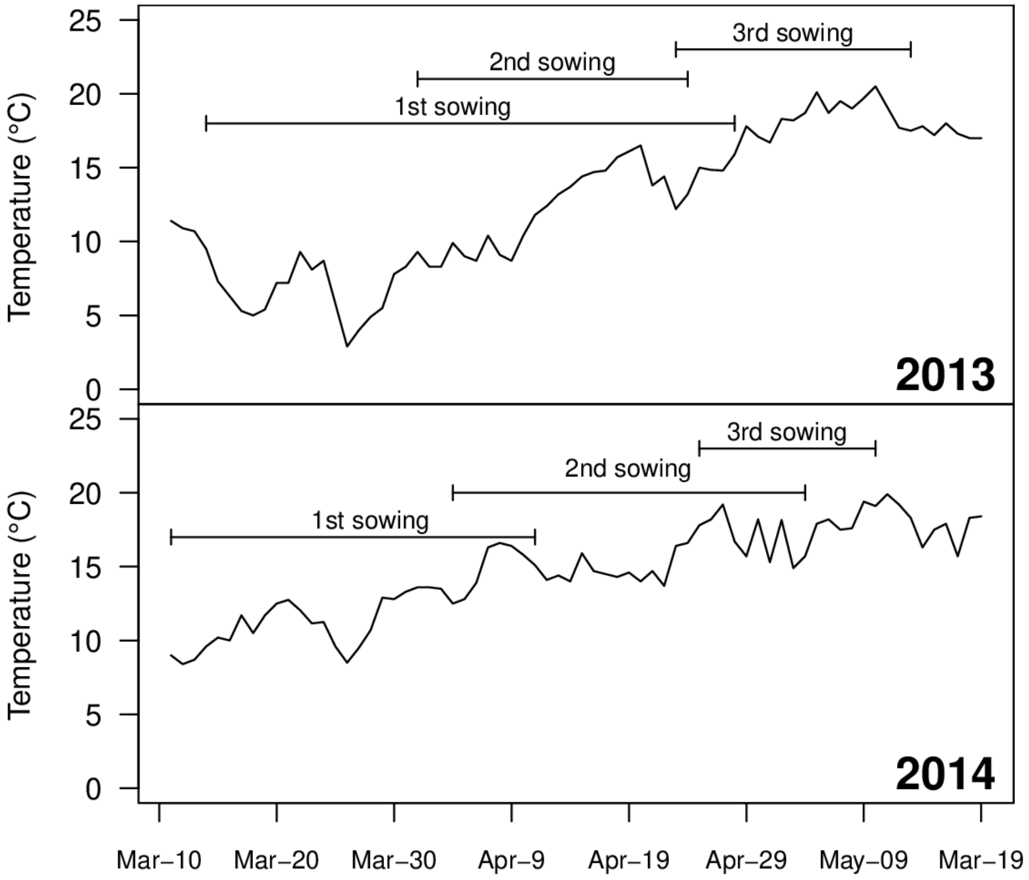

Figure 1. Daily mean soil temperatures from 10th March to 19th May 2013 and 2014. For each year and each seeding date, a period of emerged seedlings counting is reported.

Emerged seedlings from the centre area were counted weekly and then removed without soil disturbance. Counting was stopped when no new seeds emerged for two weeks. Degree days (DD) from seeding were calculated using the equation first suggested by René-Antoine Ferchault de Réaumur in 1730 and again published by McMaster (1997), Moore and Remais (2014):

$$\mathrm{DD} = \frac{(T_{MAX}+T_{MIN})}{2} - T_{BASE}$$

where if $T_{MAX} < T_{BASE}$, then $T_{MAX} = T_{BASE}$, and if $T_{MIN} < T_{BASE}$, then $T_{MIN} = T_{BASE}$.

Degree days (DD) were calculated using two T_{BASE} values. First, $T_{BASE} = 5°$ (DD5) because it was used in several published studies (Patton *et al*., 2008; Sever Mutlu *et al*., 2011; Schiavon *et al.*, 2012; Pornaro *et al.*, 2016) and second T_{BASE} = 15 °C (DD15) as reported by Giolo *et al.* (2014, 2019). A non-linear model (El-Kassaby *et al.*, 2007) was used to compute accumulated DDs for which maximum rate of germination (MGR) was found. Subsequently, an analysis of covariances was conducted using the two-way factorial design with cultivar (10 levels) and seeding time (3 levels) and either DD5 or GDD15 as covariates. The analysis was conducted separately for each year using SAS statistical software (version 9.3; SAS Institute, Cary, NC).

Results and Discussion

Sigmoidal regression curves of cumulative germination percentage of the ten cultivars tested are shown in Figure 2.

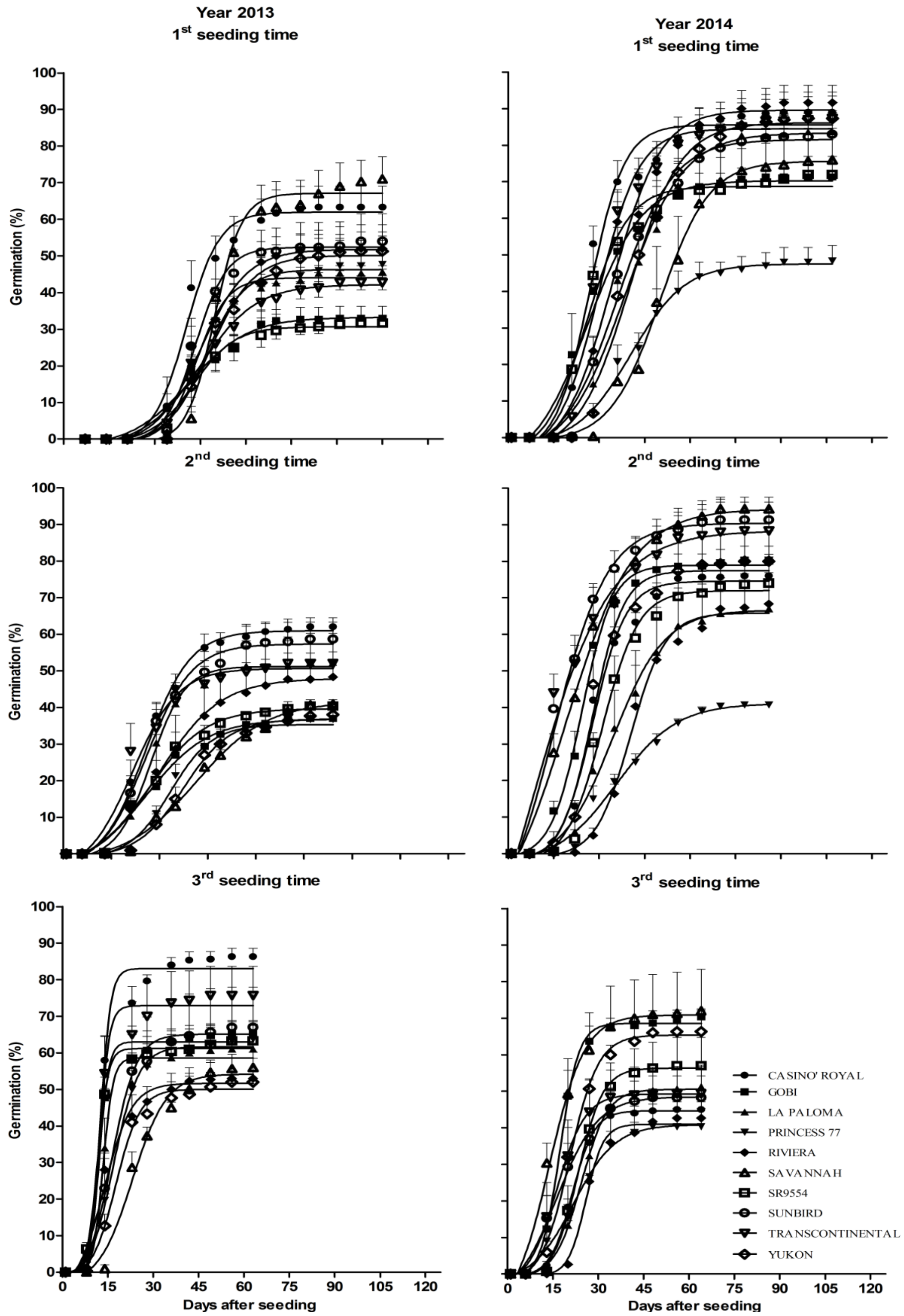

Figure 2. Cumulative germination percentage at a 7-day interval of ten seeded bermudagrass cultivars at three seeding dates in 2013and 2014. Vertical bars represent the upper bound standard error.

The number of days from seeding (DFS) to reach 25% and 38% germination determined based on the non-linear (sigmoidal) regressions are listed in Tables 1 and 2 together with DD5 and DD15.

Table 1. Number of days from seeding (DFS) to reach 25% germination and degree days calculated with a base temperature of 5 (DD5) and 15 °C (DD15) in 2013 and 2014 (mean of ten bermudagrass cultivars).

Seeding date	**DFS**	**DD5**	**DD15**
	2013		
March 13	48	130.1	38.8
April 3	34	110.9	60.5
April 24	15	49.9	43.3
	2014		
March 10	31	161.3	49.6
March 31	25	124.2	71.9
April 22	21	98.7	78.2

Table 2. Number of days from seeding (DFS) to reach 38% germination and degree days calculated with a base temperature of 5 (DD5) and 15 °C (DD15) in 2013 and 2014 (mean of six bermudagrass cultivars).

Seeding date	**DFS**	**DD5**	**DD15**
	2013		
March 13	53	148.4	54.6
April 3	42	138.3	84.0
April 24	18	59.5	51.9
	2014		
March 10	36	183.7	62.2
March 31	28	138.0	82.2
April 22	25	19.5	97.3

The 70% germination, which is considered the minimum acceptable level for seeded bermudagrasses (The Council of the European Economic Community, 1966), was reached by most of the tested cultivars only at the first two seeding dates in 2014 (Figure 2). In both experimental years, all ten cultivars reached 25% germination at each seeding date, but only six of them reached 38% germination at each seeding dates. Except Princess 77, germination was higher in 2014 than in 2013 at the first two seeding dates. At the third seeding date, most of the cultivars had higher germination in 2013 than in 2014.

The results of the analysis of variances are listed in table 3. Analyses indicate that DD affects germination and emergence significantly. The model fit statistics for the analyses of covariances are displayed in table 4. Both the Bayesian information criterion (BIC) and the Akaike information criterion (AIC) are smaller for DD15 compared to DD5 in 2013 and 2014. These results indicate that despite both DD affecting the germination outcome significantly (table 3), DD15 explains germination more accurately than DD5 (table 4).

Table 3. P-values for results of the analysis of variance testing the effects of cultivars and time of seeding and their interactions on bermudagrass germination calculated as Degree Days (DD) using a base temperature of either 5°C (DD5) or 15°C (DD15).

Fit Statistics	**2013**		**2014**	
	DD5	**DD15**	**DD5**	**DD15**
Cultivar (CV)	0.1728	0.2019	<.0001	<.0001
Time of Seeding (TS)	<.0001	<.0001	0.0740	0.0059
CV*TS	0.0043	0.0142	0.9448	0.9579
DD	0.0017	0.0037	0.0166	0.0231

Table 4. Model fit statistics for the analysis of covariances model with Degree Days calculated with either 5°C (DD5) or 15°C (DD15) as base temperature for 2013 and 2014 as a covariate.

Fit Statistics	**2013**		**2014**	
	DD5	**DD15**	**DD5**	**DD15**
-2 Res Log-Likelihood	100.45	99.16	83.07	81.17
AIC (smaller is better)	164.45	163.16	147.07	145.17
AICC (smaller is better)	245.68	244.39	228.30	226.40
BIC (smaller is better)	230.93	229.64	213.55	211.65
CAIC (smaller is better)	262.93	261.64	245.55	243.65
HQIC (smaller is better)	190.40	189.11	173.02	171.12
Pearson Chi-Square	8.89	9.10	6.63	6.70
Pearson Chi-Square / DF	0.15	0.15	0.11	0.11

Our results corroborate previous studies by Deaton and Williams (2013) and Giolo *et al.* (2014, 2019). The authors also reported significant differences in germination between bermudagrass genotypes as affected by different temperature conditions. Similar to our findings, the authors demonstrated that the accuracy of the algorithm used to calculate DD is strongly influenced by the T_{BASE} used in the formula.

Richardson *et al.* (2004) recommended to establish bermudagrass as early as possible in spring to reduce injuries during the first winter following seeding. However, transition zone climates, spring temperatures can vary strongly between years and within years. Therefore it is challenging to define a priori the optimum time for seeding bermudagrass.

Several studies highlight the importance of soil temperatures for germination *(*Forcella *et al.*, 2000*)*. Similarly, DD calculated for the same cultivars sown at different dates should result in near equal values. However, the assumption that a soil temperature lower than T_{BASE} is to be considered equal to T_{BASE} can result in different DD if the bermudagrass is seeded during different times of the year. The model fit statistics for the analyses of covariances performed on DD5 and DD15 suggests that for bermudagrasses a T_{BASE} of 15°C provides a better estimation of cumulative DD than a T_{BASE} of 5°C.

Conclusions

The study confirms that soil temperatures strongly influence seed germination and seedling emergence and highlight significant differences in DD among bermudagrass cultivars. Our findings support the hypothesis that the sum of degree days for germination is influenced by the T_{BASE} used. Moreover, the impact of T_{BASE} varies between the temperatures occurring during the period following seeding. Based on our findings, a T_{BASE} of 15°C provides a more accurate estimation of germination degree days than a T_{BASE} of 5°C.

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Chapter 7

Conclusions

The research consisted of a series of experiments to study the influence of climate change effects on germination, seedling growth and establishment of some warm-season turf species, and it provided an overview of the main related issues. We focused on the effects of climate change in the Mediterranean region where warm-season grasses can be of great interest in increasing the sustainability of turfgrasses.

We have carried out five studies in the field and controlled environment (climatic chamber and greenhouse) to provide useful information on the effects of conditions deriving by a changing climate scenario on the crucial phase of turfgrass establishment.

New information was found useful for turf specialists to improve management practices.

The main results and findings are as follows:

<u>Effects of suboptimal temperatures</u>

The base temperature and thermal time for seven cultivars of three warm-season turfgrass species were determined, and we found that no germination occurred below 11.4 °C. These studies demonstrated that early April is the optimal seeding time for seeding bermudagrass cultivars in the Po-Venetian valley (upper transition Mediterranean zone). It permits to complete the establishment within the beginning of June with a mature turf before the cold winter.

<u>Effects of water-excess</u>

The outcomes of this thesis highlight that throughout the establishment, warm-season turf species tolerate flash floods in the early stages of the establishment when they are during seedling growth. Bermudagrass seeds and seedlings appeared to possess some short-term resilience to flood, as long as it is not prolonged. Bermudagrass can be successfully used for establishing turfgrasses under waterlogging (6-8 days of full submersion represent the threshold for symptoms of hypoxia).

Establishment

The present study demonstrated that recommended seeding rate (5.0 g m^{-2}) in comparison with reduced seeding rate (2.5 g m^{-2}) produced a significantly higher number of seedlings per unit area, and influenced the cover positively at 30 days from seeding.

Our study confirms that soil temperatures strongly influence seed germination and seedling emergence and highlights significant differences in Degree Days among bermudagrass cultivars. Also, our findings support the hypothesis that the sum of degree days for germination is influenced by the base temperature used. Furthermore, a T_{BASE} of 15°C provides a more accurate estimation of germination degree days than a T_{BASE} of 5°C, primarily used.

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