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Harvest aids for improved bermudagrass sod shelf-life and transplantation success

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Harvest aids for improved bermudagrass sod shelf-life and transplantation success

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Shelf-life and transplantation success of sodded and sprigged turfgrasses are negatively affected by disruptive harvest techniques and post-harvest handling/storage conditions. Air and light are limited inside of stacked pallets of sod or masses of sprigs/plugs, which triggers multiple processes that may lead to poor transplant success. Current research looks at the effects of several commercially available turfgrass products and cultural practices on post-harvest bermudagrass storage, its grow-in after transplantation, and harvested area recovery. Ensilation and internal heating sometimes observed in stored, full-sized pallets of sod were difficult to simulate in small-size sod masses. When storage environment and post-harvest conditions were controlled, refrigeration of stored bermudagrass slowed establishment, which is contrary to common knowledge and industry practice. Fluxapyroxad + pyraclostrobin fungicide positively affected turfgrass grow-in during field and greenhouse experiments, and in some instances hastened growth and recovery of bermudagrass.

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CHAPTER I

LITERATURE REVIEW

The shelf-life and transplantation success of sodded and sprigged turfgrasses are negatively affected by disruptive harvest techniques and post-harvest handling/storage conditions. Two primary examples include internal heating of sod masses (palleted sod or stored sprigs) and improper or delayed irrigation upon installation. Air and light are limited inside of the stacked pallets of sod, which triggers multiple processes that may lead to poor transplant success. To maintain vital physiological processes without sunlight, turfgrass increases respiration and obtains energy by metabolizing stored carbohydrates (Darrah and Powell, 1977). Furthermore, with limited access to atmospheric oxygen, favorable conditions for anaerobic microorganisms occur. Anaerobic microorganisms begin to propagate on plant biomass, using it as a source of nutrients. Their activity leads to internal heating, which significantly contributes to the stress that sod experiences (Pahlow et al., 2003). Internal heating of stacked or rolled sod and bulked sprigs during shipping/storage is a known cause of the decline in transplant success (Watschke et al., 1970). This effect is attributed principally to the respiration of the turf (Darrah and Powell, 1977), and respiration by microbes that are degrading organic matter within the “sod mass” (Watschke et al., 1970). To minimize these conditions, sod producers may harvest during reduced night/morning-time temperatures or increase soil depth so that the excess soil may serve as a heat sink or temperature buffer in harvested sod (Watschke et al., 1970). Cultural practices, such as thatch management, nitrogen fertilization prior to harvest, and mowing height, may also influence sod shelf-life (Darrah and Powell, 1977; King et al., 1982). Darrah and Powell (1977) concluded that increasing storage duration, mowing height, N fertility, or clipping residue increased storage temperatures inside stacked Kentucky bluegrass (*Poa pratensis* L.) and red fescue (*Festuca rubra* L.) sod. They also suggest that shallower soil depth of harvested sod contributes to internal pallet heating and that transplantation success was negatively affected by increased storage temperatures. Similar results were observed by King et al. (1982), where higher nitrogen fertilization (240 kg ha^{-1} compared to 0 kg ha^{-1}) was associated with higher storage temperatures (by 11.7 °C after 24 hours of storage), ethylene production (by 0.54-1.23 ppm throughout 96 hours of storage), and the number of dead leaves (by 7.5% after 48 hours of storage and by 45% after 72 hours of storage). Also, increased mowing height (5 cm vs. 2 cm) was associated with increased respiration (by 0.7-2.8% of CO_2 throughout 96 hours of storage), ethylene production (by 0.42 ppm after 6 hours of storage), and the number of dead leaves (by 11.2% after 48 hours of storage). It is unknown whether limited oxygen, increased carbon dioxide, or ethylene toxicity contributes to the injury of stored sod.

Despite being expensive, refrigeration of sod is increasingly a means of reducing sod spoilage (Brede, 2000). Other strategies to minimize effects include placing pallets in shaded or covered conditions or moistening the external surface of palleted sod to promote evaporative cooling during transport/storage.

The combination of the succinate dehydrogenase inhibiting (SDHI) fungicide fluxapyroxad and the quinone outside inhibiting (QoI) fungicide pyraclostrobin has been linked to the protection of newly sprigged ultra-dwarf bermudagrass greens in unpublished research (M. Grant, personal communication, 2017; K.M. Kalmowitz, personal communication, 2018; Lewis, 2017). The fluxapyroxad + pyraclostrobin product marketed commercially as Lexicon®

Intrinsic® (BASF Corporation, Research Triangle Park, NC) has also been demonstrated in non-peer reviewed research to increase St. Augustinegrass root growth (Martin, 2016).

Fluxapyroxad (*1H-Pyrazole-4-carboxamide,3-(difluoromethyl)-1-methyl-N-(3′,4′,5′-trifluoro[1,1′-biphenyl]-2-yl)*) is a succinate dehydrogenase inhibitor (SDHI) that blocks the electron transport in the mitochondrial complex II (Jeanmart et al., 2016). This inhibits the germination of spores, germ tubes, and the growth of mycelia within a susceptible fungus (Anonymous, 2012). Fluxapyroxad containing fungicides are foliar applied or used as a seed treatment. It is manufactured by BASF Corporation and formulated as an emulsifiable concentrate (EC) or suspension concentrate (SC) (Anonymous, 2012). As with many other SDHIs, fluxapyroxad can be applied to a variety of crops to prevent a broad range of fungal-induced plant diseases and is especially efficacious against leaf spot diseases caused by *Ascomycetes* species (Jeanmart et al., 2016). A study conducted by Chen et al. (2014) indicated that the efficacy of fluxapyroxad was significantly greater than the standard SDHI fungicide, boscalid, for controlling rice sheath blight (*Rhizoctonia solani* Kühn) at a rate six times less than boscalid. The study conducted by Xiao-hu et al. (2015) demonstrates that fluxapyroxad in soil inhibits substrate-induced respiration, total, and microbial biomass within the initial 15 days. It decreased the fungi and bacteria biomass; however, the fungi biomass decrease was more significant. Their study indicated the slow dissipation of fluxapyroxad in soil with a concentration-dependent half-life of 158 to 385 days. Non-fungicidal effects of the foliar applications of fluxapyroxad in winter wheat were studied by Smith et al. (2013). In their research, fluxapyroxad not only reduced foliar diseases but also reduced stomatal opening, which in turn led to increased water use efficiency, increased canopy size, and greater yield.

Pyraclostrobin (*carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy] methyl]phenyl]methoxy-, methyl ester*) is a strobilurin fungicide that inhibits respiration by blocking the electron transport in mitochondrial complex III (Ammermann et al., 2000). It controls a wide range of foliar fungal diseases. Numerous studies indicate that besides its fungicidal activity, pyraclostrobin causes other physiological effects on crop plants. Köehle et al. (2002) stated that the increased productivity of cereals treated with pyraclostrobin is not exclusively associated with fungal control. In the study on the effect of pyraclostrobin on grain-fill period and kernel dry matter accumulation in maize conducted by Byamukama et al. (2013), treated plants had a higher green leaf area at most locations. The absence of favorable conditions for severe disease development leads the authors to presume that the effects were caused by changes in physiological processes. To share their understanding of pyraclostrobin's non-fungicidal effect on plants, BASF published a brochure "Intrinsic™ Brand Fungicides Plant Health Research" (Anonymous, 2010). It states that pyraclostrobin inhibits not only electron transport in the mitochondria of fungi but also respiration within plant cells. This decrease in respiration may lead to an increase in both photosynthesis and stored carbon compounds. Moreover, these processes activate nitrate reductase resulting in enhanced nitric oxide synthesis. This leads to a number of positive effects: 1) nitric oxide inhibits ethylene production, which is being synthesized in a mature plant prior to leaf loss or as a reaction to stress; 2) nitric oxide increases superoxide dismutase activity, resulting in heat tolerance; 3) nitric oxide increases tolerance to infections by activating plant defense responses; 4) nitric oxide synthesis increases nitrogen utilization by the plant.

A study by Joshi et al. (2014) investigated the effect of pyraclostrobin on nodulation and nitrogenase activity in soybean. Soybean treated with variable rates of pyraclostrobin showed an increase in leghemoglobin content as well as enhanced nitrogenase activity that resulted in enhanced nitrogen fixation. The authors concluded that pyraclostrobin may be applied to increase soybean growth, nitrogen assimilation, and ultimately soybean yield.

The means of extending turfgrass shelf-life and increasing its transplantation success are still poorly studied. The goal of the set of studies is to evaluate the effects of refrigeration and products reported or advertised as possible post-transplantation sod health enhancers on bermudagrass.

CHAPTER II

FIELD RESEARCH ON ENHANCED BERMUDAGRASS POST-HARVEST ESTABLISHMENT

Abstract

Field studies were conducted in 2018 and 2019 on ‘Latitude 36’ hybrid bermudagrass (*Cynodon dactylon* × *C. transvaalensis*). Treatments included: the experimental bio-nutritional plant extract ACA-3434, the fungicides fluxapyroxad + pyraclostrobin, fluopyram + trifloxystrobin, azoxystrobin, and the plant growth regulator trinexapac-ethyl. Sod was harvested, stacked to a height of 12 layers, and stored for 70 hours; treated and untreated sod was stored at ambient field temperature (28 °C average). To evaluate storage refrigeration on sod establishment, an additional untreated check was also stored at 4°C refrigeration. Sod was then installed on an adjacent prepared native soil and the treatment effects on bermudagrass transplantation were measured visually, by spectral reflectance, and by root analysis. Our methods failed to simulate heating inside stored sod masses, and none of the treatments affected internal storage temperatures. Nevertheless, refrigeration, and in some cases fluxapyroxad + pyraclostrobin fungicide application, increased plant health characteristics of transplanted sod in the first days after installation. In addition, the results of the current study suggest that none of the treatments affect the recovery of areas from which sod was harvested. Albeit often unrealistic, refrigeration of harvested sod may be the best practice to extend sod shelf-life. When applied prior to harvest, fluxapyroxad + pyraclostrobin fungicides provided slight improvements

in post-installation sod health; however, little is known about the mechanism by which this occurs.

Introduction

Storage-related stress of warm-season grass sod, like bermudagrass [*Cynodon dactylon* (L.) Pers.], is poorly studied relative to cool-season grasses, such as Kentucky bluegrass. This may be in part due to its higher tolerance to storage-related stress compared to cool-season grasses, which is owed principally to its ability to tolerate higher temperatures as a C4 plant (Du et al., 2011). Research has been biased towards the more sensitive turfgrass species; however, storage-related stress also affects warm-season species, albeit perhaps in different ways.

Research has linked the use of certain biostimulants and plant growth regulators to increased sod shelf-life (Zhang et al., 2003; Heckman et al., 2001a; Heckman et al., 2001b). Zhang et al. (2003) reported that foliar application of seaweed (*Ascophyllum nodosum* Jol.) extract with humic acid (93% ai), propiconazole, or combination of both treatments, may reduce heat injury and improve quality of transplanted tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] sod.

Heckman et al. (2001a) found that applications of trinexapac-ethyl (a gibberellin biosynthesis inhibitor commonly used for plant growth regulation) may reduce Kentucky bluegrass sod storage temperatures by 3°C after 12 hours of storage, which positively affects sod shelf-life. A similar study by Heckman et al. (2001b) concluded that Kentucky bluegrass sod treated with trinexapac-ethyl two weeks prior to harvest was 10°C cooler in the center of a pallet after 48 hours of storage compared to the untreated check. This led to greater tensile strength (by 30%) and better quality of the treated sod after 24 hours of storage.

Some common fungicides are also thought to increase post-harvest sod health. Goatley and Schmidt (1991) stated that propiconazole, a triazole or demethylation inhibiting (DMI) fungicide, applied one week before harvest enhanced Kentucky bluegrass sod tensile strength by 23% compared to other DMI fungicides.

It is relatively common for sod producers to apply the QoI fungicide, azoxystrobin, during warm-season sod production due to its low cost and broad-spectrum preventative disease control. However, there are no published reports of benefits beyond pathogen control before sod harvest. Conversa et al. (2014) studied the effects of the azoxystrobin on the post-harvest shelf-life of baby spinach (*Spinacia oleracea* L.) and demonstrated the responses of the plant to the pre-applied fungicide application under different N fertilization rates. Azoxystrobin application decreased total weight loss during 14-day storage, increased the total chlorophyll content (in particular chlorophyll a), and decreased electrolyte leakage. It also improved nitrogen uptake under low N fertilization rates and reduced nitrate content in the leaves under high N fertilization rate. Authors suggest that these effects are due to the QoI fungicide's ability to activate NADH-nitrate reductase, citing the study of nitrate reductase activity in leaf tissue after application of kresoxim-methyl conducted by Glaab and Kaiser (1999).

Based on reviewed prior research, our objective was to evaluate refrigeration, several commercially available turfgrass fungicides, a bio-nutritional experimental plant extract, and a commonly used plant growth regulator on sod storage, its grow-in after transplantation, and harvested area recovery.

We hypothesized that the application of the tested products or refrigeration of sod may reduce the storage-related stress, decrease internal heating during storage, and enhance transplantation success.

Materials and methods

The research was conducted at the Mississippi State University R.R. Foil Plant Science Research Center near Starkville, MS during the summers of 2018 and 2019 on ‘Latitude 36’ hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt Davy] established from sprigs in June of 2017 and grown in fully that same year. The site contained a native Marietta fine sandy loam (fine-loamy, siliceous, active, Fluvaquentic Eutrudepts) soil with pH 6.2 (1:1 soil/H_2O). Plots were reel-mown weekly at 1.3 cm with clippings returned (Tri-King Reel Mower, Jacobsen®, Augusta, GA) during active growth.

The sod was harvested 16 July in 2018 and 5 August in 2019. The study evaluated the effects of eight treatments (Table 2.1) within a completely randomized design (four replications) with two runs (in 2018 and 2019). The experimental units were 1 × 3 m in size.

Treatments were applied in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer (Figure 2.1). Except for one post-applied treatment, all were applied 25 and 4 days prior to harvest, which was in close accordance with the recommendations based upon prior research of fluxapyroxad + pyraclostrobin (K.M. Kalmowitz, personal communication, 2018; Martin, 2016).

When applied prior to harvest, treatments were incorporated into the canopy by post-application irrigation with 8 mm of overhead irrigation. As an exception, trinexapac-ethyl was applied three hours later when turf had dried, as it is absorbed by the plant through the leaves and can be washed off by rain or irrigation within the first hour after application, according to the Primo Maxx® (Syngenta Crop Protection LLC., Greensboro, NC) label (Syngenta Crop Protection, 2015). An additional treatment consisted of fluxapyroxad + pyraclostrobin applied immediately after sod installation and again 22 days later.

The sod (40 × 50 cm rectangles) was harvested at a soil depth of 2 cm using a Ryan walk-behind sod harvester (Schiller Grounds Care Inc., Johnson Creek, WI) (Figure 2.2), stacked to a height of 12 layers and either left in the field at ambient temperature (Figure 2.3) or refrigerated at 4°C for 70 hours within a walk-in cooler (Figure 2.4).

In preparation for sod installation, the area was prepared by applying glyphosate (Roundup PowerMax®, 1,000 g ai ha^{-1}, Monsanto, St. Louis, MO) twice, on a 3-week interval. The area was cultivated using a Blecavator™ 5V175 (Blec Global Ltd., Peterborough, UK), fumigated with dazomet soil fumigant (Basamid® G, 300 kg ha^{-1}, AMVAC Chemical Corp., Los Angeles, CA) and kept under a black plastic cover for an additional three weeks before being uncovered and leveled one week before sod installation.

The sod pieces and experimental units were installed in the same order as harvested for ease of logistics and to reduce in-plot variation due to stacking order. The post-application of fluxapyroxad + pyraclostrobin treatment was applied immediately after installation, followed by irrigation (20 mm) of the sod and the harvested area (Figure 2.5). Irrigation was resumed 24 hours later and continued twice weekly (15 mm each event) unless rainfall had occurred or was imminent (± 24 hours) until fully recovered from installation. All turf was maintained with adequate fertility according to soil test reports. A 13-13-13 balanced fertilizer (Woods Farm Supply, Byhalia, MS) was applied two weeks prior to harvest (equivalent to 49 kg N ha^{-1}).

The internal temperature of harvested sod and external ambient temperature were measured (°C) using methods described by Schmidt et al. (2010). Temperature sensors were fabricated with type-T thermocouple wire (Omega Engineering, Inc., Stamford, CT). Thermocouple wire junctions were twisted together and soldered, and a plastic surface coating was applied to protect against moisture and corrosion. For internal sod mass temperature measurements, sensors were inserted into the center of each sod mass (directly in the center between sod pieces 6 and 7). Ambient temperature was measured at the data logger location in close proximity to the sod stacks as seen in figure 2.3. Measurements were routed through relay multiplexers (AM16/32A, Campbell Scientific, Logan, UT) and recorded on five minute intervals with data loggers (CR-1000, Campbell Scientific Inc., Logan, UT). Temperature sensors were calibrated in a water bath (IsoTemp 3013D, Fisher Scientific, Pittsburgh, PA) against a NIST-traceable thermometer.

Treatment effects on both bermudagrass post-installation establishment and harvest area regrowth were evaluated by visually assessing percent green cover 1 and 4 days after installation (DAI), as we were unable to visually detect any differences 7 DAI and onward. The treatment effects on transplanted bermudagrass establishment and harvest area regrowth were also assessed by spectral reflectance 1, 4, 7, 14, and 21 DAI using a Holland Scientific RapidScan CS45 handheld crop sensor (Holland Scientific Inc., Lincoln, NE) held 110 cm above, and perpendicular to, the canopy (Figure 2.6). Three subsamples on a 1-m linear transect were recorded in the center of each experimental unit, with approximately 30-cm spacings between each measurement. The calculated spectral indexes (Table 2.2) included: normalized difference vegetative index (NDVI), ratio vegetation index (RVI), and chlorophyll index – red edge (CI-RE).

In order to assess root growth and architecture of installed sod, 10 cm diameter core samples were excavated at 10 cm depth with a golf green cup-cutter (Figure 2.7) 2, 5, and 8 weeks after installation from four treatments: untreated check, refrigerated sod, pre-applied fluxapyroxad + pyraclostrobin, and post-applied fluxapyroxad + pyraclostrobin. These treatments were chosen based upon unpublished preliminary study results. Other treatments were excluded for root analysis due to expense. The core samples were washed free of soil, and new-grown roots were separated from those of the sod layer. Roots were patted dry with paper towels and frozen (-17°C) for further analysis. The root architecture was measured using the method described by Begitschke et al. (2018). Root samples were shaken in 50-ml conical tubes (Thermo Fisher Scientific Inc., Waltham, MA) in 5% sodium hexametaphosphate (The Chemistry Store.com, Cayce, SC) solution for 3-4 hours. Samples were then dispersed on a waterproof transparent tray, scanned, and digitized (600 dots cm^{-1}). Digital images were analyzed using WinRHIZO Pro (Regent Instruments Inc., Sainte-Foy, Quebec City, Canada). The root samples were placed in paper envelopes and kept in a plant dryer (75°C) for three days prior to measuring root dry-mass. Root dry-mass and root length are discussed, as these were the only root parameters that differed due to treatment.

Data were subject to analysis of variance ($\alpha=0.05$) using SAS (Version 9.4; SAS Institute Inc., Cary, NC), and means were separated within PROC GLM using the Student-Newman-Keuls method ($\alpha=0.05$).

Results and discussion

The internal sod mass temperature was not observed to be in excess of ambient air temperature. Prior research indicates that soil depth of harvested sod may affect internal sod mass heating, as the soil separates and isolates layers of vegetative mass and serves as a “heat sink” (Darrah and Powell, 1977; King et al., 1982; Watschke et al., 1970). Heckman et al. (2001b) observed internal heating by stacking forty 102 × 102 cm sod layers, which is a considerably larger sod mass than our own twelve 40 × 50 cm sod layers. In prior unpublished research, we tested various stacking configurations, as well as plastic wrapping, in order to promote an ensiling effect. In those un-replicated studies, we observed significant fermentation (noted by pungent smell) but no internal sod heating. In the present study, sod was similarly odorous, but no sod heating was observed. Our stacking configurations were chosen in order to accommodate available space and practicality of hand harvest, transport, and transplantation.

Figure 2.8 illustrates the internal temperature of stored sod masses kept at ambient and refrigerated conditions, as well as ambient temperature outside the sod mass in 2018 and 2019. In both years, the difference between internal temperatures of sod masses left in the field fluctuated ± 0.5°C. Statistical analysis was conducted at different points in time, such as daytime peak- and nighttime low-temperatures; however, at no time were treatment differences observed. These data suggest that none of the treatments affected internal sod storage temperature, including trinexapac-ethyl application, which has previously been shown to decrease the temperature of palleted Kentucky bluegrass sod by 10°C after 48 hours of storage (Heckman et al., 2001b). Besides the difference in the sod pallet design [18 layers of sod (102 × 102 cm in size) against 12 layers (40 × 50 cm in size)], the biggest difference between our studies that could explain the difference in obtained results is the turfgrass species subjected for the research. Bermudagrass compared to Kentucky bluegrass that was used in the Heckman et al. study is a C4 plant and is able to withstand greater water and heat stresses (Du et al., 2011).

The treatment effects on percent green cover of post-storage transplanted sod differed between years of the study; however, the same trends were observed across years. That is, refrigeration resulted in the greater green cover of transplanted sod, 1 DAI; however, by 4 DAI and onward, percent green cover failed to differentiate between any of the treatments (Table 2.3). During our study, we also monitored the regrowth of the harvested area. At no time were treatment differences observed.

The treatment effects on NDVI differed between the two years. When assessed 1 DAI in 2018, refrigeration and pre-applied fluxapyroxad + pyraclostrobin resulted in NDVI values that were greater than those of the untreated (Table 2.4). The NDVI of refrigerated sod was higher than that of pre-applied fluxapyroxad + pyraclostrobin treatment. The effects of other treatments did not differ from the untreated, 1 DAI. Refrigeration and pre-application of fluxapyroxad + pyraclostrobin were the only two treatments with NDVI values greater than the untreated, at 4 and 7 DAI, in 2018. It is important to note that the pre-application of fluxapyroxad + pyraclostrobin resulted in NDVI values similar to those of refrigeration, 4 and 7 DAI. There were no treatment differences when NDVI was assessed 14 DAI and onward in 2018. In 2019, only refrigeration resulted in higher NDVI values compared to the untreated, 1 and 4 DAI. There were no treatment differences at 7 DAI and onward in 2019.

The treatment effects on RVI were similar across years. When data were pooled, refrigeration resulted in greater RVI than all other treatments, 1 DAI (Table 2.5). No treatment differences were observed beyond 1 DAI.

The treatment effects on CI-RE differed between the two years. In 2018, when assessed 1 DAI, the CI-RE of refrigerated sod was greater than that of the untreated (Table 2.6). When assessed 4 DAI, refrigerated sod and pre-applied fluxapyroxad + pyraclostrobin resulted in greater CI-RE compared to that of the untreated. No treatment differences regarding CI-RE were observed at 7 DAI and onward in 2018. In 2019, when assessed 1 and 4 DAI, refrigeration resulted in greater CI-RE compared to that of the untreated. No treatment differences within CI-RE were observed when evaluated 7 DAI and onward in 2019.

These results suggest that refrigeration increased sod post-transplantation establishment in the first 4–7 days after installation. In some cases, pre-applied fluxapyroxad + pyraclostrobin had similar effects and increased spectral reflectance indexes of transplanted sod compared to the untreated check. Conversa et al. (2014) observed similar effects of azoxystrobin, a fungicide of the same family as pyraclostrobin, on baby spinach storage, when azoxystrobin application increased total chlorophyll concentration measured after 14 days of storage. In our study, however, azoxystrobin treatment effects did not differ from the untreated check.

The root length and dry-mass values differed between study years and are discussed separately. In 2018, eight weeks after installation, the post-installation application of fluxapyroxad + pyraclostrobin resulted in greater root mass compared to pre-applied fluxapyroxad + pyraclostrobin and the untreated check (Table 2.7). In 2019, refrigeration increased both root dry-mass (by 0.089 g) (Table 2.7) and length (by 916.6 cm) (Table 2.8) compared to the untreated, two weeks after installation. These were the only differences observed within root analysis. That we failed to replicate treatment effects on root growth in both years might be explained by the differences in the environment. However, these results suggest that refrigeration, and post-applied fluxapyroxad + pyraclostrobin may stimulate root system establishment.

Our methods failed to simulate heating inside stored sod masses, and none of the treatments affected internal storage temperatures. Based on our results, refrigeration, and in some cases fluxapyroxad + pyraclostrobin fungicide application, increased plant health characteristics of transplanted sod; however, those characteristics were not perceived by visual rating, and vegetative indexes demonstrated improvements only from 1 to 7 DAI, but not beyond. Treatment effects may have been more pronounced if sod masses had experience greater stress during storage and transplantation, for example, if internal heating would have been observed. In order to increase the storage-related stress, prior studies used larger sod masses (Heckman et al., 2001a; Heckman et al., 2001b) or even stored sod masses artificially heated to 40°C environment (Zhang et al., 2003).

Either refrigeration or fluxapyroxad + pyraclostrobin fungicide application would impose substantial production costs. Yet, their inclusion may be justified in situations where high-quality sod with a more rapid establishment is required, such as on athletic fields or golf course putting greens. In addition, results suggest that none of the treatments affect the recovery of the harvested area. Without more pronounced benefits to bermudagrass sod production, it is difficult to expect the rapid implementation of either refrigeration or fluxapyroxad + pyraclostrobin fungicide application as general best management practices.

An experimental compost and kelp extract (ACA-3434; Aquatrols, Paulsboro, NJ) did not affect storage and did not increase sod establishment characteristics, despite resulting in increased turf vigor and recovery of transplanted sod during drought stress in unpublished research at Mississippi State University.

Future research should focus on determining the nature of fluxapyroxad + pyraclostrobin containing fungicide effects on turfgrass post-harvest establishment. It is yet unknown whether these positive effects are associated with possible plant stress-protective attributes or prophylactic fungicidal properties. There is also a need to evaluate methods of simulating conditions that sod experiences during storage and transport. Variables like sod stacking configuration, transportation method, and varietal differences may affect sod shelf-life and transplantation success. Our small-pallet methods failed to replicate internal heating effects that have been observed in studies of cool-season sod storage (Goatley and Schmidt, 1991; Heckman et al., 2001a; Heckman et al., 2001b; Darrah and Powell, 1977; King et al., 1982; Watschke et al., 1970). Large pallet size studies are expensive due to material and space requirements, and they are logistically difficult to design due to variation within and between experimental units. Yet, it is difficult to study the subject of sod without adequately replicating actual transportation variables. Sod depth is another shortcoming of our study. Our soil depth was 2 cm in order to limit variation in sod strength and reduce losses by tearing/shearing. The tearing of shallow cut sod (1 cm) after 70 hours of storage was a critical issue in preliminary studies, leading to sod that could not be handled for transplantation.

Conclusions

Other than refrigeration, none of the treatments affected visual assessments of transplant success. However, refrigeration, and in some cases fluxapyroxad + pyraclostrobin fungicide application, increased vegetative indexes that have previously been associated with improved plant health. These benefits were short-lived (1 to 4 DAI) but may translate to enhanced rooting and recovery from post-harvest storage and transplantation. In addition, the results of the current study suggest that none of the treatments affect the recovery of areas from which sod was harvested. Albeit often unrealistic, refrigeration of harvested sod may be the best practice to extend sod shelf-life. When applied prior to harvest, fluxapyroxad + pyraclostrobin fungicides provided slight improvements in post-installation sod health; however, little is known about the mechanism by which this occurs.

Tables and Figures

Table 2.1 List of field research treatments, products, and application dates

Treatment / Trade name	Active ingredient (ai)	Product rate ha^{-1}	g ai ha^{-1}	Application dates	
				2018	2019
Untreated	-	-	-	-	-
Refrigeration	-	-	-	-	-
ACA-3434[a]	bio-nutritional extract	9.6 L	-	21 June, 11 July	11 July, 31 July
Lexicon® Intrinsic®	fluxapyroxad + pyraclostrobin	1.6 L	257.1 +512.9	21 June, 11 July	11 July, 31 July
Exteris® Stressgard®	fluopyram + trifloxystrobin	13.2 L	163.4 +263.6	21 June, 11 July	11 July, 31 July
Heritage®	azoxystrobin	1.2 kg	6.1	21 June, 11 July	11 July, 31 July
Primo Maxx®	trinexapac-ethyl	0.8 L	96.0	21 June, 11 July	11 July, 31 July
Lexicon® Intrinsic® [b]	fluxapyroxad + pyraclostrobin	1.5 L	257.1 +512.9	19 July, 9 Aug	8 Aug, 29 Aug

[a] Products were applied 25 and 4 days prior to harvest (unless stated otherwise) in a water carrier volume of 561 L ha^{-1} via a CO$_2$ pressurized backpack sprayer on 'Latitude 36' hybrid bermudagrass 3 m^2 experimental units. The study was conducted at Mississippi State University in 2018 and 2019.

[b] Applied immediately and 21 days after installation.

Table 2.2 Spectral reflectance indexes

Index	Abbreviation	Equation[a]	References
Normalized difference vegetation index	NDVI	$(R_n - R_r)/(R_n + R_r)$	Tucker, 1979
Ratio vegetation index	RVI	R_n/R_r	Pearson and Miller, 1972
Chlorophyll index – red edge	CI−RE	$R_n/R_{re} - 1$	Gitelson et al., 2003

[a] R_n – reflectance at the near infrared wavelength (760–900 nm); R_r – reflectance at the red wavelength (630–690 nm), R_{re} – reflectance at the red wavelength (700–730 nm). Spectral reflectance was recorded with the Holland Scientific RapidScan CS45 handheld crop sensor held 110 cm above and perpendicular to the canopy of ‘Latitude 36’ hybrid bermudagrass. Three subsamples on a 1-m linear transect were recorded in the center of each experimental unit, with approximately 30-cm spacings between each measurement.

Table 2.3 Visual percent green cover of transplanted bermudagrass sod after 70 hours of storage

Treatment/Product[a]	**Active ingredient**	**Days after installation**			
		2018		**2019**	
		1	**4**	**1**	**4**
Untreated	-	26 b[c]	55 a	68 bc	80 ab
Refrigeration	-	66 a	76 a	95 a	95 a
ACA-3434	bio-nutritional extract	35 b	58 a	68 bc	79 b
Lexicon® Intrinsic®	fluxapyroxad + pyraclostrobin	38 b	71 a	69 bc	85 ab
Exteris® Stressgard®	fluopyram + trifloxystrobin	25 b	48 a	75 b	91 ab
Heritage®	azoxystrobin	29 b	61 a	64 c	86 ab
Primo Maxx®	trinexapac-ethyl	23 b	48 a	68 bc	80 ab
Lexicon® Intrinsic®[b]	fluxapyroxad + pyraclostrobin	29 b	56 a	69 bc	84 ab

[a] 'Latitude 36' hybrid bermudagrass treated 25 and 4 days prior to harvest (unless stated otherwise) with the following products in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer. The study was conducted at Mississippi State University in 2018 and 2019. Harvest dates were 16 July, 2018 and 5 August, 2019.

[b] Applied immediately after installation and again 21 days later.

[c] Means followed by the same letter in a column are not significantly different. Means were separated using the Student-Newman-Keuls method (α=0.05).

Table 2.4 Normalized difference vegetative index (NDVI) values of transplanted bermudagrass sod after 70 hours of storage

Treatment/ Product[a]	**Active ingredient**	**2018**					**2019**				
		Days after installation					**Days after installation**				
		1	**4**	**7**	**14**	**21**	**1**	**4**	**7**	**14**	**21**
Untreated	-	0.327 c[c]	0.399 b	0.434 b	0.786 a	0.800 a	0.476 bc	0.644 b	0.658 a	0.745 a	0.791 a
Refrigeration	-	0.455 a	0.484 a	0.510 a	0.836 a	0.827 a	0.679 a	0.716 a	0.746 a	0.801 a	0.833 a
ACA-3434	-	0.345 c	0.416 b	0.492 ab	0.847 a	0.845 a	0.471 c	0.646 b	0.680 a	0.760 a	0.797 a
Lexicon® Intrinsic®	fluxapyroxad + pyraclostrobin	0.393 b	0.480 a	0.504 a	0.804 a	0.803 a	0.526 b	0.658 b	0.688 a	0.765 a	0.824 a
Exteris® Stressgard®	fluopyram + trifloxystrobin	0.333 c	0.410 b	0.461 ab	0.815 a	0.847 a	0.521 bc	0.674 b	0.716 a	0.773 a	0.779 a
Heritage®	azoxystrobin	0.333 c	0.419 b	0.471 ab	0.827 a	0.843 a	0.494 bc	0.653 b	0.673 a	0.748 a	0.809 a
Primo Maxx®	trinexapac-ethyl	0.330 c	0.391 b	0.450 ab	0.831 a	0.832 a	0.478 bc	0.620 b	0.646 a	0.744 a	0.827 a
Lexicon® Intrinsic®[b]	fluxapyroxad + pyraclostrobin	0.352 c	0.418 b	0.458 ab	0.822 a	0.848 a	0.493 bc	0.666 b	0.704 a	0.764 a	0.776 a

[a] ‘Latitude 36’ hybrid bermudagrass, treated 25 and 4 days prior to harvest (unless stated otherwise) with the following products in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer. Sod was transplanted on to prepared native soil. The study was conducted at Mississippi State University in 2018 and 2019. Harvest dates were 16 July, 2018 and 5 August, 2019.

[b] Applied immediately after installation and again 21 days later.

[c] Means followed by the same letter in a column are not significantly different. Means were separated using the Student-Newman-Keuls method (α=0.05).

Table 2.5 Ratio vegetation index (RVI) values of transplanted bermudagrass sod after 70 hours of storage

Treatment/ Product[a]	**Active ingredient**	**Days after installation**				
		1	**4**	**7**	**14**	**21**
Untreated	-	2.195 b[c]	2.936 a	3.194 a	8.695 a	9.247 a
Refrigeration	-	3.347 a	3.701 a	4.109 a	10.968 a	11.052 a
ACA-3434	-	2.261 b	3.024 a	3.604 a	11.124 a	11.668 a
Lexicon® Intrinsic®	fluxapyroxad + pyraclostrobin	2.547 b	3.368 a	3.684 a	10.014 a	10.913 a
Exteris® Stressgard®	fluopyram + trifloxystrobin	2.303 b	3.092 a	3.596 a	9.995 a	11.541 a
Heritage®	azoxystrobin	2.247 b	3.044 a	3.425 a	10.500 a	11.947 a
Primo Maxx®	trinexapac-ethyl	2.205 b	2.798 a	3.171 a	10.058 a	11.900 a
Lexicon® Intrinsic®[b]	fluxapyroxad + pyraclostrobin	2.312 b	3.092 a	3.555 a	9.800 a	11.536 a

[a] 'Latitude 36' hybrid bermudagrass, treated 25 and 4 days prior to harvest (unless stated otherwise) with the following products in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer. Sod was transplanted on to prepared native soil. The study was conducted at Mississippi State University in 2018 and 2019. Harvest dates were 16 July, 2018 and 5 August, 2019. Data did not differ between 2018 and 2019; therefore, data are pooled across years.

[b] Applied immediately after installation and again 21 days later.

[c] Means followed by the same letter in a column are not significantly different. Means were separated using the Student-Newman-Keuls method (α=0.05).

Table 2.6 Chlorophyll index – red edge (CI-RE) values of transplanted bermudagrass sod after 70 hours of storage

Treatment/ Product[a]	**Active ingredient**	**2018**					**2019**				
		Days after installation					**Days after installation**				
		1	**4**	**7**	**14**	**21**	**1**	**4**	**7**	**14**	**21**
Untreated	-	0.314 bc[c]	0.383 c	0.519 a	1.349 a	1.315 a	0.399 b	0.670 b	0.901 a	1.105 a	1.385 a
Refrigeration	-	0.416 a	0.455 ab	0.614 a	1.531 a	1.476 a	0.693 a	0.815 a	1.088 a	1.324 a	1.544 a
ACA-3434	-	0.324 bc	0.411 bc	0.606 a	1.658 a	1.651 a	0.410 b	0.688 ab	0.950 a	1.145 a	1.334 a
Lexicon® Intrinsic®	fluxapyroxad + pyraclostrobin	0.358 b	0.477 a	0.622 a	1.450 a	1.502 a	0.457 b	0.698 ab	0.960 a	1.171 a	1.515 a
Exteris® Stressgard®	fluopyram + trifloxystrobin	0.320 bc	0.407 bc	0.564 a	1.490 a	1.678 a	0.471 b	0.762 ab	1.048 a	1.228 a	1.286 a
Heritage®	azoxystrobin	0.300 c	0.403 bc	0.569 a	1.573 a	1.660 a	0.429 b	0.692 ab	0.909 a	1.136 a	1.438 a
Primo Maxx®	trinexapac-ethyl	0.323 bc	0.386 c	0.564 a	1.544 a	1.611 a	0.427 b	0.662 b	0.874 a	1.126 a	1.623 a
Lexicon® Intrinsic®[b]	fluxapyroxad + pyraclostrobin	0.328 bc	0.412 bc	0.561 a	1.447 a	1.673 a	0.434 b	0.746 ab	1.045 a	1.195 a	1.340 a

[a] 'Latitude 36' hybrid bermudagrass, treated 25 and 4 days prior to harvest (unless stated otherwise) with the following products in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer. Sod was transplanted on to prepared native soil. The study was conducted at Mississippi State University in 2018 and 2019. Harvest dates were 16 July, 2018 and 5 August, 2019.

[b] Applied immediately after installation and again 21 days later.

[c] Means followed by the same letter in a column are not significantly different. Means were separated using the Student-Newman-Keuls method (α=0.05).

Table 2.7 Root dry-mass in grams of transplanted bermudagrass sod after 70 hours of storage

Treatment/ Product[a]	Active ingredient	Weeks after installation					
		2018			2019		
		2	5	8	2	5	8
Untreated	-	0.070 a[c]	0.443 a	0.660 b	0.038 b	0.130 a	0.131 a
Refrigeration	-	0.112 a	0.504 a	1.084 ab	0.127 a	0.154 a	0.189 a
Lexicon® Intrinsic®	fluxapyroxad + pyraclostrobin	0.082 a	0.332 a	0.723 b	0.075 ab	0.121 a	0.186 a
Lexicon® Intrinsic®[b]	fluxapyroxad + pyraclostrobin	0.076 a	0.367 a	1.191 a	0.080 ab	0.133 a	0.142 a

[a] 'Latitude 36' hybrid bermudagrass, treated 25 and 4 days prior to harvest (unless stated otherwise) with the following products in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer. Sod was transplanted on to prepared native soil. Golf green cup-cutter was used to excavate 10 cm diameter, 10 cm depth core samples for root analysis. The study was conducted at Mississippi State University in 2018 and 2019. Pre-storage harvest dates were 16 July, 2018 and 5 August, 2019.

[b] Applied immediately after installation and again 21 days later.

[c] Means followed by the same letter in a column are not significantly different. Means were separated using the Student-Newman-Keuls method (α=0.05).

Table 2.8 Total root length in centimeters of transplanted bermudagrass sod after 70 hours of storage

Treatment/ Product[a]	**Active ingredient**	**Weeks after installation**					
		2018			**2019**		
		2	**5**	**8**	**2**	**5**	**8**
Untreated	-	437.3 a[c]	516.8 a	903.9 a	376.5 b	1072.5 a	1024.7 a
Refrigeration	-	623.6 a	670.4 a	1266.5 a	1293.1 a	1476.1 a	1399.5 a
Lexicon® Intrinsic®	fluxapyroxad + pyraclostrobin	568.4 a	578.3 a	1050.6 a	813.3 ab	1255.7 a	1348.2 a
Lexicon® Intrinsic®[b]	fluxapyroxad + pyraclostrobin	534.4 a	458.0 a	1234.0 a	877.1 ab	1239.9 a	1198.9 a

[a] ‘Latitude 36’ hybrid bermudagrass, treated 25 and 4 days prior to harvest (unless stated otherwise) with the following products in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer. Sod was transplanted on to prepared native soil. Golf green cup-cutter was used to excavate 10 cm diameter, 10 cm depth core samples for root analysis. The study was conducted at Mississippi State University in 2018 and 2019. Pre-storage harvest dates were 16 July, 2018 and 5 August, 2019.

[b] Applied immediately after installation and again 21 days later.

[c] Means followed by the same letter in a column are not significantly different. Means were separated using the Student-Newman-Keuls method (α=0.05).

Figure 2.1 Product application using CO_2 pressurized backpack sprayer.

Treatments were applied in a water carrier volume of 561 L ha^{-1}.

Figure 2.2 Ryan walk-behind sod harvester

Sod (40 × 50 cm rectangles) was harvested at a soil depth of 2 cm.

Figure 2.3 Storage of sod masses in field conditions

Sod was stacked to a height of 12 layers, kept in the field for 70 hours. Internal sod temperature was monitored by Type T thermocouple wire sensors inserted into the center of each sod mass.

Figure 2.4 Storage of sod masses in walk-in cooler

Sod was stacked to a height of 12 layers, kept in the walk-in cooler at 4°C for 70 hours. Internal sod temperature was monitored by thermocouples inserted into the center of each sod mass.

Figure 2.5 Post-transplantation irrigation

After the transplantation, the sod and the harvested area were irrigated at 20 mm.

Figure 2.6 Spectral reflectance measurement

Spectral reflectance was assessed 1, 4, 7, 14, and 21 days after installation using Holland Scientific RapidScan CS45 handheld crop sensor held 110 cm above and perpendicular to the canopy.

Figure 2.7 Core sample excavation

Golf green cup-cutter was used to excavate 10 cm diameter core samples at 10 cm depth for root analysis.

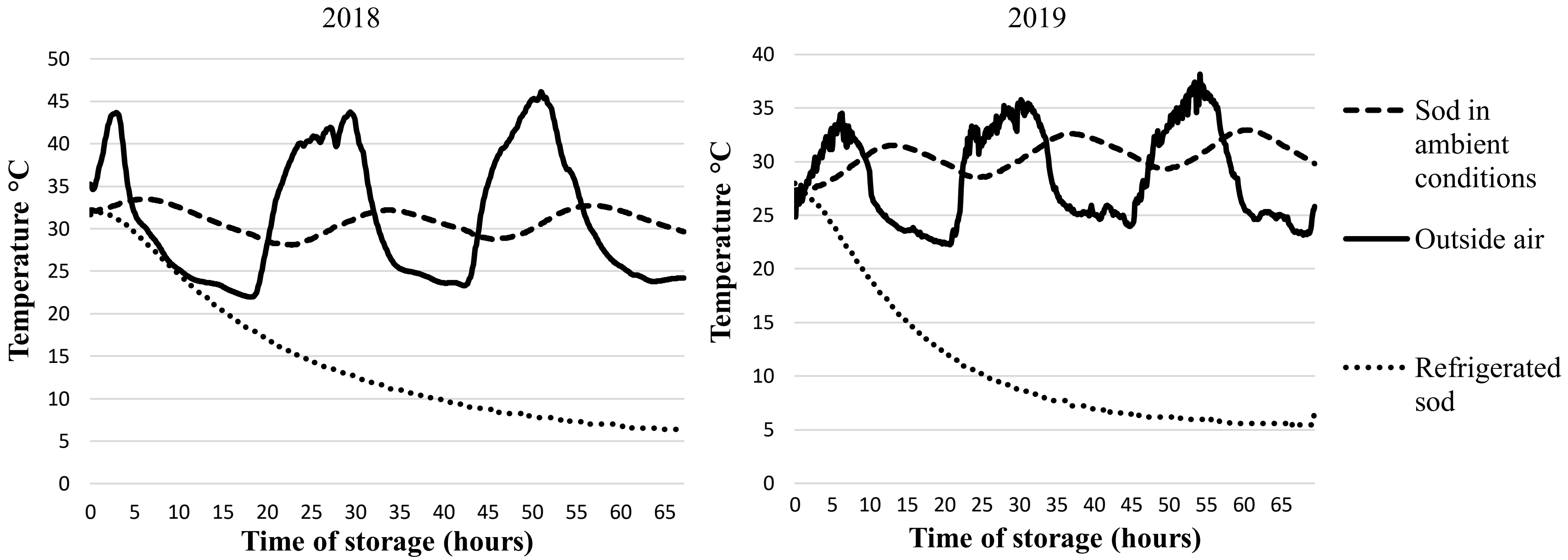

Figure 2.8 Mean storage temperatures in field research

‘Latitude 36’ hybrid bermudagrass sod was harvested (40×50 cm rectangular pieces, 2 cm harvest depth) on 16 July, 2018 and 5 August, 2019 and was stacked in 12 layers. Sod was stored in field conditions or refrigerated for 70 hours. Thermocouples were placed between layers 6 and 7 in the center of the sod mass. The study was conducted at Mississippi State University.

CHAPTER III

FLUXAPYROXAD + PYROCLOSTROBIN FUNGICIDE EFFECTS ON ULTRA-DWARF BERMUDAGRASS ESTABLISHMENT

Abstract

Fluxapyroxad + pyraclostrobin containing fungicide has been linked to the protection of transplanted sod and sprigs. Our objective was to evaluate fluxapyroxad + pyraclostrobin fungicide applications, storage under refrigerated environment, and their combinational effect on transplantation success of vegetatively established ultra-dwarf bermudagrass. The research evaluated the effects of the fungicide applied 21 and 2 days prior to harvest of ‘Champion’ ultra-dwarf hybrid bermudagrass. Turfgrass was harvested as sod, washed of soil, and 5 cm diameter plugs were removed and stored at 4 or 32°C. After 72 hours of storage, plugs were transplanted into 14.6 cm pots containing sand-peat (9:1 ratio) soil. Pots were maintained in a greenhouse. Green cover was assessed weekly by digital image analysis. Pots were harvested 3, 5, and 8 weeks after installation for root analysis. No internal mass heating was observed during storage at either temperature. Although green cover, and root analysis data at some harvest timings, differed between runs, a similar trend was observed: refrigeration at 4°C during storage slowed vegetative establishment by increasing the time required for cover and by decreasing root volume in the first 3-5 weeks after initiation. We conclude that refrigeration may significantly add to the stress experienced by stored ultra-dwarf hybrid bermudagrass. However, fluxapyroxad + pyraclostrobin fungicide applied 21 and 2 days prior to sod harvest may help turfgrass to

overcome that cold-induced stress. The application of this fungicide prior to harvest resulted in a more rapid establishment of refrigerated turfgrass and increased root dry-mass measured 8 weeks after installation. These results suggest that pre-application with fluxapyroxad + pyraclostrobin fungicide is a viable option for the improved vegetative establishment of ultradwarf bermudagrass. Future research should focus on determining the nature of fluxapyroxad + pyraclostrobin fungicide plant protective activity and its effects on storage when internal heating occurs.

Introduction

Hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt Davy] is a widely grown turfgrass in southern states and warm climate countries due to its high temperature and low precipitation tolerance (Hanna et al., 2013). Also, it prefers low mowing heights, which makes it an ideal grass for a golf course putting green. It is propagated vegetatively, primarily by sprigs or sod. Post-harvest storage of vegetatively propagated turfgrass can contribute to reduced transplantation success. Inside a stored sod mass, such as a pallet of stacked sod, or mass of sprigs/plugs, there is limited access to light and atmospheric air. In these conditions, turfgrass increases respiration by metabolizing stored carbohydrates (Darrah and Powell, 1977). In addition, increased activity of anaerobic microorganisms leads to internal heating (Pahlow et al., 2003). Health and quality of turfgrass decrease under these conditions. Sod producers, therefore, are highly motivated to reduce plant transplantation stress and ensure the quick and successful establishment of transplanted turfgrass.

Field research conducted during 2018 and 2019 (presented in Chapter II) tested several common turfgrass fungicides, plant growth regulators, and plant health-promoting products for effects upon sod storage and post-storage transplantation. Fluxapyroxad + pyraclostrobin fungicide (Lexicon™ Intrinsic®, BASF Corporation, Research Triangle Park, NC) increased plant health characteristics (NDVI, CI-RE, and root dry-mass) of transplanted bermudagrass sod. This fungicide combination has been linked to the protection of newly sprigged ultra-dwarf bermudagrass greens in previous unpublished research (M. Grant, personal communication, 2017; Lewis, 2017; K.M. Kalmowitz, personal communication, 2018). Fluxapyroxad + pyraclostrobin fungicide has also been demonstrated in non-peer reviewed research to increase St. Augustinegrass root growth (Martin, 2016).

A brochure published by BASF for its pyraclostrobin containing product series (Anonymous, 2010) contains important information on the effects of this active ingredient. It states that pyraclostrobin inhibits not only electron transport in the mitochondria of fungi but also respiration within plant cells, increasing stored carbon compounds within a plant and its photosynthetic activity. Additionally, one of pyraclostrobin's numerous effects is decreased plant production of ethylene in response to stress.

To further analyze the effects of fluxapyroxad + pyraclostrobin on the vegetative establishment of hybrid bermudagrass, we hypothesized that the application of fluxapyroxad + pyraclostrobin before harvest and refrigeration of sod may result in enhanced stress tolerance, less internal heating during storage, and greater transplantation success. Thus, the objectives of this greenhouse study were to examine the effects of fluxapyroxad + pyraclostrobin and refrigeration on ultra-dwarf hybrid bermudagrass storage and plant stress tolerance.

Materials and methods

Research was conducted at Mississippi State University in 2019 on a research putting green grassed with 'Champion' ultra-dwarf bermudagrass. The green was reel-mown five times weekly at 1.3 cm (Toro Triplex Model 3150-04357, Toro Co., Bloomington, MN). Before study initiation, the site was sand top-dressed three times per year with approximately 10 mm topdressing sand. No topdressing or aerification was performed in the six months before harvest. Maintenance also included routine insect and weed control. During the six months before harvest, no fungicides were applied to the research putting green. The field portion of the experiment was a completely randomized design (3 field replications) with two runs in time. Treatments included an untreated check and fluxapyroxad + pyraclostrobin (257 + 513 g ai ha^{-1}) applied in a water carrier volume of 560 L ha^{-1} with a CO_2 pressurized backpack sprayer 21 and 2 days prior to harvest. Experimental units were 1.5 × 0.8 m. Untreated plots were covered with plywood during applications (Figure 3.1). Immediately following the application, plywood was removed, and 8 mm of overhead irrigation was applied.

Turfgrass was harvested as sod in two 100 cm long and 40 cm wide lanes per treatment using a walk-behind Ryan Jr. Sod Cutter (CGC Inc., Johnson Creek, WI) on 10 June (run 1) and 23 August (run 2), 2019. The depth of harvest was 2.5 cm. Harvested sod (one piece per experimental unit) was washed of excess soil, and 5 cm diameter plugs were removed (Figure 3.2) and stored in two 3.8 L Great Value™ zip-to-lock plastic bags (Walmart Inc., Bentonville, AR). One plastic bag from each experimental unit [50 plugs (1.1-1.5 kg) per bag] was stored in a climate-controlled chamber maintained at 32°C, and another bag was stored in a walk-in cooler maintained at 4°C (Figure 3.3). Both storage conditions were absent of light. Within each plastic bag, temperatures were monitored with the sensors fabricated with type-T thermocouple wire (Omega Engineering, Inc., Stamford, CT) as described in Chapter II. The sensors were placed inside the plastic bags and the temperatures were recorded once every five minutes by two data loggers (CR-1000, Campbell Scientific Inc., Logan, UT) equipped with relay multiplexers (AM16/32A, Campbell Scientific, Logan, UT). Temperature sensors were calibrated in a water bath (IsoTemp 3013D, Fisher Scientific, Pittsburgh, PA) against a precision thermometer. These methods are described by Schmidt et al. (2010). Plugs were stored for 72 hours.

After storage, nine plugs from the center of each plastic bag were transplanted into 14.6 cm diameter and 1.38 L volume pots containing sand-peat (9:1 ratio) soil (Figure 3.4). Pots were grown in a climate-controlled greenhouse (33°27'11.0"N, 88°47'40.0"W) maintained at 30 °C dark/light temperature. The plugs were reel-mown weekly at 1.0 cm mowing height and equally fertilized (57 kg nitrogen ha^{-1}, 19 kg phosphorus ha^{-1}, 38 kg potassium ha^{-1}, plus microelements [B, Cu, Fe, Mn, Mo, Zn]) bi-weekly (Miracle-Gro© Water Soluble All Purpose Plant Food fertilizer, Scott's Miracle-Gro Products Inc., Port Washington, NY). Pots were irrigated daily or as needed in order to maintain growth and appearance.

Pot green cover was assessed using digital image analysis weekly until all pots reached 100% green cover. Images were processed in TurfAnalyzer (Green Research Services, LLC) computer program, which detects the percentage of green pixels in a digital image (Figure 3.5). Similar methods are described by Karcher and Richardson (2003) and Begitschke et al. (2018). In order to exclude differences in lighting, a 0.31 m^3 light box was used, which fully covers the pot and illuminates samples with four helical 10 W soft white lamp bulbs (GE Lighting, Cleveland, OH) (Figure 3.6). A neon pink foam board (Elmer's Products, Inc., Westerville, OH) was cut to the size of the pot in order to occlude the background of each image (Begitschke et al., 2018). Images were captured with a digital camera (Nikon D90 with AF-S DX NIKKOR 18-105 mm attachable lens; Nikon Inc., Melville, NY). The digital camera was white-balanced, and settings were as follows: shutter speed 1/250, aperture size f5.6 and 500 International Standards Organization (ISO) sensitivity.

Plants were destructively harvested in order to allow root analysis 3, 5, and 8 weeks after installation (3 pots of each experimental unit per harvest). Plants were washed free of sand, and roots were harvested. Root analysis was performed similarly to methods of Begitschke et al. (2018). Roots were patted dry with paper towels and frozen (-17°C) for further analysis. Root samples were shaken in 50-ml conical tubes (Thermo Fisher Scientific Inc., Waltham, MA) in 5% sodium hexametaphosphate (The Chemistry Store.com, Cayce, SC) solution for 3-4 hours. Samples were then dispersed on a waterproof transparent tray, scanned, and digitized (600 dots cm^{-1}). Digital images were analyzed within WinRHIZO Pro (Regent Instruments Inc., Sainte-Foy, Quebec City, Canada). Root samples were placed in paper envelopes and kept in a plant dryer (75°C) for 72 hours prior to measuring root dry-mass. Out of all indexes that WinRHIZO Pro software calculated, only root volume will be discussed due to a lack of differences within the rest of the indexes.

Green cover data were subject to analysis of variance ($\alpha=0.05$) within SAS PROC GLM (Version 9.4; SAS Institute Inc., Cary, NC). Data were analyzed as a completely randomized design, with replication in time (run) considered a fixed effect. Data were subject to nonlinear sigmoidal variable slope regression within GraphPad Prism (Version 7.04; GraphPad Software Inc., San Diego, CA). Curves were compared using pairwise F-tests ($\alpha=0.05$). Time to reach 50% green cover was estimated and compared using 95% confidence intervals. Root volume and dry-mass means were separated within SAS PROC GLM using Student-Newman-Keuls method ($\alpha=0.05$).

Results and discussion

Green cover data differed due to run and are therefore discussed separately. Refrigeration generally increased time to cover. These results were evidenced by F-tests performed to compare various treatment responses (Table 3.1). For instance, in run 1 of the study, plugs stored at 32°C covered faster than those stored at 4°C (Figure 3.7). Untreated plugs that were refrigerated covered the slowest of all treatments in run 1. However, when turfgrass was treated with the fluxapyroxad + pyraclostrobin fungicide prior to refrigeration, plugs covered as quickly as those stored at 32°C. In run 2, fungicide-treated plugs stored at 32°C covered faster than all other treatments (Figure 3.8). Unlike the results of run 1, despite plugs being treated with the fungicide prior to refrigeration, they still established slower than untreated plugs stored at 32°C.

To further compare trends of vegetative establishment, days to reach 50 percent green cover were calculated. In run 1, untreated plugs stored at 32°C reached 50 percent green cover faster than all other treatments (Figure 3.9). Similarly, treated plugs stored at 32°C reached 50 percent green cover faster than untreated plugs that were refrigerated. These same trends are evident in run 2, where treated plugs stored at 32°C reached 50 percent green cover faster than those treated and refrigerated (Figure 3.10).

When plugs were harvested 3 weeks after installation (WAI), root dry-mass data did not differ between the runs and, therefore, were pooled (Table A1). Root dry-mass did not differ due to treatment (Table 3.2). Similarly, root dry-mass data obtained 5 WAI were pooled across runs (Table A2) and no differences were observed between treatments (Table 3.3). Root dry-mass data differed between runs, 8 WAI (Table A3), and are presented separately (Table 3.4). In run 1, plugs treated with fluxapyroxad + pyraclostrobin had greater root dry-mass compared to the untreated, 8 WAI. In run 2, no differences between the treatments were observed within root dry-mass.

Root volumes observed 3 WAI did not differ between runs (Table A4) and were pooled. Plugs stored at 32°C had greater root volumes than refrigerated plugs, 3 WAI (Table 3.5). Root volumes observed 5 WAI differed between runs (Table A5) and are presented separately. In run 1, plugs stored at 32°C had greater root volumes than refrigerated plugs (Table 3.6). In run 2, root volumes failed to differ due to treatments. Root volumes observed 8 WAI differed between runs (Table A6); however, root volumes failed to differ due to treatments in either run (Table 3.7).

Despite bermudagrass greens establishment through sprigs being common industry practice, our methods utilized plugs. During preliminary studies, sprigging resulted in a high degree of variation, despite our best attempts to control for uniformity of sprig characteristics (node counts, internode lengths, and visual inspection of health). The methods presented herein attempted to standardize vegetative propagation using uniform-sized plugs that had been harvested with as little disruption or injury as possible.

Our methods failed to simulate internal heating, as temperatures within stored sod plugs did not exceed the temperatures within either environmental chamber or refrigerator (Figure 3.11-3.14). A slight rise in internal mass temperature occurred 50 hours after storage initiation in run 1 (Figure 3.11), which might only be explained by the malfunction of the growth chamber. With regards to internal mass temperature, no differences in treatments were observed in either run.

These results are indicative of the complexity of ensilation within stored sod masses described by others (Watschke et al., 1970; Darrah and Powell, 1977; Pahlow et al., 2003). Methods of this study largely eliminated the possibility of soil layers that separate vegetative mass in pallets to inhibit internal heating, as prior research stated (Watschke et al., 1970; Darrah and Powell, 1977; King et al., 1982). In the current experiment, sod pieces were washed of excess soil prior to plug excavation and storage. There may be other requirements that need to be met in order to simulate ensilation and internal heating within stored sod masses besides isolation from outside air, light, and thin soil layer. This issue should prompt further research of soil and foliar microbial communities; however, that was not an objective of our research.

Refrigeration slowed the vegetative establishment and increased time to cover, which contradicts our hypothesis. Although refrigerated plugs were visually greener after storage than those stored at 32°C, their growth was stunted. We conclude that low temperatures during storage may contribute to the stress experienced by non-dormant turfgrass during transplantation. However, fluxapyroxad + pyraclostrobin fungicide appears to help refrigerated turfgrass overcome that cold-induced stress and establish as quickly as that stored at 32°C. This may be explained by the ability of pyraclostrobin to inhibit ethylene production caused by stress (Anonymous, 2010).

Conclusions

Despite reduced soil content and isolation from outside air and light, this study was not able to recreate the internal heating of stored turfgrass. These results indicate that sod masses smaller than a full-size pallet do not tend to experience the same stresses, such as ensiling processes and internal heating. Future research should determine what triggers those negative processes inside stored sod masses. No differences were observed between treatments in terms of internal mass temperature throughout the storage in either run of the experiment.

Contrary to the hypothesis of the study, refrigeration at 4°C during storage slowed vegetative establishment by increasing the time required for cover and by decreasing root volume in the first 3-5 weeks after turfgrass installation. We conclude that refrigeration may significantly add to the stress experienced by stored turfgrass. However, fluxapyroxad + pyraclostrobin fungicide applied 21 and 2 days before sod harvest may help turfgrass to overcome that cold-induced stress. The application of this fungicide prior to harvest resulted in faster establishment of refrigerated turfgrass and increased root dry-mass by week 8 after installation. These results suggest that pre-application with fluxapyroxad + pyraclostrobin fungicide is a viable option for the improved vegetative establishment by sprigs, plugs, or sod. Future research should focus on determining the nature of fluxapyroxad + pyraclostrobin effects upon plant protection activity and its effects on storage when internal heating occurs.

Tables and figures

Table 3.1 Pairwise F-test comparisons of percent green cover sigmoidal variable slope regression curves of greenhouse-grown bermudagrass plugs

	Run							
	1				**2**			
	NT-E[a]	**NT-R**	**T-E**	**T-R**	**NT-E**	**NT-R**	**T-E**	**T-R**
NT-E	-	<0.0001[b]	0.0805	0.0003	-	0.4487	0.0045	0.6881
NT-R	<0.0001	-	<0.0001	0.0005	0.4487	-	0.0456	0.0237
T-E	0.0805	<0.0001	-	0.4412	0.0045	0.0456	-	<0.0001
T-R	0.0003	0.0005	0.4412	-	0.6881	0.0237	<0.0001	-

[a] NT – Untreated; T – Turfgrass treated with fluxapyroxad + pyraclostrobin fungicide 21 and 2 days prior to 5 cm plugs excavation in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer; E – Stored at 32°C air temperature; R – Stored at 4°C air temperature. The storage time was 72 hours. The diameter of pots was 14.6 cm.

[b] P values calculated within GraphPad Prism (α=0.05).

Table 3.2 Root dry-mass of 3-week old greenhouse-grown 5 cm diameter bermudagrass plugs

Treatment	**Mean root dry-mass (g)**
Untreated, stored at 32°C[a]	0.0757 a[c]
Untreated, stored at 4°C	0.0417 a
Treated[b], stored at 32°C	0.0675 a
Treated, stored at 4°C	0.0741 a

Harvest of greenhouse-grown bermudagrass plugs conducted 3 weeks after installation at 3 pots per plot. The study was conducted at Mississippi State University in 2019.

[a] 50 bermudagrass plugs (5 cm in diameter) were stored for 72 hours in 3.8 L sealed plastic bags prior to transplantation into pots maintained in the greenhouse.

[b] Treatment consisted of fluxapyroxad + pyraclostrobin application 21 and 2 days prior to plugs excavation in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer.

[c] Means followed by the same letter in a column are not significantly different. Means were separated using the Student-Newman-Keuls method (α=0.05).

Table 3.3 Root dry-mass of 5-week old greenhouse-grown 5 cm diameter bermudagrass plugs

Treatment	Mean root dry-mass (g)
Untreated, stored at 32°C[a]	0.2145 a[c]
Untreated, stored at 4°C	0.2014 a
Treated[b], stored at 32°C	0.2201 a
Treated, stored at 4°C	0.2134 a

Harvest of greenhouse-grown bermudagrass plugs conducted 5 weeks after installation at 3 pots per plot. Data did not differ across runs and are pulled across them. The study was conducted at Mississippi State University in 2019.

[a] 50 bermudagrass plugs (5 cm in diameter) were stored for 72 hours in 3.8 L sealed plastic bags prior to transplantation into pots maintained in the greenhouse.

[b] Treatment consisted of fluxapyroxad + pyraclostrobin application 21 and 2 days prior to plugs excavation in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer.

[c] Means followed by the same letter in a column are not significantly different. Means were separated using the Student-Newman-Keuls method (α=0.05).

Table 3.4 Root dry-mass of 8-week old greenhouse-grown 5 cm diameter bermudagrass plugs

Run			
1		2	
Treatment	Root dry-mass (g)	Treatment	Root dry-mass (g)
Untreated [a]	0.5806 b[b]	Untreated, stored at 32°C[c]	0.3720 a
Treated	0.6926 a	Untreated, stored at 4°C	0.3148 a
		Treated, stored at 32°C	0.3864 a
		Treated, stored at 4°C	0.3540 a

Harvest of greenhouse-grown bermudagrass plugs conducted 8 weeks after installation, when 100 percent green cover was reached, at 3 pots per plot. The study was conducted at Mississippi State University in 2019.

[a] Treatment consisted of fluxapyroxad + pyraclostrobin application 21 and 2 days prior to plugs excavation in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer.

[b] Means followed by the same letter in a column are not significantly different. Means were separated using the Student-Newman-Keuls method (α=0.05).

[c] 50 bermudagrass plug sod masses (5 cm in diameter) were stored for 72 hours in 3.8 L sealed plastic bags prior to transplantation into pots maintained in the greenhouse.

Table 3.5 Root volume of 3-week old greenhouse-grown 5 cm diameter bermudagrass plugs

Treatment	Mean root volume (cm^3)
Stored at 4°C[a]	0.5550 b[b]
Stored at 32°C	1.2513 a

Harvest of greenhouse-grown bermudagrass plugs conducted 3 weeks after installation at 3 pots per plot. Prior to installation, Data did not differ across runs and are pulled across them. The study was conducted at Mississippi State University in 2019.

[a] 50 bermudagrass plugs (5 cm in diameter) from each plot were harvested and stored for 72 hours in 3.8 L sealed plastic bags prior to transplantation into pots maintained in the greenhouse.

[b] Means followed by the same letter in a column are not significantly different. Means were separated using the Student-Newman-Keuls method (α=0.05).

Table 3.6 Root volume of 5-week old greenhouse-grown 5 cm diameter bermudagrass plugs

Run			
1		2	
Treatment	Root dry-mass (g)	Treatment	Root dry-mass (g)
Stored at 4°C[a]	2.6752 b[b]	Untreated, stored at 32°C	4.1864 a
Stored at 32°C	3.7324 a	Untreated, stored at 4°C	3.1504 a
		Treated[c], stored at 32°C	5.5756 a
		Treated, stored at 4°C	4.3064 a

Harvest of greenhouse-grown bermudagrass plugs conducted 5 weeks after installation at 3 pots per plot. The study was conducted at Mississippi State University in 2019.

[a] 50 bermudagrass plugs (5 cm in diameter) were stored for 72 hours in 3.8 L sealed plastic bags prior to transplantation into pots maintained in the greenhouse.

[b] Means followed by the same letter in a column are not significantly different. Means were separated using the Student-Newman-Keuls method (α=0.05).

[c] Treatment consisted of fluxapyroxad + pyraclostrobin application 21 and 2 days prior to plugs excavation in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer.

Table 3.7 Root volume of 8-week old greenhouse-grown 5 cm diameter bermudagrass plugs

Run			
1		**2**	
Treatment	**Root dry-mass (g)**	**Treatment**	**Root dry-mass (g)**
Untreated, stored at 32°C[a]	12.8866 a[b]	Untreated, stored at 32°C	7.0966 a
Untreated, stored at 4°C	7.8598 a	Untreated, stored at 4°C	12.6776 a
Treated[c], stored at 32°C	14.4113 a	Treated, stored at 32°C	7.3560 a
Treated, stored at 4°C	13.3380 a	Treated, stored at 4°C	4.8305 a

Harvest of greenhouse-grown bermudagrass plugs conducted 8 weeks after installation, when 100 percent green cover was reached, at 3 pots per plot. The study was conducted at Mississippi State University in 2019.

[a] 50 bermudagrass plugs (5 cm in diameter) were stored for 72 hours in 3.8 L sealed plastic bags prior to transplantation into pots maintained in the greenhouse.

[b] Means followed by the same letter in a column are not significantly different. Means were separated using the Student-Newman-Keuls method (α=0.05).

[c] Treatment consisted of fluxapyroxad + pyraclostrobin application 21 and 2 days prior to plugs excavation in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer.

Figure 3.1 Fluxapyroxad + pyraclostrobin application

Applications were in a water carrier volume of 560 L ha^{-1} using CO_2 pressurized backpack sprayer 21 and 2 days prior to harvest. Plywood was used to cover untreated plots.

Figure 3.2 Plug excavation from harvested sod

Sod pieces (2.5 cm in depth) were washed of excess soil. Plugs were 5 cm in diameter.

Figure 3.3 Storage of sod masses in an environmental chamber

50 plugs from each field experimental unit were placed in a 3.8 L plastic bag, sealed with a temperature sensor inside and stored in an environmental chamber set at 32°C. 50 more plugs from each field experimental unit were stored similarly in an environmental chamber set at 4°C. The storage time was 72 hours.

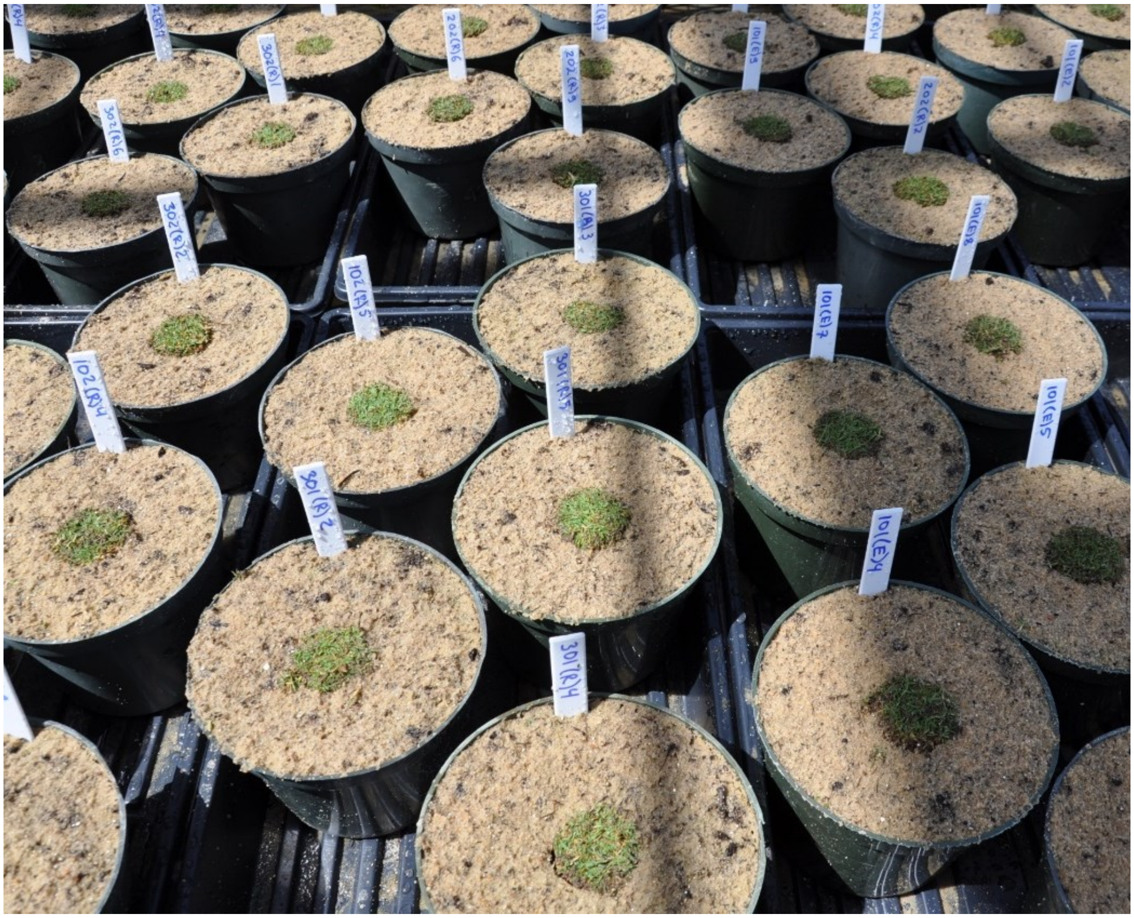

Figure 3.4 Plugs transplanted into pots

From each plastic bag, plugs were transplanted into 14.6 cm diameter and 1.38 L volume pots containing sand-peat (9:1 ratio) soil with 9 pot replications.

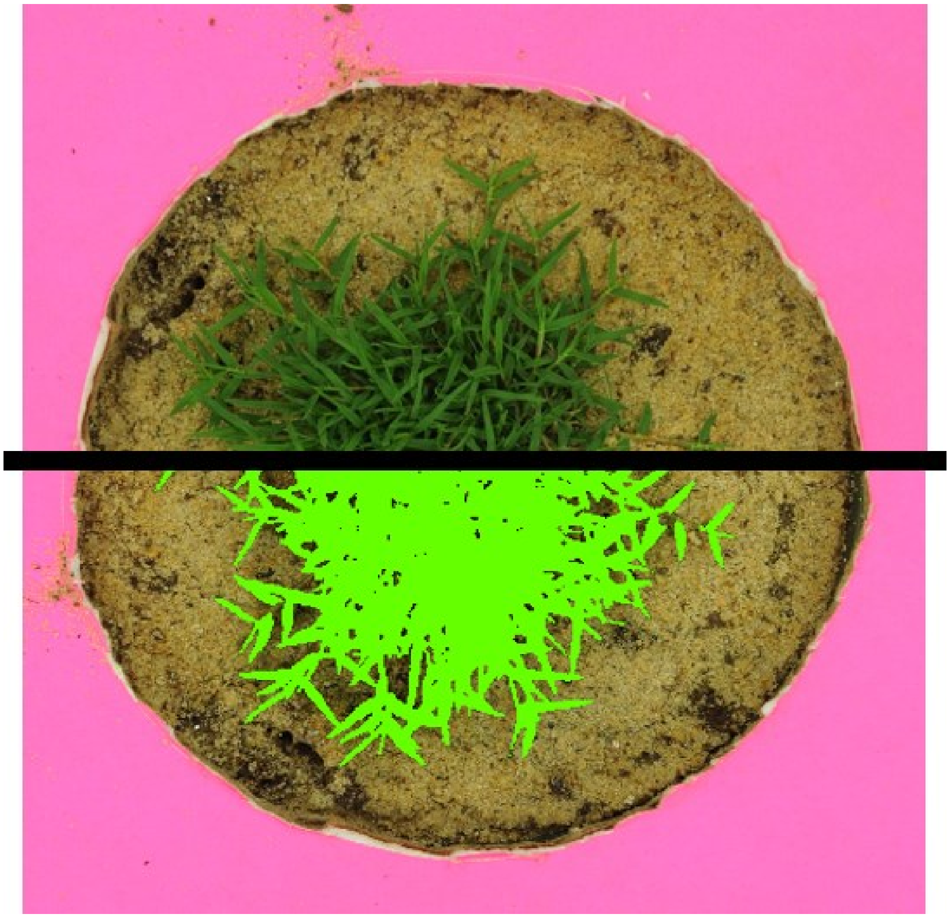

Figure 3.5 Digital image analysis for green cover

Digital images were analyzed for green cover using TurfAnalyzer computer program. The upper half of the image is a raw photograph; the lower half illustrates which pixels were counted as a green cover by marking them with a light green color.

Figure 3.6 Process of obtaining digital images using the lightbox

The lightbox has a volume of 0.31 m^2. It fully covers a pot and is illuminated with four helical 10 W soft white lamps. The camera is placed on the top in the center.

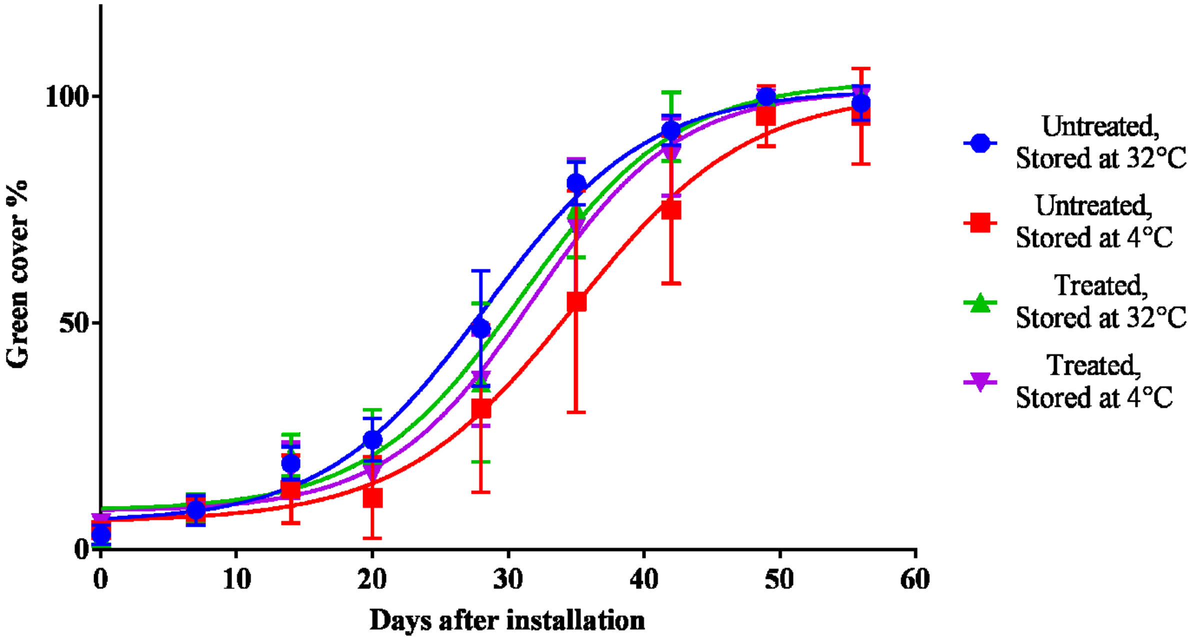

Figure 3.7 Percent green cover sigmoidal variable slope regression (run 1)

Data obtained within GraphPad Prism. Error bars represent standard deviations. Green cover of establishing 5 cm diameter bermudagrass plugs was assessed by digital image analysis within TurfAnalyzer software. The diameter of pots was 14.6 cm. Treatment consisted of two fluxapyroxad + pyraclostrobin applications 21 and 2 days prior to harvest in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer. The study was conducted at Mississippi State University in 2019. The date of harvest was 10 June, 2019. The storage time was 72 hours.

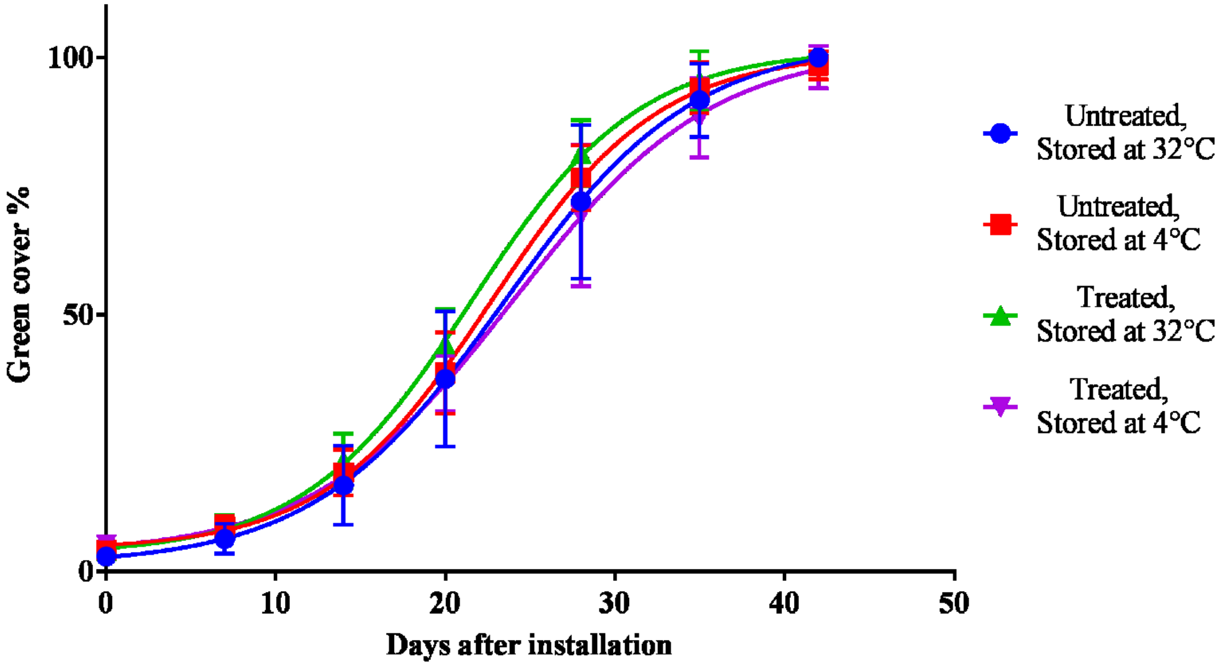

Figure 3.8 Percent green cover sigmoidal variable slope regression (run 2)

Data obtained within GraphPad Prism. Error bars represent standard deviations. Green cover of establishing 5 cm diameter bermudagrass plugs was assessed by digital image analysis within TurfAnalyzer software. The diameter of pots was 14.6 cm. Treatment consisted of two fluxapyroxad + pyraclostrobin applications 21 and 2 days prior to harvest in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer. The study was conducted at Mississippi State University in 2019. The date of harvest was 23 August, 2019. The storage time was 72 hours.

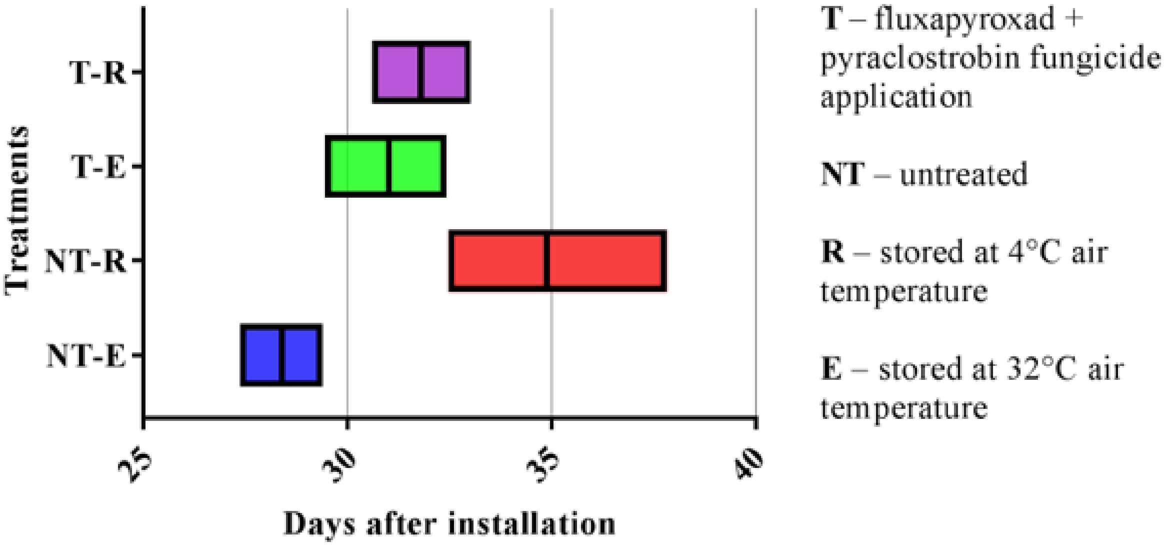

Figure 3.9 Days to reach 50 percent green cover for greenhouse-grown bermudagrass plugs (run 1)

The bars represent estimates for days to reach 50 percent green cover by 5 cm diameter bermudagrass plugs based on 95 percent confidence intervals predicted by a sigmoidal variable slope regression model within GraphPad Prism. The diameter of pots was 14.6 cm. The green cover was assessed by digital image analysis within TurfAnalyzer software. Treatment consisted of two fluxapyroxad + pyraclostrobin applications 21 and 2 days prior to harvest in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer. The storage time was 72 hours. The study was conducted at Mississippi State University in 2019. The date of harvest was 10 June, 2019.

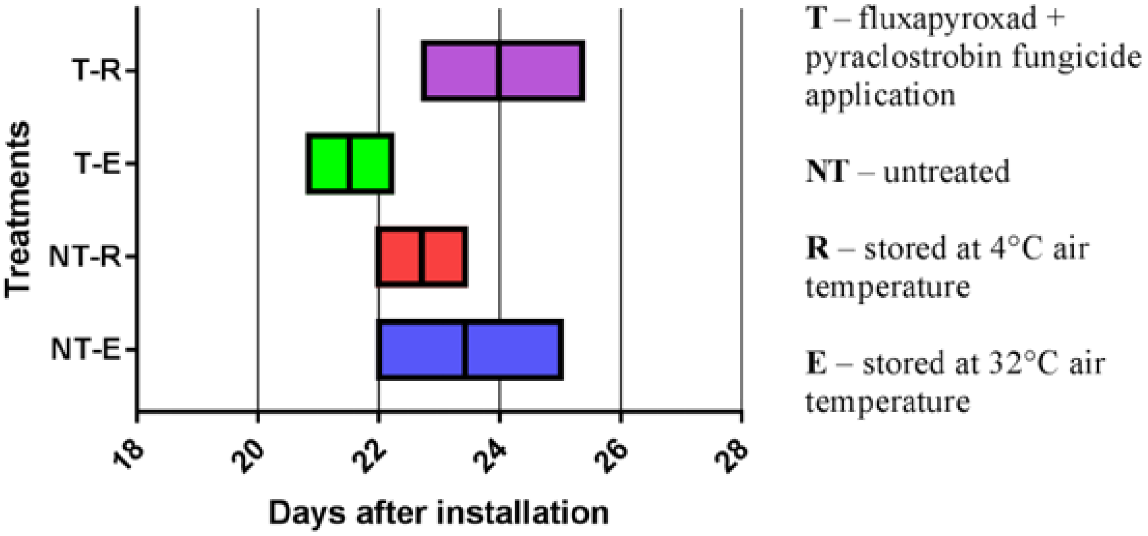

Figure 3.10 Days to reach 50 percent green cover for greenhouse-grown bermudagrass plugs (run 2)

The bars represent estimates for days to reach 50 percent green cover by 5 cm diameter bermudagrass plugs based on 95 percent confidence intervals predicted by a sigmoidal variable slope regression model within GraphPad Prism. The diameter of pots was 14.6 cm. The green cover was assessed by digital image analysis within TurfAnalyzer software. Treatment consisted of two fluxapyroxad + pyraclostrobin applications 21 and 2 days prior to harvest in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer. The storage time was 72 hours. The study was conducted at Mississippi State University in 2019. The date of harvest was 23 August, 2019.

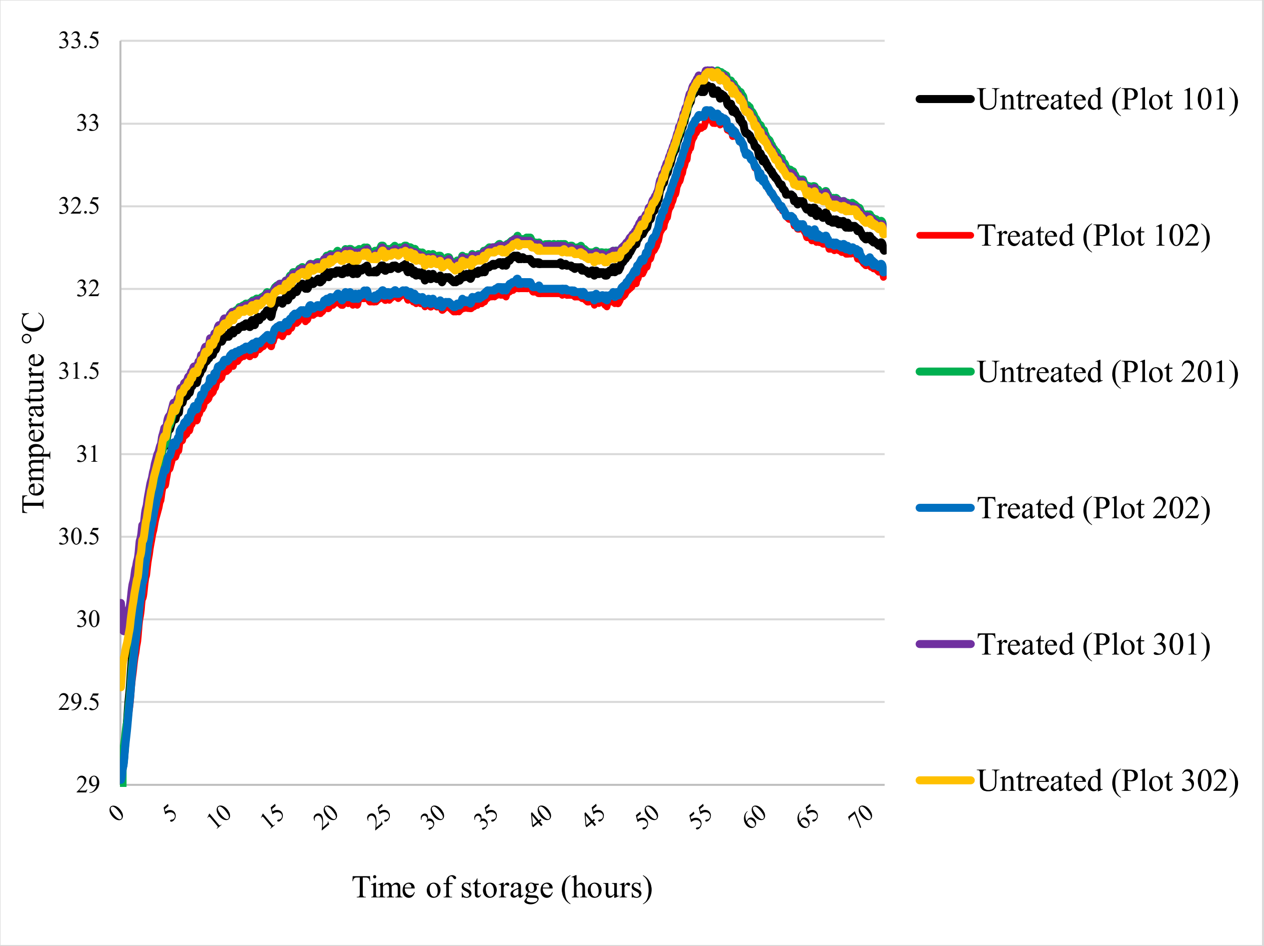

Figure 3.11 Internal temperatures of sod masses stored at 32°C (run 1)

Sod masses consisted of 50 bermudagrass plugs (5 cm in diameter) stored in 3.8 L sealed plastic bags in an environmental chamber. Treatment consisted of two fluxapyroxad + pyraclostrobin applications 21 and 2 days prior to harvest in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer. The study was conducted at Mississippi State University in 2019. The date of harvest was 10 June, 2019.

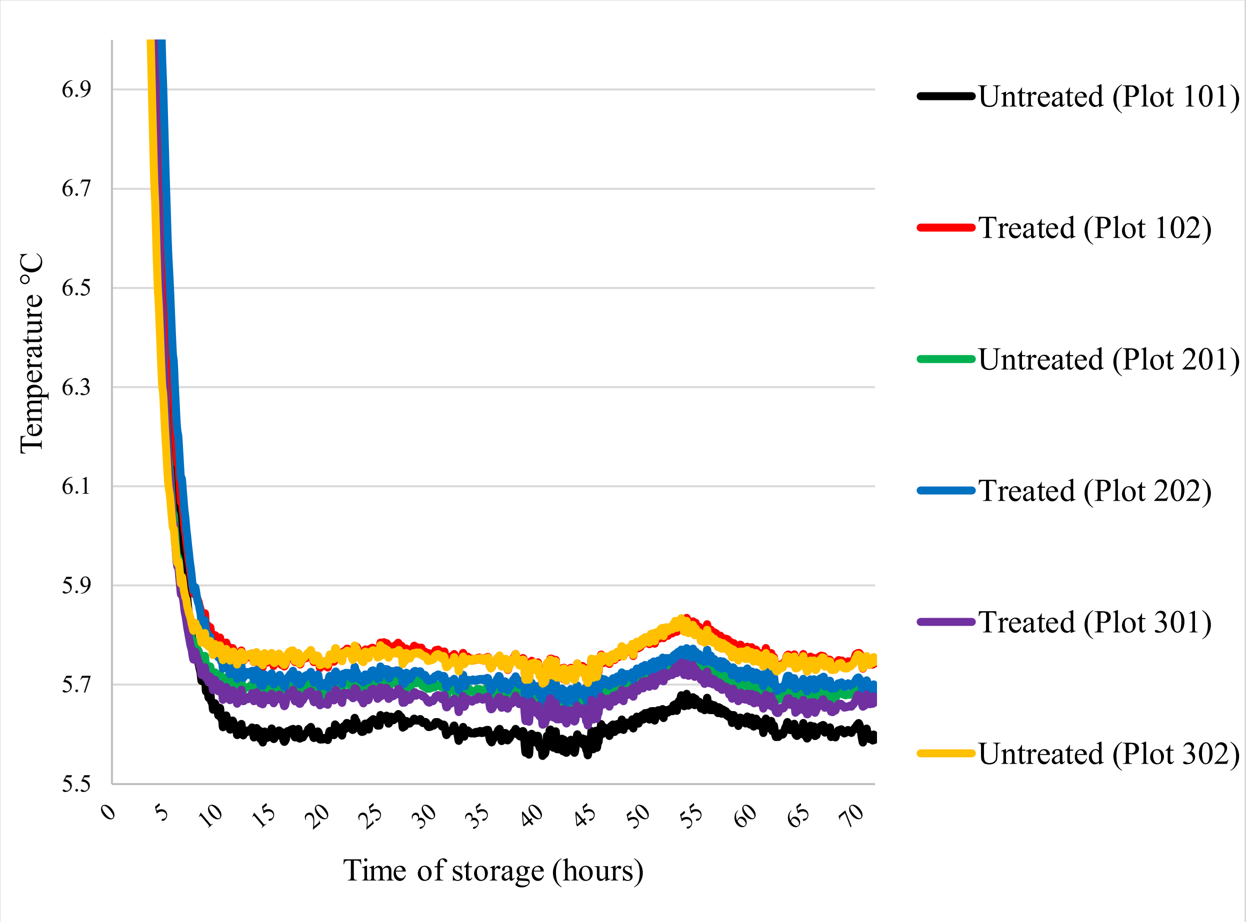

Figure 3.12 Internal temperatures of sod masses stored at 4°C (run 1)

Sod masses consisted of 50 bermudagrass plugs (5 cm in diameter) stored in 3.8 L sealed plastic bags in a walk-in cooler. Treatment consisted of two fluxapyroxad + pyraclostrobin applications 21 and 2 days prior to harvest in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer. The study was conducted at Mississippi State University in 2019. The date of harvest was 23 August, 2019.

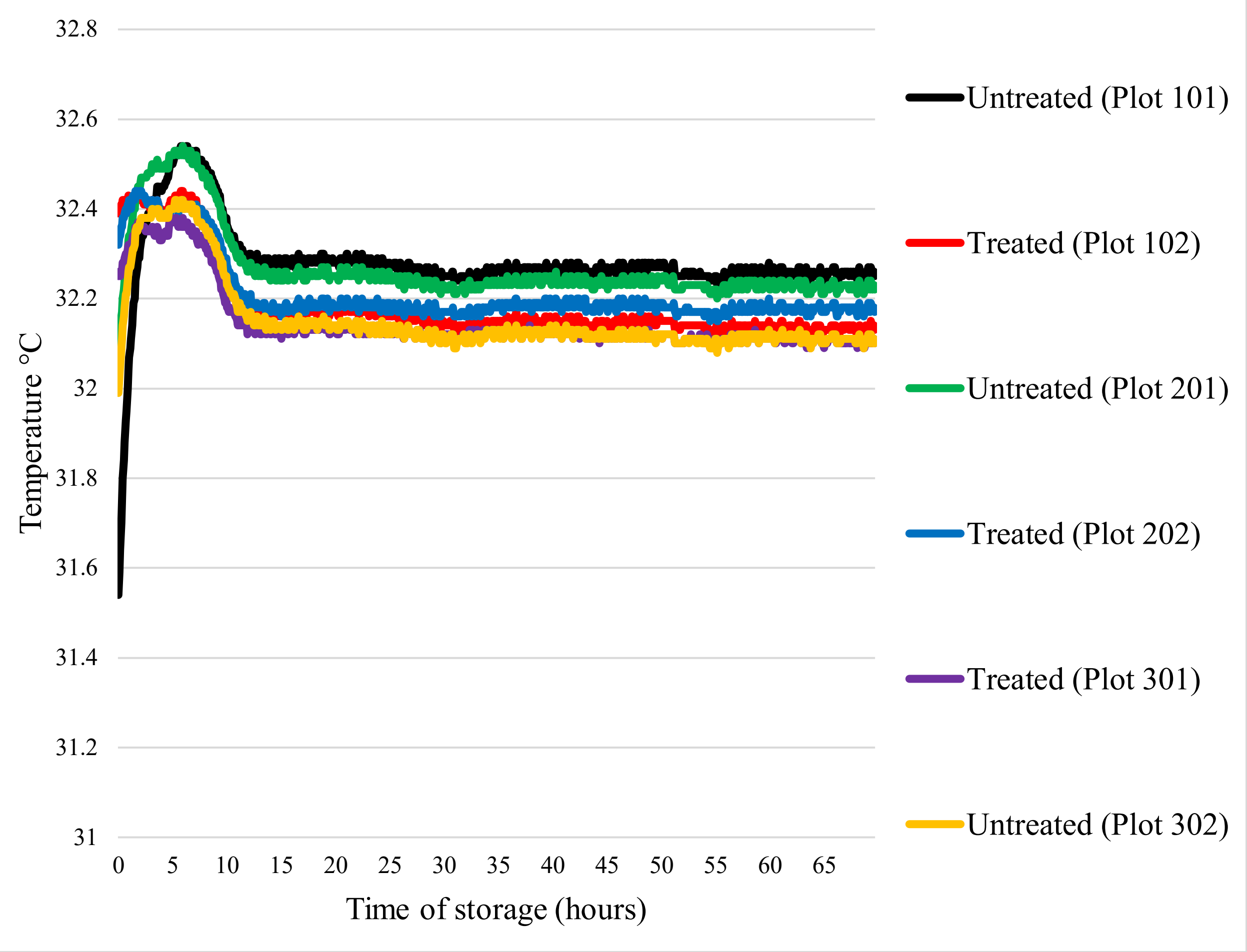

Figure 3.13 Internal temperatures of sod masses stored at 32°C (run 2)

Sod masses consisted of 50 bermudagrass plugs (5 cm in diameter) stored in 3.8 L sealed plastic bags in an environmental chamber. Treatment consisted of two fluxapyroxad + pyraclostrobin applications 21 and 2 days prior to harvest in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer. The study was conducted at Mississippi State University in 2019. The date of harvest was 10 June, 2019.

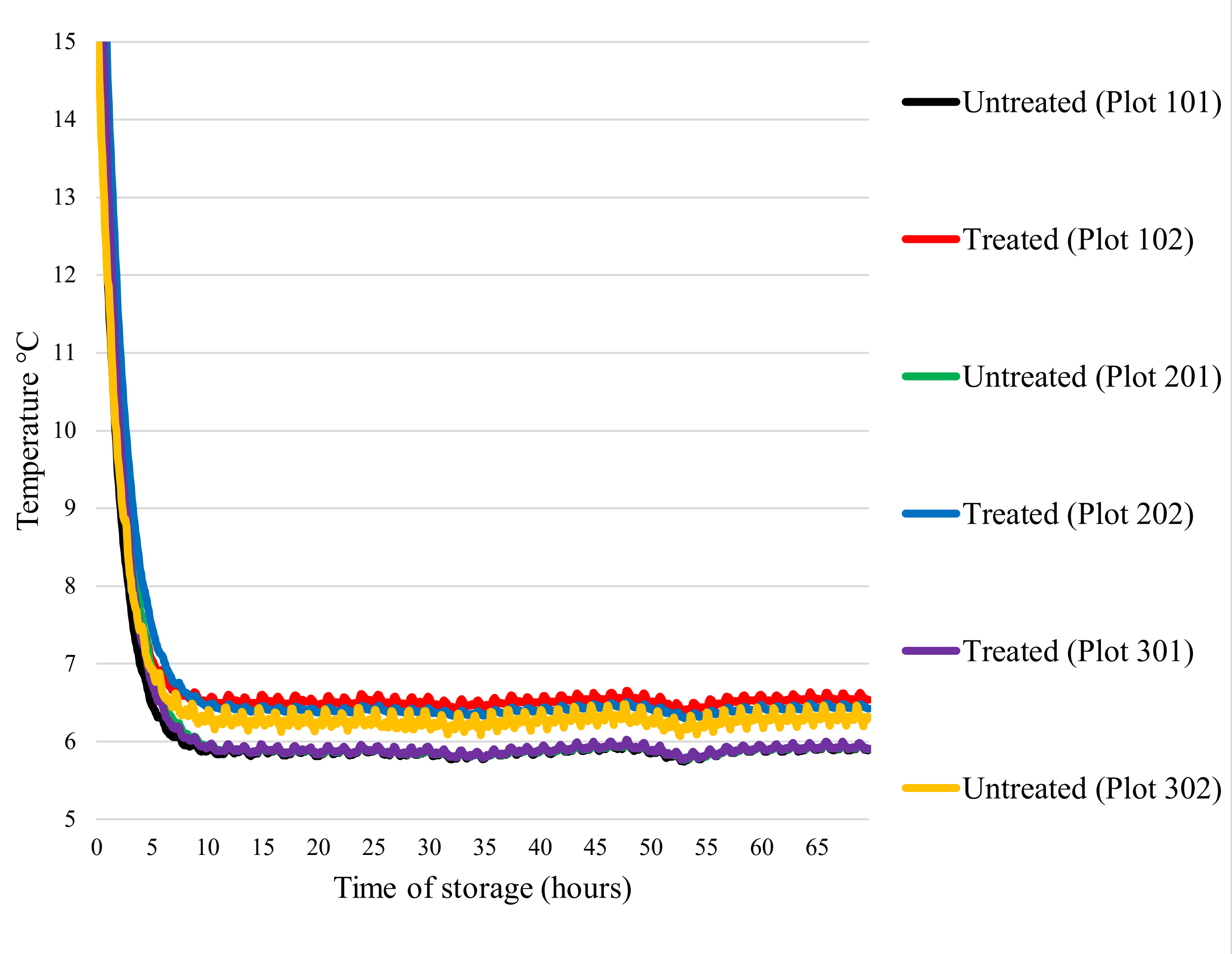

Figure 3.14 Internal temperatures of sod masses stored at 4°C (run 2)

Sod masses consisted of 50 bermudagrass plugs (5 cm in diameter) stored in 3.8 L sealed plastic bags in a walk-in cooler. Treatment consisted of two fluxapyroxad + pyraclostrobin applications 21 and 2 days prior to harvest in a water carrier volume of 561 L ha^{-1} via a CO_2 pressurized backpack sprayer. The study was conducted at Mississippi State University in 2019. The date of harvest was 23 August, 2019.

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APPENDIX A

ANALYSIS OF VARIANCE FOR GREENHOUSE-GROWN BERMUDAGRASS ROOT ANALYSIS DATA

Table A.1 Root dry-mass data analysis of variance (α=0.05) (Harvest 1)

Factor	t-value
Fungicide treatment[a]	0.4658
Storage[b]	0.2439
Fungicide treatment × Storage	0.3509
Run	0.0763

Harvest 1 of greenhouse-grown bermudagrass plugs conducted 3 weeks after installation at 3 pots per plot. The study was conducted at Mississippi State University in 2019. Data did not differ across runs and are pulled.

[a] Treatment with fluxapyroxad + pyraclostrobin fungicide against untreated.

[b] Storage for 72 hours at 32°C air temperature against storage at 4°C air temperature.

Table A.2 Root dry-mass data analysis of variance (α=0.05) (Harvest 2)

Factor	t-value
Fungicide treatment[a]	0.7450
Storage[b]	0.7112
Fungicide treatment × Storage	0.2573
Run	0.8888

Harvest 2 of greenhouse-grown bermudagrass plugs conducted 5 weeks after installation at 3 pots per plot. The study was conducted at Mississippi State University in 2019. Data did not differ across runs and are pulled.

[a] Treatment with fluxapyroxad + pyraclostrobin fungicide against untreated.

[b] Storage for 72 hours at 32°C air temperature against storage at 4°C air temperature.

Table A.3 Root dry-mass data analysis of variance (α=0.05) (Harvest 3)

Factor	t-value	
	Run	
	1	2
Fungicide treatment[a]	0.1894	0.3993
Storage[b]	0.2644	0.7709
Fungicide treatment × Storage	0.4586	0.2140
Run	0.0208	

Harvest 3 of greenhouse-grown bermudagrass plugs conducted 8 weeks after installation at 3 pots per plot. The study was conducted at Mississippi State University in 2019.

[a] Treatment with fluxapyroxad + pyraclostrobin fungicide against untreated.

[b] Storage for 72 hours at 32°C air temperature against storage at 4°C air temperature.

Table A.4 Root volume data analysis of variance (α=0.05) (Harvest 1)

Factor	t-value
Fungicide treatment[a]	0.8821
Storage[b]	0.0055
Fungicide treatment × Storage	0.5961
Run	0.6733

Harvest 1 of greenhouse-grown bermudagrass plugs conducted 3 weeks after installation at 3 pots per plot. The study was conducted at Mississippi State University in 2019. Data did not differ across runs and are pulled.

[a] Treatment with fluxapyroxad + pyraclostrobin fungicide against untreated.

[b] Storage for 72 hours at 32°C air temperature against storage at 4°C air temperature.

Table A.5 Root volume data analysis of variance (α=0.05) (Harvest 2)

Factor	t-value	
	Run	
	1	2
Fungicide treatment[a]	0.0584	0.2236
Storage[b]	0.0394	0.2927
Fungicide treatment × Storage	0.7960	0.8441
Run	0.0012	

Harvest 2 of greenhouse-grown bermudagrass plugs conducted 5 weeks after installation at 3 pots per plot. The study was conducted at Mississippi State University in 2019.

[a] Treatment with fluxapyroxad + pyraclostrobin fungicide against untreated.

[b] Storage for 72 hours at 32°C air temperature against storage at 4°C air temperature.

Table A.6 Root volume data analysis of variance (α=0.05) (Harvest 3)

Factor	t-value	
	Run	
	1	2
Fungicide treatment[a]	0.1894	0.3993
Storage[b]	0.2644	0.7709
Fungicide treatment × Storage	0.4586	0.2140
Run	0.0208	

Harvest 3 of greenhouse-grown bermudagrass plugs conducted 8 weeks after installation at 3 pots per plot. The study was conducted at Mississippi State University in 2019.

[a] Treatment with fluxapyroxad + pyraclostrobin fungicide against untreated.

[b] Storage for 72 hours at 32°C air temperature against storage at 4°C air temperature.