# Cold tolerance in warm season turfgrasses

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# Abstract

Warm season ($C_4$) turfgrasses are a popular choice for sports and public venues in tropical, subtropical, arid and semiarid climates due to their spreading characteristics, multiple stress resistance, including water deficit and heat tolerance, and faster establishment. However intolerance of low temperature is the key limitation to their use in temperate regions. The New Zealand turf industry has a growing interest in warm season ($C_4$) grasses due to their water use efficiency under heat stress and summer dormancy of cool season ($C_3$) grasses, especially in the upper parts of the North Island. Twelve commercially available cultivars of four warm season grass genera (*Cynodon, Zoysia, Paspalum and Pennisetum*) were established in a glasshouse and ten cultivars in field at Palmerston North, New Zealand, using seeds and stolon cuttings. This phase of the project was carried out from November 2012 to January 2015, with three major aspects of turf function measured. Established plots were scored for quality attributes (colour, texture, uniformity, ground cover and overall quality) as prescribed by NTEP (National Turf Evaluation Program, USA). Field plots became dormant and began browning in late autumn. Browning progressed and became more visible by the end of winter. Glasshouse plots displayed better overall turf quality than field plots except for seeded *Cynodon* varieties which showed susceptibility to Anthracnose fungal attack. Vegetative *Cynodon* varieties (Agridark, Windsor green and Santa Ana) performed well along with Sea spray (*Paspalum vaginatum*). Regal Staygreen (*Pennisetum clandestinum*) proved more cold tolerant than other varieties but, being coarse textured, cannot attain high acceptance in the turf industry. A subsequent experiment was focused on detailed morphology and growth pattern of these varieties. It was observed that glasshouse plots developed fewer roots per node and a lower total root mass compared with those grown in field conditions. In field plots stolon structures were more compact with a high number of horizontal stolons. Rhizome appearance differed between the glasshouse and the field and during the first year of establishment only vegetatively established *Cynodon* varieties developed rhizomes under field conditions and only Agridark in the glasshouse. However, during the next growing season all varieties in the field, except Zenith, had formed rhizomes. Seeded couches failed to produce rhizomes in the glasshouse even after their 2nd growing season. Detailed study of stolon morphology confirmed findings on turf mat quality from visual scoring, and identified a pattern of ecological interest in that varieties of the genera *Cynodon* and *Zoysia* formed compound or triplet nodes, with root, branch and internode formation allocated to different leaves.

A second phase of the research investigated cold tolerance in warm season turf grasses and the response of four varieties from three different warm season turf species Agridark and Windsor Green (*Cynodon dactylon*), Sea Spray (*Paspalum vaginatum)*, and Zenith (*Zoysia japonica*) when exposed to low but non freezing temperatures. This experiment aimed to identify low temperature tolerance thresholds at various exposure durations, to help turf mangers define temperature tolerance of available varieties. Plants were established in trays in a glasshouse and were exposed to a series of progressively decreasing temperatures (16/10°C, 12/8°C, 10/6°C, 8/4°C and 6/2°C, day/night) with 2 weeks at each temperature step, or to sudden, short exposure to the same temperatures for 2 weeks. Colour change during the various combinations of low temperature exposure, and recovery after damage were observed along with measurement of selected physiological indices including proline, malondialdehyde (MDA) and carbohydrate accumulation. It was found that longer exposure with gradually lowered temperature was more detrimental to plants than sudden, short exposure. Seashore paspalm (Sea spray) exhibited better colour retention during cold exposure than the other three varieties in this experiment. Levels of proline and MDA in leaf and stolon tissue, and carbohydrate status tended to return towards pre-stress levels when plants were placed in a glasshouse for recovery from these cold-stress challenges.

The ecological significance of the triplet stolon structure is unclear but deserves further study. Understanding that cold damage is a cumulative process rather than a sudden event when a threshold is reached, will be helpful to development of recommendations for turf industry use of $C_4$ grasses in temperate climates.

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# CHAPTER 1

# Introduction

## 1.1 Emergence of the modern turf industry

Turfgrass is an essential component of landscaped ecosystems and symbolizes beauty and utility in integration with plants and other features (Roberts et al., 1992). Turf is grown at various venues (such as lawns at residential units, public and community parks, sports fields, city green belts, golf courses and many commercial properties) for functional, recreational and aesthetic benefits (Duble, 1996). Turfgrass enhances aesthetic appeal of a landscape, stimulates mental health with positive societal impact, public unity, enhanced productivity and enhanced living standards (Kaplan and Kaplan, 1989; Ulrich, 1986). Sports activities are believed to contribute significantly to societal health in the modern world (Stiles et al., 2009). Turfgrass provides an economical surface for recreation and outdoor sports. The unique cushioning effect provided by turfgrass minimizes the risk of injuries to players in games like soccer and rugby (Orchard, 2002; Beard & Green, 1994). Therefore a large proportion of the area under turf comprises sports and athletic fields (Morris, 2003).

Turfgrasses are separated into cool season ($C_3$) grasses and warm season ($C_4$) grasses based on their photosynthetic pathways (Christians, 2011), and the two groups also have distinct ecological distributions and temperature adaptations. Cool season grasses include among others the genera *Agrostis, Festuca* and *Lolium*, historically widely used in New Zealand (NZ). This group is often considered to be resource hungry and to have comparatively high maintenance and input requirements. Susceptibility to heat stress is a basic limitation of cool season or $C_3$ grasses in warmer climates. $C_3$ grasses exhibit low water use efficiency due to increased evapotranspiration rates under high temperatures resulting in a water imbalance that in more extreme conditions can lead to dehydration (Jiang and Huang, 2001).

Warm season turfgrasses primarily belong to the genera *Cynodon, Eremochloa, Paspalum, Pennisetum,* and *Zoysia* (Busey, 1989) and members of these genera typically have horizontal creeping stems, resulting in a spreading growth habit. Warm season turfgrasses are widely grown in tropical regions of the world and even sometimes in temperate regions, where hot summers are experienced (Geren et al., 2009). They exhibit higher levels of heat and drought tolerance due to their morphology, growth pattern, internal cell metabolism and photosynthetic efficiency (Zhou and Abaraha, 2007). Intolerance of low temperature is a limiting factor for most of the warm season grass species (Thomas et al., 2009). Cold temperature damage in warm season turfgrasses lowers the aesthetic appeal. Their growth usually stops below 16ºC, while foliage starts turning brown below 10ºC (Anderson et al., 2002).

*Cynodon* (often referred to by the common names Bermuda grass, or couch grass) is the most widely cultivated genus among the warm season group of grasses (Taliaferro, 2003) and is extensively used for golf courses and athletic surfaces (Munshaw et al., 2006) in southern USA and many other countries worldwide where summer temperatures exceed 25°C. The most commonly encountered species is *C. dactylon* (L.) Pers., but *C. transvaalensis* Burtt Davy with a number of sterile hybrid crosses of these two species, are also used in the industry. Other genera, especially *Zoysia* and *Paspalum* are now attracting industry attention as some members of these genera display particular adaptations such as shade and salinity tolerance (Duncan, 1996).

The turf industry typically produces and manages lawns and other land areas for landscape, public and recreational purposes (Nutter, 1965). The industry started to develop in its present structure after World War II and grew dramatically in the next three decades (Ralph and Busey, 1987). The turf industry as an entity evolved radically during the latter half of 20th century with USA becoming the hub for research and development. The United States Department of Agriculture (USDA) and the United States Golf Association (USGA) were two of the main organizations that influenced this transformation of the pre-existing traditional turf culture (Jenkins, 1994). Research into warm season turf species is now well documented in scientific literature from USA, China and Australia, to name three countries that have strong links with New Zealand, but also in many other countries in Europe, Asia, Africa and South America. Turf breeders seek to develop turfgrass cultivars that can offer acceptable growth and surface quality under a wide range of atmospheric and soil conditions. The ultimate goal for any turfgrass breeding programme is the development of cultivars with smaller shoot size than forage grasses, and with the capacity to form a finely structured turf. A turf variety should also have reduced maintenance requirements, but ability to tolerate biotic (disease, pest, traffic) and abiotic (drought, cold, heat, salinity) stress factors (Duncan and Carrow, 1999; Lee et al., 2004).

The turf industry in New Zealand was founded on cool season grasses from the United Kingdom (UK), particularly species of *Agrostis, Festuca, Lolium*, and *Poa*. The presence of warm season turfgrass species was noted in the 19th and early 20th centuries (*C. dactylon – 1871, Paspalum dilatatum* Poir. *– 1896, Pennisetum clandestinum* Hochst. Ex. Chiov. *– 1940*) (Field and Forde, 1990), and *P. clandestinum* in particular has become spontaneously dominant in household lawns in many coastal and northern areas of New Zealand. However, there was generally little interest in using $C_4$ grasses in sown turf initially. With increasing urbanisation and indications of a warming climate, the period since 2000 has seen an increased interest in warm season turfgrasses ($C_4$) by the NZ turf industry, especially for the more northern areas of the North Island, due to their drought tolerance and high water use efficiency. However, warm season turfgrasses are often intolerant of low temperature and susceptible to winter injury.

## 1.2 Thesis objectives

This project was designed with a primary interest to evaluate warm season turfgrass varieties available in NZ for their cold tolerance characteristics. Data collection was targeted towards describing aspects of the growth morphology and physiology of selected warm season turfgrasses. Envisaged outcomes were development of management practices for the New Zealand turf industry to reduce the occurrence of problems such as winter browning in warm season turfgrasses. The project was a joint venture between the Institute of Agriculture and Environment, Massey University and the New Zealand Sports Turf Institute (NZSTI), to evaluate a range of warm season turf species for their turf characteristics and cold tolerance under glasshouse and field conditions. The project comprised two phases:

### 1.2.1 Phase 1: Assessment of agronomic and mat forming properties of some commonly used warm season turfgrasses

The core objectives of the study were:

- To assess the mat quality for a range of warm season turfgrasses protected from and exposed to frost and evaluation of each variety in terms of visual attributes.

- To compare different cultivars for their agronomic/morphological characteristics under frost free (glasshouse) and frost exposed (field) growing conditions.

### 1.2.2 Phase 2: Response evaluation of selected varieties from Phase 1 under controlled exposure to low temperature

A follow-up experiment was designed to explore detailed physiological responses to different levels of cold exposure to selected varieties from the first phase. Key aims were to:

- Explore the level of cold tolerance among selected warm season turfgrasses under controlled conditions to precisely define their damage threshold levels for industry extension purposes.

- To develop an understanding about morphological changes occurring during cold exposure and comparison of discoloration and re-greening rates.

- To evaluate different physiological changes (e.g., levels of sugars) taking place in plants during cold exposure for an improved understanding of the physiology of cold tolerance.

- To determine the rate of recovery from cold stress (“green up”) under optimal growing conditions (spring).

## 1.3 Thesis structure

The thesis comprises seven chapters. Following this brief introduction, Chapter 2 provides a review of literature with insights into turf culture and history focused towards warm season turfgrasses and their potential use in the New Zealand turf industry. Turfgrass qualitative and quantitative attributes and cold stress physiology are also explored in Chapter 2.

Chapter 3 is the first data chapter and provides qualitative assessment of turf quality traits of the investigated warm season turfgrass species (10 cultivars from 5 species) selected for initial evaluation. Chapter 4 is complementary to Chapter 3 and presents data describing above and below ground growth and morphology of selected turfgrass varieties under the two different growth environments investigated (glasshouse and field).

In view of the $C_4$ grass creeping growth habit being so different from the erect tufted growth habit of the $C_3$ grasses traditionally used in New Zealand, Chapter 4 and Chapter 5 provide a detailed investigation of $C_4$ turfgrass morphology. Chapter 4 includes a combination of quantitatively measured and visually observed characteristics. In the course of measurements in Chapter 4 it was noted that there appeared to be different plant anatomy (Niklas and Kutschera, 2009) in certain genera for the organisation of phytomers in the construction of stolons. As this fundamental morphology difference has been only occasionally and briefly reported in the literature, morphology data to explore this point were collected and are presented in Chapter 5.

Chapter 6 describes an experiment designed to explore temperature thresholds at which chilling damage occurs. The chilling damage experiment included selected cultivars from those evaluated in the turf quality and plant morphology studies reported in Chapters 3, 4, and 5. Plant growth dynamics, leaf colour change, and some physiological status indicators were evaluated at various points in a regime of progressively decreasing temperatures.

The thesis concludes with a brief summary of the main findings and a general discussion of future research requirements in Chapter 7.

## References

Anderson, J. A., Taliaferro, C. M., Martin, D. L. (2002) Freeze tolerance of Bermuda grasses: Vegetatively propagated cultivars intended for fairway and putting green use, and seed-propagated cultivars. *Crop Science* 42: 975–977.

Beard, J. B., Green, R. L. (1994) The role of turfgrasses in environmental protection and their benefits to humans. *Journal of Environmental Quality* 23: 452–460.

Busey, P. (1989) Progress and benefits to humanity from breeding warm-season grasses for turf. *Contributions from Breeding Forage and Turfgrasses* 14: 49–70.

Christians, N. (2011) Fundamentals of Turfgrass Management (4th ed.). John Willey and sons, Inc. Hoboken, New Jersey, USA.

Duble, R. L. 1996. Turfgrasses, Their Management and Use in the Southern Zone. Texas A&M University Press, College Station, TX.

Duncan, R. R., Carrow, R. N. (1999) Turfgrass molecular genetic improvement for biotic/edaphic stress resistance. *Advances in Agronomy* 67: 233–305.

Duncan, R. R. (1996) The environmentally sound turfgrass of the future. *USGA Green Section Record* 34: 9–11.

Field, T. R. O., & Forde, M. B. (1990). Effects of climate warming on the distribution of C4 grasses in New Zealand. *Proceedings of the New Zealand Grassland Association* 55: 47–50.

Geren, H., Avcioglu, R., Curaoglu, M. (2009) Performances of some warm-season turfgrasses under Mediterranean conditions. *African Journal of Biotechnology* 8: 4469–4474.

Jenkins, V. (1994). *The lawn: a history of an American obsession*. Smithsonian Institution press, Washington DC.

Jiang, Y., & Huang, B. (2001). Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation. *Crop Science 41*: 436–442.

Kaplan, R., Kaplan, S. (1989) The Experience of Nature: A Psychological Perspective. Cambridge University Press, New York.

Lee, G. J., Carrow, R. N., Duncan, R. R. (2004) Salinity tolerance of selected seashore paspalums and bermudagrasses: root and verdure responses and criteria. *Horticulture Science* 39: 1136–1142.

Morris, K. N. (2003) The National Turfgrass Research Initiative. National Turfgrass Federation, Inc. National Turfgrass Evaluation Program, Beltsville Agricultural Research Center, Beltsville, Maryland, 20705 USA.

Munshaw, G. C., Ervin, E. H., Shang, C., Askew, S. D., Zhang, X., Lemus, R. W. (2006) Influence of late season iron, nitrogen and seaweed extract on fall color retention and cold tolerance of bermudagrass cultivars. *Crop Sc*ience 46: 273–283.

Niklas, K.J., Kutschera, U. (2009) The evolutionary development of plant body plans. *Functional Plant Biology* 36: 682–695.

Nutter, G. C., Watson, J. R. (1969). The turfgrass industry. Turfgrass Science, In Hansan, A. A., Juska F.V. (Eds.). Turfgrass science. (9–26) American Society of Agronomy, Wisconsin, USA.

Orchard, J. (2002) Is there a relationship between ground and climatic conditions and injuries in football?. *Sports Medicine* 32: 419–432.

White, R. W., Busey, P. (1987) History of turfgrass production in Florida. In *Proceedings of Florida State Horticulture Society* 100: 167–174.

Stiles, V. H., James, I. T., Dixon, S. J., Guisasola, I. N. (2009) Natural Turf Surfaces. *Sports Medicine* 39: 65–84.

Taliaferro, C. M. (2003) Bermudagrasses. In Casler, M. D., & Duncan, R R. (Eds.). Turfgrass biology, genetics, and breeding. (235–256) Hoboken, N.J.: John Wiley.

Thomas, R. S., Rodgers, C. A., VanDyke, R., Williams, D. W., and Phillips, T. D. (2009) The Inheritance of Cold Tolerance and Turf Traits in a Seeded Bermuda grass Population. *Crop Science* 49: 1489–1495.

Ulrich, 1986 Ulrich, R. S. (1986) Human responses to vegetation and landscapes. *Landscape and Urban Planning* 13: 29–44. http://dx.doi.org/10.1016/0169-2046(86)90005

Zhou, S., Abaraha, A. (2007) Response to heat stress in warm season and cool season turfgrass cultivars. *Scientific Research and Essays* 2: 95–100.

# CHAPTER 2

# Review of Literature

## 2.1 Grass to turfgrass

Grasses belong to a major group within the flowering plants, or Angiosperms, that are known as monocotyledons because there is only a single embryonic first leaf or cotyledon inside the seed. More than 10,000 individual species of grasses exist in the plant kingdom, and they exhibit a great diversity in form, growth and habitat (Christians, 2011), including among others, maize, wheat, rice, and the bamboos. Turfgrasses are recognized for a compact horizontal growth with mat forming ability, and are capable of maintaining a high density when subject to regular mowing and significant treading, traffic, or other forms of wear (Roberts et al., 1992). Almost 50 grass species worldwide fit these criteria (Christians, 2011).

The historic use of grasses is referred to in many parts of the Bible (Roberts et al., 1992). Some artists' depictions of the "Garden of Eden", though tree-dominated, also show a green surface covered with grass like vegetation. The modern concept of turf can be traced back to early domestication of plants and animals when grazed lands were used as natural playgrounds. Historical records hint at development of vast and luxurious pleasure gardens around imperial palaces in China about 157–87 B.C. (Huffine and Grau, 1969). Similar development of gardens has been reported after this era in Persia, India and Europe (Spain, Italy, and France). However the emergence of ball games (about 5000 years ago) has had more influence on the evolution of turfgrasses to their current form. The awareness of turfgrass husbandry practices grew rapidly after 15th century with development of golf (Casler and Duncan, 2003; Roberts et al., 1992).

Modern turfgrass varieties emerged through three major forces of selection, described by Darwin as natural, unconscious, and methodical selection (Casler and Duncan, 2003). Biotic and abiotic stress factors help grasses to develop and colonize over a large fraction of Earth's terrestrial surface. Species and local ecotypes with natural adaptation to conditions in the geographic region where they occur, including resistance to cold, salt or drought stress have emerged independently of any human activity (Duncan and Carrow, 1999). By developing such adaptations, grasses have colonised a range of habitats including coastal strips, alpine slopes, river plains, deserts and wetlands and in each case have developed specific adaptations to prevailing conditions. A good example, is North African germplasm of tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort and cocksfoot *Dactylis glomerata* L. which tends to have a summer dormancy that is recognised as a water conservation adaptation to help overcome summer moisture deficit (Volaire and Norton, 2006, and references therein).

The unconscious selection process involves the early domestication of livestock and grasses. Perennial grasses present in grazed lands display a number of defence strategies including dwarfism, axillary bud utilisation to generate a branching growth habit, and the presence of rhizomes and stolons which both facilitate spreading and protect plant tissue from consumption by browsing animals (Casler and Duncan, 2003). Continued defoliation of grazing lands is assumed to have resulted in a shift towards a short stature and high tiller density (Roberts et al., 1992). Besides defoliation by grazing animals,  other factors such as trampling and fire would have contributed selection pressure, so that ecotypes subject to these selection pressures ultimately evolved the ability to grow as a mat of intertwined stems on the soil surface (Busey, 1977). Methodical selection processes were initially based upon selection and propagation of wild cultivars with required characters. Selections from old turf grounds were used as planting material with a broad adaptation to certain stress factors. These selections were then passed through recurrent selection processes to choose the best individual cultivars (Casler and Duncan, 2003; Moore and Moser, 1995).

Western civilizations in the early eighteenth century domesticated wild grass species for turf purposes (Bormann, 1993). Cool season grass genera like *Agrostis, Festuca, Lolium* and *Poa* are widely reported as earlier selections from grazed lands in different regions of Europe and North America (Beard, 1998; Busey 1989). European botanists collected and successfully disseminated materials from colonized areas resulting in naturalization of non-native, long living, turf species (Duncan and Carrow, 1999). In New Zealand anecdotal information available in turf industry circles suggests that as recently as the 1980s, many sports ground managers were using the same seed lines of browntop (*Agrostis capillaris* L.), as were being sold to hill country sheep farmers for pasture use, for new sowings of cricket wickets and outfields and local body parks and reserves grounds used for weekend sports.

In the second half of the 20th century, breeding of turfgrasses was initiated by agronomists working on pasture grasses (Seagle and Iverson, 2002). Turf breeders are trying to develop turfgrass cultivars that can offer acceptable growth and surface quality for a wide scenario of atmospheric and soil conditions. Dwarf cultivars with ability to tolerate biotic (disease, pest, traffic) and abiotic (drought, cold, heat, salinity) stress factors with less maintenance requirements are the ultimate goals for any breeding program (Lee et al., 2004). In New Zealand, ‘Grasslands Egmont’ browntop and ‘Grasslands Cook’ Chewing fescue are examples of such selection of pasture grasses aimed at producing varieties specifically for turf use (Rumball and Robinson, 1982; Rumball, 1982). This point is further discussed below.

## 2.2 Turfgrass classification

From a turf industry perspective, adaptability to cold climate is a major abiotic stress tolerance factor helpful in classification of turfgrasses and defining their distribution in different regions (Casler, 2006). On this basis turfgrasses are often grouped within turf industry circles into cool season grasses and warm season grasses, also known respectively as $C_3$ and $C_4$ grasses. The cool season grasses are mostly of European geographic origin and have the $C_3$ photosynthetic pathway, while the warm season grasses are typically of tropical or subtropical geographic origin and possess the $C_4$ photosynthetic pathway (Christians, 2011). Hence, the two groups also have distinct ecological distributions and temperature adaptations. The $C_3$ and $C_4$ terminology is derived from the identity of the first photosynthetic products which are 3-carbon and 4-carbon molecules respectively (Ehleringer and Cerling, 2002). These two photosynthetic pathways respond differently to available atmospheric $CO_{2.}$ $C_4$ plants achieve a higher photosynthetic rate and resource utilization than $C_3$ plants (Shay & Kubien, 2013) by increased $CO_2$ concentration around the enzyme Rubisco. The $C_4$ pathway is an energy expensive pathway but eliminates photorespiration, and also results in reduced stomatal conductance, giving a strong advantage for drought tolerance, (Sage, 2004; Sage and McKown, 2006). The $CO_2$ enrichment process of $C_4$ plants enables them to synthesize 2.4 g sugar compared with 1.9 g in $C_3$ plants for each MJ of light energy intercepted by the plant’s leaf surface area (Christians, 2011), while the reduced stomatal conductance and lower water use allow $C_4$ plants to tolerate high temperatures (>20°C) better than $C_3$ plants, and maintain growth longer in water deficit conditions before stored soil moisture is depleted. Alternatively, under irrigation, $C_4$ grass turf has lower water requirement than a $C_3$ grass turf, which can be an attractive consideration when planning maintenance of a playing surface or golf course in a modern city, since a saving of 100 mm irrigation water applied in a growing season translates to 1ML $ha^{-1}$.

Taxonomically, all grasses are members of the botanical family Poaceae, and the commonly used cool season turfgrasses are members of the Poeae tribe within that family, while the major warm season turfgrasses are members of the Paniceae.

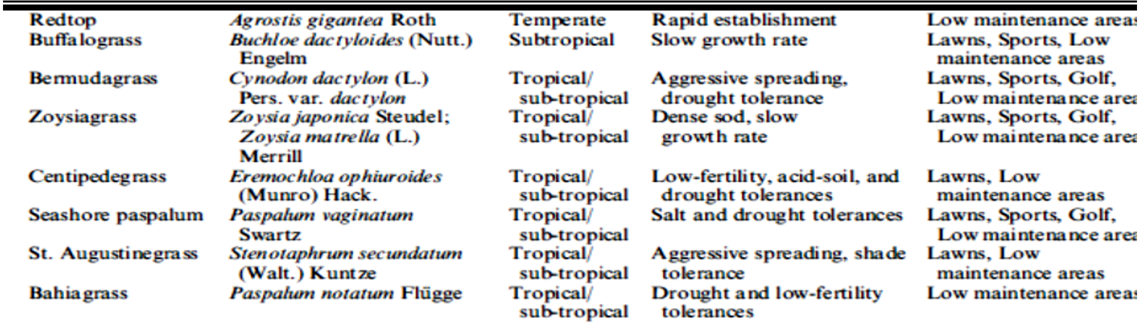
## 2.3 Warm season turfgrasses

The warm season turfgrasses are widely distributed throughout the regions with warmer temperatures including, humid, sub-humid, arid and semi-arid climates. (Beard, 1973). Optimum air temperature suitable for warm season turfgrasses ranges between 17–35ºC (Fry and Huang, 2004). They tend to become dormant when temperature drops below 10ºC or during heavy frosting (Beard, 1982). Despite this limited tolerance of cool temperatures, they are often found in regions with a continental climate, where they thrive in the warm conditions of summer but cease leaf production and lose colour in winter. Important warm season turfgrasses include, but are not limited to species of the genera *Cynodon*, *Zoysia*, *Buchloe*, *Paspalum*, *Pennisetum*, *Eremochloa*, and *Stenotaphrum* (Fry and Huang, 2004; Juska et al., 1969). Most of the $C_4$ grasses with high functional quality grown for sports turf surfaces are propagated vegetatively (ie using sprigs or cuttings obtained from an established turf) rather than by seed (Geren et al., 2009). For species where both vegetatively propagated and seeded varieties are available, the vegetatively propagated varieties are generally considered within industry circles to be superior in performance, compared to seeded varieties.

### 2.3.1 Bermuda grass (*Cynodon*)

Bermuda grass is the most widely grown and adopted warm season turf species. It is commonly known as couch grass, green couch grass and Indian doab (Teliaferro, 2003). Many commercial selections and seeded varieties are available for a range of climates and specific uses with good variation in form and habitat. Bermuda grass originated and evolved in Eastern Africa. Biotic and abiotic stresses including environment and grazing pressure from larger herds of African wild mammals resulted in the grass to evolving a deep root system with plenty of lateral growth (stolons) and tramping resistance (Beard, 1998). The genus *Cynodon* has nine different species with greater genetic variation. *C. dactylon* (L.) Pers. and *C. transvaalensis* Burtt Davy are the important turf types and most of the commercial varieties are crosses between these two species, which are generally sterile and need to be vegetatively propagated (McCarty and Miller, 2002).

**Table 2.1 Important warm season turfgrass species with their uses and characteristics** (Extracted from Casler, 2006).



| **Common name** | **Latin name** | **Region** | **Traits** | **Uses** |
|---|---|---|---|---|
| Redtop | *Agrostis gigantea* Roth | Temperate | Rapid establishment | Low maintenance areas |
| Buffalograss | *Buchloe dactyloides* (Nutt.) Engelm | Subtropical | Slow growth rate | Lawns, Sports, Low maintenance areas |
| Bermudagrass | *Cynodon dactylon* (L.) Pers. var. *dactylon* | Tropical/ sub-tropical | Aggressive spreading, drought tolerance | Lawns, Sports, Golf, Low maintenance area |
| Zoysiagrass | *Zoysia japonica* Steudel; *Zoysia matrella* (L.) Merrill | Tropical/ sub-tropical | Dense sod, slow growth rate | Lawns, Sports, Golf, Low maintenance area |
| Centipedegrass | *Eremochloa ophiuroides* (Munro) Hack. | Tropical/ sub-tropical | Low-fertility, acid-soil, and drought tolerances | Lawns, Low maintenance areas |
| Seashore paspalum | *Paspalum vaginatum* Swartz | Tropical/ sub-tropical | Salt and drought tolerances | Lawns, Sports, Golf, Low maintenance area |
| St. Augustinegrass | *Stenotaphrum secundatum* (Walt.) Kuntze | Tropical/ sub-tropical | Aggressive spreading, shade tolerance | Lawns, Low maintenance areas |
| Bahiagrass | *Paspalum notatum* Flügge | Tropical/ sub-tropical | Drought and low-fertility tolerances | Low maintenance areas |

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### 2.3.2 Alternative species

*Zoysia* grass is another very important member of the warm season turfgrasses. This genus is known to be native from western Pacific Rim along the western coasts of Indian Ocean. This genus comprises 11 known species with a distribution map spreading from New Zealand to Japan and French Polynesia through to Mauritius. Species of *Zoysia* grass (*Zoysia japonica* Steud., *Zoysia matrella* (L.) Merr., *Zoysia pacifica (*Goudsw.) M. Hotta & Kuroki) are used in the turf industry, especially in the Southeast Asian tropics, in countries such as Malaysia and Indonesia. Among the *Zoysia* species, *Z. japonica* is considered most tolerant to low temperature stress and to have a superior shade tolerance compared to *Cynodon* (Zhang et al., 2009; Engelke and Anderson, 2003). *Zoysia* has a good reputation in the turf industry as it has good turf quality characters and low maintenance requirements compared to some other warm season grasses (White et al., 2001).

*Paspalum vaginatum* Sw. or seashore paspalum is a more recent inclusion in lists of turfgrass species, predicted to become much more widely used in the turf industry in the 21st century (Duncan and Carrow, 2000). The grass is attracting attention among turf scientists and industry stakeholders as it displays resistance to multiple abiotic stresses, including soil salinity, drought, cold and shade (comparable to Bermuda grass) tolerance (Duncan, 2003).

## 2.4 Emergence of the global warm season turfgrass industry

As defined by Watson et al., 1992: the “Turfgrass industry in its broader aspect is a group of specialized individuals and organizations sharing their common interest in production, development and maintenance of green space”. The turf industries in various countries are dynamic in nature and their definition encompasses various factors including geographic location, scale of operation and demand for products, manpower, investment and governmental policies (Nutter, 1965). The origins of the turf industry are closely aligned with the history of sports turf usage particularly with the ball games; initially golf (Watson et. al., 1992), and more recently sports such as football. Evolution of the turf industry was slow in the first half of the 20th century but very steady (Beard, 1982). However, it has experienced tremendous growth during the latter half of 20th century, encompassing the farming and maintenance of targeted species for environmental, aesthetic and recreational utilities (Shearman, 2006). The international turf industry is expected to grow and change rapidly during the 21st century, with an increased emphasis on environmental protection and best management practices (Seagle and Iverson, 2002).

### 2.4.1 Turfgrass evolution in the USA

Turfgrasses gained their current status as products of an established industry with an extensive range of private, private, commercial, and government users only during the past five decades in USA (Roberts et al., 1992). At present turfgrass is the fourth largest ‘crop’ in USA covering more than 50 million acres (Milesi et al., 2005) with an estimated annual economic turnover of up to 60 billion USD. It is estimated that 2% of total land area in USA is under turf cover (Milesi et al., 2005) and it has been estimated that more than 90% of American citizens use or come into contact with turfgrass, in some way, in their daily life.

### 2.4.2 The Australian turf industry

The key developmental period for the Australian turf industry is considered to have been between World Wars I and II. Rapid urbanization and the increased need for sophisticated and well developed sports centres are considered the main drivers of this expansion (Loch et al., 2016). According to the Turf Producers Association (TPA) around 4,400 hectares of turf sod is produced for sale, with a total annual value of $300 million and the Turf maintenance sector has over $500 million annual turnover. Queensland's share of the total turf production is 38%, New South Wales has a 33% share, and Victoria and Western Australia account for 15 and 11% of production, respectively (TPA 2017). A survey conducted during 2006-2007 showed that about 85% of the total area under turf production in Australia comprised warm season turfgrass species, of which couches (*Cynodon sp.),* buffalo grass (*Buchloe dactyloides* (Nutt.) Engelm) and Kikuyu (*Pennisetum clandestinum* Hochst. ex Chiov.) make up 91% (Haydu et al., 2008).

### 2.4.3 The New Zealand turf industry

The New Zealand turf industry is also growing in volume very rapidly. Many public institutes and private organizations are working in research, training, production and maintenance sectors of the industry. According to a study conducted in New Zealand during 2006, an estimated area of 122,328 hectares was planted in turf, of which schools comprise about 48% (58,217 ha). Council administered areas were estimated at 30% (36,326 ha) followed by 20% (25,361 ha) for golf courses. Approximately 48% (58,139 ha, previously reported 60,000 ha in 2000) of the total area under turf was managed for sports and amenity use only (Haydu et al., 2008; Haydu et al., 2006; Way, 2001).

The New Zealand sports turf industry has been assessed as having $33 billion worth of resources including land, buildings and equipment. Schools were estimated as the largest stakeholder comprising 70% of total assets followed by city councils at 20% and golf courses at 6% respectively. Almost 94% of turf industry business is dependent on domestic customers. In 2006, the New Zealand turf industry was reported to have a financial turnover of $NZ 356.6 million for operational expenditure. The major expense was the salaries of 24,000 people employed by the industry under different categories (NZSTITO, 2011). According to a recent report by SPARC (Sports and Recreation New Zealand), 92% of young people and 83% of adults surveyed indicated active participation in sports (Dalziel, 2011).

New Zealand turf culture is dominated by cool season grasses. Settlers from United Kingdom introduced amenity grasses early in 20th century (e.g. species of *Agrostis*, *Festuca* and *Lolium*). Initially browntop (*Agrostis capillaris* L.) and fine fescues (*Festuca rubra* L.) were extensively used to establish amenity and sports areas. *Festuca rubra* was introduced in the 19th century, and a growth form lacking creeping rhizomes and with high shoot density, now known as *F. rubra* subsp. *commutata*, was identified on a farm in the South Island, owned by a Mr Chewings, who sold seed and promoted it as a superior type. The variety was widely sown around New Zealand, and significant volumes of seed were exported to Europe and USA, where it remains well known in turf industry circles (Rumball, 1983). The name Chewings fescue is now widely recognised in Europe and the USA, and even listed in Merriam-Webster online dictionary (https://www.merriam-webster.com/dictionary/chewings%20fescue). The first New Zealand breeding program for cool season turfgrasses was started at the Department of Scientific and Industrial Research, Grasslands Division (DSIR Grasslands, now AgResearch) in 1973 and they released five varieties of cool season grasses, developed by selection of pasture types for shoot density and short stature. Chewings fescue was one of these. Descriptive notes on ‘Grasslands Cook’ Chewings fescue were published by Rumball (1982), and the wider turfgrass development programme at that time is described by Rumball (1983). However, the DSIR Grasslands turf varieties were not widely adopted by the turf industry, and during the 1980s many groundkeepers in New Zealand began importing planting material from USA and Europe. Additionally, from the 1990s establishment of a plant variety rights scheme in New Zealand facilitated the development of commercial breeding of turf varieties. PGG Wrightson is a Christchurch-based company that has been active in turfgrass breeding and evaluation, with interest centred on but not confined to the Australasian market.

In the last two decades, the New Zealand turf industry has developed an interest in warm season grasses due to summer dormancy and poor heat and moisture deficit tolerance of cool season grasses, especially in the upper parts of the North Island. Kikuyu (*P. clandestinum*) was introduced to New Zealand in the 20th century to improve summer pasture production in northern North Island regions. In these areas, Kikuyu has been found to spontaneously displace $C_3$ grass species and is now widespread as a major species in many urban lawns and roadside berms, but because of its coarse growth habit it has not generally been used in turf applications. One of the warm season turf species commonly employed in New Zealand is Indian doab (*C. dactylon*), also known as "couch" in the turf industry. Interest in this species is especially strong for golf courses and sports fields. Seeded (Princess-77, Yukon, Southern Star, La Paloma) and non-seeded couches (Agridark, Windsor Green, Legend, Santa Ana) are available for the turf market in New Zealand (Hunt, 2011). However seeded varieties of *Z. japonica (*Zenith and Compadre*),* *P. vaginatum* (Sea Spray) and *P. clandestinum* (Regal Staygreen) are also available in the market. Warm season turf species, being efficient in water and nutrient use, in addition to their high temperature tolerance, can potentially have a large future role in the New Zealand sports turf industry.

## 2.5 Turfgrass quality attributes

The aesthetic appearance of turf venues is a high priority for turf managers, and is often demanded by users as well, even in those situations where there is an intense use or unfavourable weather conditions. Very objective criteria are required for determining the grass type to be employed under any particular condition, in order to match varieties established with environmental and soil conditions and available resources (Carrow et al., 2010). Quality assessment or visual field assessment in turfgrasses is a type of ranking system that varies with species, season, growing conditions and the evaluator (Krans and Morris, 2007).

Turfgrass quality is the combination of qualitative traits and visual appearance. Turfgrass quality is measured as the combined visual effect of density, uniformity, texture, smoothness, growth habit and colour (Beard and Beard, 2005). A scale widely used for visual field assessment in turfgrasses is scoring at 1–9 where 1 is the poorest denomination and 9 as the best (Krans and Morris, 2007). Turfgrass evaluators judge the turfgrass quality on the basis of visual observations. Turfgrass quality assessment ratings differ from individual to individual. It is recommended that only one evaluator should perform the procedure throughout one study (Bell et al., 2009; Morris, 2003). Qualitative ratings of turfgrasses vary to a large extent depending upon variety/species, seasonal changes, cultural and management practices (Morris and Shearman, 2014).

## 2.6 Turfgrass morphology

All grasses share a segmental morphology whereby growth units called phytomers are continuously formed at an apical meristem (Sharman, 1947), and progress through a series of development phases producing first a leaf, then a bud within the leaf axil which may initiate to form a branch. There may or may not be elongation of the stem between successive leaves. Leaves normally undergo programmed senescence after a defined life span, at which point root primordia may form and generate adventitious roots, which take several leaf appearance intervals to fully develop. Unlike familiar plants such as shrubs or trees where the seedling stem persists for the life of the plant and expands by secondary thickening; in grasses the constancy of shoot form over time comes from coordinated cycling of the component phytomers through their development stages. The ‘stem’ may either be a ‘pseudostem’ which is actually a whorl of leaves with the youngest emerging from the centre and the oldest shed from the exterior when the leaf dies, or where internode elongation has occurred the stem may be a stolon on or just above the soil surface or an underground rhizome. There are many studies of grass morphology, often in the context of optimising herbage intake by animals. Among these, for ryegrass, Silsbury (1970) has described the coordination of phytomers within the grass shoot or tiller, while Fulkerson and Donaghy (2001) described leaf turnover, noting that there are typically three live leaves at any one time in a ryegrass shoot. A comprehensive study of the fate of individual phytomers in *Poa pratensis* plants over a series of years was published by Etter (1951). The author is not aware of similar studies of grass morphology at the phytomer level in $C_4$ grasses.

Warm season turfgrasses primarily belong to the genera *Cynodon, Eremochloa, Paspalum, Pennisetum,* and *Zoysia* (Busey, 1989) and members of these genera typically display a spreading nature, since the internode regions of their phytomers are normally elongated to form stolons or rhizomes. In modern turf breeding many commercial $C_4$ grass cultivars do not have the capacity to produce seeds, often as a result of development through interspecies hybridisation or artificially induced polyploidy. Turf managers frequently report that seeded varieties exhibit poor mat quality, an open growth habit and less traffic resistance, compared to vegetatively propagating varieties of the same species (Geren et al., 2009).

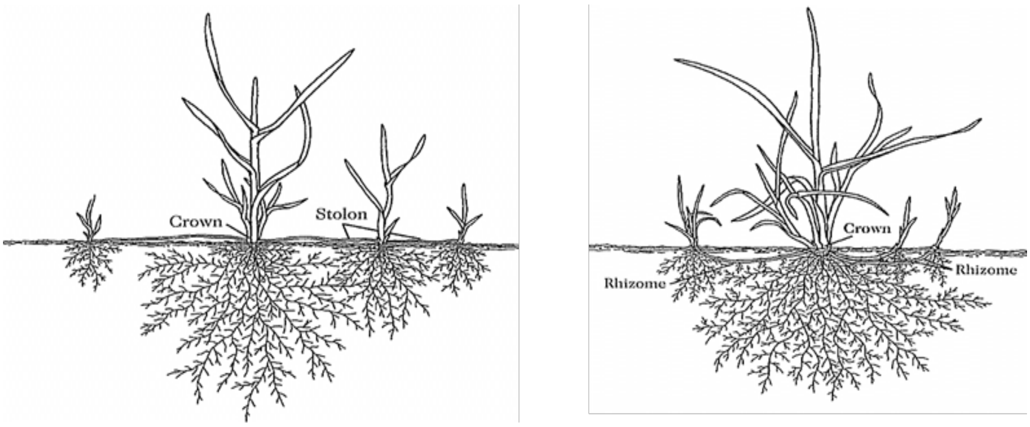


**Fig. 2.1 Structure of grasses with stolons and rhizomes (from Christians, 2011).**

## 2.7 Temperature tolerance of cool and warm season grasses

Cool season grasses have an optimal growth temperature ranging from 16°C to 24°C, temperatures, and above this range results in decreased metabolism (Du et al., 2009). With extremely high temperatures and drought conditions they undergo dormancy, cease to grow and turn brown in colour (Beard, 1994).

The $C_4$ pathway naturally occurs mainly close to the equator under warm and sunny conditions. It involves complex metabolic and anatomical differences, compared to $C_3$ plants, which allow efficient water use and retain productive capacity under high light intensity and temperatures (Beard, 1998). Warm season or $C_4$ turfgrasses are widely grown in tropical regions of the world and even sometimes in temperate regions, where hot summers are experienced (Geren et al., 2009). The $C_4$ grasses are able to tolerate high temperatures (i.e. warmer maximum summer temperatures ranging from 25 – 38°C) and water deficiency very well. Tolerance to low temperature is one major limiting factor for most of the warm season turf species (Thomas et al., 2009). Cold temperature damage in warm season turfgrasses lowers their aesthetic appeal. Their growth usually stops below 16°C, while foliage starts turning brown below 10°C (Anderson et al., 2002).

Since playing fields and parks are key amenities in urban infrastructure, the need for a sustainable turf surface always keeps managers under pressure. The tension between the desire for scheduled events to go ahead without cancellation, and the need to avoid damage and maintain visual appearance, is a central consideration nowadays when developing maintenance practices (Cisar, 2004). Damaged or dormant grasses lose their aesthetic and environmental values and are considered undesirable in sports and athletic fields. The costs of redevelopment of injured or dead turf can be a significant burden on municipal maintenance budgets (Anderson et al., 2007). There is, therefore, an increasing interest in superior varieties capable of survival and growth under both extremes of temperature. Turf breeders have made substantial progress with increasing stress tolerance in warm season grasses (Anderson et al., 2007). Research work with warm season turfgrasses has been carried out in many countries but mainly concentrated in USA, Australia and China.

Lack of tolerance to chilling temperatures is the most often investigated performance trait for selection and usage of warm season species (Bermuda grass, *Zoysia* and seashore paspalum) under cooler climates (Stefaniak et al., 2009; Kopec et al., 2007; Casler, 2006; Anderson et al., 2003; Beard, 1982). Warm season grasses are usually considered to be intolerant to both sudden and prolonged exposure to low temperatures. Low temperature exposure caused by rapid temperature drop (frosting) has been referred to as 'direct' cold exposure, and exposure to low temperatures for a prolonged duration is termed 'indirect' cold exposure (Fry, 1990). Cold temperature exposure results in chilling injury leading to dormancy, chlorosis and necrosis, and such damage is followed by death in more sensitive species. Frost kill occurs because of disruption of cell membranes and other components due to expansion on freezing of solutes. Overall reduction in chlorophyll accumulation, photosynthesis, metabolite translocation, enzyme activity and protein synthesis can be observed (Sanghera et al., 2011; Samala et al., 1998; Salisbury and Ross, 1992).

Winter field performance is a typical assessment method for cold hardiness in grasses. One measure of cold hardiness is the $LT_{50}$ (LT denotes lethal temperature), which refers to the low temperature exposure that will kill 50% of plants in a grass population (Zhang et al., 2009). Uncertainty over what kinds of fluctuations in temperature may be most damaging to plants limits the interpretive power of field experiments. *In vitro* experiments using controlled environment cabinets and freeze chambers provide better information and understanding of cold stress mechanisms (Anderson et al., 2005).

*Cynodon* (Bermuda grass, couch grass) is the most common and widespread genus among the warm season group of grasses (Taliaferro, 2003) and is extensively used for golf courses and athletic surfaces. However *Cynodon* varieties often lack winter hardiness and so are vulnerable to frost damage or winter kill (Munshaw et al., 2006). The cold tolerance level of different Bermuda grass cultivars was evaluated by Anderson et al. (2002) using a laboratory freeze method and found a great variation in tolerance levels based on cultivar type and usage. Varieties particularly recommended for fairways were more tolerant compared with those used for greens.

Anderson et al. (2007) observed that vegetatively propagated Bermuda grass had a lower $LT_{50}$ (–6.2°C to –11.3°C) than seeded varieties (–5.3°C to –8.7°C). Similarly Zhang et al., 2006 compared two seeded cultivars of *Cynodon* (Riviera and Princess-77) for their level of cold tolerance. $LT_{50}$ was lower (–8.3°C) for Riviera compared to Princess–77 (–6.3°C). Tolerance to cold, while partly mediated by physiological factors, is also influenced by grass morphology. A positive correlation can be found with stolon length, stolon size and rhizome length (Ahring et al., 1975). Bermuda grass cultivars with increased cold tolerance are mostly selections of clonal (vegetatively propagating) material from cooler regions which were further hybridized with superior mat forming cultivars (Samudio and Brede, 2002).

As noted above, *Zoysia* is one of the genera of $C_4$ grsses used in the turf industry. Species, with the three species most commonly used by the industry being (*Z. japonica, Z. matrella,* and *Z. pacifica*. *Z. japonica* is considered most tolerant to low temperature stress and to have a superior shade tolerance compared to *Cynodon* (Zhang et al., 2009; Engelke and Anderson, 2003). Exposure to air temperatures below 10°C is problematic for common *Z. japonica* and can cause leaf discoloration and increase in carbohydrate and proline contents (Wei et al., 2008). Patton and Reicher, 2007 evaluated *in vitro* freeze tolerance of different *Zoysia* varieties, and in that work a cultivar Diamond displayed inferior performance ($LT_{50}$ = –8.4°C) compared with Meyer and Zenith ($LT_{50}$ = –11.5°C).

## 2.8 Physiology of cold tolerance

Intolerance to low temperature is the key factor limiting the use of warm season ($C_4$) grasses (Bush, 2000). Warm season grasses start to turn dormant when exposed to temperatures below 15°C followed by browning of the leaves at 10°C (Beard, 1973). This intolerance can cause chilling injury (discoloration, chlorosis, and necrosis) known as winter browning. Low temperature thresholds vary greatly among species and varieties depending upon genetic and environment interactions (Stier, 2007).

The circumstances where damage is triggered rapidly below a certain threshold temperature or occurs slowly and cumulatively in response to temperatures that would not be low enough to cause immediate symptoms, and where a protective effect from ‘conditioning’ may occur, are not well clarified in the existing research literature. It is generally assumed that gradual but prolonged exposure would not prove as lethal as a sudden freezing exposure. However, there are reports that continuous exposure to low temperature above the sudden damage threshold can kill a significant proportion of an existing population. Low temperature exposure, whether sudden or gradual, results in several physiological and morphological changes in the plants, leading to reduced transpiration rates, changes in concentration or induction of particular enzymes, production of proteins related to stress tolerance and carbohydrate translocation (Bocian et al., 2011; Hisano et al., 2008). Prior exposure to non-lethal low temperatures (cold acclimation) triggers the expression of many physiological and metabolic responses and enables the plant to cope with cold stress (Cyril, 2002; Dionne, 2001).

### 2.8.1 Carbohydrates

An increase in non-structural carbohydrate concentration is often correlated with low temperature tolerance in warm season turfgrasses, and this effect involves substances responsible for membrane stabilization and limiting crystallization. Concentrations of soluble carbohydrates in buffalo grass vary significantly in samples taken at different times of the year (Bell et al., 2002). There is a strong association between higher concentrations of non-structural carbohydrates and cold tolerance (Huang et al., 2014; Bush et al., 2000). DiPaola and Beard (1992) have also reported higher levels of sugars in *Zoysia* compared with *Cynodon*, and linked this to improved survival. Different varieties of *Cynodon* also showed variation in total non-structural carbohydrate contents during and after cold acclimation (Zhang et al., 2006).

### 2.8.2 Proline

C4 grasses are usually susceptible to chilling injury (1°C–12°C), which may cause membrane damage and loss to plant proteomic activity, decreased photosynthesis and respiration (Stier, 2007). Proline is an amino acid widely studied as an indicator of response to abiotic stress, mainly drought and cold stress. Many scientists have reported an increase in proline concentration during exposure to low temperature in their experiments performed under various conditions on warm season turfgrasses (Munshaw et al., 2006; Zhang et al., 2006 & 2011).

Increased proline content in turfgrasses is often correlated with increased cold tolerance. Patton et al. (2007) studied 13 different genotypes of *Zoysia* and find out their difference in cold tolerance in relation to proline and carbohydrate accumulation. They concluded that proline levels increase in *Zoysia* during cold acclimation but it was found that proline is less beneficial to plants as temperature drops. Kauffman (2010) found Diamond as the most hardy variety when compared with TifEagle, Champion, and SeaDwarf and reported an increase in proline contents. Patton et al. (2007) further confirmed that a rise in carbohydrate and proline contents help plants to tolerate cold temperatures.

### 2.8.3 Malondialdehyde (MDA)

The cell membrane is a primary site of cold injury and any alteration in its composition can influence cell metabolism and fluid exchange, and especially a transformation to a rigid state can reduce its permeability (Huang et al., 2014). Increase in fatty acid desaturation (FAD) is related to improved cold tolerance in plants (Wang et al., 2003) and cold susceptible plants increase lipid desaturation during cold acclimation for better membrane permeability (Taiz and Zeiger, 1991). Malondialdehyde (MDA) formation as a response to lipid peroxidation is considered a precursor to environmental stress (Wang et al., 2010). With prolonged exposure to chilling stress, MDA levels tend to increase in leaves (Wang et al., 2009).

## 2.9 Experimental programme

Considering the above discussion, a research programme was planned that would first provide data relevant to greenkeepers and grounds supervisors in New Zealand on the performance in the field at Palmerston North of a selection of warm season turfgrass species and varieties available in New Zealand. An intended outcome of the planned research was to identify, from the results of that study, research questions specific to performance of warm season turfgrasses in New Zealand that could be the focus of some detailed follow up research.

## References

Ahring, R. M., Huffine, W. W., Taliaferro, C. M., Morrison, R. D. (1975) Stand establishment of Bermuda grass from seed. *Agronomy Journal* 67: 229–232.

Anderson, J. A., Taliaferro, C. M. Martin, D., Wu, Y. Anderson, M. (2007) Bermudagrass freeze tolerance. *Turfgrass and Environmental Research Online* 6: 1–7.

Anderson, J. A., Taliaferro, C. M., Martin, D. L. (2002) Freeze tolerance of Bermuda grasses: Vegetatively propagated cultivars intended for fairway and putting green use, and seed-propagated cultivars. *Crop Science* 42: 975–977.

Anderson, J. A., Taliaferro, C. M., Martin, D. L. (2003) Longer exposure durations increase freeze damage to turf Bermudagrasses. *Crop Science* 43: 973–977.

Anderson, J., Taliaferro, C., Anderson, M., Martin, D., Guenzi, A. (2005) *USGA Turfgrass and Environmental Research Online* 4: 1–7.

Anderson, J., Taliaferro, C., Martin, D. (2002) Freeze tolerance of Bermudagrasses: Vegetatively propagated cultivars intended for fairway and putting green use, and seed propagated cultivars. *Crop Science* 42: 975–977.

Anderson, J., Taliferro, C., Martin, D., Wu, Y., Anderson, M. (2007) Bermudagrass freeze tolerance. *USGA Turfgrass and Environmental Research Online* 6: 1–7.

Beard, J. B., Beard, H. J. (2005) Beard's turfgrass encyclopedia for golf courses, grounds, lawns, sports fields. Michigan State University Press.

Beard, J. B. (1998) The origins of turfgrass species. *Golf Course Management* 66: 49–55.

Beard, J. B., Green, R. L. (1994) The role of turfgrasses in environmental protection and their benefits to humans. *Journal of Environmental Quality* 23: 452–460.

Beard, J. B. (1973) Turfgrass: Science and Culture. Prentice-Hall, Inc., Englewood

Beard, J. B. (1982) Turf Management for Golf Courses. Macmillan Publishing Co., NY.

Bell, G. E., Martin, D. L., Koh, K., Han, H. R. (2009) Comparison of turfgrass visual quality ratings with ratings determined using a handheld optical sensor. *Hort Technology* 19: 309–316.

Bell, G. E., Martin, D. L., Wiese, S. G., Dobson, D. D., Smith, M. W., Stone, M. L., Solie, J. B. (2002) Vehicle-mounted optical sensing. *Crop Science 42*: 197–201.

Bocian, A., Kosmala, A., Rapacz, M., Jurczyk, B., Marczak, Ł., Zwierzykowski, Z. (2011) Differences in leaf proteome response to cold acclimation between Lolium perenne plants with distinct levels of frost tolerance. *Journal of Plant Physiology* 168: 1271–1279.

Bormann, H. F., Balmori, D., Geballe, G. T. (1993) Redesigning the American lawn: A Search for Environmental Harmony. Yale University Press.

Busey, P. (1977) Turfgrasses for the 1980's. In *Proceedings of Florida State Horticultural Society* 90: 111–114.

Busey, P. (1989) Progress and benefits to humanity from breeding warm-season grasses for turf. Contributions from breeding forage and turfgrasses, Publ. 15. 49–70. doi:10.2135/cssaspecpub15.c5.

Bush, E., Wilson, P., Shepard, D., McCrimmon, J. (2000) Freezing tolerance and non-structural carbohydrate composition of carpetgrass (Axonopus affinis Chase). *Hort Science 35*: 187–189.

Carrow, R. N., Krum, J.M., Flincroft, I., Cline, V. (2010) Precision turfgrass management: challenges and field applications for mapping turfgrass soil and stress. *Precision Agriculture* 11: 115–134. DOI 10.1007/s11119-009-9136-y.

Casler, M. D. (2006) Perennial grasses for turf, sport and amenity uses: evolution of form, function and fitness for human benefit. *The Journal of Agricultural Science* 144: 189–203 http://dx.doi.org/10.1017/S0021859606006137.

Casler, M. D., Duncan, R. R. (2003) Origins of the Turfgrasses. In: Casler, M.D., & Duncan, R. R. (Eds.). *Turfgrass biology, genetics, and breeding*. Hoboken, N.J.: John Wiley.

Christians, N. (2011) Fundamentals of Turfgrass Management (4th ed.). John Willey and sons, Inc. Hoboken, New Jersey, USA.

Cisar, J. L. (2004) Managing turf sustainably. “New directions for a diverse planet". *Proceedings of the 4th International Crop Science Congress, Brisbane, Australia. www.cropscience.org.au/icsc2004/pdf/2137_cisar.pdf.*

Cyril, J., Duncan, R.R., Baird, W.V. (2002) Changes in membrane polar lipid fatty acids of seashore paspalum in response to low temperature exposure. *Crop Science* 42: 2031 –2037.

Dalziel, P. (2011) The Economic and Social Value of Sport and Recreation to New Zealand, Research Report No. 322. Lincoln University, Christchurch, New Zealand. http:// www.sportnz.org.nz/Documents/Research/value%20of%20sport/RR-Value-22w-FINAL-logo.pdf.

Dionne, J., Castonguay, Y., Nadeau, P., Desjardins, Y. (2001) Freezing tolerance and carbohydrate changes during cold acclimation of green-type annual bluegrass (Poa annua L.) ecotypes. *Crop Science* 41: 443–451.

DiPaola, J. M., Beard, J. B. (1992) Physiological effects of temperature stress. In (eds.) Waddington, D. V., Carrow, R. N., Shearman, R. C. *Turfgrass*, American Society of Agronomy 231–267.

Fulkerson, W. J., Donaghy, D. J. (2001) Plant-soluble carbohydrate reserves and senescence-key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Australian Journal of Experimental Agriculture* 41: 261-275.

Du, H., Wang, Z., Huang, B. (2009) Differential responses of warm-season and cool-season turfgrass species to heat stress associated with antioxidant enzyme activity. *Journal of American Society of Horticultural Sciences* 134: 417–422.

Duncan, R. R., Carrow, R. N. (2000) *Seashore paspalum: The environmental turfgrass.* John Wiley & Sons.

Duncan, R. R. (2003) Seashore paspalum (*Paspalum vaginatum* Swartz). In, Casler M. D., Duncan, R. R. (Eds.), *Turfgrass biology, genetics, and breeding*. John Wiley & Sons, Hoboken, NJ, 295–308

Duncan, R. R., Carrow, R. N. (1999) Turfgrass molecular genetic improvement for biotic/edaphic stress resistance. *Advances in Agronomy* 67: 233–305.

Ehleiringer, J. R., Cerling, T. E. (2002) The Earth sytem: biological and ecological dimensions of global environmental change. In. Mooney, H. A. and Canadell, J. G. (Eds.), Encyclopedia of global environmental change. John Wiley & Sons, Ltd. 186–190.

Engelke, M., Anderson, S. (2003) Zoysiagrasses. In: Casler, M.D., & Duncan, R R. (Eds.). *Turfgrass biology, genetics, and breeding*.) Hoboken, N.J.: John Wiley. 271–286

Etter A.G. (1951) How Kentucky bluegrass grows. *Annals of the Missouri Botanical Garden* 38: 293–375.

Fry, J., Huang, B. (2004) Applied turfgrass science and physiology. J. Wiley and sons, INC. Hoboken New Jersey

Fry, J. D. (1990) Cold temperature tolerance of bermudagrass. *Golf Course Management* 58: 26–32.

Fulkerson, W. J., Donaghy, D. J. (2001) Plant-soluble carbohydrate reserves and senescence - key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Australian Journal of Experimental Agriculture* 41: 261–275.

Geren, H., Avcioglu, R., Curaoglu, M. (2009) Performances of some warm-season turfgrasses under Mediterranean conditions. *African Journal of Biotechnology* 8: 4469–4474.

Haydu, J., Way, B., Hodges, A. (2006) New Zealand sports turf industry survey & future skills analysis project. Tertiary Education Commission, Wellington, New Zealand.

Haydu, J., Way, B., Hodges, A., Cisar, J. L,, Aldous, D. E. (2008) Economic challenges confronting New Zealand’s sports turf industry. In J. C. Stier, L. Han & D. Li (Eds.), *Proceedings of second international conference on turfgrass science and management for sports fields, Acta Horticulturae (ISHS)* 783: 349–356 http://www.actahort.org/books/ 783/783_37.htm.

Hisano, H., Kanazawa, A., Yoshida, M., Humphreys, M. O., Iizuka, M., Kitamura, K., Yamada, T. (2008) Coordinated expression of functionally diverse fructosyltransferase genes is associated with fructan accumulation in response to low temperature in perennial ryegrass. *New Phytologist* 178: 766–780.

Huang, B., DaCosta, M., Jiang, Y. (2014). Research advances in mechanisms of turfgrass tolerance to abiotic stresses: from physiology to molecular biology. *Critical reviews in plant sciences* 33: 141–189.

Huffine, W.W., Grau, F.V. (1969) History of turf usage In Hanson, A. A., & Juska, Felix Victor (Eds.). Turfgrass science. (1-5) Madison, Wisconsin. American Society of Agronomy.

Hunt, L. (2011) 100% playability technical report on Auckland’s sports fields retrieved from http://wellington.govt.nz/~/media/your-council/meetings/Committees/Strategy%20and%20Policy%20Committee/2012/05/21/files/21_may_2012_minutes_tabled_information_tuesday_22_may_2012_part_2.pdf.

Juska, F. V., Cornman, J. F., Hovin, A. W. (1969) Turfgrasses under cool, humid conditions. In Hanson A. A., Juska, F. V. Eds. *Turfgrass Science* 491–512.

Kauffman, J. M. (2010). Investigating cold hardiness and management practices of warm-season putting green species in the transition zone. PhD dissertation, University of Tennessee. http://trace.tennessee.edu/utk_graddiss/811.

Kopec, D. M., Walworth, J. L., Gilbert, J. J., Sower, G. M., Pessarakli, M. (2007) ‘SeaIsle 2000’ Paspalum putting surface response to mowing height and nitrogen fertilizer. *Crop Science* 99: 133–140.

Krans, J. V., Morris, K. (2007). Determining a profile of protocols and standards used in the visual field assessment of turfgrasses: A survey of national turfgrass evaluation program-sponsored university scientists. *Applied Turfgrass Science*, 4: 0–0

Lee, G. J., Carrow, R. N., Duncan, R. R. (2004) Salinity tolerance of selected seashore paspalums and bermudagrasses: root and verdure responses and criteria. *Horticulture Science* 39: 1136–1142.

McCarty, L. B., Miller, G. (2002) Managing bermudagrass turf: Selection, construction, cultural practices, and pest management strategies. Sleeping Bear Press, Chelsea, Michigan.

Milesi, C., Running, S. W., Elvidge, C. D., Dietz, J. B., Tuttle, B. T., Nemani, R. R. (2005) Mapping and modelling the biogeochemical cycling of turfgrasses in the United States. *Environmental Management* 36: 426–438.

Moore, K. J., Moser, L. E. (1995) Quantifying Developmental Morphology of Perennial Grasses. *Crop Science* 35: 37–43.

Morris, K. N., Shearman, R. C. (2014) NTEP turfgrass evaluation guidelines. In *NTEP turfgrass evaluation workshop*, Beltsville, MD (1–5).

Morris, K. N. (2003) The National Turfgrass Research Initiative. National Turfgrass Federation, Inc. National Turfgrass Evaluation Program, Beltsville Agricultural Research Center, Beltsville, Maryland, 20705 USA.

Munshaw, G. C., Ervin, E. H., Shang, C., Askew, S. D., Zhang, X., Lemus, R. W. (2006) Influence of late season iron, nitrogen and seaweed extract on fall color retention and cold tolerance of bermudagrass cultivars. *Crop Sc*ience 46: 273–283.

Nutter, G. C. (1965) Turf-grass is a 4 billion dollar industry. *Turfgrass Times* 1(1): 1–2.

NZSTITO. (2011) The New Zealand turf industry strategic training plan 2011–1013. New Zealand Turf Industry Training Organization, Palmerston North, New Zealand.

Patton, A. J. Reicher, Z. J. (2007) Zoysiagrass species and genotypes differ in their winter injury and freeze tolerance. *Crop Science* 47: 1619–1627.

Patton, A. J., Cunningham, S. M., Volenec, J. J., Reicher, Z. J. (2007) Differences in freeze tolerance of Zoysiagrasses: II. Carbohydrate and proline accumulation. *Crop Science* 47: 2170–2181.

Roberts, C.E., Huffine, W. W., Grau, F. V., Murray, J. J. (1992) Turfgrass Science, Historical Overview In Waddington, D. V., Carrow, Robert N., Shearman, Robert C. (Eds.). Turfgrass. (1–27) Madison, Wis., USA: American Society of Agronomy: Crop Science Society of America : Soil Science Society of America.

Rumball, W. (1982) 'Grasslands Cook' Chewings fescue (*Festuca rubra* L.) – bred for amenity areas. *New Zealand Journal of Experimental Agriculture* 10:167–168.

Rumball, W. (1983) Amenity grasses. In G.S. Wrattand H.C. Smith (Ed.) Plant breeding in New Zealand. (pp. 271–276) Butterworths of New Zealand, CPO Box 472, Wellington, New Zealand

Rumball, W., Robinson, G.S. (1982) 'Grasslands Egmont' amenity browntop (*Agrostis capillaris* L. syn A. tenuis Sibth.). *New Zealand Journal of Experimental Agriculture* 10: 175–177.

Sage, R. F. (2004) The evolution of C4 photosynthesis. *New Phytologist* 161: 341–370.

Sage, R. F., McKown, A. D. (2005) Is $C_4$ photosynthesis less phenotypically plastic than $C_3$ photosynthesis. *Journal of Experimental Botany* 57: 303–317.

Salisbury F.B., Ross C.W. (1992) Plant Physiology (4th ed.) Belmont, CA: Wadsworth Publishing Co.

Samala, S., Yan, J., Baird, W. V. (1998) Changes in polar lipid fatty acid composition during cold acclimation in 'Midiron' and U3' bermudagrass. *Crop Science* 38: 188–195.

Samudio, S.H., Brede, A. D. (2002). Registration of 'Southern Star' Bermudagrass Registration by CSSA. *Crop Science* 42: 669–670. doi: 10.2135/cropsci2002.6690

Sanghera, G. S., Wani, S. H., Hussain, W., Singh, N. B. (2011) Engineering cold stress tolerance in crop plants. *Current Genomics* 12: 30–43.

Seagle, E. and Iverson, M. (2002) Characteristics of the turfgrass industry in 2020: A Delphi Studywith Implications for Agricultural Education Programs. *Journal of Southern Agricultural Education Research* 52: 1–13.

Sharman B.C. (1947) The biology and developmental morphology of the shoot apex in the Gramineae. *New Phytologist* 46: 20–34.

Shay, P. E., Kubien, D. S. (2013) Field analysis of photo protection in co-occurring cool climate C3 and C4 grasses. *Physiologia Plantarum* 147: 316–328. doi: 10.1111/j.1399-3054.2012.01662.x

Shearman, R. C. (2006) Fifty years of splendor in the grass. *Crop Science* 46: 2218–2229. doi:10.2135/cropsci2005.12.0467gas.

Silsbury J.H. (1970) Leaf growth in pasture grasses. *Tropical Grasslands* 4:17–39.

Stefaniak, T. R., Rodgers, C. A., Van Dyke, R., Williams, D. W., Phillips, T. D. (2009) The inheritance of cold tolerance and turf traits in a seeded bermudagrass population. *Crop Science* 49: 1489–1495.

Stier, J. C. (2006, August). Understanding and managing environmental stresses of turfgrass. In *XXVII International Horticultural Congress-IHC2006: International Symposium on Horticultural Plants in Urban and Peri-Urban 762:* 63-80.

Taiz, L., Zeiger, E. (1991) Plant Physiology The Benjamin. *Cummings Redwood City*, *565*.

Taliaferro, C. M. (2003) Bermudagrasses. In Casler, M. D., Duncan, R. R. (Eds.). *Turfgrass biology, genetics, and breeding.* (235-256) Hoboken, N.J.: John Wiley.

Thomas, R. S., Rodgers, C. A., VanDyke, R., Williams, D. W., and Phillips, T. D. (2009) The inheritance of cold tolerance and turf traits in a seeded bermuda grass population. *Crop Science* 49: 1489–1495.

TPA (2017) Facts and figures. https://www.turfaustralia.com.au/aboutus/facts-figures accessed on 6 June 2017.

Volaire, F, Norton, M. (2006) Summer dormancy in perennial temperate grasses. *Annals of Botany* 98: 927–933.

Wang, Y., Li, J., Wang, J., Li, Z. (2010) Exogenous H2O2 improves the chilling tolerance of manilagrass and mascarenegrass by activating the antioxidative system. *Plant growth regulation* 61: 195–204.

Wang, Y., Yang, Z. M., Zhang, Q. F., Li, J. L. (2009) Enhanced chilling tolerance in Zoysia matrella by pre-treatment with salicylic acid, calcium chloride, hydrogen peroxide or 6-benzylaminopurine. *Biologia Plantarum 53*: 179.

Watson, J. R., Kaerwer, H. E., Martin, D. P. (1992) The Turfgrass Industry. *Turfgrass, Agronomy Monograph* (Turfgrass):29–88. doi: 10.2134/agronmonogr32.c2.

Way, B. (2001) New Zealand Turf 2000. *New Zealand Turf Management Journal* 16: 14–17.

Wei, S., Zhou, Y., Li, D. and Sun, Z. (2008). Cold sensitivity and biochemical adjustments in Zoysiagrass under low temperature stress. In. Stier, J. C. et al., (Eds.,), *Proceedings of second International Conference on Turfgrass*. *Acta Horticulturae (ISHS)* 783: 195–206.

White, R. H., Engelke, M. C., Anderson, S. J., Ruemmele, B. A., Marcum, K. B., Taylor, G. R. (2001) Zoysiagrass water relations. *Crop Science* 41: 133–138.

Zhang, X., Wang, K., Ervin, E. H., Waltz, C., Murphy, T. (2011) Metabolic changes during cold acclimation and deacclimation in five bermudagrass varieties. I. Proline, total amino acid, protein, and dehydrin expression. *Crop Science* 51: 838–846.

Zhang, Q., Fry, J., Pan, X., Rajashekar, C., Bremar, D., Engelke, M., Wang, X. (2009) Cold acclimation of *Zoysia japonica* and *Z. matrella* and changes in rhizome abscisic acid levels. *International Turfgrass Society Research Journal* 11: 883–892.

Zhang, X., Ervin, E. H., LaBranche, A. J. (2006) Metabolic defence responses of seeded bermudagrass during acclimation to freezing stress. *Crop Science* 46: 2598–2605.

# CHAPTER 3

# Quality Attributes of Different Warm-Season Turfgrasses

## 3.1 Introduction

Turfgrass is an important and integral part of modern urban infrastructure. Turf has recreational uses, and often is an element in the landscaping of commercial, residential and public premises. Turf provides aesthetic appeal, confers environmental benefits and offers a safe playable surface for various sport activities (Duble, 1996; Beard and Green, 1994; Ulrich, 1986). Cool-season ($C_3$) grasses have historically been the mainstay of the New Zealand turf industry, and a range of varieties of *Lolium, Festuca* and *Agrostis* are widely grown across the country. However, cool-season grasses suffer from heat stress and lose their vigour and aesthetic appeal under high temperatures and/or drought conditions. Consequently, there are problems related to summer dormancy when these species are grown at venues in warm areas, especially in the northern parts of the North Island (Bay of Plenty, Auckland Region, Northland and parts of Waikato). Warm-season ($C_4$) grasses are gaining popularity in such areas because of their superior heat and drought tolerance.

Different venues have different performance requirements for turf, reflecting the needs of their main users. Hence, objective criteria can be used to determine which grass type is best suited to a particular venue to match the specific requirements of the users, the available resources and environmental and soil conditions (Carrow et al., 2010). Continuous efforts in selection and breeding have not only widened the range of available germplasm resources, but also given rise to a wide range of commercially available turfgrass varieties that have been specifically bred for different purposes and venues. There are strong user demands for a year-round green and playable surface, which presents a challenge for turf researchers and growers, especially under high-use and/or unfavourable growth conditions.

Warm-season grasses have some advantages over cool-season grasses in summer since they have a unique morphology, high photosynthetic efficiency and reduced water requirements (Zhou and Abaraha, 2007; Volterrani et al., 1997). However, they tend to be frost-sensitive, and winter performance can be an issue. Thus, research to identify varieties with superior performance in traits such as colour retention and surface homogeneity during autumn, winter, and early spring will provide useful information for turf growers and managers.

The aesthetic appearance of turf is a high priority for turf managers and users, even in situations of intensive use and unfavourable weather conditions. Objective criteria can be used to determine which grass type is best suited to particular conditions, to match varieties to the environmental and soil conditions and available resources (Carrow et al., 2010). Warm-season turfgrass species use water and nutrients efficiently and tolerate high temperature; therefore, they have the potential for widespread use in the New Zealand sports industry.

Given the need for objective data on the winter performance of the various commercially available warm-season turfgrasses, a range of $C_4$ grasses was established in plots in the field and glasshouse, and monitored over the autumn, winter and spring in 2013 and again in autumn in 2014. The specific objectives of this study were as follows:

1- To observe a selection of $C_4$ species and varieties through an autumn-winter-spring (cool season) cycle under temperature-protected (glasshouse) and frost-exposed (open field) conditions to better understand their ecophysiology;
2- To record visual attributes such as colour retention and mat quality through the cool-season cycle;
3- To compare seeded and non-seeded $C_4$ turfgrasses to assess their suitability for growth under cool-climate conditions.

## 3.2 Materials and methods

### 3.2.1 Turf establishment

Twelve varieties (Table 3.1) of four warm season turf genera (*Cynodon, Zoysia, Pennisetum* and *Paspalum*) were established in a heated glasshouse at the Plant Growth Unit (PGU), Massey University, Palmerston North and temperature was controlled at 18°Cmin/25°Cmax. Ten varieties were also established in the field at the New Zealand Sports Turf Institute (NZSTI), State Highway 57, Palmerston North, using a sand carpet of 50-mm depth over the existing soil surface at both trial sites. For both trials, varieties were planted in a randomized complete block design (RCBD) with four replicates (Table 3.2). Uniform water supply was provided with automatic overhead sprinkler system in the glasshouse and with popup sprinklers in the field. Plots in both the glasshouse and the field were supplemented with NPK fertiliser and later on with Peters liquid feed every 2 weeks.

Seeds or stolons were used to establish the plots. Four varieties of Cynodon (AgriDark, Legend, Windsor Green and Santa Ana) were established using stolons from fully developed grass sods. To rule out variation in establishment rate caused by differences in stolon size, mature stolon fragments that were each three nodes long were planted in parallel lines approximately 6 cm apart. Seeds of three Cynodon varieties (Princess 77, Yukon and La Paloma) and Sea Spray, Zenith and Regal Staygreen were sown at the standard rate recommended by the breeder (Table 3.1).

Temperature data were collected in the glasshouse throughout the experiment. Field temperature data collected at the nearest location (AgResearch, Palmerston North) were obtained from the National Institute of Water and Atmospheric Research (NIWA) database. After planting in November–December 2012, plots were allowed to establish for 3 months. Field plots of seeded varieties were covered with polypropylene germination mats to facilitate seed germination. The mats were removed in early January 2013 after seedling emergence.

**Table 3.1 Warm-season turfgrass varieties, sources establishment method, and seed rate**

| # | Species | Variety | Source | Method | Seed rate |
|---|---|---|---|---|---|
| **1** | ***Cynodon dactylon*** | Princess-77 | PGG Wrightson | Seed | 10 g/m$^2$ |
| **2** | ***Cynodon dactylon*** | Yukon | PGG Wrightson | Seed | 15 g/m$^2$ |
| **3** | ***Cynodon dactylon*** | La Paloma | PGG Wrightson | Seed | 15 g/m$^2$ |
| **4** | ***C. dactylon* × *transvaalensis*** | AgriDark | Cervadon Ltd. | Vegetative | 400 Stolon pieces/m$^2$ |
| **5** | ***C. dactylon* × *transvaalensis*** | Santa Ana | Hololio Turf Farm | Vegetative | 400 Stolon pieces/m$^2$ |
| **6** | ***Cynodon dactylon*** | Windsor green | Supplier A | Vegetative | 400 Stolon pieces/m$^2$ |
| **7** | ***Cynodon dactylon*** | Legend | Supplier A | Vegetative | 400 Stolon pieces/m$^2$ |
| **8** | ***Paspalum vaginatum*** | Sea Spray | PGG Wrightson | Seed | 10 g/m$^2$ |
| **9** | ***Zoysia japonica*** | Zenith | PGG Wrightson | Seed | 10 g/m$^2$ |
| **10** | ***Zoysia japonica*** | Compadre | PGG Wrightson | Seed | 10 g/m$^2$ |
| **11** | ***Pennisetum clandestinum*** | Regal Staygreen | PGG Wrightson | Seed | 08 g/m$^2$ |
| **12** | ***Pennisetum clandestinum*** | Local kikuyu | Wild collection | Vegetative | 400 Stolon pieces/m$^2$ |

*****Stolon pieces were three internode long fragments from mature stolon.

## Table 3.2 Randomized layout plan

| | | | | | | |
|---|---|---|---|---|---|---|
| **Glasshouse (PGU)** | | | | | | |
| **1.Zenith (ZN)** | **R1** | **2.La Paloma (LP)** | | **1.W Green (WG)** | **R2** | **2. Princess 77 (P77)** |
| **3. Compadre** | | **4.Agri Dark (AD)** | | **3. Santa Ana (SA)** | | **4. R Staygreen (RS)** |
| **5.Princess 77(P77)** | | **6.Sea Spray (SS)** | | **5. Compadre** | | **6. Zenith (ZN)** |
| **7. Legend** | | **8.Yukon (YK)** | | **7. Local Kikuyu** | | **8. Yukon (YK)** |
| **9. Local Kikuyu** | | **10.Santa Ana (SA)** | | **9.Agri Dark (AD)** | | **10.La Paloma (LP)** |
| **11.R Staygreen(RS)** | | **12.W Green (WG)** | | **11. Legend** | | **12. Sea Spray (SS)** |
| | | | | | | |
| **1. Zenith (ZN)** | **R3** | **2. Legend** | | **1.Agri Dark (AD)** | **R4** | **2. Zenith (ZN)** |
| **3. Local Kikuyu** | | **4. Staygreen (RS)** | | **3.La Paloma (LP)** | | **4. Yukon (YK)** |
| **5. Agri Dark (AD)** | | **6. Compadre** | | **5. Santa Ana (SA)** | | **6. W Green (WG)** |
| **7. Sea Spray (SS)** | | **8. Princess77(P77)** | | **7. Legend** | | **8. Compadre** |
| **9. W Green (WG)** | | **10. Yukon (YK)** | | **9.Princess 77 (P77)** | | **10. Local Kikuyu** |
| **11.La Paloma(LP)** | | **12. Santa Ana(SA)** | | **11.R Staygreen(RS)** | | **12. Sea Spray (SS)** |
| **Field Plots (NZSTI)** | | | | | | |
| **1. R Staygreen(RS)** | **R1** | **2. Santa Ana (SA)** | | **1. Zenith (ZN)** | **R2** | **2. W Green (WG)** |
| **3. Zenith (ZN)** | | **4. Agri Dark (AD)** | | **3. Agri Dark (AD)** | | **4. Princess 77 (P77)** |
| **5. Sea Spray (SS)** | | **6. W Green (WG)** | | **5. Sea Spray (SS)** | | **6. La Paloma (LP)** |
| **7. Yukon (YK)** | | **8. La Paloma (LP)** | | **7. Santa Ana (SA)** | | **8. Yukon (YK)** |
| **9. Legend (LG)** | | **10.Princess77(P77)** | | **9.R Staygreen(RS)** | | **10. Legend (LG)** |
| | | | | | | |
| **1. Yukon (YK)** | **R3** | **2.R. Staygreen(RS)** | | **1. Zenith (ZN)** | **R4** | **2. La Paloma (LP)** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **3. Zenith (ZN)** | | **4. La Paloma (LP)** | | **3.Princess 77 (P77)** | | **4. RStaygreen (RS)** |
| **5. Agri Dark (AD)** | | **6. Santa Ana (SA)** | | **5. Agri Dark (AD)** | | **6. Legend (LG)** |
| **7. Legend (LG)** | | **8.Princess 77 (P77)** | | **7. Sea Spray (SS)** | | **8. Santa Ana (SA)** |
| **9. Sea Spray (SS)** | | **10.W. Green (WG)** | | **9. Yukon (YK)** | | **10. W. Green (WG)** |

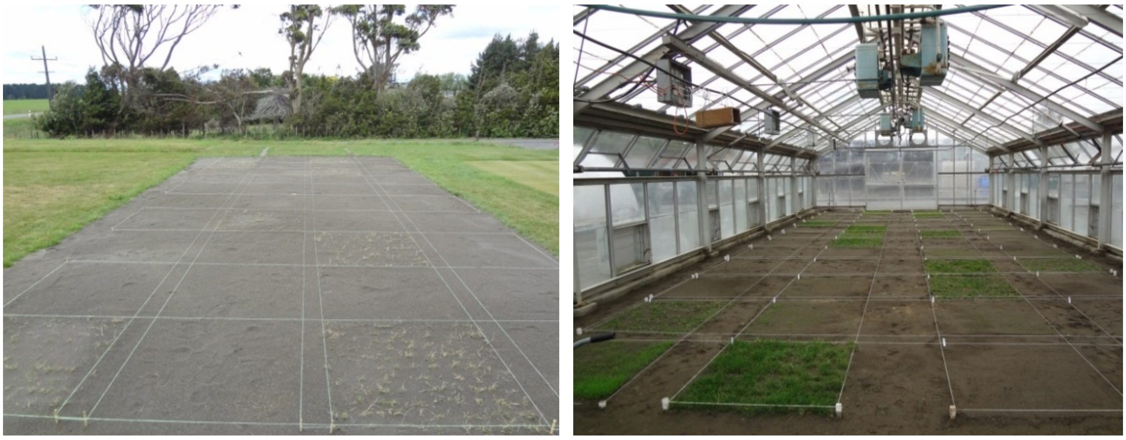


**Fig. 3.1 Establishment of field plots (left) and glasshouse plots (right)**

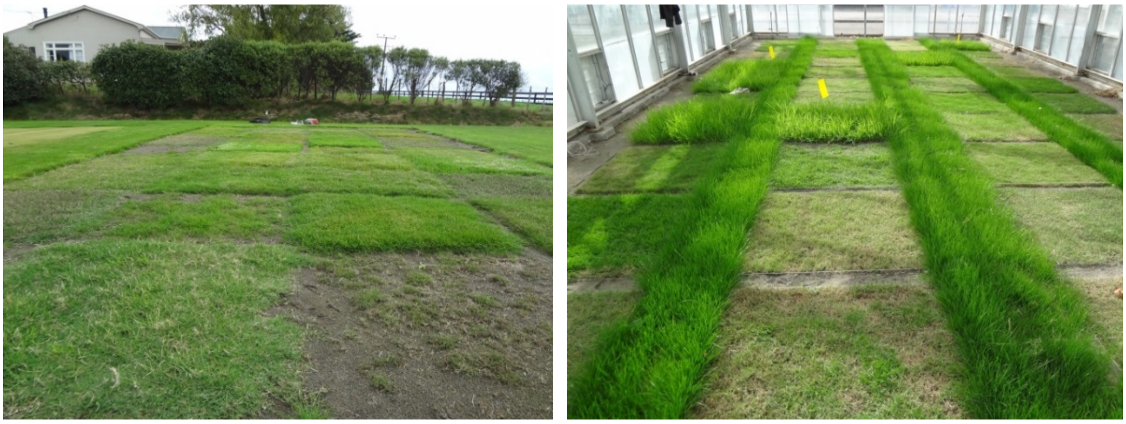


**Fig. 3.2 Established field plots (left) and glasshouse plots (right) in March 2013.**

### 3.2.2 Data collection

After establishment, the plots were evaluated in March, May, and July (autumn and winter) in 2013, and then again in the following winter May and July, 2014 to score overall quality, ground cover, density, texture, uniformity, and colour using standards as prescribed by the NTEP (National Turf Evaluation Program, USA) (Morris, n.d.). Qualitative attributes including visual quality, density, texture and colour was scored on a visual scale of 1–9 with 9 as the best or highest value and 1 as the lowest value. In the case of texture, 9 = fine, and 1 = coarse. Ground cover was scored on a percentage basis with 100 as a full cover.

The field trial site was established with 10 varieties. Entries 10 and 12 (Compadre *Zoysia* grass, and local kikuyu) were omitted from the field plots and subsequently, to keep the data collection uniform and comparable, it was decided not to collect data for these two treatments within the glasshouse either. Data for entry 7, Legend *Cynodon*, though collected, are also not presented, as it emerged from discussion with the plant variety rights owner later in the trial that they did not consider they had given permission for the inclusion of their material, which had been obtained in good faith from an Auckland supplier.

### 3.2.3 Data analysis

Data were analysed using independent sample t-tests ($P \leq 0.05$) to determine statistically significant differences between the two locations. One-way ANOVA was used ($P \leq 0.05$) to detect significant differences among varieties at each location in each month. Analyses were performed using Microsoft Excel 2010 and SPSS 21 (Statistical Package for Social Sciences). ANOVA tables are presented in the appendices.

## 3.3 Results

There were clear differences among varieties for all the scored attributes at both locations. The turfgrasses in the glasshouse showed relatively vigorous growth and better percentage groundcover during early stages of establishment, whereas those in open field plots grew slowly with a more compact growth habit (short internodes and small leaves). The seeded varieties, especially La Paloma, Yukon and Zenith showed poor survival after the germination covers were removed in the first week of January 2013. In the establishment phase, the warm-season turfgrasses were sensitive to desiccation and to overnight temperatures below 10°C, which occurred in the field in Palmerston North even in mid- January. Seeded varieties of *Cynodon* and Zenith (*Z. japonica)* were most sensitive to these stresses. The cold-sensitivity of these varieties was reflected by their slow mat development and decreased percentage ground cover in winter. Among the *Cynodon* varieties, the vegetatively propagated varieties established faster than the seeded varieties, and maintained good density during winter in 2013 both in the glasshouse and in the field, but lost colour at both sites. Regal Staygreen formed the thickest mat in in the field (but not in the glasshouse), but had a coarse texture. Seeded Princess-77 and Sea Spray showed similar performance to that of the vegetatively established varieties.

Plots were well established by the end of their second summer in 2013/14, with a better ground cover percentage and compact mat, than in their first winter. By most of the criteria scored, the C4 varieties could be considered persistent. Winter browning appeared a little later in autumn 2014 than 2013, possibly because of less extreme temperatures during the autumn season compared to 2013 (Fig. 3.3). Overall, in the second summer, plots exhibited a better turf quality and improved visual characteristics.

### 3.3.1 Quality

At the end of summer in 2013, most varieties had established good quality swards both in the field and the glasshouse. The only exceptions were Regal Staygreen, which showed a lower quality score in the glasshouse because of increased stolon elongation in the low-light conditions, and Zenith, which showed a lower score in the field as a result of slow establishment and issues arising from that (Fig. 3.5-A). The quality scores of all varieties decreased during May, especially for seeded *C. dactylon* varieties in the glasshouse. In general, the turfgrasses showed similar quality scores in the field and glasshouse, but Zenith showed significantly better quality scores in the glasshouse than in the field, and vice versa for La Paloma (Fig 3.5-B). In the field, cold temperatures during winter in 2013 led to browning off and an associated deterioration in turf quality, but the severity differed among varieties. Santa Ana, AgriDark and Regal Staygreen retained good quality during winter under field conditions (Fig 3.5-C), and AgriDark, Santa Ana, and Sea Spray showed the best results in the glasshouse during winter. A fungal infestation in the glasshouse in April was the main cause of quality differences among varieties, and the seeded *Cynodon* varieties were most severely affected. The following autumn (2014), plots showed overall improved visual quality with seeded couches as best performers at both locations (Fig 3.5-D, E). Quality deterioration was observed with the onset of winter, especially under field conditions and Zenith was again noted to be the most susceptible variety, with low scores for several turf characters (Fig 3.5-D, E). Regal Staygreen showed persistence to the previous results and retained its scoring under field conditions.

### 3.3.2 Ground cover

Groundcover development tended to be slightly better in the glasshouse than in the field. There was also a better rate of groundcover development in the vegetatively propagated *Cynodon* varieties than in the seeded *Cynodon* varieties. The groundcover development of Sea Spray (*P. vaginatum*) and Regal Staygreen (*P. clandestinum*) was comparable to that of the vegetative *Cynodon* varieties, while Zenith (*Z. japonica*) showed good groundcover development in the glasshouse but not in the field (Fig. 3.6-A). For some varieties, especially the seeded *Cynodon* varieties, the percentage ground cover decreased during winter in 2013 (Fig 3.6-B, C).

Regal Staygreen showed a stable growth pattern under field conditions and maintained a good ground cover percentage throughout the experimental period. All varieties except for seeded *Cynodon* varieties formed groundcover in the first few months after establishment, and maintained the percentage groundcover under glasshouse conditions. Before the second winter (2014) most of the varieties attained full ground cover except some seeded varieties (Fig 3.5-D, E). In general, the varieties evaluated could be regarded as persistent in that they retained their ground cover percentage through winter, especially under glasshouse conditions but some decrease in ground cover scores in winter did occur (Fig 3.6, E), with Zenith once again being the most affected variety and scored as most susceptible to winter injury.

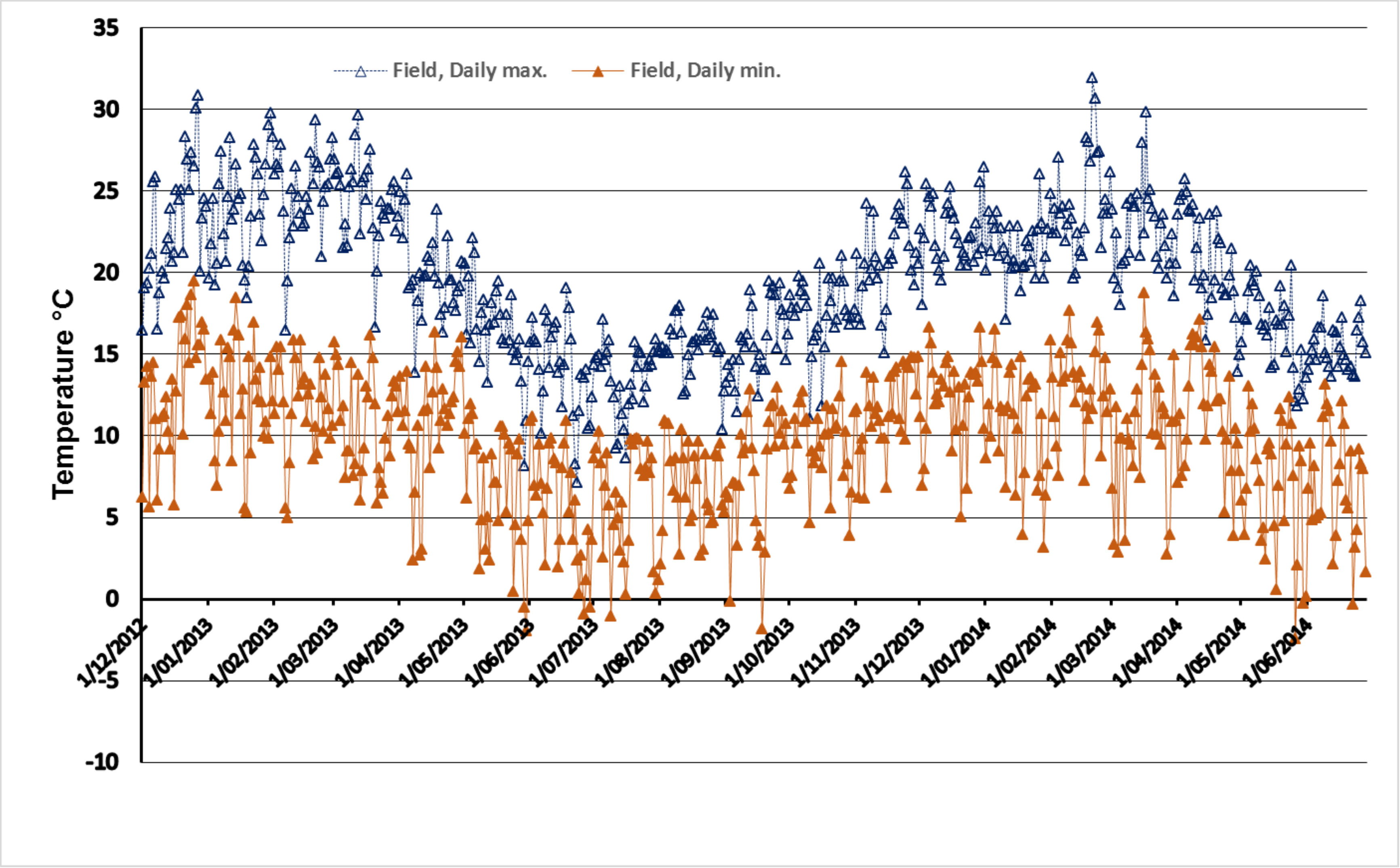


**Fig. 3.3** **Daily minimum/maximum temperatures in the field during summer, autumn, and winter in 2012-14.**

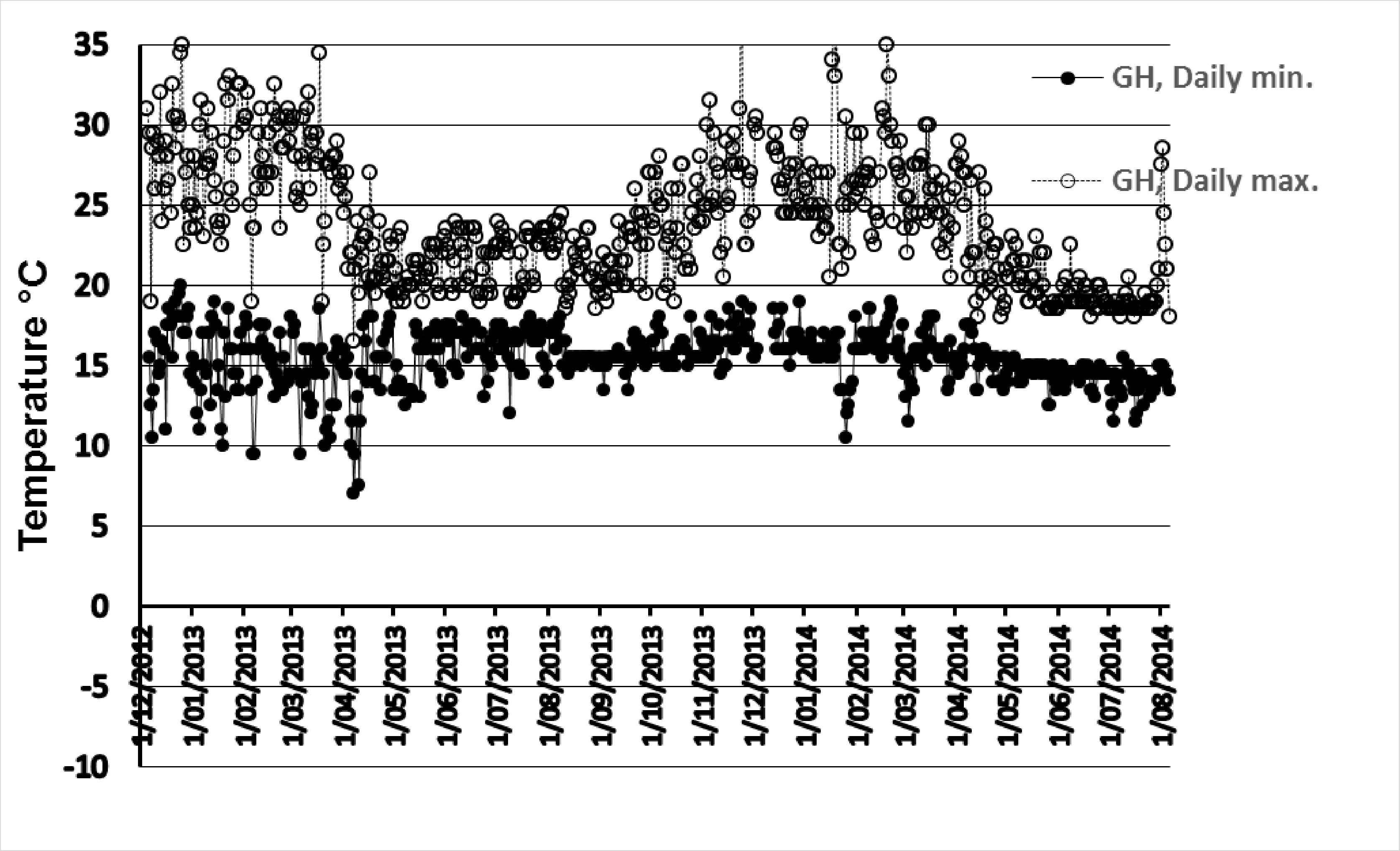
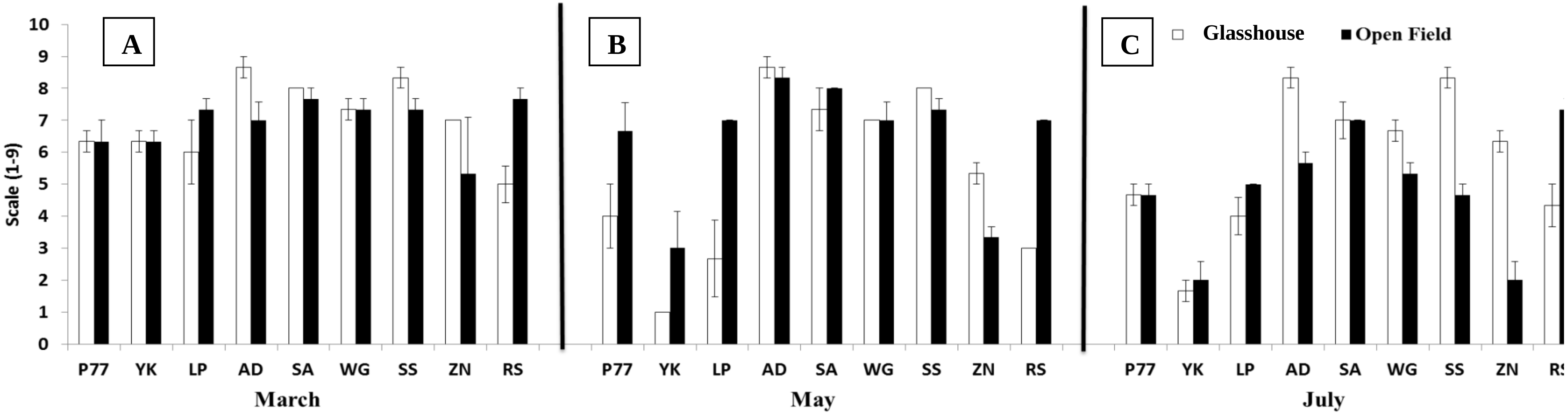
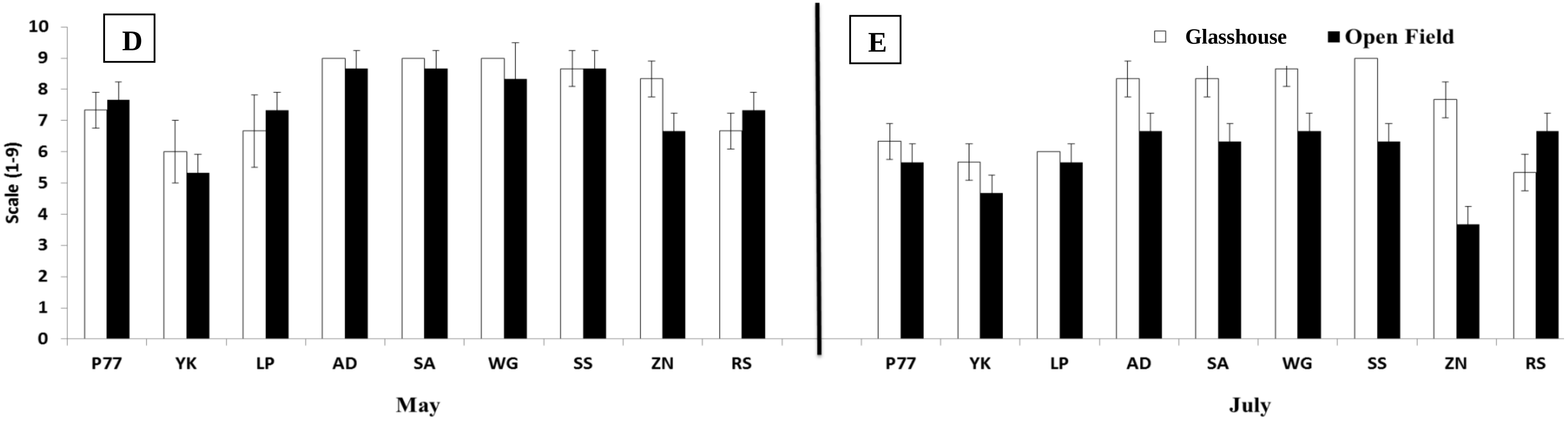


**Fig. 3.4  Daily minimum/maximum temperatures in the glasshouse (GH) during 2012-13.**



Quality-2013



Quality-2014

**Fig: 3.5 Quality (overall visual appearance) of turf mat in glasshouse and open field conditions during Autumn/Winter 2013-2014.** P77=Princess-77, YK=Yukon, LP=La Paloma, AD=AgriDark, SA=Santa Ana, WG=Windsorgreen, SS=Sea Spray, ZN=Zenith, RS=Regal Staygreen.

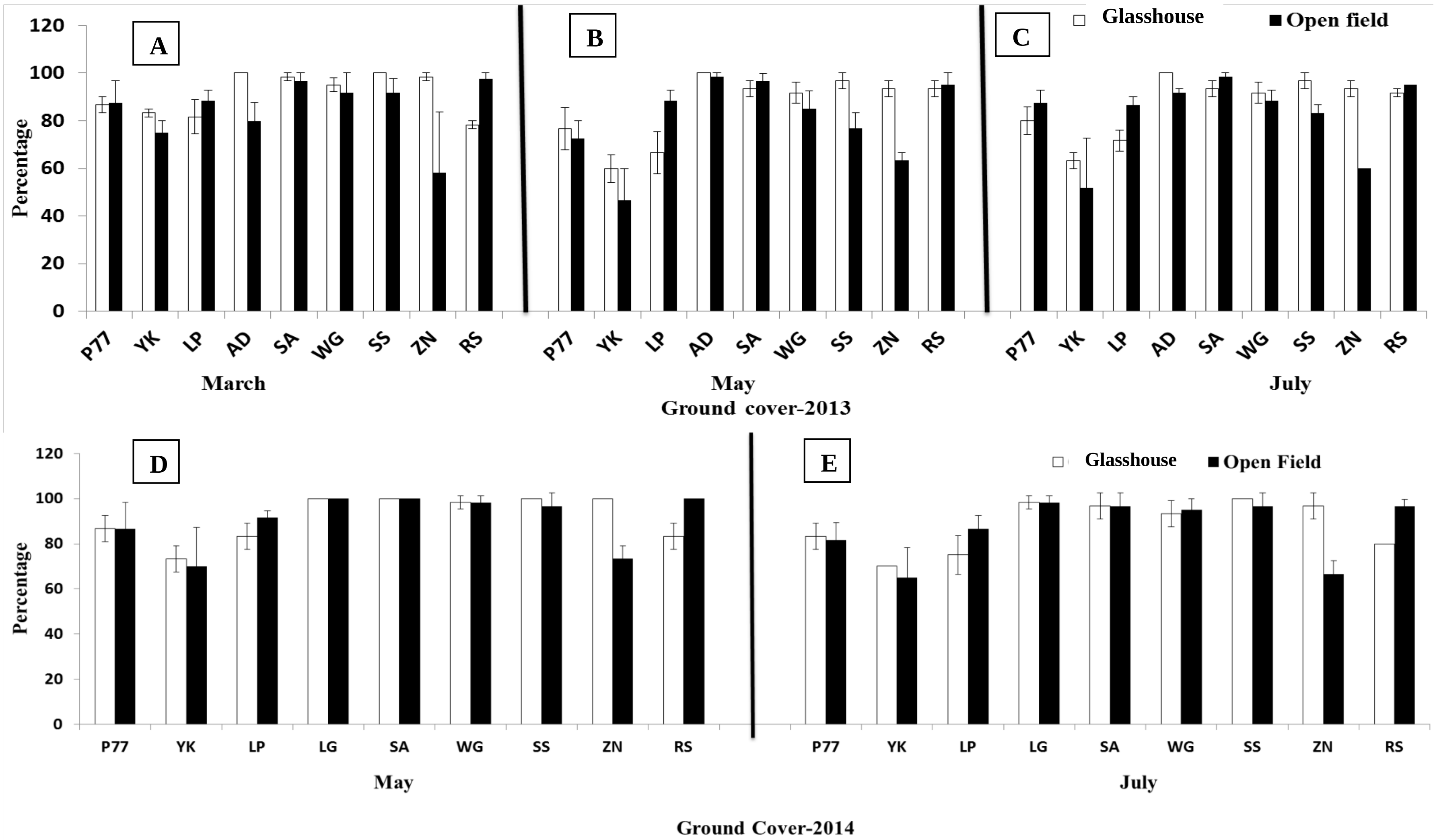


**Fig: 3.6 Ground cover percentage of turf mat in glasshouse and open field conditions during Autumn/Winter 2013-2014.** P77=Princess-77, YK=Yukon, LP=La Paloma, AD=AgriDark, SA=Santa Ana, WG=Windsorgreen, SS=Sea Spray, ZN=Zenith, RS=Regal Staygreen.

### 3.3.3 Density

At the end of summer (March 2013), there was little difference in turf density between glasshouse and field plots. The only exceptions were Zenith, which showed higher density in the glasshouse after its better establishment there, and Regal Staygreen (*P. clandestinum*), which showed higher density in the field due to reduced stolon internode elongation (Fig 3.7-A, B, C). With falling temperatures from May to July in 2013, the varieties that lost percentage groundcover also lost density (Fig 3.7-B, C).

The groundcover and density were closely linked, and appeared to provide a useful measure of cold-sensitivity in these experiments. In the following autumn (2014) (Fig 3.7- D, E) better turf density was observed than in the previous season, however, Zenith stood out as having very low density under field conditions. The turf mat of Zenith field plots was never fully developed, in contrast with the glasshouse plots. As had occurred in the previous year, turfgrasses retained their density under glasshouse conditions but a slight decrease in density was observed in field plots. Regal Staygreen was an exception, and this variety showed better performance and higher scores under field conditions in both years, than in the Glasshouse (Fig 3.7).

### 3.3.4 Texture

The vegetative varieties of *Cynodon* (AgriDark, Santa Ana and Windsor green) and Sea Spray had the finest texture. Zenith and Regal Staygreen were coarse-textured, especially under glasshouse conditions (Fig 3.2-D, E, F) but showed better performance under field conditions. This was especially true for Regal Staygreen, the leaves of which were longer and coarser in the glasshouse than in the field. Most varieties showed a coarser texture under glasshouse conditions than under field conditions, except for AgriDark and Santa Ana, which showed no statistically significant difference in texture between the glasshouse and field plots.

Similar trends in texture scores were found during the 2014 winter observations. Again, Zenith and Regal Staygreen exhibited a coarser growth habit in the glasshouse than in field plots, while in the turf plots grown under field conditions (Fig 3.8-D, E). Turf varieties exhibited overall finer texture under field conditions except Princess 77, which showed a slightly better texture in the glasshouse.

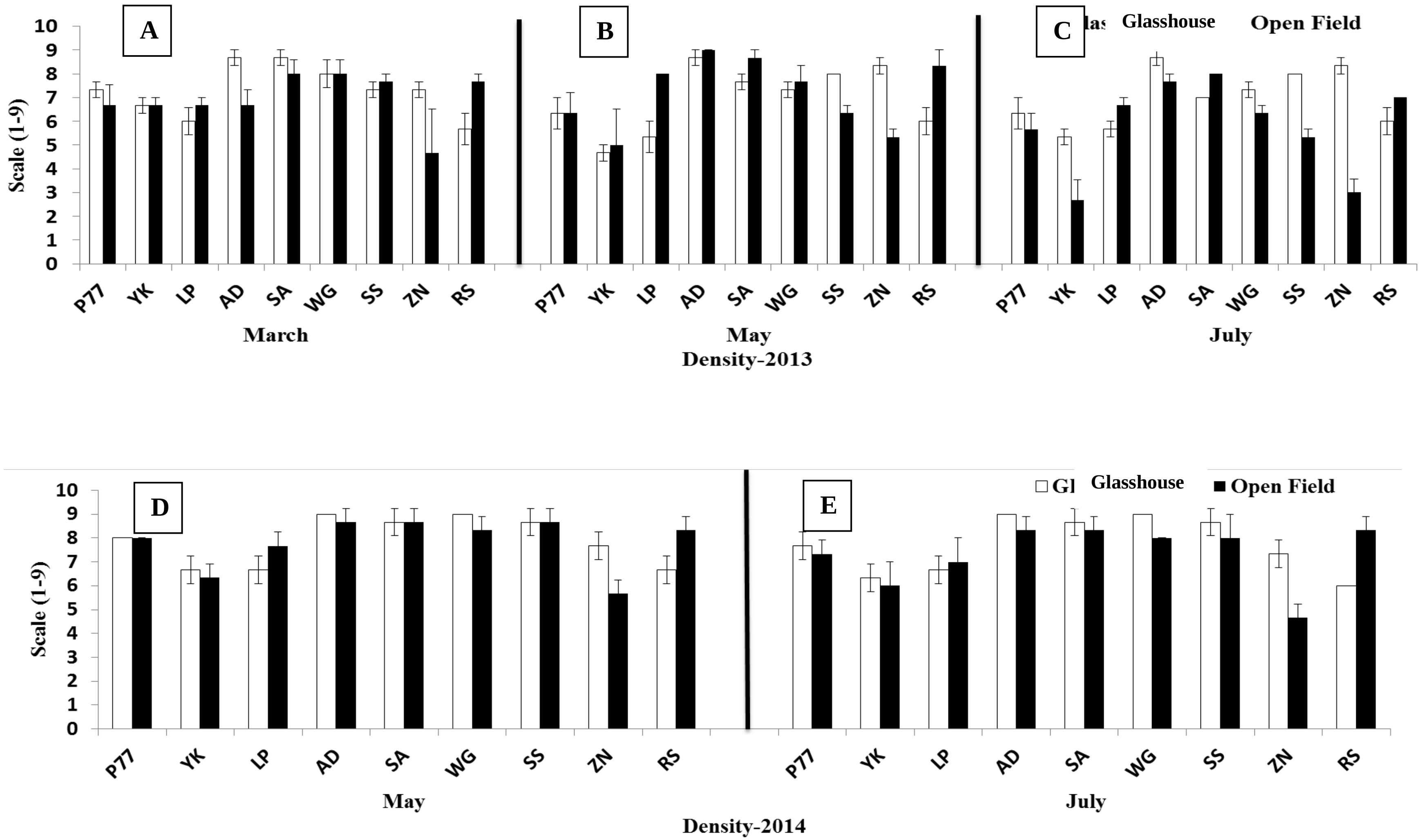
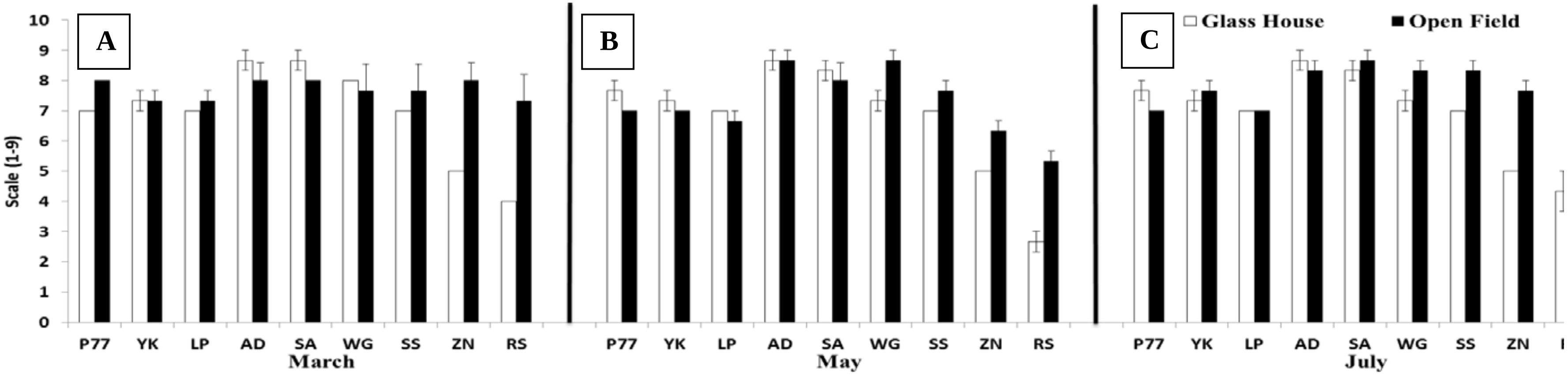
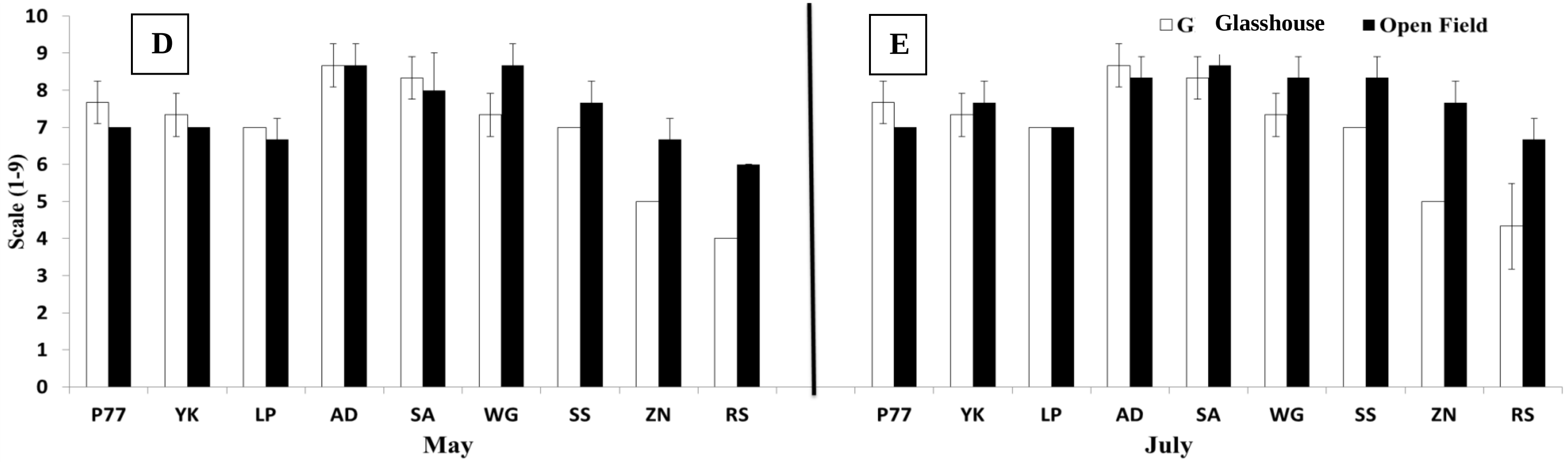


**Fig: 3.7 Density of turf mat under glasshouse and open field during Autumn/Winter 2013-2014.**
P-77=Princess-77, YK=Yukon, LP=La Paloma, AD=AgriDark, SA=Santa Ana, WG=Windsorgreen, SS=Sea Spray, ZN=Zenith, RS=Regal Staygreen

**Glasshouse**



Texture-2013



Texture-2014

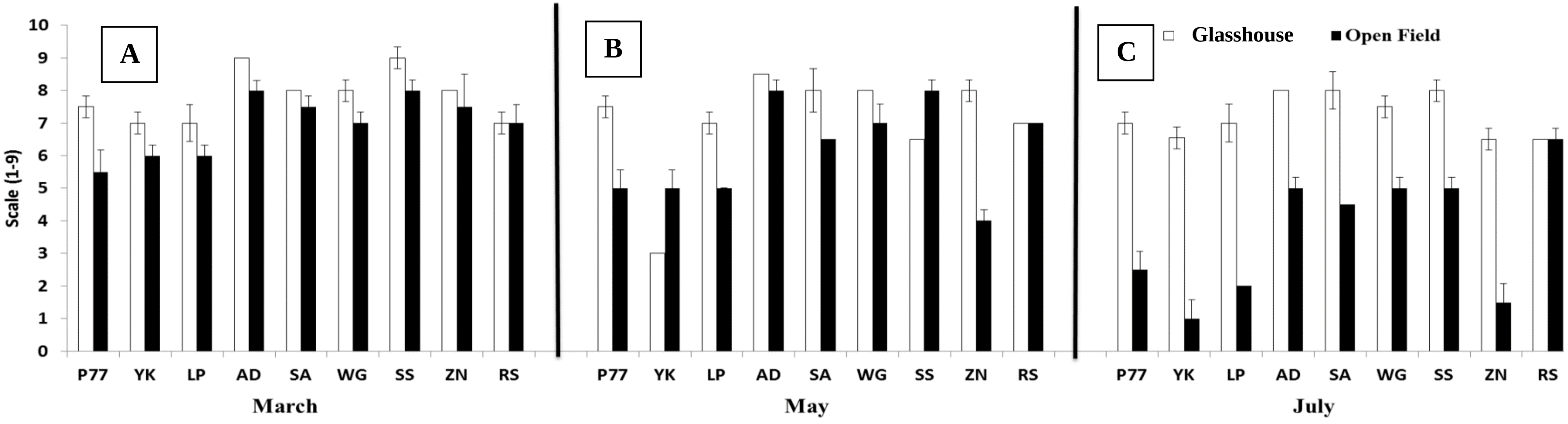
**Fig: 3.8 Texture of turf mat in glasshouse and open field conditions during Autumn/Winter 2013-2014.**
P77=Princess-77, YK=Yukon, LP=La Paloma, AD=AgriDark, SA=Santa Ana, WG=Windsorgreen, SS=Sea Spray, ZN=Zenith, RS=Regal Staygreen.

### 3.3.5 Colour

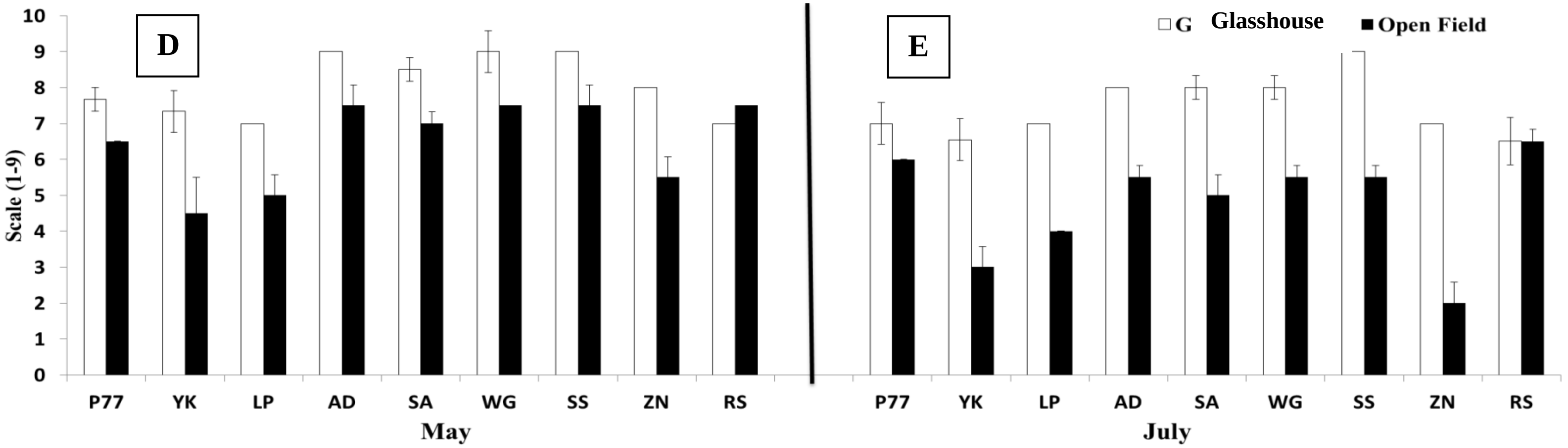
Among the tested varieties, AgriDark, Windsor Green, Santa Ana and Sea Spray showed comparatively better colour retention over the winter months than the other varieties, despite wide variations (Fig. 3.9). Zenith was the most cold-sensitive variety, and browned off earlier than the other varieties during winter. Colour degradation was more visible in field plots than in the glasshouse as the temperature decreased, especially for Zenith and the seeded *Cynodon* varieties. Gradual colour degradation can be observed in the results with the progression in winter period especially in the field plots (Fig. 3.12), however colour changes were gradual and comparatively less severe under glasshouse conditions (Fig. 3.11). Some varieties, especially seeded *Cynodon* varieties, showed some colour degradation in glasshouse plots as well as the field plots. For example the colour score for Yukon in May 2013 in the glasshouse fell to 3, with some recovery in July (Figs. 3.9, 3.11).

A very similar pattern was observed during the second winter. The turfgrasses grown under glasshouse conditions retained their colour, though with some degree of colour degradation. Higher colour degradation was noted in field plots with similar behaviour of varieties and Yukon and La Paloma as most susceptible among the *Cynodons*. Zenith was most affected of all the varieties under field conditions (Fig. 3.9) and Regal Staygreen maintained a good green colour as in the previous year. Performance of all three vegetative produced *Cynodon* varieties (Agridark, Santa Ana and Windsor green) was similar, and consistent in both year’s data. Colour is a primary indicator of turf playability and aesthetic appearance, and is often an important consideration in selection of a variety for a particular venue. Hence, these results may be helpful for development of guidelines for the use of these tested varieties in different areas of New Zealand.

Turf plots were photographed in their third summer to observe the green up and recovery from cold damage (Fig. 3.13). All of the varieties survived the cold weather but showed signs of browning and winter dormancy to a varied degree. Agridark, Santa Ana, Zenith and Regal Staygreen showed signs of green up in early spring. Santa Ana was a good performer along with Agridark.



Turf Color-2013



Color-2014

**Fig: 3.9 Colour variations of turf mat in glasshouse and open field conditions during Autumn/Winter 2013-2014.** P77=Princess-77, YK=Yukon, LP=La Paloma, AD=AgriDark, SA=Santa Ana, WG=Windsorgreen, SS=Sea Spray, ZN=Zenith, RS=Regal Staygreen.

**May 2013**

**July 2013**

**Fig. 3.10 Progression in cold damage of experimental plots during 2013 under field conditions**

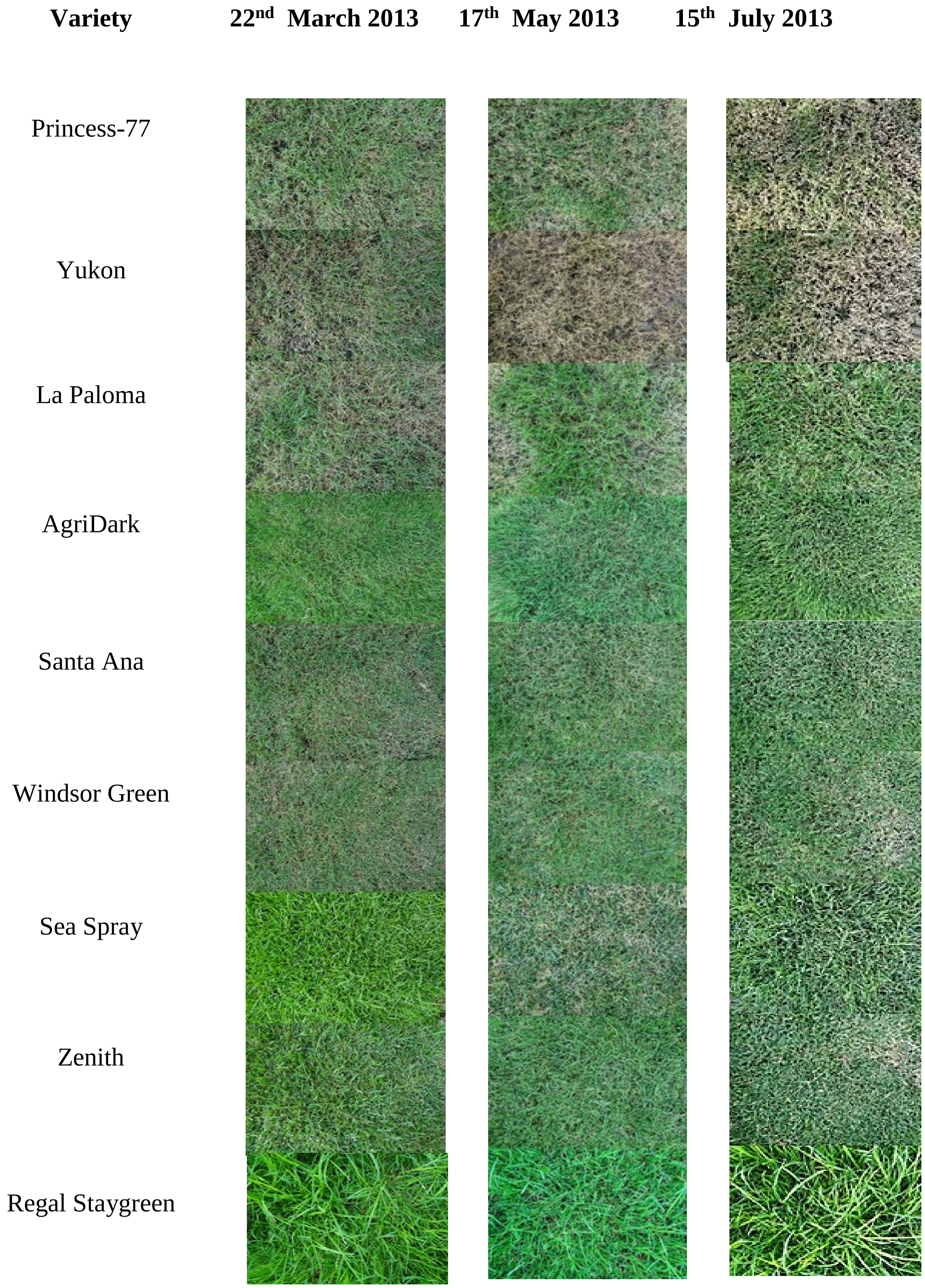


**Fig: 3.11** **Differences in appearance of experimental plots from early autumn to mid-winter (2013) under glasshouse conditions.**

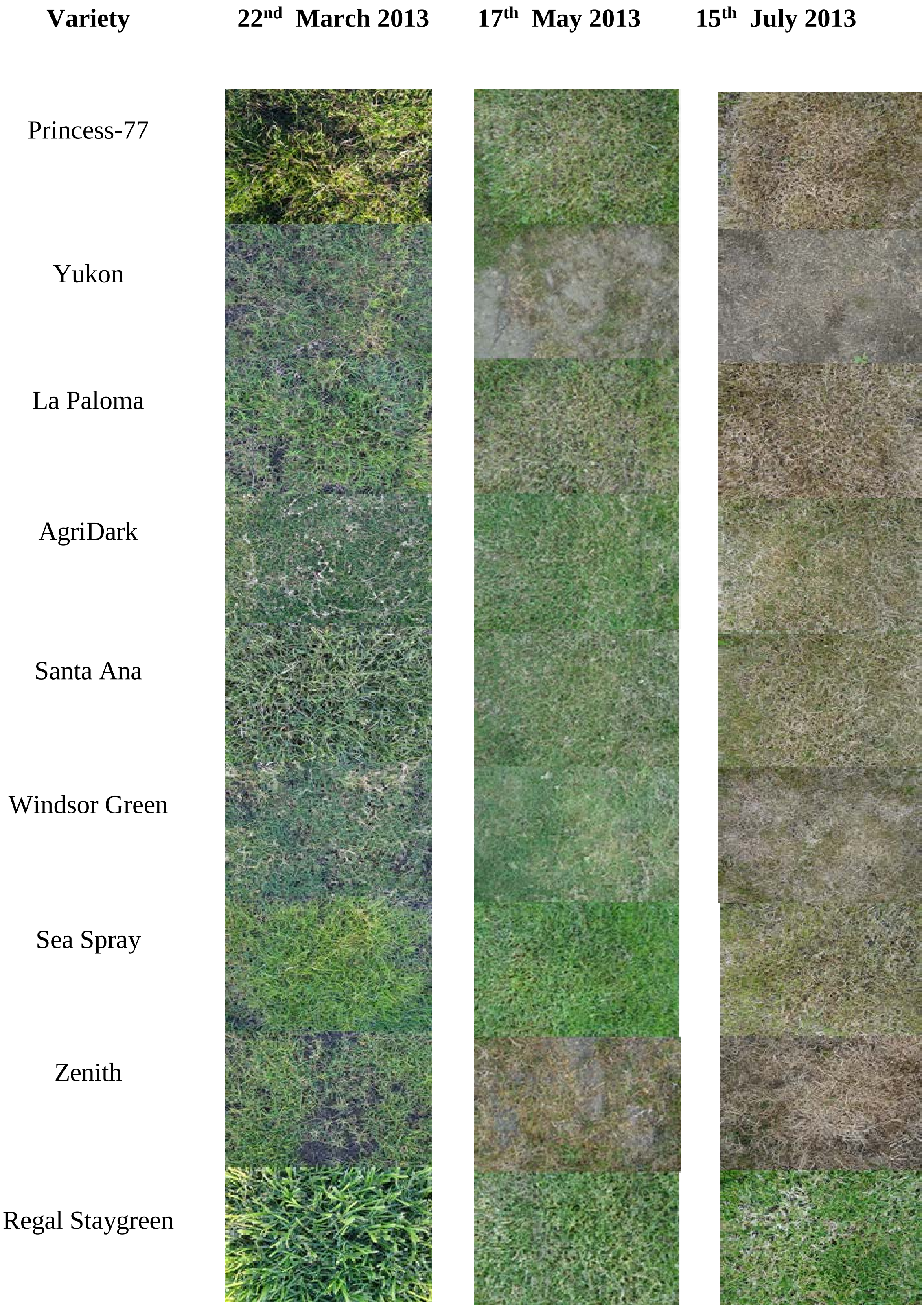


**Fig: 3.12 Differences in appearance of experimental plots from early autumn to mid-winter (2013) under field conditions.**

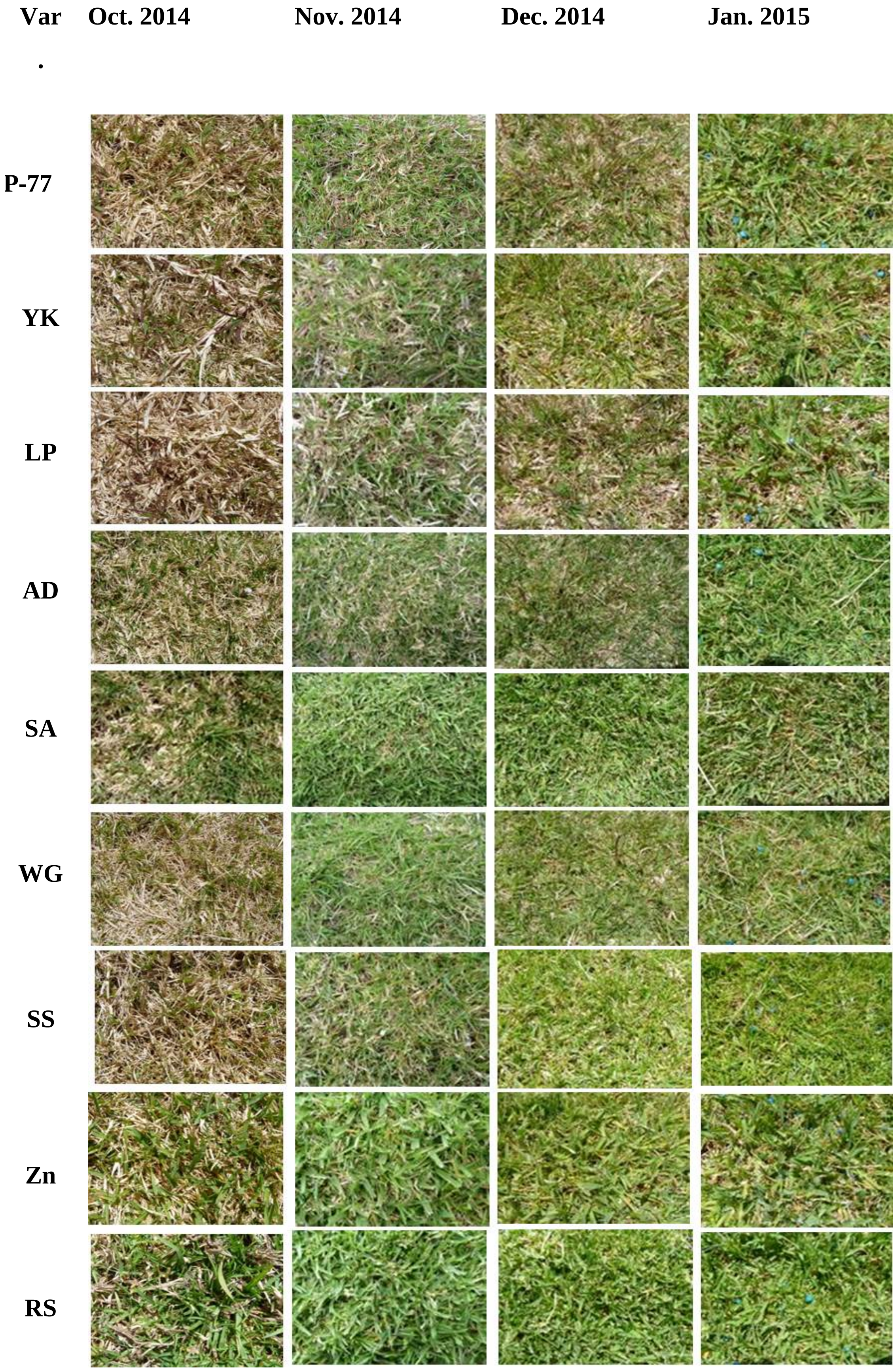


**Fig: 3.13 Visual status of field plots in third summer, showing recovery of colour after winter (October 2014) and retention of colour through summer 2014–2015.**
P-77=Princess 77, YK=Yukon, LP=La Paloma, AD=Agridark, SA=Santa Ana, WG=W.Green, SS=Sea Spray, ZN=Zenith, RS=R.Staygreen

**Photos taken by Dr Andrew Mitchell**

## 3.4 Discussion

The turfgrasses in this study were established during the spring and summer of 2012–2013. Nine different turf varieties of warm season turfgrasses were used in the experimental plots (Table 3.1) collected from different sources. Two different establishment methods were employed (seed, stolon) according to the genetic characteristics and available resources for the different varieties. Daily irrigation was needed, especially on sunny days, when establishing the seeded turfgrasses on a sand carpet. In Palmerston North, overnight temperatures in mid-summer appear to be low enough to seriously check the growth of cold-sensitive turfgrass varieties. In this study, the overnight temperatures during mid-January 2013 were as low as 7–8°C. The varieties most affected by these low temperatures (and possibly also by concurrent desiccation) were Yukon and Zenith, which showed poor survival after removal of the germination covers. Zenith was found most susceptible to low temperature injury.

### 3.4.1 Quality

Bermuda grass (*C. dactylon* varieties) and *Zoysia* are the most popular warm-season turf species because of their finer quality and low maintenance requirements, compared to other C4 grasses. Both are generally established vegetatively, but seeds are commercially available for many varieties for easy and fast establishment (Patton et al., 2004). The quality of turfgrasses can vary widely depending on the species, time of year, and management practices (Morris and Shearman, 2014), which determine their aesthetic appeal and functional values. As shown in the figures and pictures, the quality of the turfgrasses studied here was strongly affected by the decrease in temperature in autumn, and most varieties showed a gradual decrease in quality as the temperatures decreased and with prolonged exposure to low temperatures. Roche et al. (2010) reported similar results from a trial in Australia and concluded that the quality and colour of *Cynodon* and *Paspalum* turfgrasses were significantly affected by decreasing temperatures in winter. They also concluded that Seashore paspalum generally showed better quality and maintained this quality for longer during the winter. Geren et al. (2009) trialled 11 different $C_4$ turfgrass varieties and found that *Cynodon* and *Paspalum* varieties showed high scores for most quality attributes during two growing seasons. The *Paspalum* variety in this study, Sea Spray, also showed high scores for most quality attributes, with good groundcover, a fine texture, and good colour retention over winter. All three *Cynodons* established through vegetative means showed superior quality during the experiment compared to varieties established through seed.

### 3.4.2 Groundcover

In general, groundcover development was better in the glasshouse than in the field. Temperature fluctuation and weather events with temperature dropping below tolerance level may restrict or slow down growth under field conditions which created growth differences in field and Glasshouse plots where temperature was controlled to a minimum (Fig 3.3, 3.4). The vegetative *Cynodon* varieties established groundcover more rapidly than did the seeded varieties, and subsequently maintained good density, especially AgriDark and Santa Ana (the two *C. dactylon* × *transvaalensis* varieties). The groundcover development of Sea Spray was comparable to that of the vegetative *Cynodon* varieties. The faster groundcover establishment of the vegetative *Cynodon* varieties and Sea Spray may be attributed to their rapid and vigorous stolon formation. The seeded *Cynodon* and *Zoysia* varieties showed poor groundcover development and lower percentage groundcover, and some varieties, especially the seeded *Cynodon* varieties, showed significant decreases in groundcover during winter after browning off which indicates winter damage to foliage and loss of vegetative cover. Regal Staygreen was one of the exceptions and performed well under field conditions with a significantly better cover and stable attributes. However the growth was very dispersed and etiolated in the glasshouse which may be due to shade intolerance or response to high humidity conditions.

### 3.4.3 Texture

Once fully established, vegetative varieties of *Cynodon* and Sea Spray exhibited a fine texture, most seeded *Cynodon* varieties showed a medium to fine texture, and Zenith and Regal Staygreen showed a coarse leaf texture. In general, the turfgrasses grown under glasshouse conditions tended to show a coarser texture than that of field-grown turfgrasses. The field-grown turfgrasses showed a higher root density and earlier rhizome formation after establishment, compared with those grown in the greenhouse. Texture is largely genetically determined, but is also affected by environmental factors.

In the glasshouse, Regal Staygreen showed irregular growth, and the plants were elongated with longer leaves. This was attributed to etiolation of stems and leaves, triggered by growth conditions of lower light and increased humidity, compared to field swards. These conditions may also have contributed to the trend towards coarser texture of the other studied turfgrasses in that environment.

Texture of grasses is usually considered to be a genetic factor but as discussed above environmental factors including but not limited to humidity, shade and temperature play an important role in the growth, and hence sward characteristics and appearance of turfgrasses. Experiments would need to be conducted using precisely controlled levels of light and humidity for the different turfgrass varieties to obtain a better understanding of the influence of these factors.

### 3.4.4 Colour

Among all the tested varieties, Regal Staygreen showed the best colour retention during autumn and winter under field conditions and results were consistent over both years. It would be a high-quality surface for low maintenance areas such as roadsides and roughs but would have limited use in some turf applications, because of its coarse nature. As mentioned earlier in Section 3.3.5, glasshouse plots maintained a better colour and showed less degradation during winter months which is to be expected in view of the controlled temperature limits compared with field grown plots. Zenith was the most cold-sensitive variety, and showed the earliest browning off among all the tested varieties as well as low scores for density and cover, and cannot be recommended for use in temperate climates such as Palmerston North, where some winter frost is expected. Patton and Reicher (2007) also found that *Z. japonica* is susceptible to winter injury, along with other species of *Zoysia*. Colour variation is used as an indicator to threshold for winter performance in turfgrasses however along with temperature genetic characteristics, nutrition and use of a particular venue plays important role in final appearance and suitability of any turf type. As mentioned earlier in Section 3.3.5, glasshouse plots maintained a better colour and showed less degradation during winter months which is to be expected in view of the glasshouse minimum temperature being held above 15°C during winter, apart from some exceptions in April 2013, and the daily maximum typically being in the range 19–25°C (Fig 3.4). From the literature these temperatures would not have been expected to cause stress (Section 2.7). The colour loss in some seeded couch plots in the glasshouse in May 2013 was considered at the time to be caused by a fungal infection, with symptoms of Anthracnose infestation diagnosed. However, in April there were some nights when the glasshouse heating did not start as the temperature fell overnight (The problem was quickly rectified.) and inspection of temperature data shows that the temperature of the main body of air in the glasshouse reached as low as 7.5°C (Fig. 3.4). Data from another sensor placed near the ground against a glasshouse wall (data not presented) indicated that some plots would have experienced temperatures a degree lower (6.5°C). Hence it may be that the cumulative effect of several stresses, including the two overnight chilling events in April, and lower light levels in the glasshouse through incomplete solar radiation transmission (not quantified) was a trigger for the fungal infection, and colour loss. From a turf management perspective it is helpful to understand that factors such as colour loss may reflect the cumulative effect of several stresses, and that the impact on the seeded couches might have been exacerbated by their slower establishment after sowing, compared to varieties established vegetatively.

## 3.5 Summary

The aim of screening several different turfgrasses and recording their visual attributes (e.g. quality, colour) was to provide reliable information for turf managers and green keepers. On the basis of the data collected in these trials, it is concluded that the vegetatively propagated *Cynodon* varieties performed significantly better than the seeded *Cynodon* varieties in many quality attributes. Seashore paspalum is a new turfgrass variety that showed promising performance attributes in this trial. The main results of this study can be summarized as follows:

- The turfgrasses in the glasshouse were affected by a fungal (*Anthracnose*) infestation during April 2013. Seeded varieties of *Cynodon* were badly affected by the fungus, but vegetatively produced varieties were more resistant, especially Agridark. *Zoysia*, *Paspalum* and *Pennisetum* also showed resistance to the fungus attack.
- Sea Spray performed well, as indicated by vigorous stolon growth, bright green colour, and reasonable cold tolerance, including retention of colour beyond May. It developed a good mat with excellent turf attributes, compared with most of the vegetatively established *Cynodon* varieties.

- Compared with other varieties, Regal Staygreen (kikuyu) showed superior cold tolerance and retained its green colour better during winter. Its coarse leaf texture meant that it is unsuitable for fine playing surfaces, but it may be suitable for turf applications where fine texture is not a requirement, such as roadsides or golf course roughs.
- Among the tested varieties, Zenith *(Z. japonica*) was the most cold-sensitive, making it unsuitable for use as a turfgrass in cool climates like Palmerston North. However, it may be suitable for use in warmer districts such as Northland.
- Recovery or green up was faster and earlier in Agridark and Santa Ana. Zenith and Regal Staygreen also showed signs of early green up compared to other varieties. All the seeded couches performed relatively poorly in terms of recovery from cold stress.

Published material from this chapter is presented in Appendices 3.3 and 3.4.

## References

Beard, J. B., Green, R. L. (1994) The Role of Turfgrasses in Environmental Protection and Their Benefits to Humans. *Journal of Environmental Quality* 23: 452–460.

Carrow, R. N., Krum, J.M., Flincroft, I., Cline, V. (2010) Precision turfgrass management: challenges and field applications for mapping turfgrass soil and stress. *Precision Agriculture* 11: 115–134. DOI 10.1007/s11119-009-9136-y.

Duble, R. L. 1996. Turfgrasses, their management and use in the Southern zone. Texas A&M University Press, College Station, TX.

Geren, H., Avcioglu, R., Curaoglu, M. (2009) Performances of some warm-season turfgrasses under Mediterranean conditions. *African Journal of Biotechnology* 8: 4469–4474.

Morris, K.N., Shearman, R. C. 1998. NTEP turfgrass evaluation guidelines. National Turfgrass Evaluation Program. In *NTEP turfgrass evaluation workshop, Beltsville, MD* (pp. 1–5. http://www.ntep.org/pdf/ratings.pdf (accessed 11 April 2017).

Morris, K. N. (2003) The National Turfgrass Research Initiative. National Turfgrass Federation, Inc. National Turfgrass Evaluation Program, Beltsville Agricultural Research Center, Beltsville, Maryland, 20705 USA.

Patton, A. J. Reicher, Z. J. (2007) Zoysiagrass species and genotypes differ in their winter injury and freeze tolerance. *Crop Science* 47: 1619–1627.

Patton, A. J., Hardebeck, G. A., Williams, D. W., Reicher, Z. J. (2004) Establishment of bermudagrass and zoysiagrass by seed. *Crop Science* 44: 2160–2167.

Roche, M. (2010) Management Guidelines for New Warm-Season Grasses in Australia. Horticulture Australia limited http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.652.8262&rep=rep1&type=pdf

Ulrich, R. S. (1986) Human responses to vegetation and landscapes. *Landscape and Urban Planning* 13: 29–44 http://dx.doi.org/10.1016/0169-2046(86)90005-8.

Volterrani, M., Grossi, N., Pardini, G., Miele, S., Gaetani, M., Magni, S. (1997) Warm season turfgrass adaptation in Italy. *International Turfgrass Society Research Journal*, *8*: 1344–1354.

Zhou, S., Abaraha, A. (2007). Response to heat stress in warm season and cool season turfgrass cultivars. *Scientific Research and Essays*, *2*: 95–100.

# CHAPTER 4

# Morphological Attributes of Different Warm Season Turfgrasses

## 4.1 Introduction

An understanding of plant morphology is helpful to develop effective management plans against different stress factors. Many grass species, especially cool season grasses exhibit what is referred to as ‘bunch type’ growth, with more or less vertically orientated tillers developing from a main crown and daughter or branch tillers orientated in the same direction as their parent to form tufts. The way in which plant parts are organised for function as a whole has been referred to as a ‘body plan’ (Niklas and Kutschera, 2009). A variation of the plant body plan is that in some grasses a bud can grow laterally to form a stem along the soil surface known as a stolon, alternatively these lateral stems can grow belowground, in which case they are called rhizomes. Rhizomes lack chlorophyll and their leaves are typically reduced to scales at each node. After a period of underground extension growth, their tip may sometimes turn upwards and produce leaves or a flower after emerging some distance from the shoot that generated it. These specialized reproductive stems help plants to spread horizontally and survive under unfavourable or stress conditions. Warm season turf typically displays a spreading nature, since they typically possess stolons or rhizomes, or both. The horizontal stems migrate across the soil surface by internode elongation and are capable of producing roots and shoots at individual nodes. Along with other differences it is clear that warm season turfgrasses usually exhibit deeper root systems compared with cool season grasses (Christians, 2011). However, while the horizontally spreading growth habit of the warm season grasses gives them different turf mat characteristics from the $C_3$ grasses, historically more familiar in New Zealand, there has been little research that quantitatively describes the contributions of leaf and stolon or rhizome material to the turf mat.

Warm season or $C_4$ turfgrasses are widely grown in tropical regions of the world and even sometimes in temperate regions, where hot summers are experienced (Geren et al., 2009). In modern turf breeding, many commercial $C_4$ grass cultivars do not have the capacity to produce seeds, often as a result of development through interspecies hybridisation or artificially induced polyploidy. Turf managers frequently report that seeded varieties exhibit poor mat quality, an open growth habit and less traffic resistance, compared to vegetatively propagating varieties of the same species (Geren et al., 2009).

*Cynodon* (bermudagrass, couchgrass) is the most common and wide spread genus among the warm season group of grasses (Taliaferro, 2003) and is extensively used for golf courses and athletic surfaces but *Cynodon* varieties often lack winter hardiness and so are vulnerable to frost damage or winter kill (Munshaw et al., 2006). Tolerance level to cold, while partly mediated by physiological factors, is also influenced by grass morphology. A positive correlation can be found with stolon length, stolon size and rhizome length (Ahring et al., 1975). Bermuda grass cultivars with increased cold tolerance are mostly selections of clonal (vegetatively propagating) material from cooler regions which were further hybridized with superior mat forming cultivars (Samudio and Brede, 2002). As mentioned in Section 2.5.2, three species of Zoysiagrass (*Zoysia japonica, Zoysia matrella, Zoysia pacifica*) are used in the turf industry. *Z. japonica* is considered most tolerant to low temperature stress and to have a superior shade tolerance compared to *Cynodon* (Zhang et al., 2009; Engelke and Anderson, 2003).

As discussed, there has been little previous detailed analysis of turf mat structure for $C_4$ grasses. Therefore, an experiment was designed with the following objectives:

1. Study of morphological attributes for different warm season turf species for better understanding of growth pattern, to provide information to green keepers.
2. Comparison between field grown (exposed) and glasshouse (protected) turf for growth differences, especially differences in proportion or structure of the various specialized structures.
3. To explore branching pattern and stolon morphology of different warm season turf species.

## 4.2 Materials and methods

This chapter reports a further self-contained study using turf cores collected from experimental plots described in Section 3.2.1.

### 4.2.1 Collection of turf cores

Cores of established turf were randomly collected from the ‘2012 plots’ during June 2013 and again in February 2014. Cores were extracted using a standard golf hole cup cutter (D=108 mm and A=9156 mm$^2$) from both locations (Glasshouse, Field Plots). On extraction, cores were wrapped in cling-film plastic to retain their structural integrity. Core samples were stored at 4°C for further analysis. Morphological attributes of green mass, dead mass, root mass, leaf weight, stem and stolon weight, rhizome weight, turf height, total stolon length and rhizome length were recorded on the basis of dry weight in g/m$^2$.

### 4.2.2 Data collection

Plant material within the turf cores was dissected and sorted into morphological units separately for above ground and below ground strata by using sharp scissors, the turf mat was carefully cut off at the soil surface, keeping all the aerial parts and stolons and their connections to each other intact. This turf mat section was further separated into dead mass and green mass. Any dead or decayed leaves and thatch was separated and green mass was again separated into leaves, vertical branches and stolons. Each segment was individually placed into paper bags and labelled and dried in a fan forced oven at 60ºC. Dried components were weighed individually and converted to g/m$^2$ for further analysis and presentation. To capture data on varietal differences in sward height, turf height was measured to help interpret segmental morphology data. For this purpose a ‘rising plate meter’ commonly used to assess pasture feed supply for grazing animals (Litherland et al., 2008) was used. This device measures the distance a 30 cm x 30 cm aluminium plate is lifted off the ground by the turf beneath it, in steps of 5 mm. For each plot, the recorded height was the mean of 30 ‘drops’ per plot with the rising plate meter.

### 4.2.3 Data analysis

Results were analysed using independent sample t-tests ($P \leq 0.05$) to determine any statistically significant difference between two locations and one way ANOVA was used ($P \leq 0.05$) to check the various measured morphological parameters for differences between varieties in respective months. Analyses were performed using Microsoft Excel 2010 and SPSS 21 (Statistical Package for Social Sciences). Outputs were prepared graphically and are discussed in detail below.

**Fig. 4.1 Extraction of turfgrass cores (A) and cling-film wrapped turfgrass cores (B).**

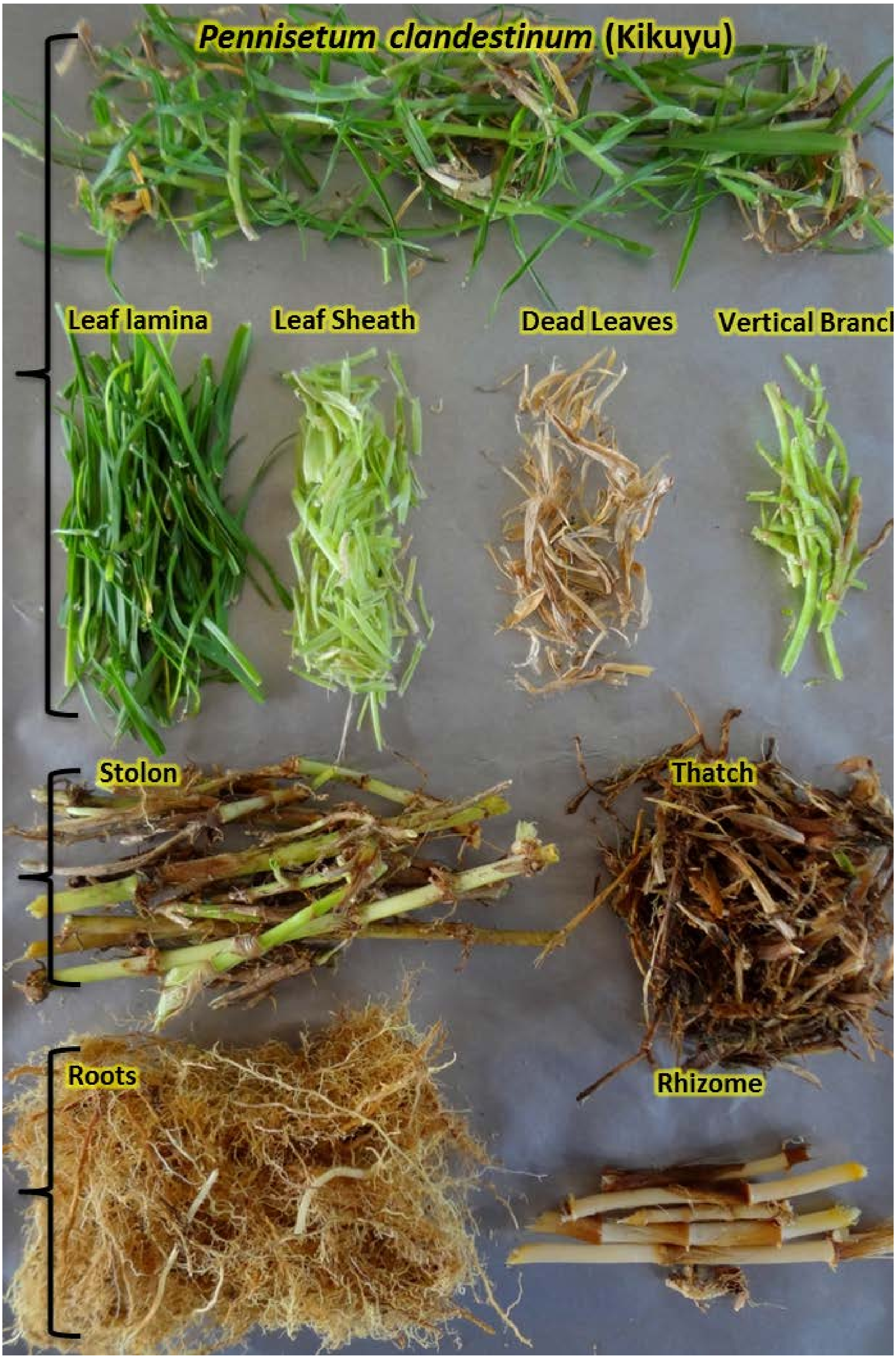

*Pennisetum clandestinum* (Kikuyu)
Leaf lamina
Leaf Sheath
Dead Leaves
Vertical Branc
Stolon
Thatch
Roots
Rhizome

**Fig. 4.2 A typical grass core dissection, in this case Regal Staygreen (*P. clandestinum*).**

## 4.3 Results

### 4.3.1 Green mass, root mass and dead mass (thatch)

#### June 2013

Figure 4.3 shows the varietal differences in total dry green mass, root mass and dead mass weight for plots in the glasshouse at PGU, Massey University (controlled conditions) and in field plots at NZSTI (outdoor conditions). Green mass was mostly higher in the glasshouse, compared to field plots except for Yukon. Further Agridark, Windsor green, Zenith and Regal Staygreen showed significantly higher green mass in the glasshouse compared to the field plots. Regal staygreen showed a significant ($P<0.05$, Appendix 4.1) difference in turf mat structure compared with all other varieties. La paloma, Santa Ana, Windsor green and Zenith could be grouped as having similar mat composition, while Yukon showed differences in structure.

All varieties (Fig. 4.3) exhibited higher dead mass in the field plots than in the glasshouse, except for Agridark. Furthermore, Regal Staygreen showed significantly ($P<0.05$) higher dead mass in the field plots, compared to the glasshouse. Plots grown under glasshouse conditions had mostly non-significant differences between varieties, however Agridark had significantly ($P<0.05$) higher accumulation of dead mass than Yukon, La Paloma, Sea spray and Regal Staygreen ($P<0.05$, Appendix 4.1). Similarly, differences among varieties in field plots were non-significant. Regal Staygreen showed higher thatch accumulation under field conditions and was significantly higher than Agridark, Windsor staygreen and Zenith.

Comparatively higher values for root mass were observed under field conditions than in the glasshouse and for most varieties the difference was statistically significant. Yukon had the lowest root mass in both field condition and glasshouse conditions (Fig. 4.3). In field plots, root mass did not differ between varieties, however in the glasshouse Zenith had significantly ($P<0.05$) higher root mass than other varieties except La paloma, Sea spray and Regal staygreen (Fig. 4.3, Appendix 4.1).

#### February 2014

Figure 4.4 shows the comparison of green mass, dead mass and root mass from samples collected in February 2014. All the varieties showed increased values for all three components compared to samples collected in June 2013. Contrasting to the previous sampling, field plots had developed higher green mass compared to the glasshouse plots. Further segregation showed (Section 4.3.3) an increase in stolon volume was the main reason for higher green mass under field conditions.

All varieties had increased thatch under field conditions, especially the vegetatively propagated *Cynodon* varieties which had a significant (P<0.05) increase compared to the other grasses. By comparison, thatch accumulation was comparatively very low in glasshouse plots, where Santa Ana accumulated maximum thatch layer followed by Agridark. Root mass had also increased under both conditions. However, all the field grown varieties developed better root mass compared to the glasshouse grown plants except for Yukon. The highest root mass was recorded by Zenith.

### 4.3.2 Stolon length and rhizome length

#### June 2013

Rhizome density was generally higher in field plots than in the glasshouse (Fig. 4.5), and differences were statistically significant for Agridark and Windsor green. Further, only Agridark showed rhizome growth underglasshouse conditions, while Agridark and the two other vegetatively propagated *Cynodon* varieties displayed rhizome growth in the field plots. The results show the rapid ability of these three varieties to develop and grow rhizome compared to others. Results of statistical analysis of stolon and rhizome density can be found in Appendix 4.2.

#### February 2014

Rhizome and stolon density was higher under field conditions (Fig 4.6) compared to June 2013 samples and glasshouse grown grasses. All the varieties, except Zenith, produced rhizomes under field conditions whereas, none of the seeded *Cynodon* varieties developed rhizomes under glasshouse conditions. Zenith developed rhizomes under glasshouse condition. Stolon density was significantly higher under field conditions especially in the vegetatively grown varieties of *Cynodon*. Santa Ana had the highest value of 1465 cm/m$^2$, followed by 1062 cm/m$^2$ recorded for Agridark. The values were significantly ($P<0.05$) lower in glasshouse plots where the highest measurement of 449 cm/m$^2$ was recorded for Agridark.

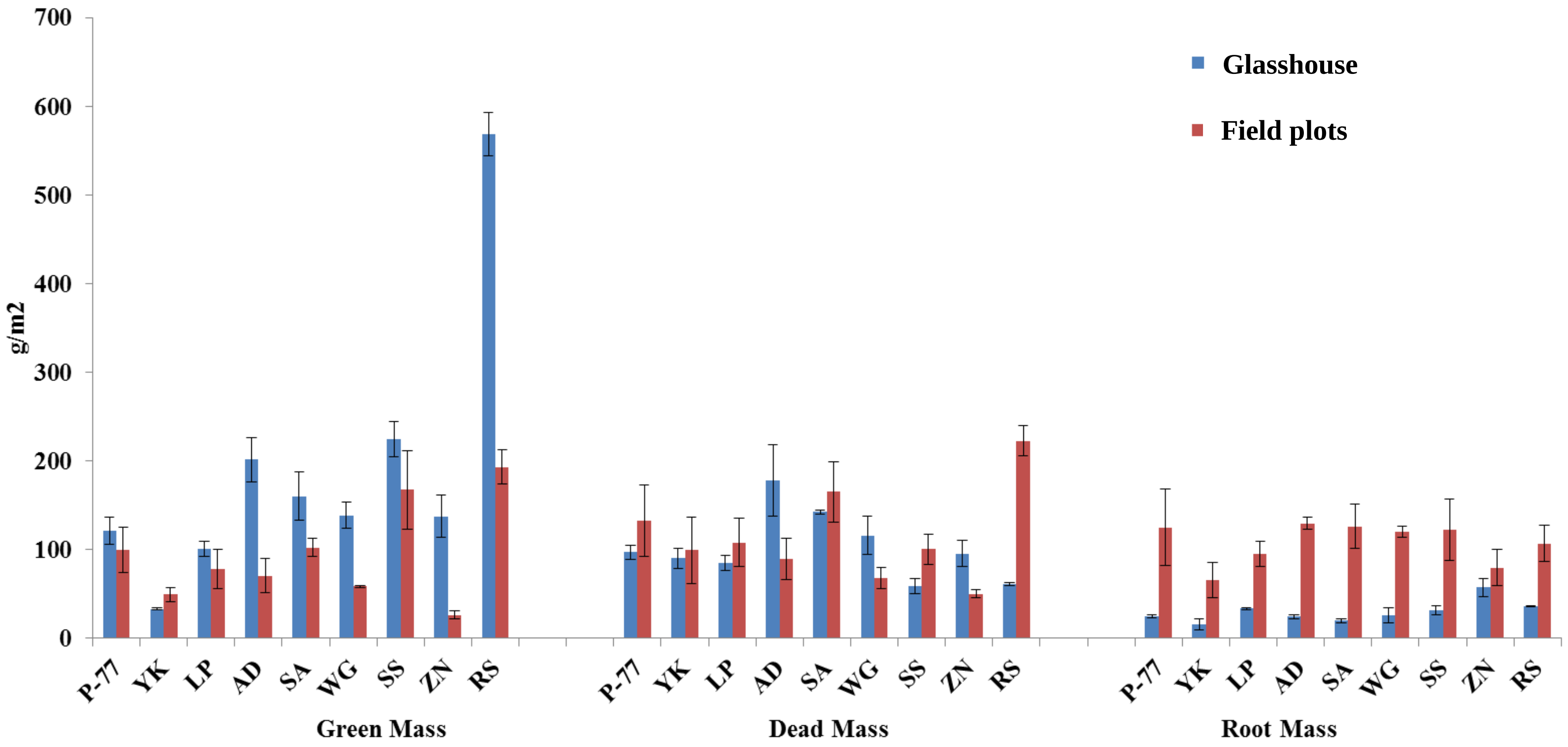


**Fig. 4.3 Dry weight comparison of green mass, dead mass and root mass in glasshouse and field plots - June, 2013**
P 77=Princess 77, YK=Yukon, LP=La Paloma, AD=Agridark, SA=Santa Ana, WG=Windsor green, SS =Sea spray, ZN = Zenith, RS=Regal Staygreen

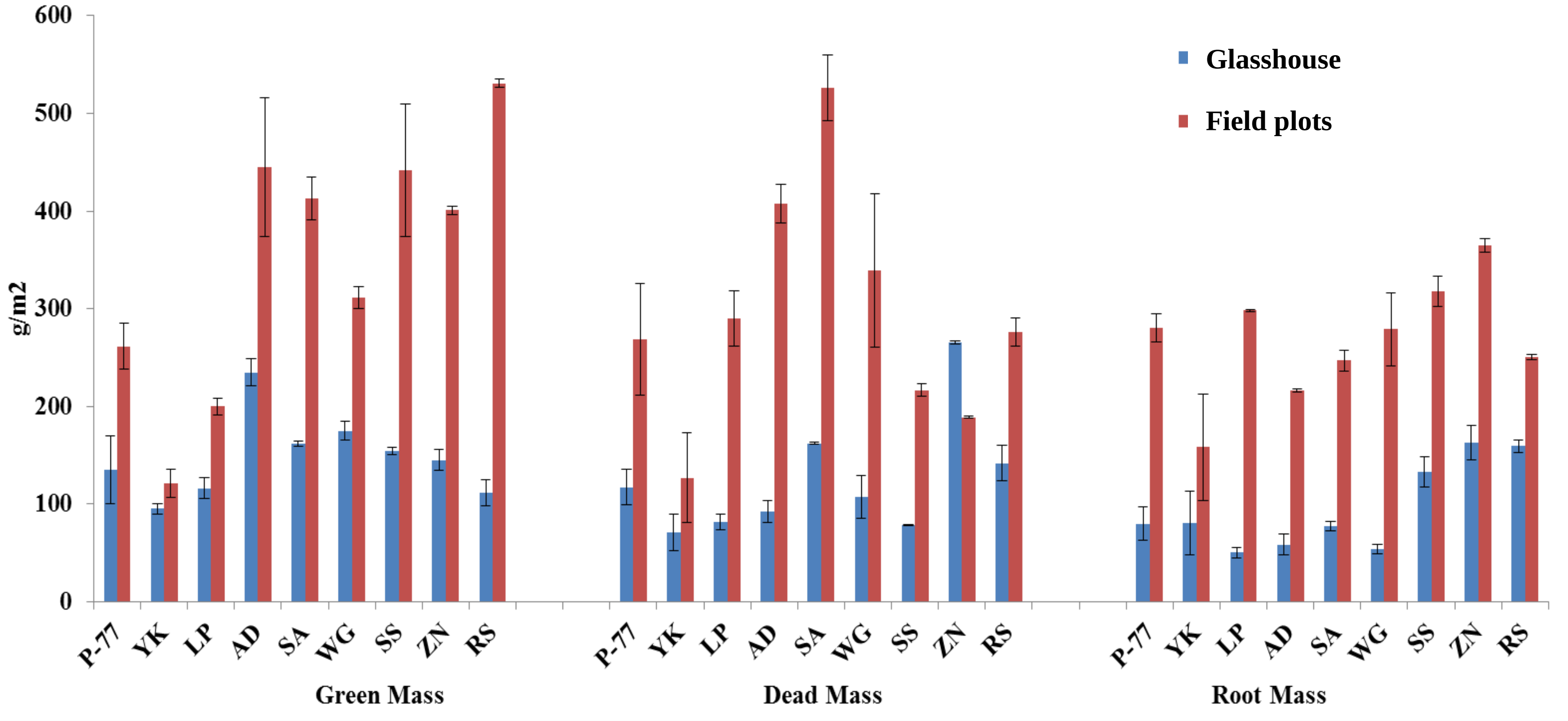


**Fig. 4.4 Dry weight comparison of green mass, dead mass and root mass in glasshouse and field plots - February, 2014**
P 77=Princess 77, YK=Yukon, LP=La Paloma, AD=Agridark, SA=Santa Ana, WG=Windsor green, SS =Sea spray, ZN = Zenith, RS=Regal Staygreen

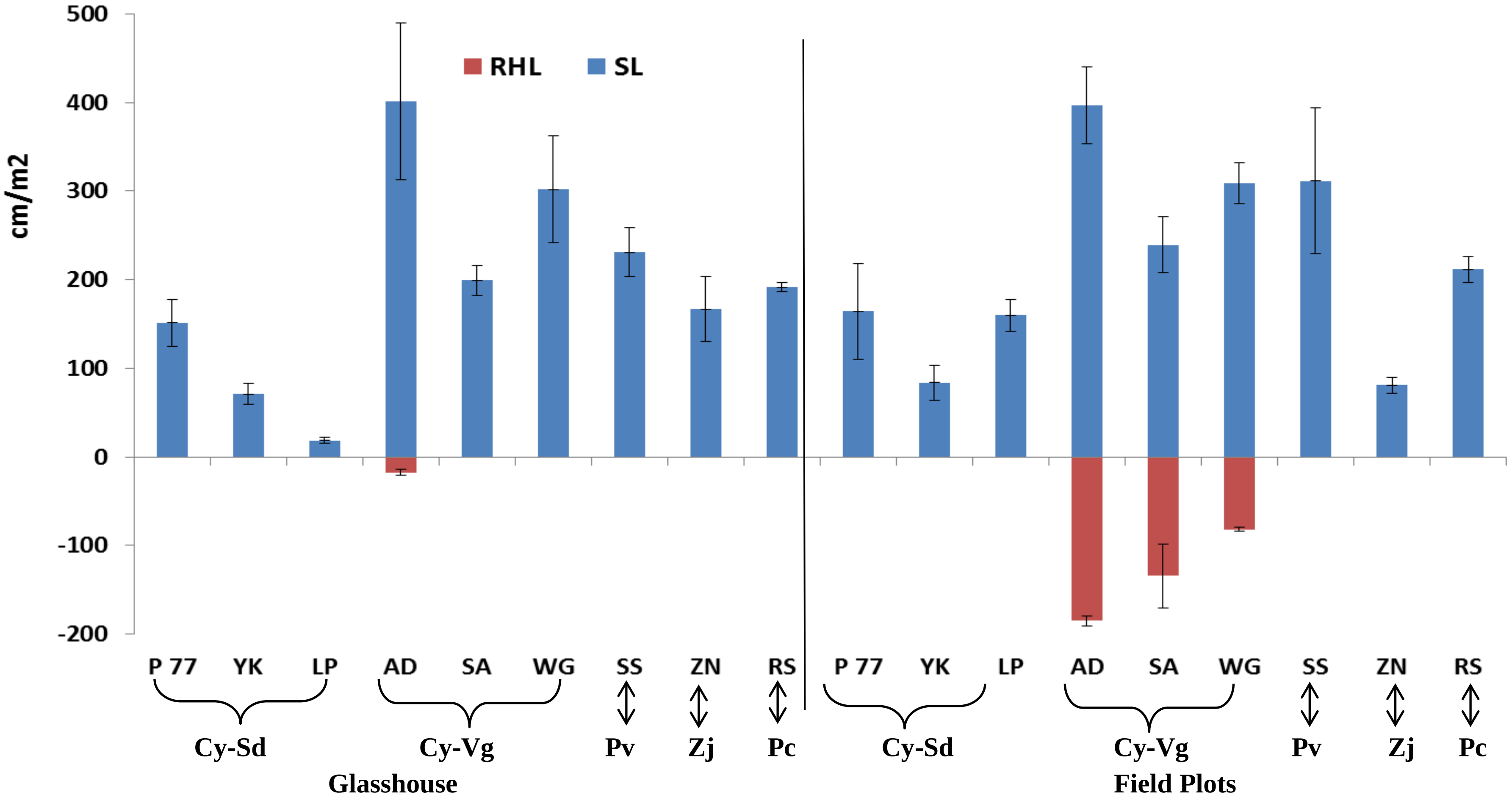


**Fig. 4.5 Comparison of stolon length and rhizome length in glasshouse and field plots June, 2013.**
RHL= Rhizome Length, S =Stolon Length and SD=Stolon diameter
Cy-Sd=Seeded *Cynodon*, Cy-Vg=Vegetative Cynodon, Pv=*Paspalum vaginatum*, Zj=*Zoysia japonica*, Pc=*Pennisetum clandestinum*
(P-77=Princess 77, YK=Yukon, LP=La Paloma, AD=Agridark, , SA=Santa Ana, WG=Windsor Green, SS=Sea Spray, ZN=Zenith, RS=Regal Staygreen)

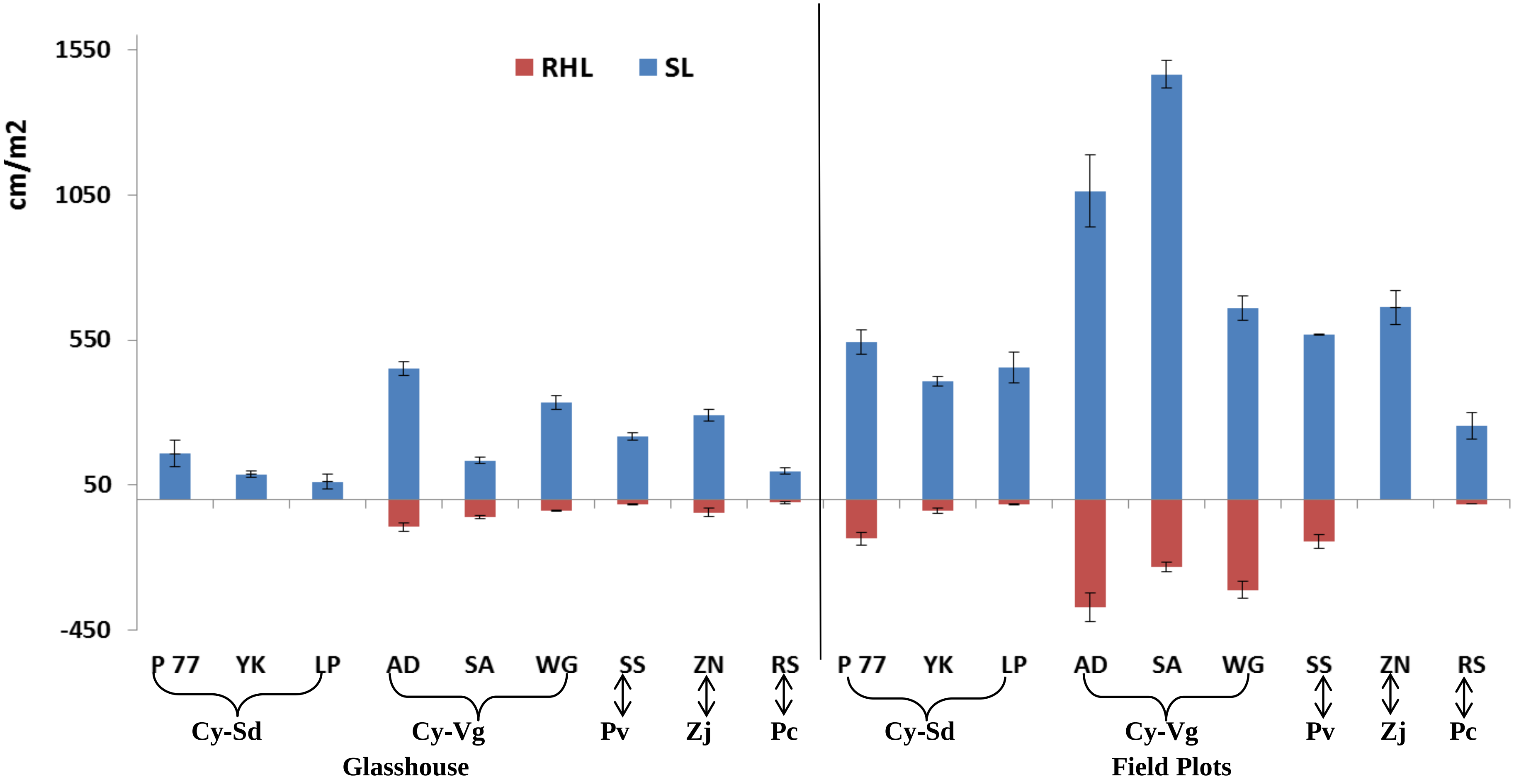


**Fig. 4.6 Comparison of stolon length and rhizome length in glasshouse and field plots - February, 2014.**
RHL= Rhizome Length, S =Stolon Length and SD=Stolon diameter
Cy-Sd=Seeded *Cynodon*, Cy-Vg=Vegetative Cynodon, Pv=*Paspalum vaginatum*, Zj=*Zoysia japonica*, Pc=*Pennisetum clandestinum* (P-77=Princess 77, YK=Yukon, LP=La Paloma, AD=Agridark, , SA=Santa Ana, WG=Windsor Green, SS=Sea Spray, ZN=Zenith, RS=Regal Staygreen)

### 4.3.3 Above ground attributes

#### *4.3.3.1 Turf height*

Turf height is an important characteristic in varietal selection for a particular venue. An evident difference was found between glasshouse and field plots for turf height. All species showed significantly greater turf height under Glasshouse conditions, compared to field plots except La Paloma (Fig. 4.7). Regal Staygreen grew tallest in the glasshouse making it undesirable for turf purposes. Regal Staygreen was tallest among the field grown varieties as well. Seeded varieties of *Cynodon* exhibited overall low turf height compared with vegetatively established varieties. Yukon was shortest in the field grown plots, while La Paloma was shortest under glasshouse conditions.

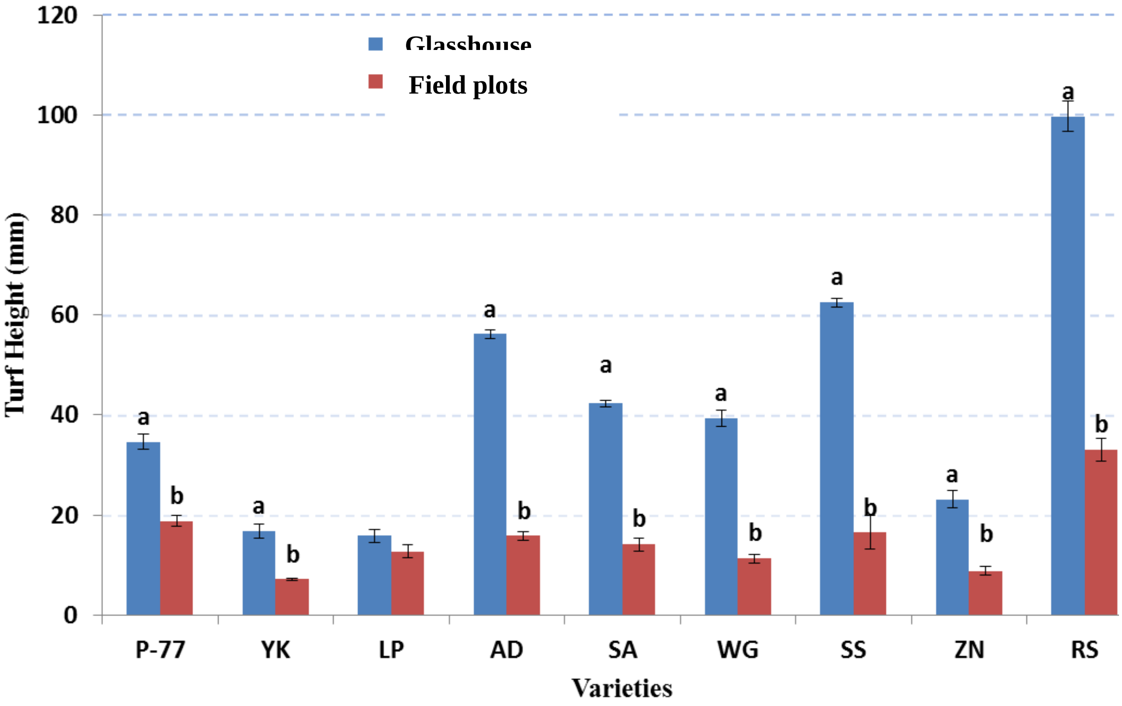


**Fig. 4.7 Comparative turf height of field and glasshouse plots (before core extraction)**

P-77=Princess 77, YK=Yukon, LP=La Paloma, AD =Agridark,, SA=Santa Ana, WG=Windsor Green, SS=Sea Spray, ZN=Zenith, RS=Regal Staygreen

#### *4.3.3.2 Stem/stolon*

**June 2013**

Compared to the glasshouse, in the open field plots, all varieties showed higher stem and stolon density values except for Zenith which accumulated more stem and stolon in the glasshouse than in the field plots (Fig 4.8).

**February 2014**

Stolon mass was dominant component in segregated turf in the February samples. All varieties showed higher stem and stolon weight under field conditions compared with glasshouse. Sea Spray had the maximum stolon weight followed by the *Cynodon* varieties under field conditions. Yukon showed the lowest stem and stolon weight under both field and glasshouse conditions (Fig. 4.9).

#### *4.3.3.3 Vertical branches and leaves*

**June 2013**

Vegetatively propagated *Cynodo*n varieties, Zenith, and Regal staygreen all formed significantly higher ($P<0.05$) vertical branch and leaf tissues under glasshouse conditions, than in the field. However, the seeded *Cynodo*n varieties, grown under field conditions produced greater vertical branch and leaf densities compared to the same varieties grown under glasshouse conditions (Fig. 4.8).

**February 2014**

All varieties showed better formation of vertical branches and leaves under field conditions except for Zenith where vertical branches were found to be higher in the glasshouse plots (Fig. 4.8). Zenith and Regal Staygreen had the highest leaf weights under both growth conditions (Fig. 4.9).

### 4.3.4 Below ground attributes

#### *4.3.4.1 Roots*

**June 2013**

Root mass was significantly ($P<0.05$) higher in all varieties grown under field conditions compared than when grown in the glasshouse. Zenith exhibited a higher mass in the glasshouse plots than other varieties but the second lowest root mass  in the field plots (Fig 4.8).

**February 2014**

By February 2014, the field plot grown grasses had developed better root mass compared with glasshouse grown one. All varieties showed higher root mass value under field conditions. Zenith showed maximum root mass under both conditions followed by Sea Spray in field and Regal Staygreen in glasshouse (Fig. 4.9).

#### *4.3.4.2 Rhizomes*

**June 2013**

In this sampling, just over 6 months after planting, it was notable that rhizomes were observed only in the vegetatively propagated *Cynodon* varieties, and mainly in field plots. Agridark was the most prolific rhizome producer, and the only variety to produce any rhizomes under glasshouse conditions.

**February 2014**

By the sampling in February 2014, all the varieties had formed rhizomes under field conditions with the exception of Zenith. Highest rhizome weight was found for Agridark followed by Windsor green. Of nine varieties included in this study only the seeded *Cynodon* varieties were found not to have developed rhizomes under glasshouse conditions (Fig 4.9.).

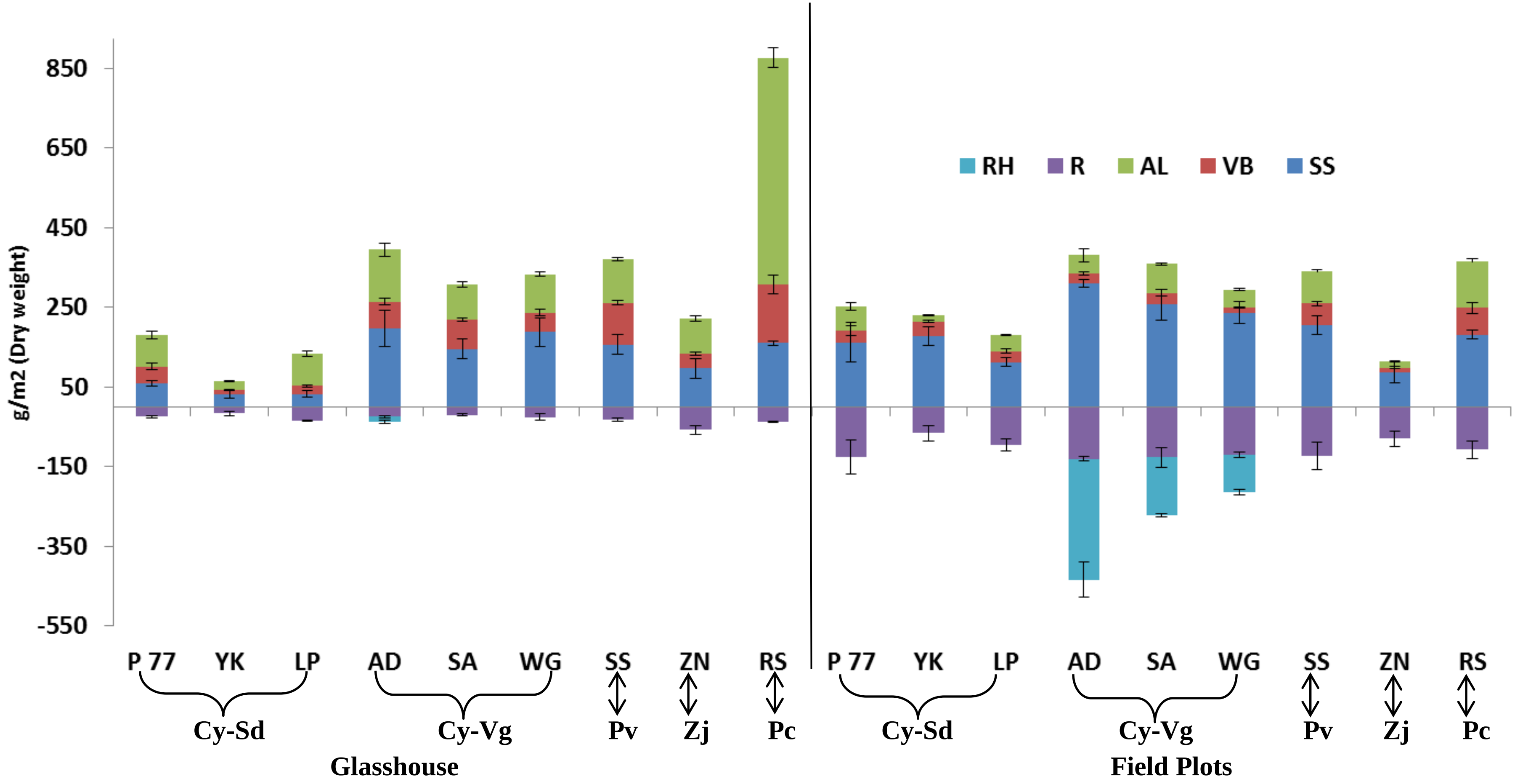


**Fig. 4.8 Dry weight comparison of structural components (leaf, vertical branches, stems/stolons, roots, and rhizomes) in June 2013.**
RH=Rhizome, R=Root, AL=Actual leaf, VB=Vertical Branches, SS=Stem/Stolon)
Cy-Sd=Seeded *Cynodon*, Cy-Vg=Vegetative Cynodon, Pv=*Paspalum vaginatum*, Zj=*Zoysia japonica*, Pc=*Pennisetum clandestinum*
P-77=Princess 77, YK=Yukon, LP=La Paloma, AD=Agridark, SA=Santa Ana, WG=W.Green, SS=Sea Spray, ZN=Zenith, RS=R.Staygreen

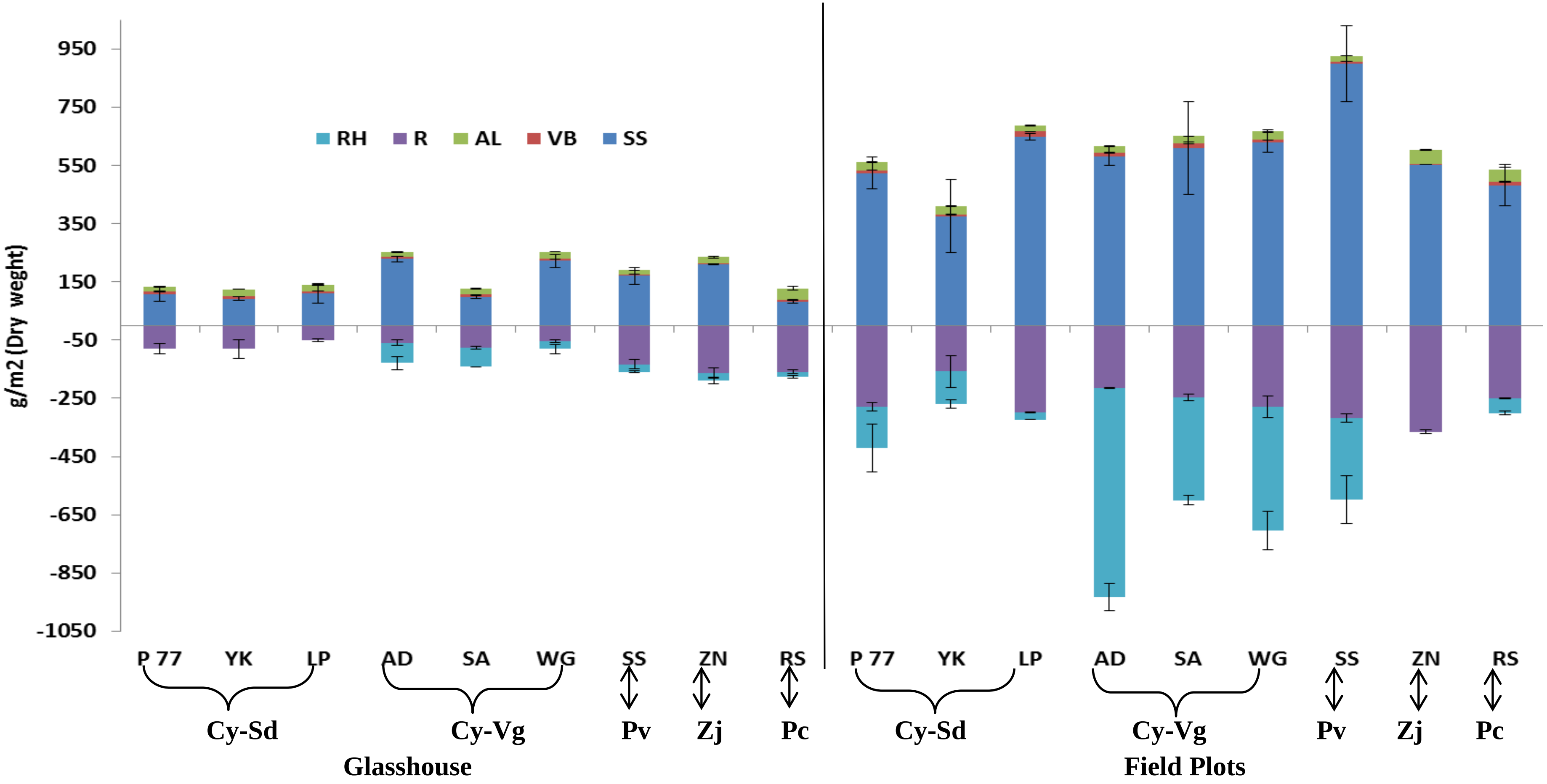


**Fig. 4.9 Dry weight comparison of structural components (leaf, vertical branches, Stem/Stolon, roots, and rhizome) in Feb 2014.**
RH=Rhizome, R=Root, AL=Actual leaf, VB=Vertical Branches, SS=Stem/Stolon)
Cy-Sd=Seeded *Cynodon*, Cy-Vg=Vegetative Cynodon, Pv=*Paspalum vaginatum*, Zj=*Zoysia japonica*, Pc=*Pennisetum clandestinum*
P-77=Princess 77, YK=Yukon, LP=La Paloma, AD=Agridark, SA=Santa Ana, WG=W.Green, SS=Sea Spray, ZN=Zenith, RS=R.Staygreen

## 4.4 Discussion

### 4.4.1 Root mass, rhizome length and dead material (thatch)

The results show that under field conditions, $C_4$ grass plants allocated more resources to development of their underground parts than the same plants grown in the glasshouse. A major difference in allocation between plant parts in different growing conditions was unexpected, but the reasons for it are not easy to discern. Once the plots were mature and well established (2nd year) it was found that field plots maintained significant values for root mass and rhizome length. The glasshouse environment had warmer temperatures (Figs. 3.3 and 3.4), and also had a spray irrigation system, and likely also higher humidity (not measured), both of which would have protected plants from water deficit. There would also have been other differences in the microenvironment between field plots and the glasshouse, such as lower insolation and UVB levels in the glasshouse. Therefore, caution is needed in considering what the environmental trigger eliciting this response might be. Root density and rooting depth play a critical role in maintaining water supply to plants. Among different varieties *Cynodon* varieties exhibited higher root mass compared to other species. In a field experiment Rimi et al. (2012) reported better root length density in *Cynodon* compared to *Zoysia* grown under same conditions. Various turfgrass species have the known ability to develop deeper root profiles in response to moisture availability (Huang, 1999). Comparatively low root mass in the glasshouse can be attributed generally to plant adaptation to regular irrigation and more stable temperature. A point to be aware of for turf managers then, is that where plants are stimulated to invest their mass mainly in shoot growth they will likely then be more vulnerable to desiccation or other periodic stress that may occur, so further study to better understand the factors responsible for variation in root allocation would be useful.

Stolons and rhizomes are considered as different organs but having the same plant ecological functions (vegetative spread and reciprocal transfer of nutrients, substrates and metabolites between roots and leaves) and therefore homologous to each other (Dong and Kroon, 1994). Even so, rhizomes will be discussed here as a below-ground structure and stolons below as above-ground structures. Rhizomes are cylindrical, straight shoots similar to stolons, but their tip travels below the soil surface and they typically have shorter internodes and therefore comparatively more number of buds per unit length compared to stolons (Dong and Kroon 1994). Stolons have the capacity to sense and react to above ground conditions and rhizomes can sense below ground conditions and have similar morphological plasticity to stolons (Hutchings, 1988). As noted above, all vegetatively grown *Cynodon* varieties developed rhizomes under field conditions but in the glasshouse only Agridark developed rhizomes, and they were few in number with shorter lengths. This result shows a functional difference and morphological plasticity between seeded and vegetatively propagated *Cynodon* varieties, with the vegetative varieties showing an ability to develop and grow rhizomes more rapidly compared to seeded *Cynodon* varieties, even under less than ideal growing conditions. Vegetatively propagated *Cynodon* varieties also have better rhizome forming ability compared with other warm season turf species tested (*Paspalam*, *Zoysia* and *Pennisetum*). It also shows that turf has strong ability to modify and develop specialized structures in accordance with the conditions they are exposed to. When looking at second year data all varieties under field conditions developed rhizomes except for Zenith, which was also found to be the most susceptible variety to cold damage. However, it produced rhizomes under glasshouse conditions which can be again considered as the threshold of morphological plasticity and that field conditions were stressful enough for this variety to underperform with regards to the development of rhizomes. Similarly, the seeded *Cynodons* failed to produce rhizomes under glasshouse conditions which can probably be attributed to their poor maturity under diffused light and humid conditions of glasshouse. Dong and Pierdominichi (1995) in an experiment on *Agrostis*, *Cynodon* and *Holcus* inferred that higher light levels influence and increase branching in both stolons and rhizomes and, stolons exhibit longer internodes under low light conditions. Low light levels were the main factor found to decrease or inhibit the initiation of rhizomes in *Cynodon* but, considerable increase in stolon length was recorded (Dong and de Kroon, 1994).

A factor not explored in this study, is the extent to which differences in rhizome formation confer positive performance attributes such as wear resistance (Shildrick, 1974) of the turf mat, or negative attributes such as increased thatch deposition. This point also merits further study.

Excess of accumulated thatch creates major issues in turf quality management (Murray and Juska, 1976) and is an undesirable character for amenity turf varieties. In this study, thatch accumulation or dead mass was found to be high in most of the varieties (Appendix 4.1) under field conditions, which indicates early senescence of leaves due to more exposed and stressing conditions. Thatch accumulation increased in all varieties with the progression to the second year of growth and it was significantly ($P<0.05$) higher under field conditions compared to glasshouse. Higher green mass under glasshouse conditions in the first year of this study could possibly be a result of diffused light conditions, stable temperature, less day and night temperature differences and high humidity.

### 4.4.2 Above -ground attributes

#### *4.4.2.1 Turf height*

Sward height and architecture played a key role in defining turf quality as discussed in the previous chapter. Turf height can be used to explore many key processes in growth and performance of turfgrasses (Stewart et al., 2001). Dense, short turf is considered ideal for most playing surfaces and for aesthetic surfaces. Vertical and etiolated growth patterns exhibited by turf plots grown in glasshouse especially in Regal Staygreen raised issues with quality and use of this particular species under low light, moist, and warm conditions, though these negative growth features might have been overcome by more frequent mowing. With respect to stolon and turf height, seeded *Cynodon* varieties showed less lateral growth and their low quality was consistent and has already been discussed in the previous chapter (3.4.1). Field performance for turfgrasses was quite uniform with the exceptionally low height in Yukon (Fig 4.5) which also has poor development and less ground cover percentage (Fig. 3.6) making it undesirable for locations such as Palmerston North. Regal Staygreen developed maximum height under field conditions and as discussed earlier (Section 3.3.4) because of its coarse leaf and stem structure, this variety is not suitable for finer turf surfaces. Among the vegetatively grown couches, Windsor green developed a good mat with comparatively low height. As described by Jiang et al. (2004) grass varieties with high turf quality and characteristics tend to retain their ranking when grown under different conditions. Results of this experiment also indicate similar changes throughout the experiment, with comparable differences in turf height between varieties under the two different growing conditions.

#### *4.4.2.2 Stem and stolon*

Stolons are very important structures in colonizing turf species which serve as growing units under stress or unfavourable conditions and also as storage organs (Dong and Kroon, 1994). Stolons are a collection of phytomers and as such bear all the normal component parts of a grass phytomer (Section 2.6): leaf, leaf sheath, nodes, internodes and auxiliary buds under the sheath (Moore and Moser, 1995). Stolons form as a result of elongation of the internode segments of the phytomers involved. As with rhizome formation discussed above, in this experiment, stem and stolon formation was also higher in field plots than glasshouse plots, and discussion above for rhizomes is also largely applicable to stolons. Exposed field plots continued to have greater development of specialized stolon structures in the second year of growth as well. As discussed previously in Sections 4.4.3.1 and 4.4.2 glasshouse plots exhibited increased turf height and upright growth pattern. This may be an effect of diffused light conditions, warmer temperatures and humid conditions. However, what is clear is that under more favourable conditions in the glasshouse, plants decreased the development of specialized structures which are helpful for stress tolerance. Results from this experiment are consistent with the findings of Dong and Pierdominichi (1995), who concluded that turf exposed to higher light levels produces more stem and stolon mass and low light levels significantly affect stolon formation in *Cynodon*.

#### *4.4.2.3 Vertical branches*

As with previously discussed above-ground parameters, a higher dry mass ratio was found in glasshouse plots for vertical branches compared with field grown plots. The species rankings were also similar, with Regal Staygreen having higher mass values which is related to the specific morphology of this species, and has stolons of much greater diameter than *Cynodon* or *Zoysia* species (Chapter 5). Lower stolon mass values were found in the species with poor development and lower ground cover percentage. Corresponding to earlier discussion of turf height (Section 4.4.3.1), Windsor Green exhibited low vertical branch mass due to its horizontal growth pattern. Low vertical branch mass in seeded couches was again due to poor development and low ground cover percentage. Horizontal growth with good ground cover is characteristic for amenity and sports turf varieties that are highly valued in turf industry circles by grounds managers. Quantitative data on this trait is scarce for stoloniferous grasses but a detailed measure for vertical growth pattern in different varieties may be helpful for green keepers regarding their variety selection decisions.

#### *4.4.2.4 Leaves*

Leaves are the primary units of carbon fixation and also have a major contribution to turf quality. Hence leaf morphology and colour and ability to retain leaf mass play a very important role in selection and evaluation of turfgrass varieties. Finer leaves with a short, narrow leaf blade are highly desirable for playing surfaces. Leaf dry mass provides a good indication of grass growth status in fully established turf plots with a uniform ground cover. This appears true in the present experiment, with low leaf mass generally attributable to establishment difficulties, as in the example of Zenith in the field plots, where it was concluded that ambient temperatures were low enough to inhibit satisfactory development of this variety. Regal staygreen attained exceptionally high leaf mass (up to 569 g/m$^2$) under glasshouse conditions. Zenith and Regal staygreen sustained higher leaf mass in second year of growth as well. Coarser and larger leaves of these grasses may be responsible for their higher leaf mass per unit area over other grass varieties grown under same conditions.

## 4.5 Summary

The main aim of this detailed morphological exploration was to provide important but less available information to the turf industry on commonly available warm season turf varieties. The data collection was complementary to the investigation of turf quality and related parameters in Chapter 3 and quantified morphological components of the turf mat which influence turf visual quality for two consecutive growing seasons. Secondly a comparison of morphology and related growth differences with turf maturity under protected and exposed conditions was reported.

Main findings can be summarized as below

- Turfgrasses grown in field conditions exhibited a well-developed root system, with a significant increase towards second year of maturity compared with those grown in the glasshouse. This morphological plasticity would be important to turf performance and further research to clarify factors controlling it would be of interest.
- Several tested grasses, especially vegetative varieties of *Cynodon*, along with Sea Spray (*P. vaginatum*), exhibited a dense rhizome or stolon mat forming capacity that would likely be considered desirable by industry practitioners. Agridark

showed excellent performance both in the field and glasshouse and exhibited higher stolon and rhizome length than other varieties followed by Santa Ana and Windsor green

- Presence of rhizomes in field varieties with an exception of Zenith was interesting and could lead to a more detailed study of seeded *Cynodons* and *Zoysia* to determine the triggers for the onset of rhizome development and favourable conditions pertaining to that.
- Results presented as qualitative scores in Chapter 3 are reinforced quantitatively in this chapter. Regal Staygreen exhibited maximum turf height and leaf mass under glasshouse conditions verifying the results discussed in Section 3.4.

## References

Ahring, R. M., Huffine, W. W., Taliaferro, C. M., Morrison, R. D. (1975) Stand establishment of Bermuda grass from seed. *Agronomy Journal* 67: 229–232.

Christians, N. (2011) Fundamentals of Turfgrass Management (4th ed.). John Willey and sons, Inc. Hoboken, New Jersey, USA.

Dong, M., de Kroon, H. (1994) Plasticity in morphology and biomass allocation in Cynodon dactylon, a grass species forming stolons and rhizomes. *Oikos* 70: 99–106. http://www.jstor.org/stable/3545704

Dong, M., Pierdominici, M. G. (1995) Morphology and growth of stolons and rhizomes in three clonal grasses, as affected by different light supply. *Vegetatio* 116(1), 25–32.

Engelke, M., Anderson, S. (2003) Zoysiagrasses. In: Casler, M.D., Duncan, R R. (Eds.). Turfgrass biology, genetics, and breeding. (271–286) Hoboken, N.J.: John Wiley.

Geren, H., Avcioglu, R., Curaoglu, M. (2009) Performances of some warm-season turfgrasses under Mediterranean conditions. *African Journal of Biotechnology* 8: 4469–4474.

Huang, B. (1999) Water relations and root activities of *Buchloe dactyloides* and *Zoysia japonica* in response to localized soil drying. *Plant and Soil* 208: 179–186.

Hutchings, M. J. (1988) Differential foraging for resources, and structural plasticity in plants. *Trends in Ecology & Evolution* 3: 200–204.

Jiang, Y., Duncan R. R., Carrow R. N. (2004) Assessment of Low Light Tolerance of Seashore Paspalum and Bermudagrass. *Crop Science* 44: 587–594. doi:10.2135/cropsci2004.5870

Litherland, A. J., Webby, R. W., Fraser, T. J., Matthew, C., McCleod, K., Walcroft, J., Clarke-Hill, W. J. (2008) Indirect measurement of pasture mass and pasture growth rate on sheep and beef pastures. In *Proceedings of the New Zealand Grassland Association* 70: 131–136.

Moore, K. J., Moser, L. E. (1995) Quantifying developmental morphology of perennial grasses. *Crop Science* 35: 37–43.

Munshaw, G. C., Ervin, E. H., Shang, C., Askew, S. D., Zhang, X., Lemus, R. W. (2006) Influence of late season iron, nitrogen and seaweed extract on fall color retention and cold tolerance of bermudagrass cultivars. *Crop Science* 46: 273–283.

Murray, J. J., Juska, F. V. (1977) Effect of management practices on thatch accumulation, turf quality, and leaf spot damage in common Kentucky bluegrass. *Agronomy Journal* 69: 365–369. doi:10.2134/agronj1977. 00021962006900030008x

Niklas, K.J., Kutschera, U. (2009). The evolution of plant body plans. *Functional Plant Biology* 36: 682–695.

Rimi, F., Macolino, S., Ziliotto, U. (2012) Rooting characteristics and turfgrass quality of three bermudagrass cultivars and a zoysiagrass. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science* 62: 24–31.

Samudio, S.H., Brede, A. D. (2002). Registration of ‘Southern Star’ Bermudagrass Registration by CSSA. *Crop Science* 42: 669–670. doi: 10.2135/cropsci2002.6690

Stewart, K. E. J., Bourn, N. A. D., Thomas, J. A. (2001) An evaluation of three quick methods commonly used to assess sward height in ecology. *Journal of Applied Ecology* 38: 1148–1154. doi:10.1046/j.1365-2664.2001.00658.x

Taliaferro, C.M. (2003) Bermudagrasses. In Casler, M.D., Duncan, R R. (Eds.). Turfgrass biology, genetics, and breeding. (235–256) Hoboken, N.J.: John Wiley.

Zhang, Q., Fry, J., Pan, X., Rajashekar, C., Bremar, D., Engelke, M., Wang, X. (2009) Cold acclimation of *Zoysia japonica* and *Z. matrella* and changes in rhizome abscisic acid levels. *International Turfgrass Society Research Journal* 11: 883–892.

# CHAPTER 5

# Stolon Morphology of Warm Season Turfgrasses

## 5.1 Introduction

The previous chapter addressed a gap in knowledge about warm season grasses by examining in detail the contribution to the turf mat of various plant components, including leaf lamina, leaf sheath, stolon or rhizome above and below ground, and roots. Turfgrasses are essentially clonal plants, and given the purposes they are used for, they are often subject to factors such as foot or vehicle traffic that can cause stress and damage to the plant. In this context, traits that enable tolerance of such stresses and ensure persistence have been the focus of selection programmes for breeding purposes. The knowledge of basic attributes regarding plant structure and the organisation of phytomers within the stolon and their contribution to the growth strategies in turfgrasses, could help to inform the selection process and help identify traits that would contribute to identification of novel and improved materials with desirable commercial characteristics.

Spatial and temporal variation in biotic and abiotic conditions of most natural habitats result in selection pressures on plant traits that can ultimately confer tolerance of or resistance to the unfavourable conditions, with positive consequences of this modified response on survival and persistence (Suzuki and Stuefer, 1999). The resulting form and structure of organs and their physical relationship to each other is defined by the plant structural blue-print (Huber and Watson, 1999), or body plan (Niklas and Kutschera, 2009). This blue-print is a species-specific suite of traits that determines the most basic structural organization or growth form of a plant, and can be thought of as a combination of phylogenetically fixed traits that determine the spatial structure and architecture (Huber and Watson, 1999). However, for a given genotype, environmental heterogeneity also triggers plastic physiological or morphological alterations that directly or indirectly enhance the capture of essential resources by plants (Hutchings and De Kroon, 1994). The evolution of C4 grasses is thought to have occurred independently under African desert climates with high grazing intensity and in tropical regions with high temperature and humidity, which ultimately determined the acquired growth form.

One outcome of the spreading clonal growth form displayed by the warm season grasses is the possibility of ‘division of labour’ through local specialisation when a plant is growing in a heterogeneous environment, and concentration of resource-acquisition duties to enhance acquisition of each resource from sites of greatest abundance. According to Stuefer (1998) division of labour in clonal plants operates as a system which enhances the outcome for the colony as a whole through the assignment of specialized tasks or a reduced number of tasks for an individual unit. The different contributions of the individual phytomers in a grass shoot to the plant growth as a whole is also a form of division of labour. The phytomer is a basic growth unit of the tiller (Moore and Moser, 1995) and is a combination of node, leaf and auxiliary bud under the leaf sheath which is responsible to give rise to root or shoot. Meristematic regions in each phytomer contain specific cell arrangements which allow the development of specialized organs (Forster et al., 2007).

The stolon is a dominant structural unit of warm season turfgrasses. Horizontally trailing stems develop an interwoven network over the soil surface which provides an extra cushion between leaves and the soil surface and results in a thick and resistant mat with increased traffic tolerance and a self-repairing capacity. Each node on a stolon indicates a junction between two phytomers, meristematic zones capable of producing new roots and shoots are typically located there. Stoloniferous plants favour a rapid local spread and colonization of open space by stolon growth as well as the fast establishment rates of clonal offspring mediated by a high degree of physiological integration, which enables survival and persistence in plant species growing in frequently disturbed habitats (Stuefer and Huber, 1999).

Each phytomer is generally assumed as a growth unit with similar behaviour, the specialization of adjacent phytomers to produce different plant functional units (Leaf, Buds, and Root) is infrequently explored in the literature. However, Sbrissia et al. (2001) visual observation of specialization in development of particular phytomers in Coastcross bermudagrass (*Cynodon* spp.), in which a root, a branch, and a leaf were each observed at regular intervals of three phytomers. This triplet structure appears to have a decisive ecological significance for foraging behaviour and to enable offspring establishment, since authors suggested first leaf would support root development, the second leaf would support shoot and the third would support stolon internode elongation (Sbrissia et al., 2001; da Silva et al., 2015).

Considering these superficial observations of specialized architecture of some of the warm season turf species, detailed measurement of samples from the glasshouse and field experiments described in Chapter 3 was carried out to determine the morphological pattern of stolon growth and structural organization in different warm season turfgrasses. The approach taken to achieve this objective was to make detailed measurement of morphological features at twelve successive phytomers in the 10 warm season turfgrass varieties from the genera *Cynodon*, *Zoysia*, *Pennisetum and Paspalum* grown under protected and exposed conditions, as described in Chapter 3.

## 5.2 Materials and methods

### 5.2.1 Sample collection

Samples were collected from actively growing turf plots established during 2012-13 under field conditions at NZSTI, Palmerston North and PGU, Massey University, Palmerston North (Section 3.2.1). Actively growing stolons were randomly selected and from multiple, spatially separated locations in individual plots. For selected stolons, 12 internodes were counted back from the stolon tip and cut with a sharp secateurs. Individual stolons were placed in zip locked bags and labelled. Collected samples were placed under refrigeration (4°C), for further measurement.

### 5.2.2 Measurement

Each stolon sample was dissected into individual phytomer units and details of morphology and status of plant organs associated with each phytomer recorded. Observations made included: internode length, bud status (quiescent or a shoot formed), leaf lamina length and width, leaf sheath length and stolon diameter for the twelve successive nodes. Internode length (mm) from end to end and stolon diameter at every third node were measured using digital vernier callipers (Digimatic CD-6”) under a binocular microscope (20X) with adjustable light source for the fine and precise measurements. Stolon diameter of measured internodes was obtained by recording a set of four measurements from different points and averaging these for a uniform result. Leaves were carefully separated from each node using a surgical scalpel and needles and dissected into leaf blade and leaf sheath. Images of specialized structures in *Cynodon* and *Zoysia* were captured using a Leica MZ12 Stereomicroscope with CCD camera at 40X magnification, at the Manawatu Microscopy Imaging Centre, Massey University, Palmerston North.

### 5.2.3 Data analysis

Means and standard errors were calculated, for the collected data. Independent sample t-tests ($P \leq 0.05$) were used to determine statistically significant differences between glasshouse and field plots. One-way ANOVA was used ($P \leq 0.05$) to detect significant differences among varieties for particular morphological units. Analyses were performed using Microsoft Excel 2010, “R” and SPSS 21 (Statistical Package for Social Sciences). Principal component analysis (PCA) and ANOVA of PC scores was carried out in Minitab version 10.51 as a means of detecting and describing the distinct morphological features of each variety when grown in the field or the glasshouse. Morphological data used in the PCA were internode lengths for the 12 internode segments per phytomer, mean stolon diameter, mean leaf lamina length and width, and mean leaf sheath length.

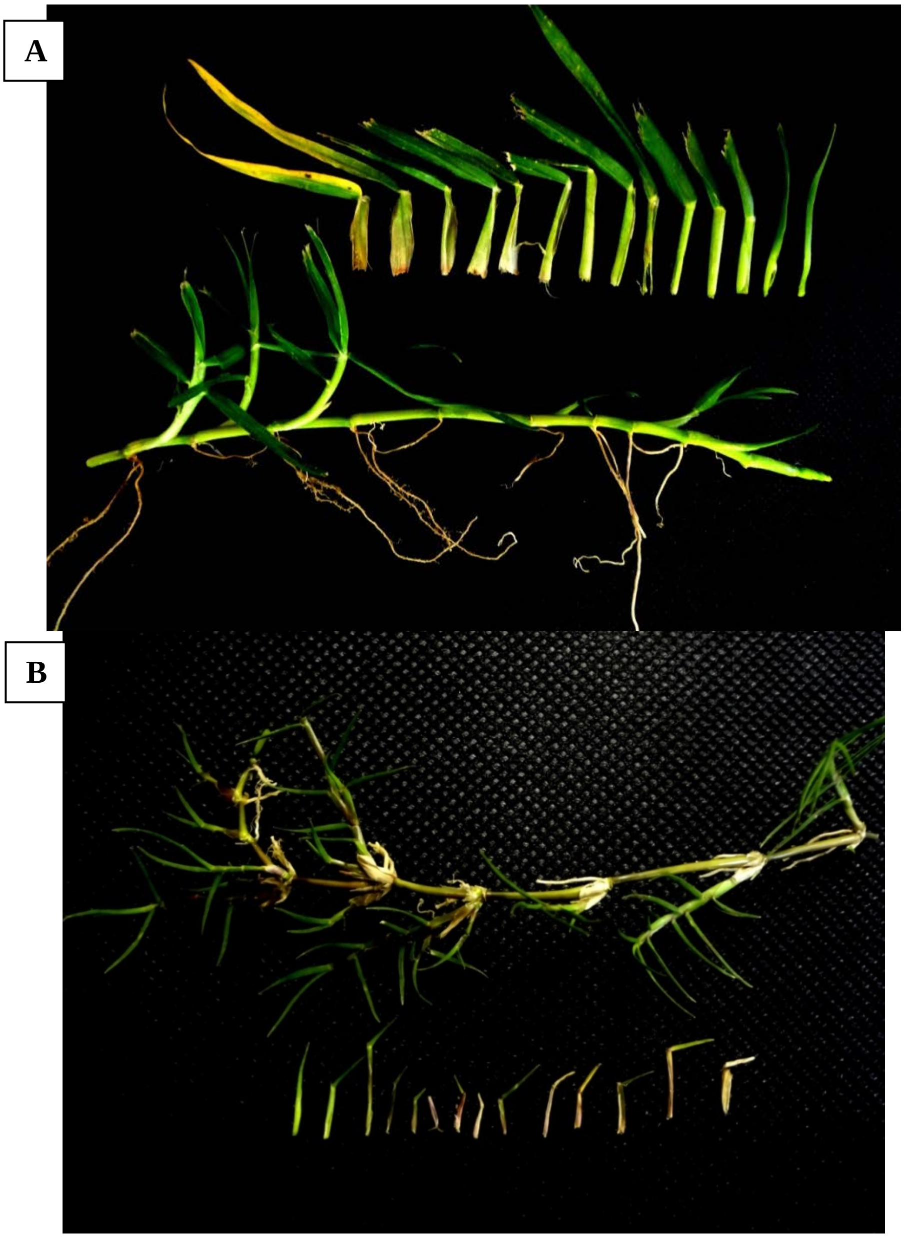


**Fig 5.1 Dissected 12-phytomer stolon segments of A) *Pennisetum* (Regal Staygreen), and B) *Cynodon* (Windsor green). Leaves under the stolon are from base to apex for 12 successive nodes including unfolded tip. In A, tip at right; in B, tip at left.**

## 5.3 Results

### 5.3.1 Internode distance

Internode distances for successive phytomers on the 12-phytomer stolon segments are shown in Fig. 5.2. It is clearly seen that *Cynodon* and *Zoysia* have internode extension at every third node, so that growth units are actually a triad of three phytomers. The visual appearance was that the two short internode segments in each triad were responsible for root and branch shoot production. The phenomenon needs further exploration for better understanding of phytomer morphology. Meanwhile *P. vaginatum* and *P. clandestinum* did not display the triad structure and had internode extension (Fig. 5.2), branch development, and root formation at each node.

Internode distance did not differ significantly between field and glasshouse plots. However it was observed that seeded *Cynodons* exhibited shorter inter node distance in the glasshouse environment and the inverse pattern was observed in vegetatively grown species except Santa Ana (Fig. 5.2). Sea Spray differed significantly from other varieties except Regal Staygreen at some but not all internode locations. The results confirm the presence of two short internodes and then a long one in *Cynodon* and *Zoysia* (Statistical test of means and F-ratios are in Appendices 5.1, 5.2).

### 5.3.2 Grass leaf attributes on a stolon

Leaf characteristics (leaf blade length, leaf blade width and leaf sheath length) were measured for 12 successive internodes in each of the grass stolon and results (averages) are presented in Fig. 5.3. All the grass varieties exhibited longer leaf blade length in the glasshouse compared with the field plots. A similar trend was seen for leaf sheath length except for P-77 which had longer sheath length in field plots.

Most varieties showed significantly longer leaf blade length (except P77, LP, SS and PS), and significantly longer leaf sheath length (except P77, LP, ZN and SS) under glasshouse conditions, when compared with field plots. In addition, SA, WG, ZN and RS exhibited significantly broader leaves in the glasshouse, while P77 and LP showed significantly broader leaves under field conditions.

### 5.3.3 Bud/branch and root occurrence

All the varieties in the experimental block showed a similar pattern of bud/branch appearance under field and glasshouse conditions however varieties lacking the triad phytomer structure (Regal Staygreen and Sea Spray) exhibited a higher number of bud/branch sites than varieties with the triad structure. Among varieties with the triad structure, Yukon had the lowest number of bud/branch sites on stolons examined for both field and glasshouse plots. In some of the younger triplet nodes in Yukon no bud primordia was visually observable.

Root appearance was quite variable in both glasshouse and field plots and higher numbers of roots were recorded under field conditions than in the glasshouse except for Sea Spray and Regal Staygreen which showed the opposite behaviour to other varieties (Fig. 5.4).

### 5.3.4 Light microscopy

Images taken through a binocular microscope with 20X magnification and later with a “Leica MZ12 Stereomicroscope” with CCD camera at 40X magnification further verified the presence of the triplet or triad structure in *Cynodon* and *Zoysia.* Internodes appear in a combination of three nodes with two very short internodes and one elongated whereas in *Pennisetum* and *Paspalum* this arrangement was not present (Figs. 5.5, 5.6).

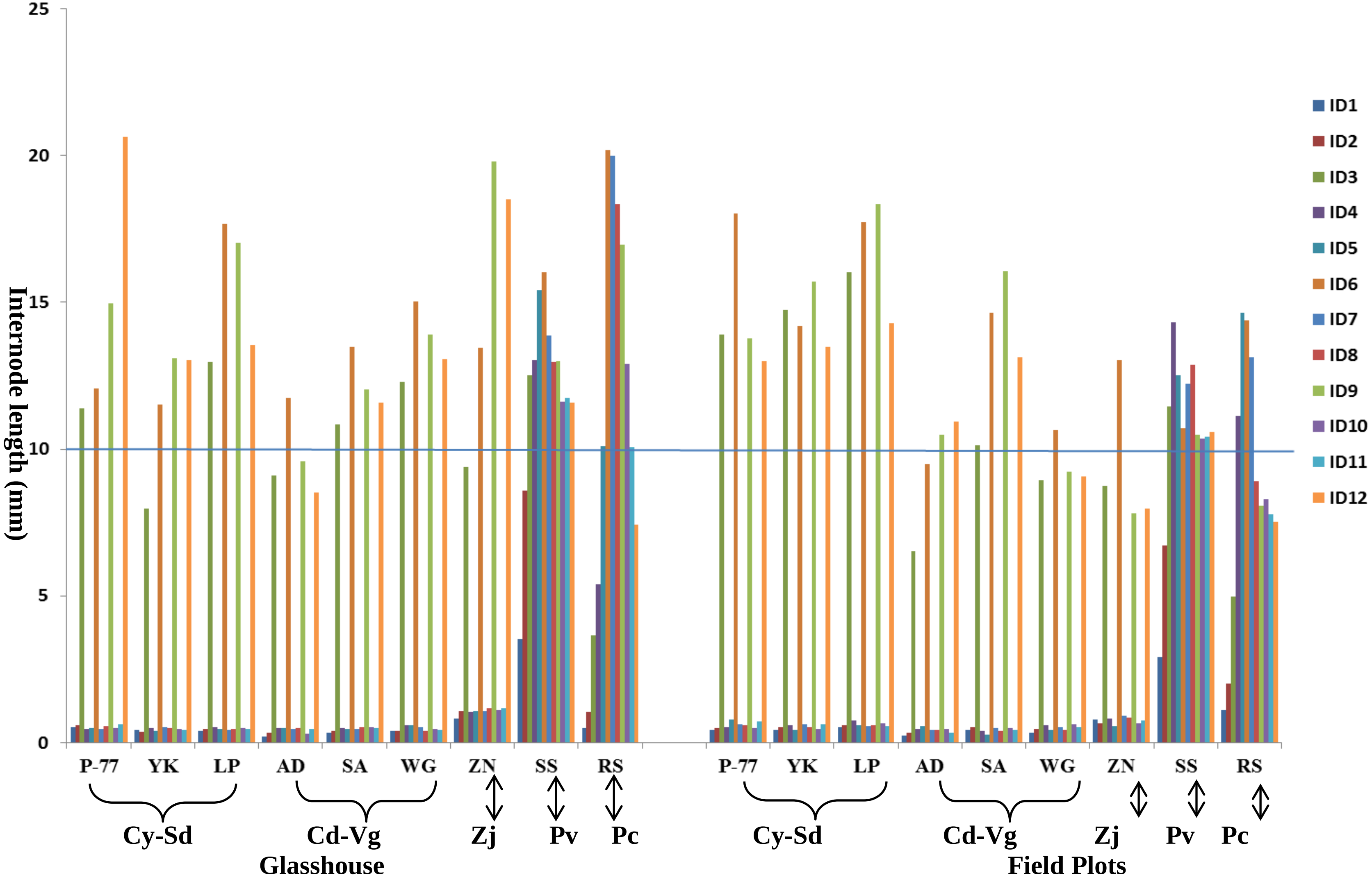


**Fig. 5.2 Stolon growth pattern of different turfgrass species on the basis of internode distance in twelve successive nodes (ID1-ID12)**
ID = Internode Distance, Cy-Sd = Seeded *Cynodon*, Cy-Vg = Vegetative Cynodon, Pv = *Paspalum vaginatum*, Zj = *Zoysia japonica*, Pc = *Pennisetum clandestinum*
P-77=Princess 77, YK=Yukon, LP =La Paloma, AD =Agridark, SA=Santa Ana, WG=W.Green, SS =Sea Spray, ZN = Zenith, RS=R.Staygreen

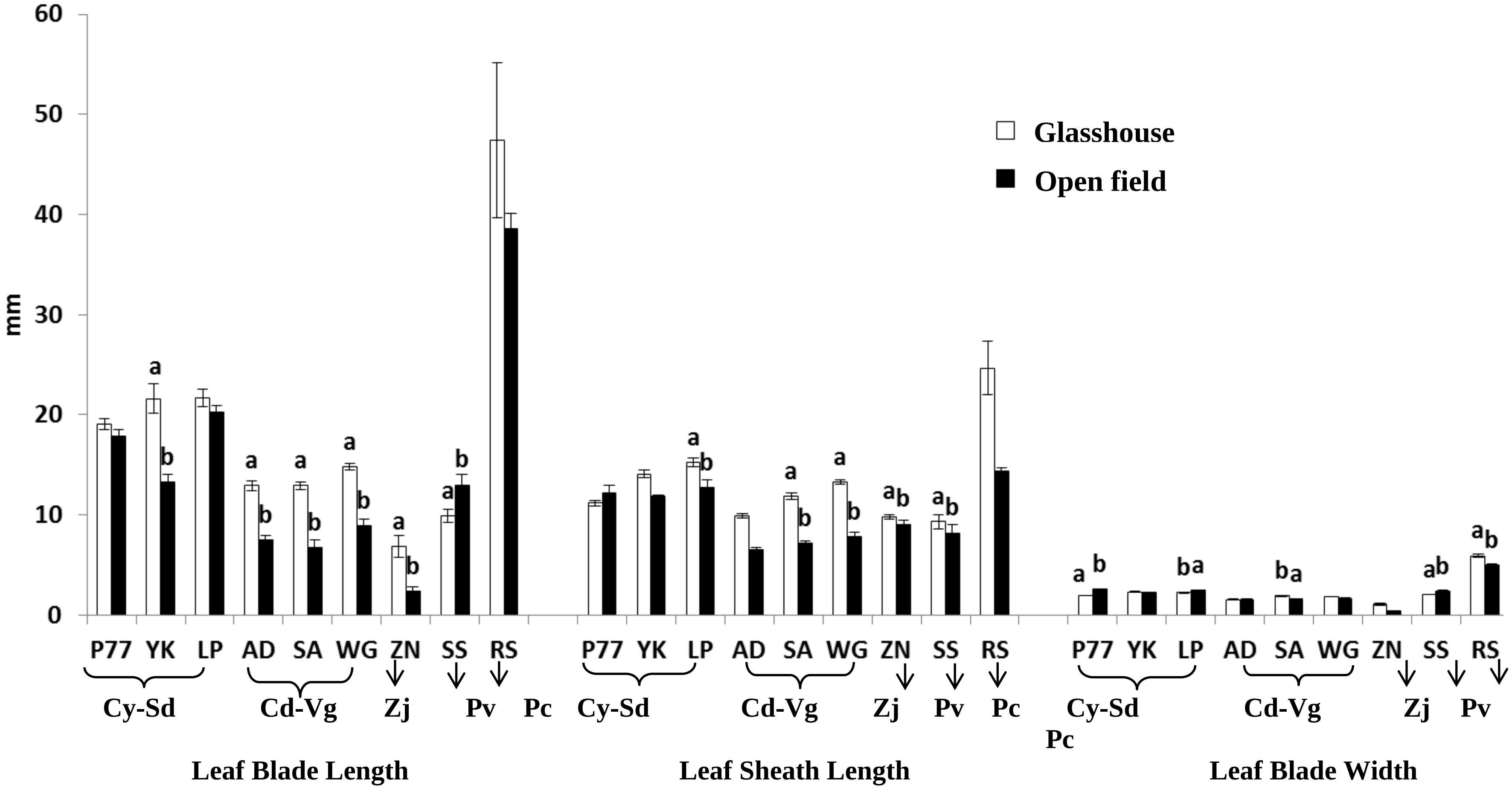


**Fig. 5.3 Comparison of leaf blade length, sheath length, and blade width in glasshouse and open field (average of twelve successive leaves on a stolon)**

Cy-Sd = Seeded *Cynodon*, Cy-Vg = Vegetative Cynodon, Pv = *Paspalum vaginatum*, Zj = *Zoysia japonica*, Pc = *Pennisetum clandestinum* P-77=Princess 77, YK=Yukon, LP = La Paloma, AD =Agridark, SA=Santa Ana, WG = W.Green, SS = Sea Spray, ZN = Zenith, RS = R.Staygreen

Lettering (“**a”** for higher value and “**b”** for lower value) is used to show the comparison between glasshouse and field plots. The effect of location is significant at $P \leq 0.05$

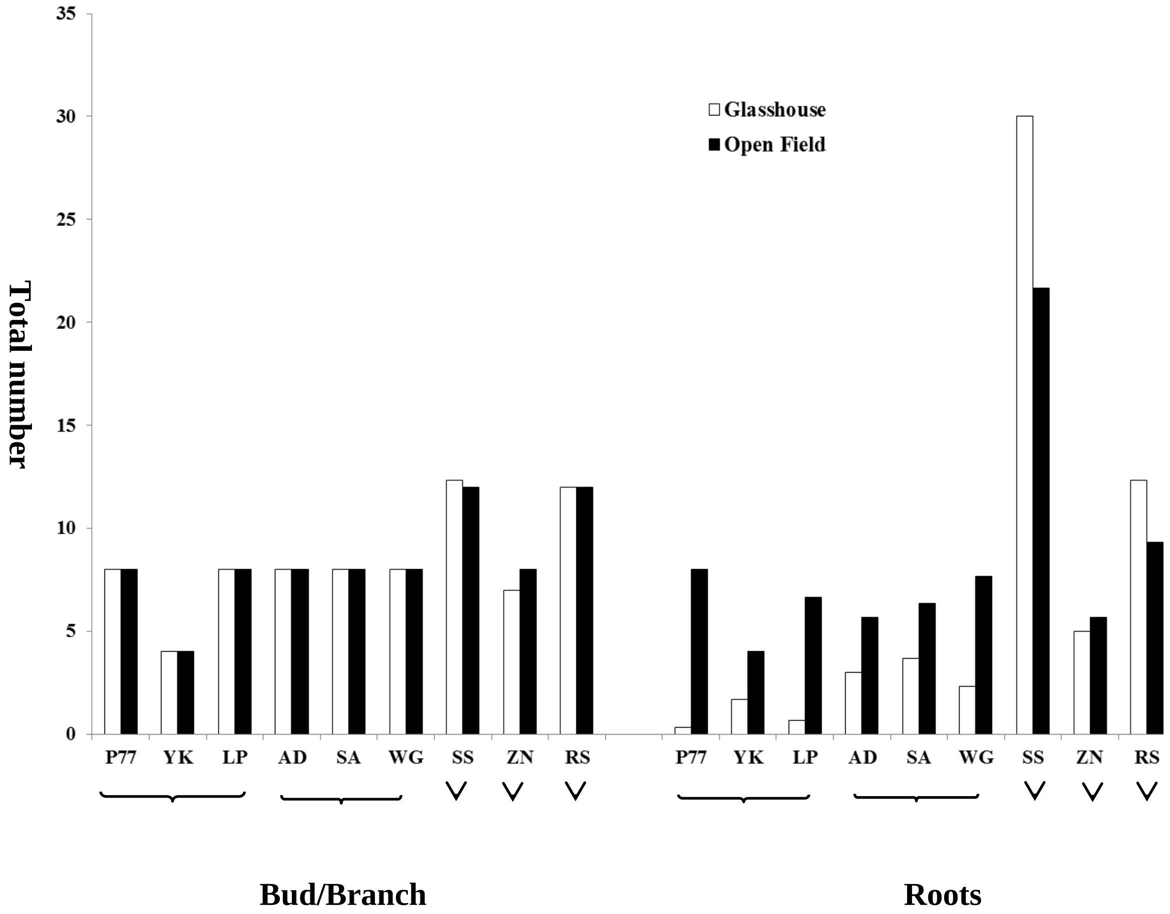


**Fig. 5.4 Total number of bud/branch and roots on a stolon for 12 successive nodes**

Cy-Sd = Seeded *Cynodon*, Cy-Vg = Vegetative *Cynodon*, Pv = *Paspalum vaginatum*, Zj = *Zoysia japonica*, Pc = *Pennisetum clandestinum*

P-77=Princess 77, YK=Yukon, LP =La Paloma, AD =Agridark, SA=Santa Ana, WG=W.Green, SS =Sea Spray, ZN = Zenith, RS=R.Staygreen

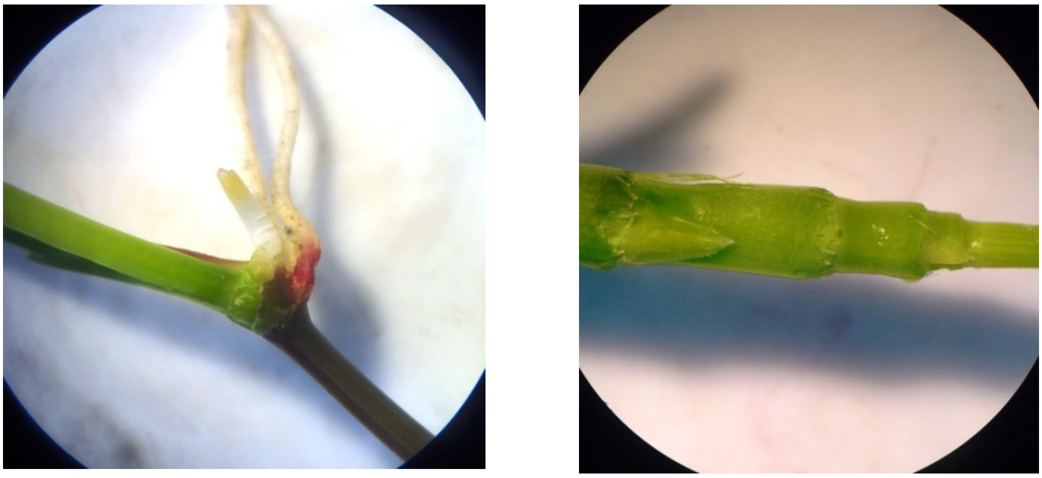


**Fig. 5.5 Triplet structure of a *Cynodon* stolon (Left), no grouping of internodes in *Pennisetum* (Right).**

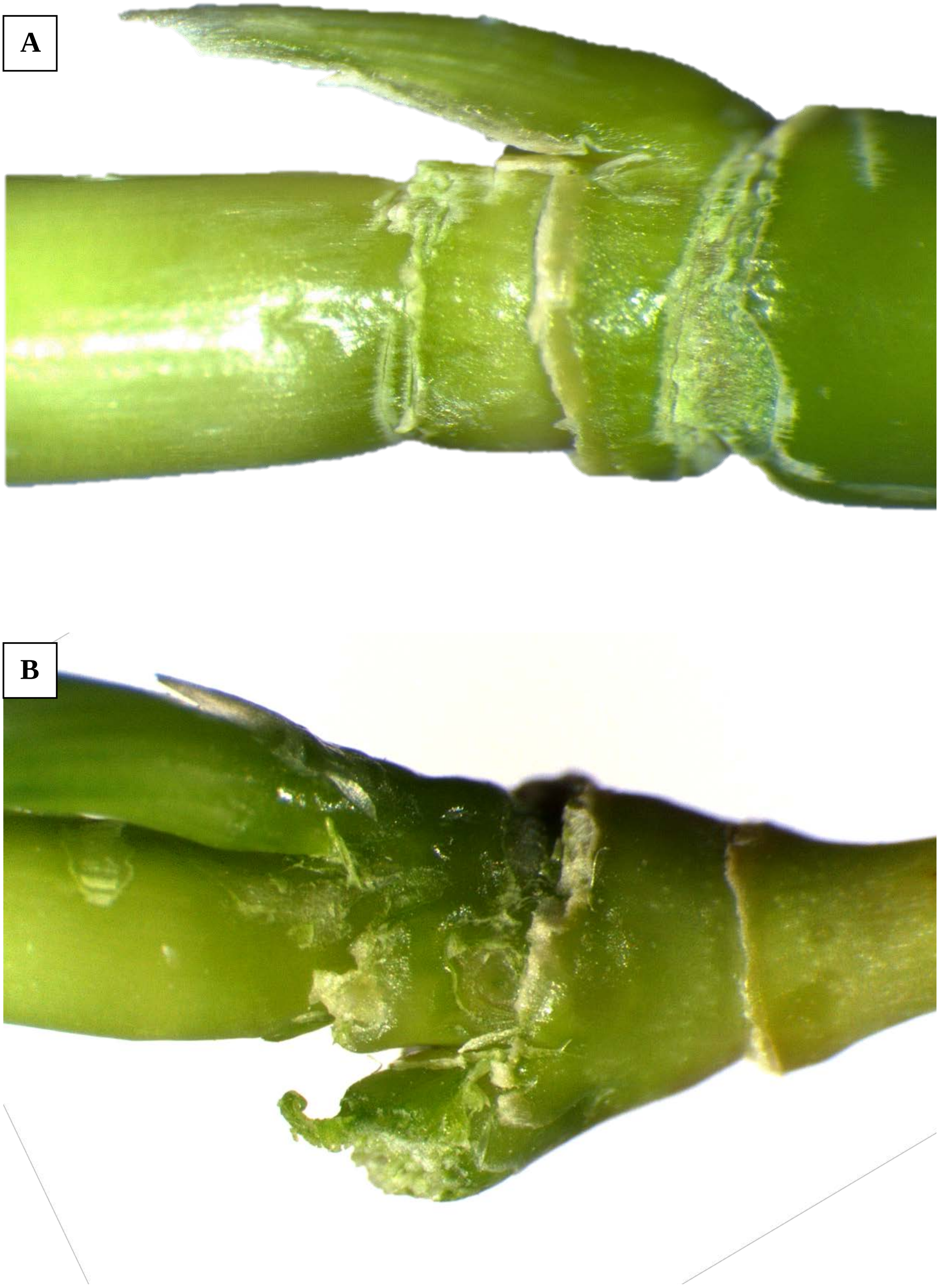


**Fig. 5.6 Triplet organisation of nodes in (A)** ***Cynodon,*** **(B)** ***Zoysia japonica***

### 5.3.5 Principal component analysis

The PCA to detect patterns among the various morphological components across species in the field or glasshouse plots generated three principal components (PCs) of interest. PC1 accounted for 46.5% of the data variation and detected the triad structure in the *Cynodon* and *Zoysia* varieties by applying a more negative coefficient to the two short internodes in each triad (Table 5.1, values in bold), and strongly separated ZN and RS from the other varieties (Figs. 5.7, 5.8). PC2 (19.3% of data variation explained) also detects the triad structure in a way that makes it score near zero and then separates non triplet grasses on leaf dimensions (Table 5.1; Figs. 5.7 & 5.8). PC3 (14% data variation explained) separates varieties on a combination of triplet structure and stolon diameter and, interestingly, makes a score separation between the three seeded (P77, YK, LP) and the three vegetatively propagated (AG, SA, WG) *Cynodon* varieties.

**Table 5.1 Eigenvalues, proportion of variation explained, and coefficients for principal components 1 to 3 of a principal component analysis designed to detect differences in morphological pattern between varieties.**

| | PC1 | PC2 | PC3 |
|---|---|---|---|
| Eigenvalue | 7.4387 | 3.0883 | 2.2381 |
| Proportion of data variation | 0.465 | 0.193 | 0.140 |
| Cumulative proportion | 0.465 | 0.658 | 0.798 |
| Morphological variable | | | |
| Internode 1 Length | **-0.197** | 0.379 | -0.14 |
| Internode 2 Length | **-0.240** | 0.382 | -0.166 |
| Internode 3 Length | 0.098 | 0.065 | -0.512 |
| Internode 4 Length | **-0.317** | 0.200 | -0.052 |
| Internode 5 Length | **-0.343** | 0.125 | 0.015 |
| Internode 6 Length | -0.126 | -0.323 | -0.356 |
| Internode 7 Length | **-0.350** | -0.053 | -0.017 |
| Internode 8 Length | **-0.350** | -0.022 | -0.046 |
| Internode 9 Length | -0.009 | -0.257 | -0.508 |
| Internode 10 Length | **-0.361** | 0.037 | -0.035 |
| Internode 11 Length | **-0.343** | 0.108 | -0.010 |
| Internode 12 Length | 0.137 | -0.010 | -0.388 |
| Stolon diameter | -0.067 | -0.139 | 0.347 |
| Leaf blade length | -0.213 | -0.372 | 0.105 |
| Leaf sheath length | -0.173 | -0.456 | -0.071 |
| Leaf lamina width | -0.260 | -0.314 | 0.114 |
| P (Variety) | <0.001 | <0.001 | <0.001 |

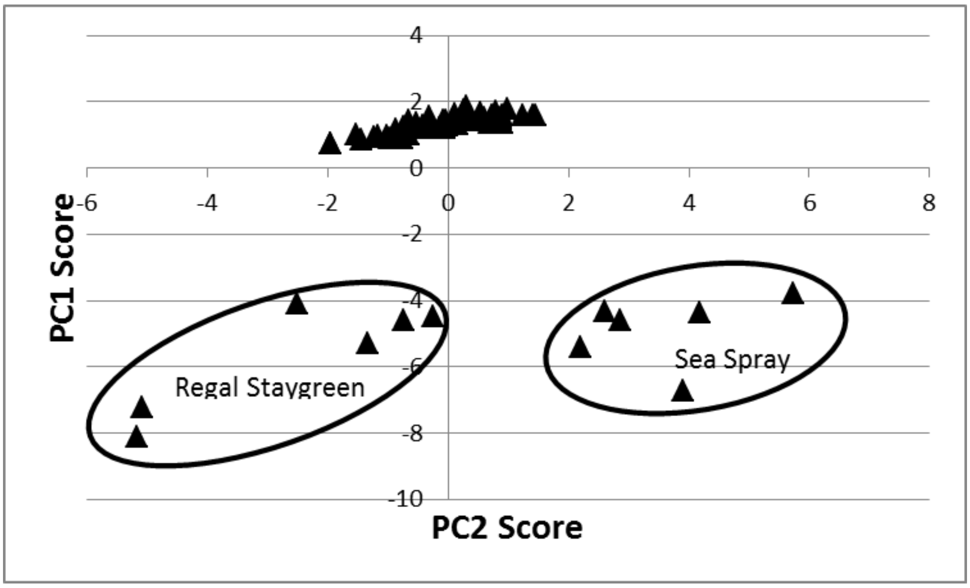


**Fig. 5.7** Plot of principal component scores for principal components 1 and 2 for 9 warm season turfgrass varieties in glasshouse and field plots.

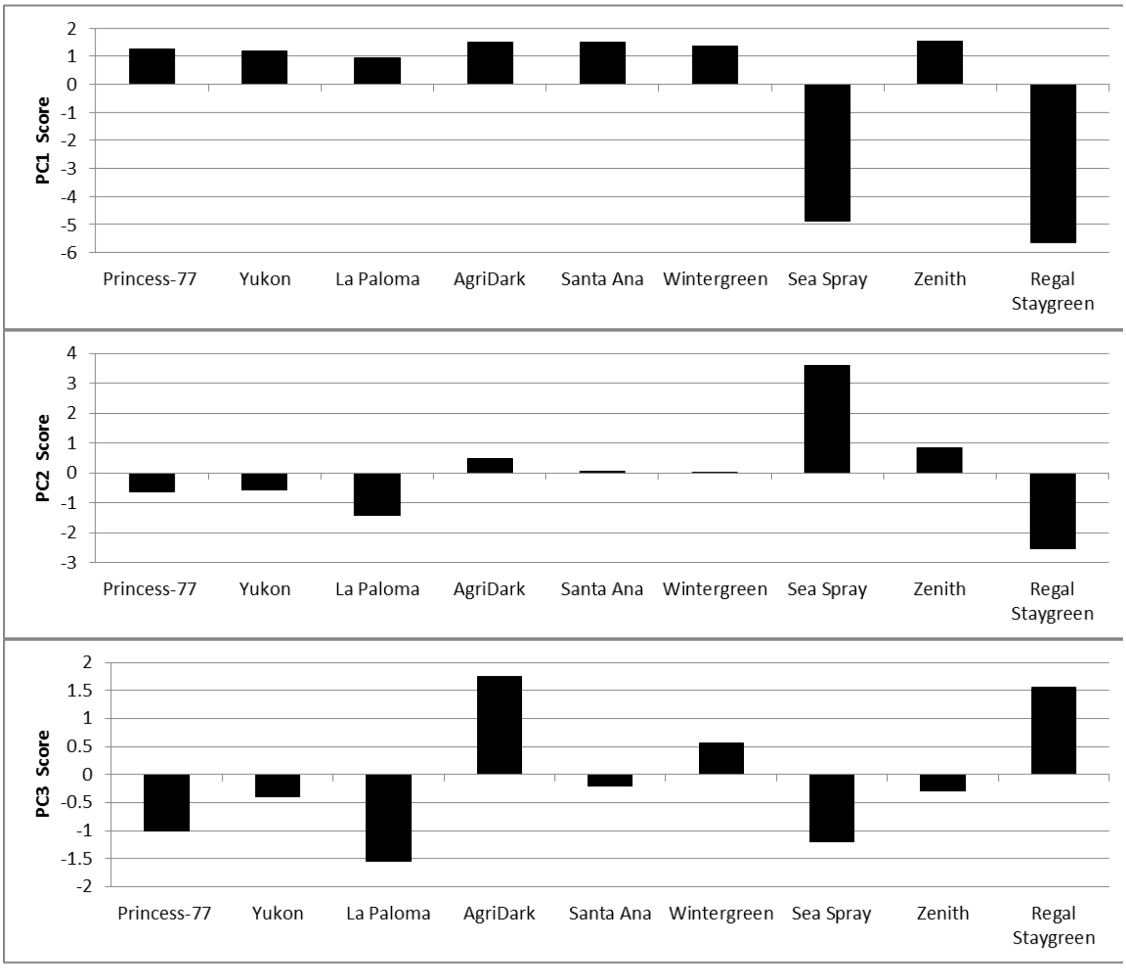


**Fig. 5.8** Mean principal component scores for 9 warm season turfgrass varieties from a principal component analysis designed to quantify morphological variation.

## 5.4 Discussion

Stolons are a morphological structure common to most of the warm season clonal turf species. They serve primary purposes of vegetative reproduction and anchorage to the parent plants, and increase the plant’s tolerance to abiotic stress. As discussed earlier, situations with high trafficking and grazing pressure may have exerted evolutionary selection pressure for development of specialized plant structures that aid survival and growth. It may also be that the stoloniferous growth habit confers ecological advantages related to resource capture in a spatially heterogeneous environment (Hutchings and de Kroon, 1994). While all the warm season grasses tested displayed a stoloniferous growth habit, there were discrete morphological differences between species and varieties, as reported in Section 5.3 and discussed below.

### 5.4.1 Internode distance

Most of the varieties exhibited longer internodes under glasshouse conditions as compared with field plots. This trend may be due to the reduced light intensity (and possibly also to spectral changes) in the glasshouse, with high soil moisture contents and air humidity which trigger cell elongation and growth. The more compact growth with shorter internodes under field conditions may reflect greater diurnal temperature fluctuation and exposure to other climatic factors.

Different species showed variations in stolon growth pattern which is interesting and is discussed in detail in Section 5.4.4 below.

### 5.4.2 Grass leaf attributes on a stolon

Leaves are the primary photosynthetic organs in turfgrasses. Leaf positioning, orientation, and leaf area index plays a very important role in appearance and growth of the plant. Furthermore, leaf texture defines the suitability of a turfgrass variety for an intended use. All the varieties showed coarser leaf texture under glasshouse conditions and exhibited longer and wider leaf blades compared to field-grown varieties. Again these differences may be attributed to plant response to low light conditions and high humidity with a more stable temperature in the glasshouse, compared to field. Regal Staygreen had longer and wider leaves at both locations than any other variety. Hence while this variety tolerated cool temperatures well, it’s genetically determined coarse morphology makes this variety undesirable for sports uses. In the case of varieties with smaller leaf size, additional to the aesthetic appeal, turfgrasses with short and narrow leaf blades or with horizontal leaf orientation have a lower evapotranspiration rate and better water use efficiency (Kim and Beard, 1986).

### 5.4.3 Bud/branch and root occurrence

Sea Spray and Regal Staygreen produced a higher number of branches than other species due to their ‘linear’ internode structure and presence of bud/shoot primordia at every internode. No differences between field- and glasshouse-grown plants were recorded for the occurrence of a bud or a branch. However, for the total number of roots on 12 successive nodes, plants from field plots were found to have a higher number of roots than glasshouse plots, except for Sea Spray and Regal Staygreen which showed the reverse trend.

These results again indicate plasticity in the allocation between plant growth modules where plants tend to develop their component parts according to their requirement. Early root development with higher number under field conditions would enable plants to compete for available resources especially water, while these parts were rather less developed under glasshouse conditions due to high moisture and humid conditions with more favourable temperature. The different behaviour shown by Sea Spray and Regal Staygreen may arise from stronger adaption to tropical conditions, possibly as a result of differing geographic origin.

### 5.4.4 Triplet morphology

As mentioned earlier (Sections 5.3.1 and 5.4.1) internode length varied among the different species of warm season turfgrasses tested. An infrequently reported and almost unexplored phenomenon was observed in *Cynodon* and *Zoysia* where internodes appear in the form of triplet structures with two short and one long internode whereas *Pennisetum* and *Paspalum* has a consistent or ‘linear’ internode pattern, where internode length varies based on maturity, but no grouping pattern between adjacent phytomers was present.

This specialized growth pattern has been reported in *Zoysia* by Engelke and Anderson (2003), referring back to the findings of Shoji (1983) that stolons of *Zoysia* have an arrangement with one long and two short internodes. Similarly Hanson et al.

(1969) mentioned the presence of long internodes alternating with one or more very short internodes in *Cynodon*. Stiff and Powell (1974) reported a similar finding in *Cynodon, Zoysia* and St. Augustine grass and referred to them as compound nodes and noticed that the number of bud/branch sites varies with the total number of leaves in a compound node. These authors also cited earlier findings of Bogdan in 1952. Sbrissia et al. (2001) and also da Silva (2015) reported a similar pattern in *Cynodon*.

The presence of the triplet structure in *Zoysia* and *Cynodon* (Fig. 5.2) is indicative of a different form having emerged during their evolution process. For the *Cynodon* and *Zoysia* it was observed that only one bud or branch is present at each segment of three nodes and usually the lower most node gives rise to the root, the middle node to the shoot and the top node elongates to provide shoot extension, and this one is the longer internode among the set of three as recorded in the data (Fig. 5.2), whereas in *Pennisetum* and *Paspalum* this pattern is not present and each node is responsible for all three functions, and a root or roots and a bud are present at each node with internode extension at each phytomer next unit.

This pattern may result from multiple evolutionary selection pressures (possibly related to survival under stress, resource exploitation in patchy environments, or control of resource allocation between leaves, roots, and internode formation) but it definitely needs further exploration and detailed insight. *Cynodon* species are known as native to African plains where heavy trafficking and grazing gave rise to the current form of many varieties. This biotic pressure along with harsh climatic conditions triggered plant plasticity to develop specialized growth structures as a triplet formation which provides division of labour between phytomers. The ecological significance of this habit needs to be explored, but it is possible dividing root formation, branch formation, and internode extension between three phytomers each with its own leaf, that intra-plant competition between the three processes is reduced and that this is advantageous when facing various forms of stress.

## 5.5 Summary

The main aim of studying stolon morphology was to identify structural differences present in warm season turf species linked to plant architecture and specialized functioning of morphological parts, in both glasshouse- and field grown plants. Differences between species were marked, key points being:

- *Cynodon* and *Zoysia* have a distinct morphological pattern that differs from *Paspalum* and *Pennisetum.* In *Cynodon* and *Zoysia*, nodes are grouped in a triplet form with two short internodes and one elongated internode. This organisation is absent in *Paspalum* and *Pennisetum* and these species rather exhibit 'linear' stolon growth. Each node in a triplet unit performs a different function, giving rise to shoot, root and stem extension while in *Paspalum* and *Pennisetum* each node is responsible for all three functions. This pattern needs further exploration and detailed stolon anatomy to verify the occurrences of root shoots and bud primordia at different nodes and their exact orientation.
- All varieties developed longer and broader leaf laminae under glasshouse conditions with the exception of Sea Spray. This generally increase in shoot size is presumed to be a response to high moisture availability, changed light conditions and more optimal temperatures, compared with the field environment. *Zoysia* exhibited very narrow and short leaf blades under field conditions which may limit the capacity for carbon fixation and ultimately reduce growth.
- A higher number of roots were recorded in stolons collected from field plots indicating plant morphological plasticity to respond in different conditions. Exposure to stressful conditions in the field triggered plants to develop roots earlier and in greater quantity, compared with optimal conditions in the glasshouse.
- Leaf and stolon attributes reported in this section reinforce the results of Chapter 3 and Chapter 4, where Regal Staygreen was reported as a coarser variety compared to other varieties. It has longer and broader leaves with long and thick internodes under both glasshouse and field conditions.

## References

da Silva, S. C., Sbrissia, A. F., Pereira, L. E. T. (2015) Ecophysiology of C4 forage grasses—Understanding plant growth for optimising their use and management. *Agriculture 5*: 598–625.

Forster, B. P., Franckowiak, J. D., Lundqvist, U., Lyon, J., Pitkethly, I., Thomas, W. T. (2007) The barley phytomer. *Annals of botany* 100: 725–733.

Hanson, A. A., Juska, F. V., Burton, G. W. (1969) Species and varieties. In Hansan, A. A., and Juska F.V. (Eds.). *Turfgrass science* (pp 9–26) American Society of Agronomy, Wisconsin, USA.

Huber, H., Lukács, S., Watson, M. A. (1999) Spatial structure of stoloniferous herbs: an interplay between structural blue-print, ontogeny and phenotypic plasticity. *Plant Ecology* 141: 107–115.

Hutchings, M. J., de Kroon, H. (1994) Foraging in plants: the role of morphological plasticity in resource acquisition. *Advances in Ecological Research* 25: 159–238.

Kim, K. S., Beard, J. B. (1988) Comparative turfgrass evapotranspiration rates and associated plant morphological characteristics. *Crop Science* 28: 328–331. doi:10.2135/cropsci1988.0011183X002800020031x

Moore, K. J., Moser, L. E. (1995) Quantifying developmental morphology of perennial grasses. *Crop Science* 35: 37–43.

Niklas, K.J., Kutschera, U. (2009) The evolutionary development of plant body plans. *Functional Plant Biology* 36: 682–695.

Sbrissia, A. F., Silva, S. C. D., Carvalho, C. A. B. D., Carnevalli, R. A., Pinto, L. F. D. M., Fagundes, J. L., Pedreira, C. G. S. (2001) Tiller size/population density compensation in grazed coastcross bermudagrass swards. *Scientia Agricola 58*: 655–665. https://dx.doi.org/10.1590/S0103-90162001000400002

Stiff, M. L., J. B. Powell. (1974) Stem Anatomy of Turfgrass. *Crop Science* 14: 181–186. doi:10.2135/cropsci1974.0011183X001400020004x

Stuefer, J. F. (1998) Two types of division of labour in clonal plants: benefits, costs and constraints. *Perspectives in Plant Ecology, Evolution and Systematics* 1: 47–60.

Stuefer, J. F., Huber, H. (1999) The role of stolon internodes for ramet survival after clone fragmentation in Potentilla anserina. *Ecology Letters* 2: 135–139

Suzuki, J. I., Stuefer, J. (1999) On the ecological and evolutionary significance of storage in clonal plants. *Plant Species Biology 14*: 11–17

Wang, Z. Y., Ge, Y. (2006) Invited review: recent advances in genetic transformation of forage and turfgrasses. *In Vitro Cellular and Developmental Biology-Plant 42*: 1–18.

# CHAPTER 6

# Evaluation of Cold Hardiness in Selected Warm Season Turfgrasses under Controlled Conditions

## 6.1 Introduction

Playing fields and landscaped areas are key amenities of urban infrastructure. For viability from an owner’s perspective, turf surfaces need to meet various community needs, such as providing space for sport or exercise, along with aesthetic values (Casler, 2006). Requirements for a turf surface are also becoming more sophisticated. Modern sports venues in major urban areas are typically designed to accommodate multiple uses (e.g. summer and winter ball sports, concerts, athletics), adding pressure to the surface and grounds men from this intensive use from tightly packed programmes. As such, any damage from sporting or public activity and from adverse weather conditions (cold or heat stress), will be highly undesirable. Any loss of turf cover due to damage will not only impose an economic cost to reinstate the surface but will also increase associated problems such as weed dominance and surface erosion.

The use of warm season ($C_4$) turfgrasses is now widespread across tropical, subtropical, arid, semi-arid parts of the world and increasingly, more temperate climates with a shorter or milder winter (Turgeon, 2008). Commercial hybrids and selections from mutated plant material with shorter internodes and medium to fine leaf textures or with dwarf stature are ideal choices for domestic, public and recreational use (Casler, 2006; Beard, 1973). Warm season grasses usually exhibit excellent levels of tolerance to drought, heat stress and wear but lack the ability to survive low or freezing temperatures.

Intolerance to low temperature is the key factor limiting greater use of warm season ($C_4$) grasses (Bush, 2000). As noted in Section 2.7, warm season grasses start to turn dormant when exposed to temperatures below 15°C, with symptoms including browning of the leaves at around 10°C in situations where temperatures continue to fall to below that level (Beard, 1973). This chilling injury (discoloration, chlorosis, and necrosis) is known as winter browning. It is observed that the low temperature tolerance threshold varies greatly among species and varieties depending upon genetic and environment interactions (Stier, 2007). A phenomenon known as ‘cold acclimation’ is also widely recognised anecdotally within the turf industry, meaning that gradual, but prolonged exposure is assumed to be less damaging than sudden exposure to freezing. However, it has also been observed that continuous exposure to low temperature can kill a significant proportion of the existing warm season grass population. Low temperature exposure (both rapid and gradual) causes several physiological and morphological changes in the plants resulting in reduced transpiration rates, changes and triggering of particular enzymes, production of proteins related to stress tolerance and carbohydrate translocation (Bocian et al., 2011; Hisano et al., 2008). Prior exposure to non-lethal low temperature (cold acclimation) triggers the expression of many physiological and metabolic responses and enables the plant to cope with longer periods of cold stress (Cyril, 2002; Dionne, 2001).

Much of the research to date on cold tolerance of warm season turfgrasses has been conducted in the USA, where there is a continental climate with much wider seasonal temperature extremes than in many countries where $C_3$ grasses are widely grown. Hence, in the USA, tolerance to freezing temperatures is the most often-investigated performance trait for selection and usage of warm season turfgrasses under cooler climates (Schiavon et al., 2015; Stefaniak et al., 2009; Kopec et al., 2007; Casler, 2006; Zhang et al., 2006; Anderson et al., 2003; Beard, 1982). Such work tends to focus on identifying germplasm with a lower temperature threshold or longer duration of exposure required for plant death to occur (Section 2.7), rather than on identifying conditions that induce or prevent winter browning where plants are under continuous or successive exposure to below zero temperatures. However, very little research has explored low temperature (above freezing) tolerance and adaptation in warm season turfgrasses.

Therefore an experiment was designed to explore the morphological and physiological responses in warm season grasses when exposed to low but non freezing temperatures. The objective was to precisely define temperature threshold limits for industry use. This work is particularly relevant to areas of New Zealand with warm to hot summers and winter temperatures involving a comparatively low incidence of frosts – these being potential adoption regions for warm season turfgrasses. Different morphological and physiological parameters are reported and discussed in the following sections of the chapter, along with recovery rate of plants after cold exposure.

## 6.2 Materials and methods

### 6.2.1 Turf establishment

The experiment included four warm season turfgrass varieties from three botanical genera, Agri Dark (*Cynodon dactylon*), Windsor Green (*Cynodon dactylon*), Sea Spray (*Paspalum vaginatum)* and Zenith (*Zoysia japonica*). Turf cores (92 $cm^2$ approx.) were collected, using a standard golf cup cutter, from the glasshouse turf plots at the PGU, Massey University, Palmerston North, established during Nov–Dec 2012 and described in Chapter 3 with selective selection from best growing areas of the plot with uniform cover percentage. Roots were trimmed to 4 cm in length and cores were individually transplanted to rectangular plastic trays (230 x 188 x 52 mm) filled with 2:1 washed sand and alluvial silt (B horizon with a silt loam texture from a floodplain soil adjacent to the Manawatu river). Plants were then allowed to grow in a heated glasshouse (25/18°C day/night) under natural photoperiod for 6 weeks. Trays were fertilized with ammonium sulphate at 0.5 kg/100 $m^2$ once after transplanting and foliar application of liquid plant fertilizer (N: P: K 20:9:17 + trace elements diluted to 1 g/L concentration) was applied weekly. Plants were watered with an overhead sprinkling system twice daily. Temperature and light levels were monitored. Plants were kept trimmed to a height of 2.5 cm with the help of handheld battery operated grass shears on a fortnightly basis until the trays are shifted to the controlled temperature room. No trimming was carried out during the cold treatment and recovery period. .

### 6.2.2 Experimental design

Once established, the trays were shifted to a controlled climate room (CTR) for cold acclimation at 16/12 °C day/night temperature with approximately 300 micromoles photons $m^{-2}$ $sec^{-1}$ at plant height provided by a bank of fluorescent light tubes with low heat production. Trays were placed on tables to make sure even supply of light is available to all the trays. Trays were randomized in their placement order to minimize the extent of location impact on different treatments. The photoperiod was controlled with automatic timers at 14 h light and 10 h dark. Temperature in the dark period was set 4°C lower in the dark period than in the light period (12/8°C light/dark), and this regime maintained for four weeks. Temperature was then dropped in 2°C steps every 2weeks (maintaining the 4°C light/dark differential) to reach final temperatures of 6/2°C day/night (Fig. 6.1). One set of plants was removed at each successive temperature drop and herbage samples were collected for chemical analysis. The plants were then returned to the heated glasshouse with controlled temperature (25°C max/18°C min) under natural photoperiod for 2 weeks and scored for green up. Another set of plants was introduced directly from the glasshouse at each temperature change after 12/8°C day/night. In this way, eight different temperature regimes involving gradual or sudden introduction to low temperature, and various durations of exposure, were created (Table 6.1). Each treatment had four replicates.

### 6.2.3 Sample collection

Fresh leaf samples were collected twice for each treatment once after exposure to cold temperature and again after the two week recovery period in the glasshouse. Leaves were cut using sharp scissors and packed in labelled zip lock bags. All samples were preserved by immediate immersion in liquid nitrogen and stored at –80°C. Samples were then freeze dried and ground to fine powder using a ball mill, for further analysis.

**Table 6.1** Table of treatments imposed during the experiment

| | CTR Temperature (Light/Dark) | | | | | |
|---|---|---|---|---|---|---|
| | **16/12°C** | **12/8°C** | **10/6°C** | **8/4°C** | **6/2°C** | |
| | 15/8/14 – 12/9/14 | 12/9/14– 26/9/14 | 26/9/14– 10/10/14 | 10/10/14– 24/10/14 | 24/10/14– 07/11/14 | 07/11/14- 21/11/14 |
| **Duration of Cold Exposure** | 4 weeks | 2 weeks | 2 weeks | 2 weeks | 2 weeks | |
| | T1 | T2 | T4 | T6 | T8 | |
| | T3 | T3 | T5 | T7 | T9 | |
| | T5 | T5 | T7 | T9 | | |
| | T7 | T7 | T9 | | | |
| | T9 | T9 | | | | |
| **Recovery Glasshouse** | | T1 | T2<br>T3 | T4<br>T5 | T6<br>T7 | T8<br>T9 |

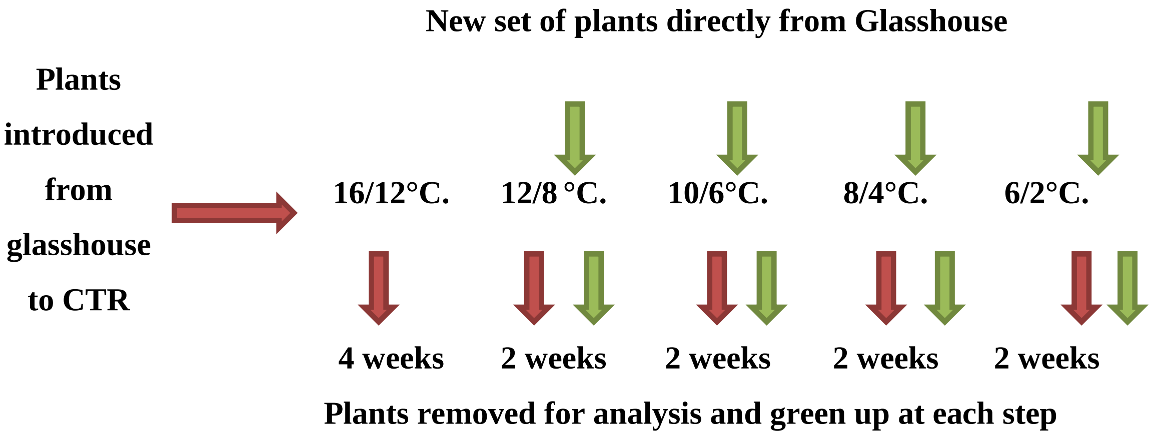


**Fig. 6.1** Flow chart for introduction to and removal of turf trays from the CTR

Fig. 6.2 Turfgrass trays during establishment in the glasshouse

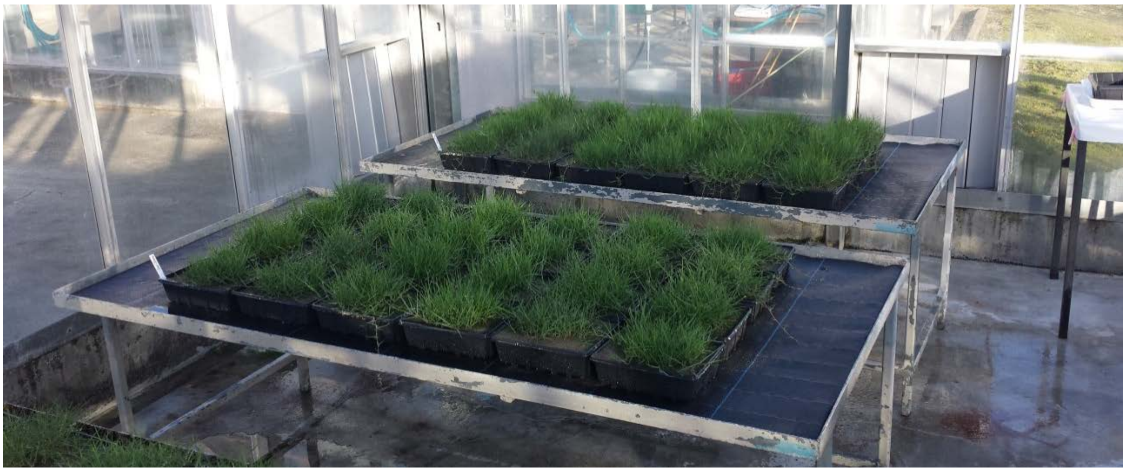


Fig. 6.3 Established turf in the glasshouse

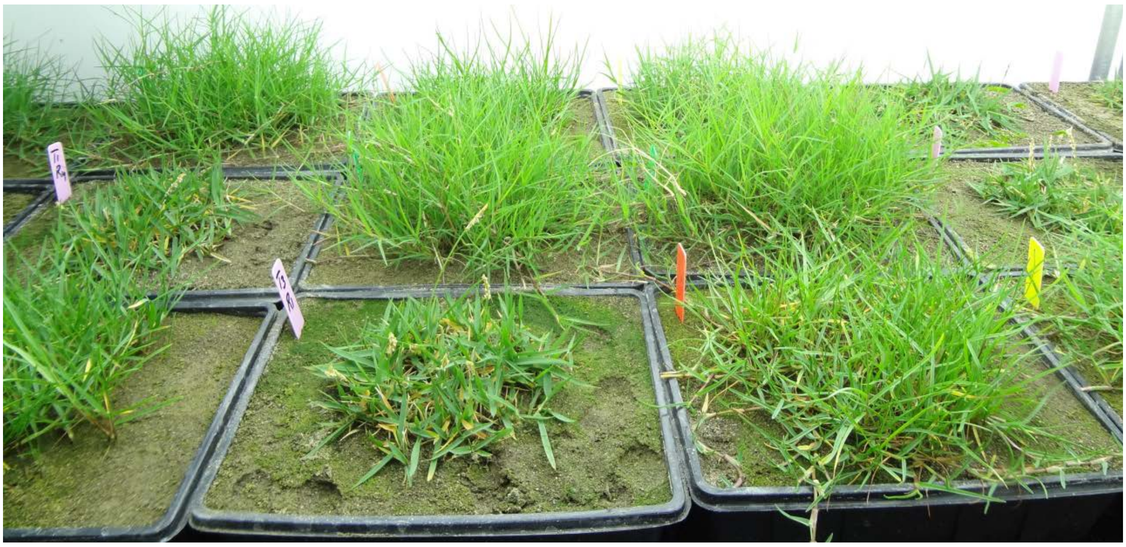


Fig. 6.4 Turf trays in the controlled temperature room (CTR)

### 6.2.4 Leaf proline contents

Leaf proline contents were determined based on the method of Magne and Larher (1992). Powdered leaf material (30 mg) was weighed into 2 ml Eppendorf tubes and mixed with 1.2 ml 3% (w/v) sulphosalicylic acid. Tubes were vortexed twice for 30 seconds and centrifuged at 10,000 rpm for 10 minutes. A 200 µl supernatant was then transferred into fresh tubes and diluted with 400 µl double distilled water. 800 µl freshly prepared ninhydrin reagent was added to the Eppendorf tubes, vortexed and placed in water bath for 60 minutes at 98°C. After completion of the reaction period, the tubes were plunged into ice to cool them and the supernatant was transferred to 5 ml tubes. Toluene (800 µl) was added to each sample and vortexed twice for 15 seconds. Samples were left for 5 minutes at room temperature for phase separation. The upper toluene phase was pipetted into a 1 ml quartz cuvette and absorbance was measured at 518 nm against a toluene blank. Proline contents were measured by comparing the absorbance against a set of proline standards (0, 5, 10, 15, 20, 25, 30 µg/ml).

### 6.2.5 MDA analysis

Plant malondialdehyde (MDA) is an indication of lipid peroxidation and therefore an indirect indication to cell damage levels. MDA was measured using MDA assay kit (Sr. # A003-3) obtained from the Nanjing Jiancheng Bioengineering Institute, China. Finely ground leaf samples (30 mg) were weighed and extracted according to the protocol accompanying the assay kit. Approximately 700 µl extract was transferred into a 1 ml quartz cuvette and absorbance was read at 532 nm and 600 nm. MDA contents were calculated using following formula:

$$MDA\ (nm/g) = \frac{OD\ Sample}{OD\ Standard} \times Standard\ conc.\,(10\ nm/ml)$$
$$\div\ Sample\ Conc.\,(g/ml)$$

Where:

OD Sample = (Sample$_{532}$ – Blank$_{532}$) – (Sample$_{600}$ – Blank$_{600}$)

OD Standard = (Standard$_{532}$ – Blank$_{532}$) – (Standard$_{600}$ – Blank$_{600}$)

Sample conc. = Plant tissue weight (g) / Extraction solution Volume (ml)

### 6.2.6 Carbohydrate content analysis

#### *6.2.6.1 Low molecular weight sugars (LMW'S)*

**Sample extraction:** Sample extraction was based on Pollock and Jones (1979). Approximately 25 mg of dried and powdered sample was placed into 2 ml Eppendorf tubes and 1 ml of 80% ethanol was added. Tubes were placed in a preheated water bath at 65°C, and shaken for 30 minutes, then centrifuged for 15 minutes at 13,000 rpm. The supernatant was pipetted out to another tube. The above procedure was repeated with the residue and the resulting supernatants were combined for analysis of LMW water soluble carbohydrates (mainly glucose, sucrose and fructose).

**Analysis (anthrone analysis):** Sample analysis was performed as described by Jermyn, et al., (1956). A 12 µl sample of supernatant was diluted with 188 µl of MilliQ water in a microcell and mixed well by pipetting. A 50 µl sample of diluted extract was transferred into a new microcell and 250 µl of freshly prepared Anthrone reagent was added. Each sample was replicated three times. Samples were incubated at 65°C in a pre-heated oven for 25 minutes and absorbance read at 620 nm. Sucrose at concentrations of 0, 10, 20, 30, 40, 50, 75 and 100 µg/ml was used as a standard.

#### *6.2.6.2 High molecular weight sugars*

**Sample extraction:** One millilitre of MilliQ water was added to the residue from LMW Carbohydrate extract and shaken for 30 minutes in a water bath at 65°C. Samples were then centrifuged for 15 minutes at 1300 rpm and the supernatant was transferred to a new Eppendorf tube. The above process was repeated and the combined supernatant was stored for analysis.

**Analysis:** A 40 µl subsample of extract was pipetted into a microcell and diluted with 160 µl of MilliQ water. A 50 µl subsample of diluted extract was transferred into a new microcell and 250 µl of freshly prepared anthrone reagent was added. Each sample was replicated three times. Initially inulin (a fructose polymer; ref) at concentrations of 0, 10, 20, 30, 40, 50, 75, 100 µg/ml was used as a standard. Samples were incubated at 65°C in a pre-heated oven for 25 minutes and absorbance read at 620 nm.

#### *6.2.6.3 Recalibration of the anthrone procedure for $C_4$ grasses*

The above procedure initially used in this experiment was designed to isolate LMW and HMW fructans in $C_3$ grasses, using sucrose and inulin as a standards, respectively. As it was later recognised that storage sugars in $C_4$ grasses differ from $C_3$ grasses, with glucose and starches predominating, a cross-calibration procedure was carried out to determine the response curves to the anthrone reagent, for sets of standards for inulin, starch and glucose, analysed under identical laboratory conditions in the same batch of samples.

### 6.2.7 Change in colour

Percent change in colour was accessed visually for each treatment. Plants were photographed using a custom built vertical tripod under similar light and exposure. Photographs were visually scored on a scale of 1-9, where 1 was severe damage and 9 was fully green coloured trays. Values presented in results are based on an average of 3 different scorings.

Variation in colour was recorded using colour, value and chroma values by comparing leaves against colour charts in a set of Munsell Colour Charts for Plant Tissues. (http://munsell.com/color-blog/plant-tissue-color-charts-update/). Three leaves from different positions of plant canopy were selected for this purpose. Leaves were matched against different green and yellow color discs from the chart and measurements were recorded for value, chroma and hue. Values from each tray were averaged for presentation in the results.

### 6.2.8 Green up or recovery

Spring green up or recovery was scored for percentage (%) change in colour based on visual observations after two weeks in glasshouse at 23/18°C day/night temperature for each treatment. Plants were photographed under similar conditions when first moved back into the glasshouse and compared for improvements in colour from photographs taken after two weeks back in the glasshouse.

### 6.2.9 Statistical analysis

A number of statistical models were explored, including some which fitted a response curve to temperature for species or other experimental design factors, but temperature responses were found to be so complex that this approach did not produce helpful description of data pattern. The model eventually adopted was to identify the fixed effect means using the GLM command in Minitab, for the 4 experimental factors:

temperature (12/8, 10/6, 8/4, and 6/2°C.), species (Agridark (AD), Sea Spray (SS), Windsor Green (WG) and Zenith (Zn)), acclimated or suddenly exposed, and stressed or recovered, and their two- and three-way interactions. Data were averaged over replicates for the GLM ANOVA and the 9 degrees of freedom of the 4-way interaction were used as the error term. Because this model produced 10 separate interaction terms, for each of 5 parameters analysed, these are not explored exhaustively in the results below but rather an attempt is made to focus on those responses with higher statistical significance that appear to best define the response patterns captured in the data.

## 6.3 Results

### 6.3.1 Proline

Comparison of proline accumulation data for the plants in the various temperature regimes (12/8°C, 10/6°C, 8/4°C, 6/2°C) allows inference about the conditions under which proline accumulation occurs. On sudden exposure to mild chilling (12/8°C, 10/6°C) in T2, and T4 proline levels were in the range 1.5–1.9 mg/g for the *Cynodon* and *Paspalum* varieties and a little higher for *Zoysia* (Table 6.2). With more severe chilling (8/4°C) proline values were elevated in the *Cynodon* and *Paspalum* varieties, but paradoxically with chilling to near freezing, proline levels tended to be lower than with mild chilling. On gradual exposure to chilling proline levels at any given temperature were notably higher than for those seen on sudden exposure to chilling, and proline levels were also elevated in the 6/2°C temperature treatment, and some elevation of proline content after prolonged chilling was also seen in *Zoysia,* When plants were placed in a 25/18°C) for recovery from chilling, values returned to those seen at the start of the experiment in plants exposed to mild cold temperature regimes (Table 6.2). The above differences are supported by relevant main effects and interactions in ANOVA (Appendix 6.1).

### 6.3.2 MDA

MDA is known for its natural existence in plants as a marker for oxidative stress. MDA accumulation under different temperature ranges and varied exposure length was evaluated and statistically analysed. Different species exhibited varied responses. Most of the interactions are statistically significant but it is difficult to discern a pattern or plant behaviour. All four species displayed lower MDA accumulation when exposed to 10/6ºC (T4) for a two week period as compared to similar duration at 12/8ºC, further decrease was observed in Agridark and Sea Spray at 8/4ºC, however Windsor green and Zenith showed elevated levels of MDA. At severe chilling 6/2ºC all varieties (except Zenith) displayed higher accumulation levels. With the prolonged exposure and gradual decrease in temperature a similar trend occurred except in Zenith where MDA levels tended to gradually decrease after 10/6°C (Table 6.3). When plants were shifted back to the glasshouse for recovery, all four species showed a significant decrease ($P<0.05$) in MDA accumulation at different levels (Appendix 6.1). All the interactions noted above were determined by ANOVA to be statistically significant (Appendix 6.1).

**Table 6.2 Proline accumulation (mg/g of dry matter) in turfgrass leaves after sudden and prolonged exposure to low temperature.**

| **Temp** | | **16/12°C** | | | | **12/8°C** | | | | **10/6°C** | | | | **8/4°C** | | | | **6/2°C** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | | | | | | **T2** | **Mean** | **SE** | | **T4** | **Mean** | **SE** | | **T6** | **Mean** | **SE** | | **T8** | **Mean** | **SE** |
| | | | | | 2 | **AD** | 1.89 | 0.325 | | **AD** | 1.58 | 0.112 | | **AD** | 2.94 | 0.017 | | **AD** | 1.48 | 0.055 |
| | | | | | | **WG** | 1.99 | 0.260 | | **WG** | 1.86 | 0.229 | | **WG** | 2.55 | 0.158 | | **WG** | 1.34 | 0.032 |
| | | | | | | **SS** | 1.56 | 0.155 | | **SS** | 1.46 | 0.053 | | **SS** | 1.92 | 0.125 | | **SS** | 1.38 | 0.080 |
| | | | | | | **Zn** | 2.65 | 0.109 | | **Zn** | 2.59 | 0.127 | | **Zn** | 2.63 | 0.176 | | **Zn** | 1.57 | 0.064 |
| | | | | | | **AD** | 1.42 | 0.046 | | **AD** | 1.25 | 0.014 | | **AD** | 1.70 | 0.257 | | **AD** | 1.27 | 0.041 |
| | | | | | | **WG** | 1.59 | 0.055 | | **WG** | 1.64 | 0.080 | | **WG** | 1.67 | 0.292 | | **WG** | 1.24 | 0.014 |
| | | | | | | **SS** | 1.46 | 0.095 | | **SS** | 1.24 | 0.007 | | **SS** | 1.18 | 0.006 | | **SS** | 1.25 | 0.023 |
| | | | | | | **Zn** | 1.59 | 0.072 | | **Zn** | 1.38 | 0.143 | | **Zn** | 1.38 | 0.128 | | **Zn** | 1.28 | 0.037 |
| | 4 | **T1** | **Mean** | **SE** | | | | | | | | | | | | | | | | |
| | | **AD** | 2.03 | 0.382 | | | | | | | | | | | | | | | | |
| | | **WG** | 2.60 | 0.202 | | **T3** | **Mean** | **SE** | | | | | | | | | | | | |
| | | **SS** | 2.54 | 0.384 | 6 | **AD** | 2.44 | 0.154 | | | | | | | | | | | | |
| | | **Zn** | 3.02 | 0.023 | | **WG** | 1.77 | 0.228 | 8 | **T5** | **Mean** | **SE** | | | | | | | | |
| | | **AD** | 1.46 | 0.067 | | **SS** | 2.17 | 0.162 | | **AD** | 2.74 | 0.104 | | | | | | | | |
| | | **WG** | 1.93 | 0.229 | | **Zn** | 2.85 | 0.053 | | **WG** | 2.26 | 0.052 | 10 | **T7** | **Mean** | **SE** | | | | |
| | | **SS** | 2.10 | 0.343 | | **AD** | 1.76 | 0.151 | | **SS** | 2.79 | 0.053 | | **AD** | 2.87 | 0.036 | | | | |
| | | **Zn** | 2.23 | 0.424 | | **WG** | 1.57 | 0.238 | | **Zn** | 2.82 | 0.013 | | **WG** | 2.66 | 0.134 | 12 | **T9** | **Mean** | **SE** |
| | | | | | | **SS** | 1.46 | 0.091 | | **AD** | 1.43 | 0.078 | | **SS** | 3.02 | 0.012 | | **AD** | 2.89 | 0.043 |
| | | | | | | **Zn** | 2.37 | 0.246 | | **WG** | 1.26 | 0.019 | | **Zn** | 3.04 | 0.024 | | **WG** | 2.95 | 0.038 |
| | | | | | | | | | | **SS** | 1.27 | 0.008 | | **AD** | 1.26 | 0.008 | | **SS** | 3.05 | 0.030 |
| | | | | | | | | | | **Zn** | 1.43 | 0.029 | | **WG** | 1.26 | 0.023 | | **Zn** | 3.04 | 0.008 |
| | | | | | | | | | | | | | | **SS** | 1.39 | 0.083 | | **AD** | 1.32 | 0.027 |
| | | | | | | | | | | | | | | **Zn** | 1.49 | 0.056 | | **WG** | 1.33 | 0.023 |
| | | | | | | | | | | | | | | | | | | **SS** | 1.49 | 0.065 |
| | | | | | | | | | | | | | | | | | | **Zn** | 1.62 | 0.029 |

The upper panel indicates values after 2 weeks exposure to the stated temperature for Agridark (AD), Windsor green (WG), Seas Spray (SS), and Zenith (Zn) in the upper 4 rows and after recovery in rows 5 – 8 (shaded). The lower panel indicates progressive lowering of temperature. Red numbers are total weeks exposure to cold temperature, and stepped tables show the sequence of temperature exposure from left to right. Proline values in each panel are those measured at the end of the indicated progressive temperature reduction and after recovery.

**Table 6.3 MDA accumulation (nmol/g of dry matter) in turfgrass leaves after sudden and prolonged exposure to low temperature.**

| Temp | 16/12°C | | | 12/8°C | | | 10/6°C | | | 08/4°C | | | 06/2°C | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | | | | T2 | Mean | SE | T4 | Mean | SE | T6 | Mean | SE | T8 | Mean | SE |
| 2 | | | | AD | 55.32 | 4.170 | AD | 55.02 | 5.157 | AD | 52.18 | 1.352 | AD | 57.73 | 2.01 |
| | | | | WG | 36.97 | 1.995 | WG | 32.23 | 3.830 | WG | 40.83 | 6.877 | WG | 55.06 | 1.11 |
| | | | | SS | 61.27 | 3.549 | SS | 58.04 | 5.335 | SS | 51.49 | 4.092 | SS | 55.95 | 1.55 |
| | | | | Zn | 44.65 | 3.607 | Zn | 37.17 | 3.420 | Zn | 40.16 | 2.221 | Zn | 37.29 | 1.84 |
| | | | | AD | 47.71 | 0.886 | AD | 49.73 | 5.075 | AD | 39.66 | 1.063 | AD | 40.98 | 0.66 |
| | | | | WG | 28.74 | 2.640 | WG | 28.00 | 2.466 | WG | 25.25 | 2.017 | WG | 29.73 | 3.17 |
| | | | | SS | 52.23 | 3.369 | SS | 49.27 | 1.832 | SS | 36.64 | 4.677 | SS | 36.91 | 3.30 |
| | | | | Zn | 31.42 | 2.505 | Zn | 31.05 | 0.309 | Zn | 33.07 | 1.025 | Zn | 29.21 | 2.32 |
| 4 | T1 | Mean | SE | | | | | | | | | | | | |
| | AD | 51.95 | 4.611 | | | | | | | | | | | | |
| 6 | WG | 40.24 | 3.134 | T3 | Mean | SE | | | | | | | | | |
| | SS | 43.39 | 2.827 | AD | 53.54 | 5.373 | | | | | | | | | |
| 8 | Zn | 44.65 | 2.511 | WG | 45.62 | 8.450 | T5 | Mean | SE | | | | | | |
| | AD | 43.38 | 5.543 | SS | 51.10 | 7.484 | AD | 52.21 | 4.757 | | | | | | |
| 10 | WG | 33.81 | 1.452 | Zn | 59.88 | 4.471 | WG | 39.53 | 1.458 | T7 | Mean | SE | | | |
| | SS | 36.83 | 2.579 | AD | 38.02 | 5.136 | SS | 48.68 | 2.103 | AD | 64.00 | 10.90 | | | |
| 12 | Zn | 36.96 | 2.579 | WG | 35.06 | 7.742 | Zn | 67.89 | 8.046 | WG | 41.91 | 2.369 | T9 | Mean | SE |
| | | | | SS | 44.07 | 6.779 | AD | 45.99 | 2.378 | SS | 46.28 | 2.652 | AD | 60.88 | 3.19 |
| | | | | Zn | 43.72 | 4.066 | WG | 31.53 | 1.924 | Zn | 64.36 | 5.123 | WG | 49.75 | 8.23 |
| | | | | | | | SS | 42.49 | 1.449 | AD | 49.92 | 5.860 | SS | 66.74 | 2.10 |
| | | | | | | | Zn | 39.84 | 1.585 | WG | 33.58 | 4.626 | Zn | 59.61 | 4.50 |
| | | | | | | | | | | SS | 40.21 | 3.174 | AD | 44.10 | 7.20 |
| | | | | | | | | | | Zn | 52.10 | 7.193 | WG | 28.60 | 4.72 |
| | | | | | | | | | | | | | SS | 50.78 | 3.27 |
| | | | | | | | | | | | | | Zn | 55.24 | 4.62 |

The upper panel indicates values after 2 weeks exposure to the stated temperature for Agridark (AD), Windsor green (WG), Seas Spray (SS), and Zenith (Zn) in the upper 4 rows and after recovery in rows 5 – 8 (shaded). The lower panel indicates progressive lowering of temperature. Red numbers are total weeks exposure to cold temperature, and stepped tables show the sequence of temperature exposure from left to right. MDA values in each panel are those measured at the end of the indicated progressive temperature reduction and after recovery.

### 6.3.3 Recalibration of the anthrone procedure for glucose and starch

It was found that starch and glucose respond similarly to the anthrone reagent under the extraction conditions used, but much less strongly than inulin (Fig. 6.5). From this result it can be inferred that both LMW and HMW analyses would have been affected by the low sensitivity of the anthrone reagent to glucose compared to fructose, when the procedure was applied to samples from $C_4$ grasses, but there should not have been any problem with incomplete starch hydrolysis during the original HMW analyses. Based on these findings, HMW sugar concentrations were recalculated from the original data based on the starch standard curves as being more appropriate for carbohydrate determinations in $C_4$ grasses.

### 6.3.4 Low molecular weight sugars

In this section the results analysis for accumulation of low molecular weight (LMW) sugars for four grass species when suddenly subjected to or gradually introduced to a range of low temperatures (12/8ºC, 10/6ºC, 8/4ºC, 6/2ºC) and exposure times are presented. When subjected to a sudden exposure plants tended to show an increase in sugar levels in proportion to the level of low temperature exposure across the range of temperatures tested: 12/8ºC, 10/6ºC and 8/4ºC (Table 6.4). Exposure to more severe chilling (6/2°C) caused a decrease in levels as compared with LMW sugar levels at 08/04ºC except for Windsor green where the trend is still towards increased accumulation at the lowest temperature. In plants subjected to gradual temperature reduction and prolonged exposure to lower temperature there was again a tendency to increase in sugar levels with greater cold exposure for the range 12/8ºC, 10/6ºC, 8/4ºC. However, sugar levels dropped at 6/2°C except for Zenith which showed highest accumulation at this temperature.

When plants were shifted to the glasshouse for recovery from cold damage, a decrease in LMW sugar levels was observed. Significant differences ($P<0.05$) were found for exposure to different temperature among different species. Most of the interactions revealed non-significant relations except recovery in plants exposed to different temperatures and among different species as well (Appendix 6.2). Due to the complex pattern of the experiment results, it was difficult to identify any consistent pattern among the interaction terms. In addition, most of them were found to be statistically non-significant.

### 6.3.5 High molecular weight sugars

Highest levels of high molecular weight (HMW) sugar accumulation were recorded at 12/8ºC for both *Cynodon* varieties and for Sea Spray. However, for Zenith they were higher at 8/4ºC. Species responses were statistically non-significant (Table 6.5). During the prolonged exposure to low temperature ranges all species showed a decline in HMW sugars at 10/6 ºC and 8/4ºC compared with the initial reading at mild cold (12/8ºC). Sugar accumulation was found to be higher in all varieties after 12 weeks of gradually lowered temperature at 6/2ºC. ANOVA verified highly significant interactions between exposure time and temperature ($P<0.001$). Agridark had the highest HMW sugar accumulation at 6/2ºC among the varieties tested.

When transferred to glasshouse plants from both exposure regimes showed a decline in high molecular weight sugar levels (Table 6.5). Minimal change in sugar levels during recovery was found in Sea Spray and even a slight increase was recorded at 6/2ºC both for sudden and gradual exposure treatments. All the results and interactions indicated above are statistically significant in the ANOVA results (Appendix 6.3)

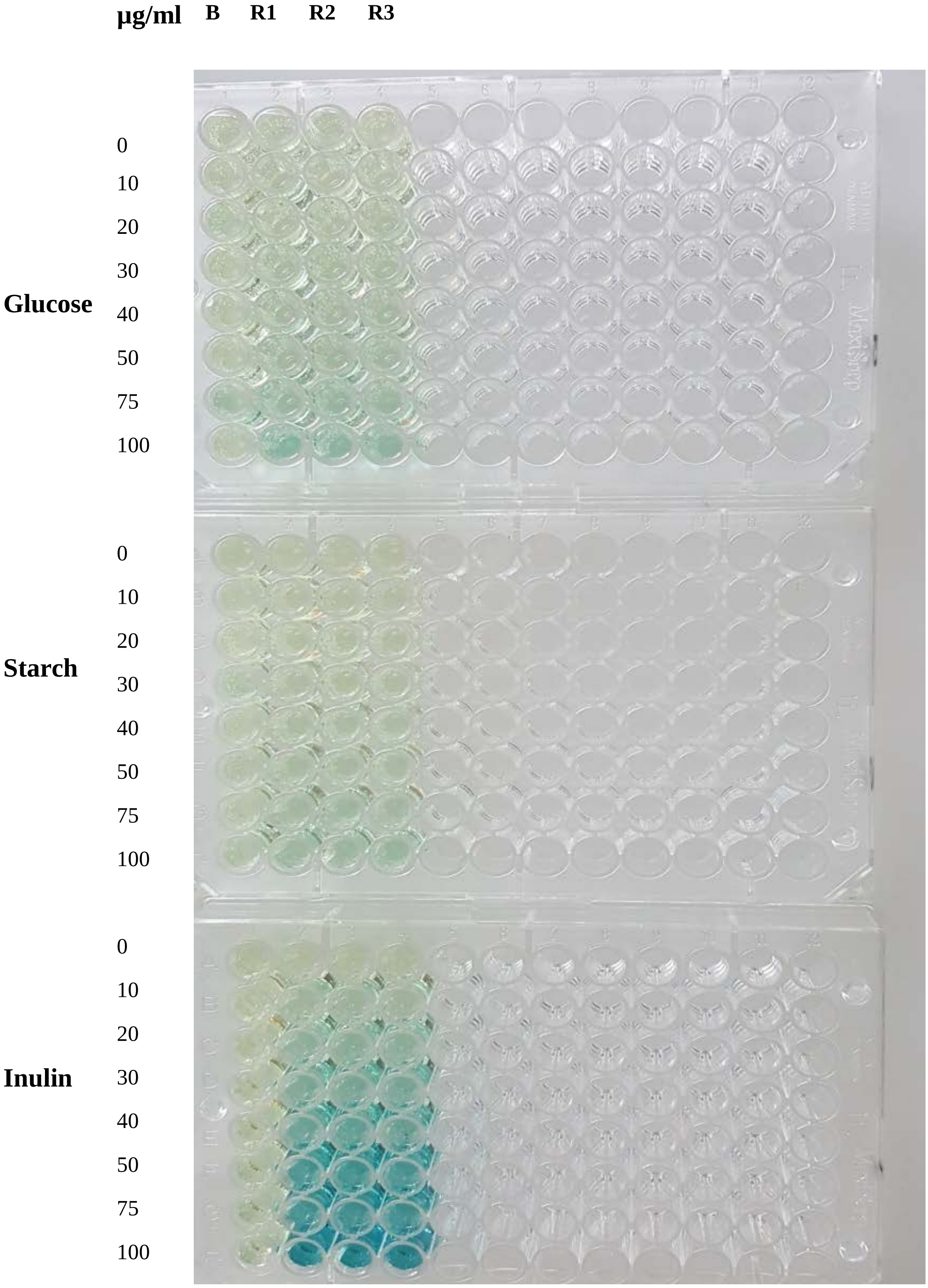


**Fig 6.5 Colour development for the anthrone reagent in the cross-calibration with glucose, starch and inulin.**

(The left hand column in each plate is a blank (B) and the next three columns are repeats (R1, R2, R3). Concentrations in successive horizontal rows from bottom to top are 0, 10, 20, 30, 40, 50, 75, and 100 µg/ml, as shown to the left of each photograph.)

**Table 6.4 Variation in low molecular weight sugar levels (mg $g^{-1}$ of dry matter) in turfgrass leaves after sudden and prolonged exposure to low temperature.**

| T( °C) | 16/12°C | | | | 12/8°C | | | | 10/06°C | | | | 08/4°C | | | | 6/2°C | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | | | | | T2 | Mean | SE | | T4 | Mean | SE | | T6 | Mean | SE | | T8 | Mean | SE |
| 2 | | | | | AD | 213.27 | 5.86 | | AD | 264.04 | 6.33 | | AD | 326.42 | 14.90 | | AD | 291.12 | 32.21 |
| | | | | | WG | 174.12 | 6.67 | | WG | 185.52 | 8.69 | | WG | 276.08 | 10.27 | | WG | 303.22 | 14.66 |
| | | | | | SS | 140.33 | 5.94 | | SS | 194.29 | 15.44 | | SS | 287.14 | 14.11 | | SS | 232.97 | 15.61 |
| | | | | | Zn | 214.22 | 6.76 | | Zn | 218.54 | 13.51 | | Zn | 304.55 | 11.57 | | Zn | 254.41 | 25.02 |
| | | | | | AD | 156.36 | 10.11 | | AD | 110.72 | 10.18 | | AD | 123.22 | 9.86 | | AD | 102.41 | 2.44 |
| | | | | | WG | 158.93 | 4.18 | | WG | 83.10 | 7.02 | | WG | 118.25 | 5.44 | | WG | 90.96 | 5.23 |
| | | | | | SS | 108.09 | 5.33 | | SS | 80.76 | 12.39 | | SS | 92.21 | 7.90 | | SS | 52.99 | 4.07 |
| | | | | | Zn | 158.83 | 7.46 | | Zn | 78.35 | 9.95 | | Zn | 98.79 | 7.13 | | Zn | 111.33 | 8.51 |
| 4 | T1 | Mean | SE | | | | | | | | | | | | | | | | |
| | AD | 168.27 | 17.74 | | | | | | | | | | | | | | | | |
| | WG | 202.32 | 12.27 | 6 | T3 | Mean | SE | | | | | | | | | | | | |
| | SS | 112.93 | 10.86 | | AD | 153.44 | 8.89 | | | | | | | | | | | | |
| | Zn | 136.48 | 21.55 | | WG | 160.35 | 13.93 | 8 | T5 | Mean | SE | | | | | | | | |
| | AD | 111.14 | 15.15 | | SS | 154.86 | 14.46 | | AD | 207.29 | 13.87 | | | | | | | | |
| | WG | 106.55 | 11.10 | | Zn | 208.37 | 8.98 | | WG | 156.78 | 6.87 | 10 | T7 | Mean | SE | | | | |
| | SS | 106.06 | 10.30 | | AD | 153.04 | 25.80 | | SS | 185.54 | 15.62 | | AD | 259.11 | 7.85 | | | | |
| | Zn | 113.52 | 23.43 | | WG | 129.58 | 10.80 | | Zn | 295.69 | 4.87 | | WG | 236.85 | 14.74 | 12 | T9 | Mean | SE |
| | | | | | SS | 118.79 | 16.04 | | AD | 114.12 | 14.79 | | SS | 235.33 | 9.42 | | AD | 173.43 | 13.62 |
| | | | | | Zn | 124.75 | 16.34 | | WG | 106.39 | 6.55 | | Zn | 383.15 | 11.09 | | WG | 129.69 | 14.42 |
| | | | | | | | | | SS | 91.98 | 22.34 | | AD | 176.69 | 9.64 | | SS | 155.96 | 7.16 |
| | | | | | | | | | Zn | 53.06 | 4.87 | | WG | 129.54 | 6.49 | | Zn | 434.61 | 16.50 |
| | | | | | | | | | | | | | SS | 81.75 | 6.23 | | AD | 82.95 | 5.81 |
| | | | | | | | | | | | | | Zn | 69.30 | 4.12 | | WG | 79.61 | 10.35 |
| | | | | | | | | | | | | | | | | | SS | 50.87 | 2.19 |
| | | | | | | | | | | | | | | | | | Zn | 51.14 | 4.22 |

The upper panel indicates values after 2 weeks exposure to the stated temperature for Agridark (AD), Windsor green (WG), Seas Spray (SS), and Zenith (Zn) in the upper 4 rows and after recovery in rows 5 – 8 (shaded). The lower panel indicates progressive lowering of temperature. Red numbers are total weeks exposure to cold temperature, and stepped tables show the sequence of temperature exposure from left to right. LMW sugars values in each panel are those measured at the end of the indicated progressive temperature reduction and after recovery.

**Table 6.5 Variation in high molecular weight sugar levels (mg g$^{-1}$ of dry matter) in turfgrass leaves after sudden and prolonged exposure to low temperature.**

| T °C | 16/12°C | | | 12/8°C | | | 10/6°C | | | 8/4°C | | | 6/2°C | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | | | | T2 | Mean | SE | T4 | Mean | SE | T6 | Mean | SE | T8 | Mean | SE |
| 2 | | | | AD | 85.93 | 14.75 | AD | 72.34 | 2.50 | AD | 58.20 | 7.08 | AD | 60.68 | 4.09 |
| | | | | WG | 79.41 | 11.81 | WG | 61.04 | 2.03 | WG | 67.34 | 9.67 | WG | 64.55 | 7.47 |
| | | | | SS | 86.58 | 10.55 | SS | 74.25 | 5.47 | SS | 87.87 | 9.63 | SS | 50.60 | 3.81 |
| | | | | Zn | 76.01 | 6.83 | Zn | 55.05 | 5.90 | Zn | 78.27 | 10.08 | Zn | 73.40 | 2.72 |
| | | | | AD | 54.93 | 11.70 | AD | 55.19 | 8.79 | AD | 39.16 | 5.07 | AD | 54.02 | 4.74 |
| | | | | WG | 77.64 | 10.88 | WG | 44.42 | 6.78 | WG | 55.56 | 6.25 | WG | 56.28 | 6.54 |
| | | | | SS | 84.29 | 6.81 | SS | 66.58 | 8.05 | SS | 73.22 | 10.01 | SS | 52.89 | 6.25 |
| | | | | Zn | 60.12 | 3.56 | Zn | 46.41 | 3.99 | Zn | 57.36 | 5.89 | Zn | 53.57 | 9.62 |
| 4 | T1 | Mean | SE | | | | | | | | | | | | |
| | AD | 90.10 | 18.52 | | | | | | | | | | | | |
| 6 | WG | 74.46 | 6.28 | T3 | Mean | SE | | | | | | | | | |
| | SS | 61.88 | 9.94 | AD | 83.70 | 4.25 | | | | | | | | | |
| 8 | Zn | 88.22 | 9.67 | WG | 74.98 | 7.30 | T5 | Mean | SE | | | | | | |
| | AD | 75.69 | 8.37 | SS | 68.62 | 5.75 | AD | 69.34 | 12.61 | | | | | | |
| 10 | WG | 74.27 | 7.05 | Zn | 83.32 | 4.12 | WG | 52.91 | 1.60 | T7 | Mean | SE | | | |
| | SS | 54.81 | 7.03 | AD | 59.41 | 2.61 | SS | 74.34 | 7.24 | AD | 78.13 | 12.05 | | | |
| 12 | Zn | 92.58 | 13.04 | WG | 59.23 | 7.86 | Zn | 74.62 | 5.66 | WG | 72.47 | 17.05 | T9 | Mean | SE |
| | | | | SS | 59.97 | 6.14 | AD | 57.29 | 5.53 | SS | 56.29 | 8.46 | AD | 122.85 | 8.87 |
| | | | | Zn | 53.83 | 4.48 | WG | 49.37 | 5.55 | Zn | 79.81 | 2.97 | WG | 103.89 | 7.72 |
| | | | | | | | SS | 63.24 | 14.31 | AD | 58.66 | 9.55 | SS | 86.58 | 12.78 |
| | | | | | | | Zn | 53.33 | 7.31 | WG | 57.37 | 11.38 | Zn | 114.55 | 26.45 |
| | | | | | | | | | | SS | 49.48 | 12.25 | AD | 106.29 | 9.24 |
| | | | | | | | | | | Zn | 44.82 | 3.88 | WG | 60.77 | 4.46 |
| | | | | | | | | | | | | | SS | 93.75 | 3.90 |
| | | | | | | | | | | | | | Zn | 41.36 | 9.72 |

The upper panel indicates values after 2 weeks exposure to the stated temperature for Agridark (AD), Windsor green (WG), Seas Spray (SS), and Zenith (Zn) in the upper 4 rows and after recovery in rows 5 – 8 (shaded). The lower panel indicates progressive lowering of temperature. Red numbers are total weeks exposure to cold temperature, and stepped tables show the sequence of temperature exposure from left to right. HMW sugar values in each panel are those measured at the end of the indicated progressive temperature reduction and after recovery.

### 6.3.5 Plant colour change during cold exposure and subsequent recovery

The degree of change in colour differed between the grass species when subjected to the range of low temperature (12/8ºC, 10/6ºC, 8/4ºC, 6/2ºC) and exposure times used in this experiment. However, all four species were affected by the low temperature exposure treatments.

Zenith (*Z. japonica*) was the most susceptible variety to cold damage with a higher percentage of discoloration than other varieties while Sea Spray (*P. vaginatum*) showed the greatest ability among tested varieties to retain colour. When subjected to sudden exposure to colder temperatures, Agridark was more affected compared to Windsor green (Table 6.6). However, during prolonged exposure treatments, initial discoloration was high for Agridark but the level of discolouration did not change greatly with time. The lowest minimum colour damage (25%) was recorded for Sea spray when exposed to chilling temperature for a prolonged period with gradually decreasing temperatures down to 6/2ºC.

When plants were shifted to the glasshouse for recovery from cold damage an overall decrease in the level of discoloration was observed, and the colour improvement was statistically significant ($P<0.05$) (Appendix 6.4). All varieties showed an improvement in colour during recovery and most of the interactions between experiment treatment factors were statistically significant.

**Table 6.6 Degree of colour change during and after exposure to low temperature based on % age and colour characteristics**

| T °C | 16/12°C | | | 12/8°C | | | 10/6°C | | | 8/4°C | | | 6/2°C | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | | | | T2 | C-V-C | %Dc | T4 | C-V-C | %Dc | T6 | C-V-C | %Dc | T8 | C-V-C | %Dc |
| 2 | | | | AD | 5GY/5/6 | 7 | AD | 5GY-5/8 | 8 | AD | 5GY-5/6 | 5 | AD | 5GY-4/6 | 10 |
| | | | | WG | 5GY/5/6 | 4 | WG | 5GY-5/6 | 5 | WG | 5GY-5/6 | 8 | WG | 5GY-4/6 | 7 |
| | | | | SS | 5GY/5/6 | 2 | SS | 5GY-5/6 | 2 | SS | 5GY-5/8 | 3 | SS | 5GY-5/6 | 5 |
| | | | | Zn | 5GY/4/4 | 10 | Zn | 5GY-4/4 | 10 | Zn | 5GY-4/4 | 12 | Zn | 5GY-4/4 | 12 |
| | | | | AD | 5GY/5/6 | 2 | AD | 5GY-5/6 | 3 | AD | 5GY-5/6 | 2 | AD | 5GY-4/6 | 3 |
| | | | | WG | 5GY/5/6 | 2 | WG | 5GY-4/4 | 2 | WG | 5GY-4/4 | 4 | WG | 5GY-4/6 | 5 |
| | | | | SS | 5GY/4/8 | 1 | SS | 5GY-5/6 | 1 | SS | 5GY-5/6 | 1 | SS | 5GY-5/6 | 2 |
| | | | | Zn | 5GY/4/6 | 3 | Zn | 5GY-4/4 | 3 | Zn | 5GY-4/4 | 5 | Zn | 5GY-4/4 | 5 |
| 4 | T1 | C-V-C | %Dc | | | | | | | | | | | | |
| | AD | 5GY-5/4 | 0 | | | | | | | | | | | | |
| 6 | WG | 5GY-5/6 | 0 | T3 | C-V-C | %Dc | | | | | | | | | |
| | SS | 5GY-5/4 | 2 | AD | 5GY-5/6 | 10 | | | | | | | | | |
| 8 | Zn | 5GY-5/4 | 4 | WG | 5GY-5/6 | 2 | T5 | C-V-C | %Dc | | | | | | |
| | AD | 5GY-5/4 | 0 | SS | 5GY-5/6 | 2 | AD | 5GY-5/6 | 20 | | | | | | |
| 10 | WG | 5GY-5/4 | 0 | Zn | 5GY-4/6 | 30 | WG | 5GY-5/6 | 30 | T7 | C-V-C | %Dc | | | |
| | SS | 5GY-5/6 | 0 | AD | 5GY-5/6 | 2 | SS | 5GY-5/4 | 5 | AD | 5GY-5/6 | 25 | | | |
| 12 | Zn | 5GY-5/4 | 1 | WG | 5GY-5/6 | 1 | Zn | 5Y-5/6 | 45 | WG | 5GY-5/6 | 25 | T9 | C-V-C | %Dc |
| | | | | SS | 5GY-5/6 | 1 | AD | 5GY-5/4 | 5 | SS | 5GY-5/4 | 15 | AD | 5GY-5/6 | 30 |
| | | | | Zn | 5GY-4/6 | 8 | WG | 5GY-5/4 | 4 | Zn | 2.5GY-5/4 | 65 | WG | 5GY-5/6 | 35 |
| | | | | | | | SS | 5GY-4/6 | 2 | AD | 5GY-5/4 | 4 | SS | 5GY-5/4 | 25 |
| | | | | | | | Zn | 5GY-4/4 | 10 | WG | 5GY-5/6 | 5 | Zn | 2.5GY-5/4 | 85 |
| | | | | | | | | | | SS | 5GY-4/6 | 2 | AD | 5GY-5/4 | 10 |
| | | | | | | | | | | Zn | 5GY-4/6 | 25 | WG | 5GY-5/6 | 15 |
| | | | | | | | | | | | | | SS | 5GY-5/6 | 8 |
| | | | | | | | | | | | | | Zn | 5GY-4/6 | 50 |

Where C-V-C = Colour- Value-Chroma and %Dc = Percent discoloration. The upper panel indicates values after 2 weeks exposure to the stated temperature for Agridark (AD), Windsor green (WG), Seas Spray (SS), and Zenith (Zn) in the upper 4 rows and after recovery in rows 5 – 8 (shaded). The lower panel indicates progressive lowering of temperature. Red numbers are total weeks exposure to cold temperature, and stepped tables show the sequence of temperature exposure from left to right. Percent color change values in each panel are those measured at the end of the indicated progressive temperature reduction and after recovery.

## 6.4 Discussion

### 6.4.1 Proline

Warm season (C4) grasses are usually susceptible to chilling injury in the temperature range of 1°C–12°C. This may cause plant membrane damage, loss of plant proteomic activity, and decreased photosynthesis and respiration (Stier, 2007). Proline is an amino acid widely studied as a response indicator to abiotic stresses including cold stress. Many scientists have reported an increase in proline concentration during exposure to low temperatures in experiments performed under a range of conditions on warm season turfgrasses (Munshaw et al., 2006; Zhang et al., 2006; Zhang et al., 2011). Increased proline content in turfgrasses is often correlated with increased cold tolerance. Different species and varieties exhibited different responses and a high level of variation in proline accumulation was associated with variations in temperature and length of exposure. Similar responses were recorded by He et al. (2010), for seashore paspalum in another experiment where a significantly higher concentration of proline was present in plants after chilling stress, when compared with normal conditions. In another experiment, Cai et al. (2004) verified that proline contents in centipede grass (*Eremochloa ophiuroides*) corresponded to decreases in temperature and played an important role in cold tolerance.

Patton et al. (2007) studied 13 different genotypes of *Zoysia* and found differences in cold tolerance related to proline and carbohydrate accumulation. They concluded that proline levels increased in *Zoysia* during cold acclimation but, proline was less beneficial to plants as temperature drops. Kauffman (2010) found the *Zoysia* cultivar Diamond, the hardiest variety among different cultivars of Bermudagrass, *Zoysia* and Seashore paspalum, followed by TifEagle, Champion, and then SeaDwarf. Cold acclimation was associated with increased proline concentration for all species/varieties except SeaDwarf.

### 6.4.2 MDA

He et al. (2010) related the increase in MDA contents to presumed oxidative injury to plant cells in seashore paspalum. They reported a 3–5 fold increase in MDA levels after two weeks of chilling stress. Lower MDA levels in plants exposed to chilling stress indicate better resistance and membrane stability (Wang et al., 2010). When plants were exposed to 10/6ºC they showed a decrease in MDA contents which may be indicative of adaption or resistance to lower temperatures but, with further decrease in temperature MDA levels tend to increase again. This may be indicative of further damage to plant membranes. Wei et al. (2008) measured the cold sensitivity and biochemical interactions in *Z. Japonica* and recorded a gradual increase in leaf MDA contents with decrease in temperature; drop in stolon MDA levels was found at temperatures lower than 12ºC. Fan et al. (2014) investigated MDA contents as an indicator of oxidative damage in cold stress and demonstrated a significant increase in leaf MDA contents in Bermudagrass. A decrease in leaf MDA contents was recorded with increased length of exposure time to low temperature and concluded this needed further investigation.

The results of this study are consistent with those of Liu et al. (2016). In their experiment to investigate combined effects of low temperature and salt stress on photosynthetic and antioxidant responses in Bermudagrass, they found an increase in leaf MDA contents during cold stress and related this to the role of low temperatures in lipid peroxidation. In other experiments, Wang et al. (2009) found *Z. matrella* exhibited gradual increase in MDA contents with increase in chilling stress in control and pre-treated (salicylic acid, calcium chloride, hydrogen peroxide) leaves, however, greater oxidative damage was observed in control treatments.

### 6.4.3 Carbohydrates

Non-structural carbohydrate concentration is often correlated with low temperature tolerance in warm season turfgrasses and reflects appearance of substances responsible for membrane stabilization and limiting crystallization. Concentration of soluble carbohydrates in *Stenotaphrum secundatum (*buffalograss) varied significantly in samples taken at different times of the year (Bell et al., 2002). Similarly, Bush et al. (2000) reported an elevation in leaf starch contents of carpet-grass during cooler months. An association between higher concentration of non-structural carbohydrates and cold tolerance has been reported in several studies by Huang et al. (2014). As with the findings in this experiment, DiPaola and Beard (1992) reported higher levels of sugars in *Zoysia* compared with *Cynodon* and related this to improved survival ability in cold temperatures. Zhang et al. (2006) reported that accumulation of non-structural carbohydrate contents during and after cold acclimation can attribute to better cold resistance in Riviera (*Z. japonica*) compared with Princess 77 (*C. dactylon*). Riviera accumulated almost double non-structural carbohydrate contents during acclimation compared to Princess 77.

In the present experiment, response patterns were complex and unravelling them is very difficult, however, there were some general responses. On sudden exposure to low temperature, there was a generally consistent response whether suddenly or gradually introduced, involving increased LMW and HMW sugar, MDA, and proline concentrations in leaf tissue, and loss of colour. Generally the ANOVA term testing sudden versus gradual exposure was more significant than the term for temperature exposed to, with greater indication of damage on gradual exposure to the same temperature (ANOVA Tables in appendices). However, contrary to the trend for increasing concentrations of measured metabolites in proportion to the temperature drop imposed, LMW sugar accumulation tended to be lower at 6/2°C than at 8/4ºC with few exceptions. Meanwhile, for HMW sugars there was increased concentration with mild low temperature exposure but not with more severe lowering of temperatures, although increase was then observed at the lowest temperature. Collectively the results suggest that for the tested varieties, plant protection systems against cold stress are able to operate down to 8/4ºC, but at 6/2ºC the cold protection mechanisms were blocked.

The shift of measured metabolite concentrations back towards normal after cold exposure was also a large and highly significant effect in most of the ANOVAs. An exception was Sea Spray (Seashore paspalum) where metabolite levels remained almost unchanged in response to low temperature exposure, and an improvement in growth was also observed. Patton et al. (2007) found higher concentrations of total reducing sugars, glucose, and proline were positively associated with *Zoysia* grass tolerance to freezing temperatures, whereas higher concentrations of starch appeared to be correlated with freezing damage among 13 genotypes of *Z. japonica.*

### 6.4.4 Discoloration

From a green keeper or groundsman’s point of view, loss of colour during winter is one of the most undesirable consequences of using warm season grasses in cooler climates. A year round, green, uniform turf surface is desirable and is more aesthetically pleasing. Low temperature tolerance varies greatly among grass species, with evident varietal differences (Beard, 1966). Traditionally, the evaluation of cold hardiness involves the recovery and regrowth during spring after winter injury (Anderson et al., 1988). When describing the understanding and management of environmental stresses for turfgrasses. Stier (2007) stated that chilling injury starts with membrane damage and the eventual loss in chlorophyll and $C_4$ grasses appear more sensitive to this type of chilling injury. Munshaw et al. (2006) studied the responses of autumn application of iron, nitrogen and sea weed extract on autumn colour retention and winter cold tolerance. Colour retention decreased for four cultivars of *Cynodon* during autumn when subjected to progressively lower temperatures whereas the cultivar, Princes-77 showed maximum colour retention. During spring the cultivars Midiron and Riviera were quickest for green recovery. In another study, *Paspalum vaginatum* had a greater rate of spring green up and better colour retention during cold stress when compared with other warm season grass varieties (Geren et al. 2009).

## 6.5 Conclusion

Reviewing all the species responses and analyses from this experiment, the main point that emerged was the general finding that the duration of exposure, rather than the minimum temperature reached, was a critical factor in determining the amount of damage sustained. Different species exhibited different patterns and responses to sudden exposure and gradual exposure to low temperature over prolonged period of time, but the prolonged period of exposure time consistently resulted in damage in all the species used.

Seashore paspalum exhibited better colour retention and high accumulation of proline when exposed to gradually lowering temperature over an extended period of time. However, MDA levels were also quite high and the role of MDA in contributing to or as an indicator of leaf tissue damage needs further investigation.

The amounts of accumulated proline, MDA, LMW sugars and HMW sugars all tended to return towards pre-cold stress levels when plants were placed in glasshouse for recovery from cold stress.

## References

Anderson, J. A., Taliaferro, C. M., Martin, D. L. (2003) Longer exposure duration increase freeze damage to turf Bermudagrasses. *Crop Science* 43: 973–977.

Anderson, J.A., M.P. Kenna, C.M. Taliaferro (1988) Cold Hardiness of ‘Midiron’ and ‘Tifgreen’ bermudagrass. *Hort Science* 23: 748–750.

Beard, J. B. (1966) Direct low temperature injury of nineteen turfgrasses. *Quarterly Bulletin of Michigun Agricultural Experiment Station* 48: 377–383.

Beard, J. B. (1973) Turfgrass: Science and Culture. Prentice-Hall, Inc., Englewood Cliffs, NJ.

Beard, J. B. (1982) Turf Management for Golf Courses. Macmillan Publishing Co., NY. 206–207.

Bell, G. E., Martin, D. L., Wiese, S. G., Dobson, D. D., Smith, M. W., Stone, M. L., Solie, J. B. (2002) Vehicle-mounted optical sensing. *Crop Science 42*: 197–201.

Bocian, A., Kosmala, A., Rapacz, M., Jurczyk, B., Marczak, Ł., Zwierzykowski, Z. (2011). Differences in leaf proteome response to cold acclimation between Lolium perenne plants with distinct levels of frost tolerance. *Journal of Plant Physiology* 168: 1271–1279.

Bush, E., Wilson, P., Shepard, D., McCrimmon, J. (2000) Freezing tolerance and nonstructural carbohydrate composition of carpetgrass (*Axonopus affinis* Chase). *Hort Science, 35:* 187–189.

Casler, M. D. (2006) Perennial grasses for turf, sport and amenity uses: evolution of form, function and fitness for human benefit. *The Journal of Agricultural Science* 144: 189–203. http://dx.doi.org/10.1017/S0021859606006137

Cyril, J., Duncan, R. R., Baird, W. V. (2002) Changes in membrane polar lipid fatty acids of seashore paspalum in response to low temperature exposure. *Crop Science* 42: 2031–2037.

Dionne, J., Castonguay, Y., Nadeau, P., Desjardins, Y. (2001) Freezing tolerance and carbohydrate changes during cold acclimation of green-type annual bluegrass (Poa annua L.) ecotypes. *Crop Science* 41: 443–451.

DiPaola, J. M., Beard, J. B. (1992). Physiological effects of temperature stress. In. Waddington D.V., Carrow, R. N., Shearman, R . C. Eds. *Turfgrass*, American Society of Agronomy 231–267.

Geren, H., Avcioglu, R., Curaoglu, M. (2009) Performances of some warm-season turfgrasses under Mediterranean conditions. *African Journal of Biotechnology* 8: 4469–4474.

He, Y., Xiao, H., Wang, H., Chen, Y., Yu, M. (2010) Effect of silicon on chilling-induced changes of solutes, antioxidants, and membrane stability in seashore paspalum turfgrass. *Acta Physiologiae Plantarum* 32: 487–494.

Hisano, H., Kanazawa, A., Yoshida, M., Humphreys, M. O., Iizuka, M., Kitamura, K., Yamada, T. (2008) Coordinated expression of functionally diverse fructosyltransferase genes is associated with fructan accumulation in response to low temperature in perennial ryegrass. *New Phytologist*, 178: 766–780.

Huang, B., DaCosta, M., Jiang, Y. (2014) Research advances in mechanisms of turfgrass tolerance to abiotic stresses: from physiology to molecular biology. *Critical Reviews in Plant Sciences* 33: 141–189.

Jermyn, M.A. 1956. A new method for the determination of ketohexoses in the presence of aldohexoses. *Nature* 177: 38–40.

Kauffman, J. M. (2010) Investigating Cold Hardiness and Management Practices of Warm-season Putting Green Species in the Transition Zone. PhD dissertation, University of Tennessee. http://trace.tennessee.edu/utk_graddiss/811.

Kopec, D. M., Walworth, J. L., Gilbert, J. J., Sower, G. M., Pessarakli, M. (2007) ‘SeaIsle 2000’ Paspalum putting surface response to mowing height and nitrogen fertilizer. *Crop Science* 99: 133–140.

Liu, A., Hu, Z., Bi, A., Fan, J., Gitau, M. M., Amombo, E., Fu, J. (2016) Photosynthesis, antioxidant system and gene expression of bermudagrass in response to low temperature and salt stress. *Ecotoxicology* 25: 1445–1457.

Magne, C., Larher, F. (1992) High sugar content of extracts interferes with colorimetric determination of amino-acids and free proline. *Analytical Biochemistry*, 200: 115–118.

Munshaw, G. C., Ervin, E. H., Shang, C., Askew, S. D., Zhang, X., Lemus, R. W. (2006) Influence of late season iron, nitrogen and seaweed extract on fall color retention and cold tolerance of bermudagrass cultivars. *Crop Sc*ience 46: 273–283.

Patton, A. J., Cunningham, S. M., Volenec, J. J., Reicher, Z. J. (2007) Differences in freeze tolerance of Zoysiagrasses: II. Carbohydrate and proline accumulation. *Crop Science* 47: 2170–2181.

Pollock, C. J., Jones, T. (1979) Seasonal patterns of fructan metabolism in forage grasses. *New Phytologist,* 83: 9–15.

Stefaniak, T. R., Rodgers, C. A., Van Dyke, R., Williams, D. W., Phillips, T. D. (2009) The inheritance of cold tolerance and turf traits in a seeded bermudagrass population. *Crop Science* 49: 1489–1495.

Stier, J. C. (2007) Understanding and managing environmental stresses of turfgrass. *Acta Horticulturae* (ISHS) 762: 63–80. http://www.actahort.org/books/762/762_6.htm.

Turgeon, A. J. (2008) *Turfgrass management* (Pearson Education, Upper Saddle River, NJ)

Wang, Y., Li, J., Wang, J., Li, Z. (2010).Exogenous H2O2 improves the chilling tolerance of manilagrass and mascarenegrass by activating the antioxidative system. *Plant Growth Regulation* 61: 195–204.

Wang, Y., Yang, Z. M., Zhang, Q. F., Li, J. L. (2009) Enhanced chilling tolerance in Zoysia matrella by pre-treatment with salicylic acid, calcium chloride, hydrogen peroxide or 6-benzylaminopurine. *Biologia Plantarum* 53: 179.

Wei, S., Zhou, Y., Li, D., Sun, Z. (2008) Cold sensitivity and biochemical adjustments in Zoysiagrass under low temperature stress. In. Stier, J. C. et al., (Eds.,), *Proceedings of second International Conference on Turfgrass*. *Acta Horticulturae (ISHS)* 783: 195–206.

Zhang, X., Ervin, E. H., LaBranche, A. J. (2006) Metabolic defence responses of seeded bermudagrass during acclimation to freezing stress. *Crop Science* 46: 2598–2605.

Zhang, X., Wang, K., Ervin, E. H., Waltz, C., Murphy, T. (2011) Metabolic changes during cold acclimation and deacclimation in five bermudagrass varieties. I. Proline, total amino acid, protein, and dehydrin expression. *Crop Science* 51: 838–846.

# CHAPTER 7

# General Discussion and Future Directions

## 7.1 Introduction

The project was undertaken to strengthen the author’s background in horticulture and professional improvement for a career in turf research and development. A well-recognised problem of cold intolerance in warm season turfgrasses was chosen for further research and exploration. Cold intolerance or winter browning is a major issue in areas with hotter summer and cold winter conditions with occurrence of frosts. The author had some previous experience in horticulture and turfgrass husbandry and was well aware of the problem of cold injury in warm season turfgrasses. The project was designed to assist with emerging needs in the New Zealand turf industry, arising from the use of warm season grasses in upper parts of the North Island.

The project was carried out in two phases. The first phase was conducted from December 2012 through to the winter of 2014, with two main objectives: 1) to assess the mat quality in terms of visual attributes for a range of warm season turfgrasses protected from and exposed to frost; 2) to compare different cultivars for their agronomic/morphological characteristics under two different growing conditions. The second phase was designed to further verify and develop a better understanding of change in appearance and physiological responses after different levels of cold exposure on selected varieties from the first phase of the project. The experiment was conducted in a purpose-designed regime of decreasing temperature in a controlled temperature room with the following main objectives: 1) to explore cold tolerance levels for selected warm season turfgrasses for a precise estimation of low temperature threshold levels; 2) to quantify physiological changes (levels of sugars, MDA and proteins) taking place during cold exposure for improved understanding of cold stress physiology; and 3) to estimate recovery rates (green up) after cold injury.

In the preceeding sections below a summary of findings is presented together with recommendations for future work.

## 7.2 Turf establishment when using warm season grasses

Twelve different cultivars of four warm season grass genera (*Cynodon, Zoysia, Pennisetum and Paspalum*) were established during Nov-Dec 2012, in the main phase 1 experiments which included a Glasshouse at Plant Growth Unit (PGU), Massey University, Palmerston and ten different cultivars in open field conditions at the New Zealand Sports Turf Institute (NZSTI) State Highway 57, Palmerston North. Plots were sown on a 50 mm deep sand carpet laid over existing soil. A randomized complete block design (RCBD) with four replicates was used at each location,. Early observations during turf establishment revealed that even during January overnight temperatures can be cold enough to impede germination and establishment of seeded warm season turf varieties in Palmerston North. It was recognized in *Cynodon* that vegetatively propagated varieties establish much more rapidly and with better resilience to an overnight cold challenge than seeded varieties. Seeded varieties of *Cynodon* were found to be susceptible to *Anthracnose* infestation while vegetatively established varieties of *Cynodon*, together with the tested varieties of *Zoysia* (Zenith), *Paspalum* (Sea Spray), and *Pennisetum* (Regal Staygreen), were resistant.

## 7.3 Qualitative traits of warm season turfgrasses

Expectations for turf surface quality vary greatly, depending whether the intended use is for ornamental turf, utility turf, or recreational turf (Turgeon, 1980). With increased user demand, the turf industry is challenged to identify options for year round turf surfaces with high quality attributes (Keith, 2008). In contrast to pasture grasses, turfgrass quality is not measured in terms of yield or animal feeding value. Turfgrass quality is measured in terms of its aesthetic appeal. It is a combined effect of colour, density, texture, uniformity and surface smoothness (Morris and Shearman, 1998).

Visual attributes (quality, texture, uniformity, ground cover and colour) for most of the commercially available warm season turfgrass germplasm in New Zealand were scored with the aim of providing reliable comparative information to the industry relevant to local climatic conditions. Some previously known information was confirmed by the data collected, along with unexpected findings and observations.

Vegetatively established *Cynodon* varieties showed superior performance, compared to the seeded varieties, and this was quantitatively evident in various data. Selection and production of vegetative *Cynodon* varieties has been ongoing for many decades, and seeded varieties have been readily available in the market for only a few years and still need a lot of improvement (Hanna and Anderson, 2008; Croce et al., 2001). Data collected in these trials confirmed that the vegetatively propagated *Cynodon* varieties exhibit rapid establishment and superior turf quality compared to seeded varieties. Sea Spray (*Paspalum vaginatum*) and Regal Staygreen (*Pennisetum clandestinum*) showed some exceptions in their establishment and performance. Sea Spray showed turf quality and characteristics comparable to vegetatively produced *Cynodon* and exhibited considerable low temperature tolerance. *P. vaginatum* is emerging as a grass for increased future use, due to its capacity to withstand multiple stresses (Duncan and Carrow, 2000) and adaptability to a variety of soils and a range of growing conditions (Duncan 1999).

Regal Staygreen was coarser in texture with less ground cover and inferior quality under glasshouse conditions. Texture was coarse in field plots compared to all other varieties but it developed a good mat with excellent colour retention throughout the winter period. This variety can be best employed in areas of less maintenance requirements. Among the tested varieties Zenith, Yukon and La Paloma stood out as low performers with slow establishment and sensitivity to low temperature.

## 7.4 Stolon and rhizome formation strategies

Turf morphology along with quality defines suitability and use of turfgrass varieties. Plant responses to biotic and abiotic stresses and their morphology have a very defined relationship. Detailed studies on warm season turfgrass morphology are not reported under New Zealand growing conditions and it was intended that availability of this knowledge will help the industry in defining future development needs for warm season turfgrass varieties for use in New Zealand, as currently germplasm is mainly imported from Australia and USA.

Plants showed higher leaf mass and a vertical growth pattern in the glasshouse where Regal Staygreen was significantly at par to all other varieties. Favourable moisture supply and temperature conditions may have induced this behaviour. However, the reduction of light intensity and possible spectral changes inside the glasshouse compared with the field environment may also be a factor promoting this growth pattern. Development of greater numbers and mass of stolons and roots in the field conditions (compared to the glasshouse) was observed and seen as a plant response, utilising their morphological plasticity, to prepare for less ideal conditions.

Differences in rhizome development were a valuable finding of this experiment, and there is little information in the literature on this aspect of *Cynodon* and *Zoysia* morphology. During the first year, vegetatively established *Cynodon* varieties produced rhizomes in the field and only one variety (Agridark) produced rhizomes in the glasshouse. However, in the second year presence of rhizomes was recorded in all species except Zenith under field conditions and in the glasshouse seeded *Cynodon* varieties failed to produce rhizomes. Further investigation is needed to better understand factors influencing the initiation and development of rhizomes.

## 7.5 Phytomer specialisation in warm season turfgrasses

Stolon formation is a key morphological feature in clonal warm season turfgrasses and indicates an evolutionary divergence from other grasses in developing specialized structures. Stolon samples were studied in detail for their morphological characteristics and leaf attributes with the main objective to explore a unique pattern of grouped nodes in *Cynodon* and *Zoysia*. Detailed examination and light microscopy confirmed the presence of triplet or compound nodes in *Cynodon* and *Zoysia* and single-phytomer nodes in *Papalum* and *Pennisetum*.

Each node in the triplet is responsible for a different function, with division of labour between stem, branch and root formation. The lower two nodes (distal from the stem tip) were found to be responsible for root and shoot emergence and top node to the stem tip undergoes node elongation to provide a spatial separation between successive sets of nodes. This phenomenon is little-reported and little-explored in the available literature. However, this morphololology is potentially of high ecological importance in understanding plant niche adaptation, it needs further investigation. Coarser leaf texture was recorded for most of the varieties under glasshouse conditions than in the field; leaves had longer and wider leaf blades and longer leaf sheaths in glasshouse-grown, than in field-grown plants. Root occurrence was significantly higher in field plots compared to the glasshouse.

Leaf and stolon attributes were described in detail in the results of Chapter 3 and Chapter 4, and Regal Staygreen (*P. clandestinum*) stands apart as a much coarser variety compared to other varieties. It has longer and broader leaves with long, large-diameter stolons under both glasshouse and field conditions.

## 7.6 Cold stress physiology

Great variation exists in winter tolerance of different warm season species but all are subject to cold injury which is a major concern in cooler climates (Gatschet et al., 1994). Most previous research has been directed at exploring survival through winter in a continental climate and little previous exploration of browning temperature is reported for less severe winter conditions where survival is expected, but with low temperature damage. Reviewing all the data from the field, glasshouse, and follow up physiological studies suggests that cold damage is a slow cumulative process with the rate depending on degree of cold, possibly influenced by both the daily maximum temperature (> 15$^{o}$C, allowing some metabolic activity) and daily minimum (which would determine damage potential). The data do not suggest that cold damage is primarily a sudden event, occurring when a certain damage threshold temperature is reached.

Species responses differed and in most cases, plants were not seriously affected by sudden temperature shocks of 2 weeks duration, whereas gradually falling temperature to values tolerated for 2 weeks, over 6–8 weeks damaged all varieties, but to a variable degree. Sea Spray showed superior cold tolerance compared to *Cynodon* varieties and retained a good turf colour along with Agridark and Windsor green. Findings further confirmed the results presented in Chapters 3, 4, and 5. It was a point of interest that accumulated levels of proline, MDA (malondialdehyde), and low (LMW) and high molecular weight (HMW) sugars returned to near-pre-stress levels during the recovery period.

## 7.7 Key findings and their applications

A number of findings in this research stand out as being of immediate interest to the turf industry in New Zealand and in similar climates internationally. Among these:

Vegetatively grown *Cynodon* turf varieties displayed an obvious advantage over seeded varieties during early establishment, better stress tolerance and rapid development of strategic structures especially stolon and rhizomes(Sec. 4.3.2). Agridark was found to be the best performer among the varieties tested during the establishment year and following summer. Turf managers can choose planting material accordingly.

The turfgrass Seashore Paspalum (*P. vaginatum,* Sea Spray) (established from seed) performed exceptionally well under both field and glasshouse growing conditions (Fig. 4.8, 4.9) and showed a reasonable level of cold stress during the low temperature exposure. This variety has unrrecognised potential. Based on results of this study, further trials with a view to increased adoption of this variety would be desirable.

Zenith (*Zoysia japonica*) was found to be obviously sensitive to cold, relative to other species or varieties tested (Sec. 3.31). Under field conditions, Zenith failed to establish a well developed mat in the first year of growth. No rhizome formation was noted in field plots in either year; however, some rhizomes developed in the glasshouse during the 2nd year (Fig. 4.9). These results indicate that Zenith should only be considered for very warm or sheltered sites in the northern part of the North Island.

*Cynodon* and *Zoysia* displayed a specialized triplet pattern of node arrangement on their stolons (Sec. 5.4.4). This growth pattern deserves further research aimed at determining its ecological significance. A possible explanation is that the triplet structures represent a colonization strategy of division of labour for rapid establishment. Alternatively they may have a role in optimising resource capture in spatially heterogeneous (patchy) environments. Previous reports suggest that first leaf would support root development, the second leaf would support shoot and the third would support stolon internode elongation (Sbrissia et al., 2001; da Silva et al., 2015).

Gradual but long term exposure to low temperature was more destructive and resulted in greater loss of turf quality compared with sudden short term chilling events. Warm season turf varieties can perform better in areas with few frosting events, compared to the regions with continuous cold nights (Sec. 6.4.4). It may be possible to develop a cumulative chilling index, similar to thermal time, or the growing degree day concept, used in crop development monitoring, and guidelines of this sort would give greater clarity on when chilling damage can be expected and for decision making on when to use cool- or warm season grasses.

## 7.8 Future research

Reduced winter browning or discolouration is a key attribute in selection of warm season turfgrasses for areas with colder winters. More work is needed to define the minimum temperature for the onset of browning in each variety, in order to provide discrete recommendations for turf managers and groundsmen. Winter damage reduces wear resistance in turfgrasses. A separate study to define damage percentage and relevant wear tolerance is needed.

The occurrence of triplet structures or compound nodes is interesting from an ecological perspective but there is a lack of published data investigating discrete functioning at each phytomer unit. A detailed experiment involving stolon anatomy in available germplasm would provide better insight into the ecological significance of this morphology variation acquired in the evolutionary process.

There were significant differences between varieties in emergence of rhizomes. Some varieties showed the presence of rhizomes in the 2nd year of growth, and variation in rhizome formation also occurred due to growing conditions. Further investigation of this phenomenon will help understanding of plant behaviour with respect to resource allocation to different organs under different conditions. An experiment with sand filled cores installed in turf plots grown under different conditions could help to provide data on rhizome occurrence and estimate factors involved in initiation of rhizomes.

A repeat cold tolerance experiment under controlled conditions with more precise temperature levels and agronomic considerations will be helpful to define the threshold level for different varieties and understand the process whereby damage potential is greater with prolonged exposure. Turf age at the time of exposure to different temperatures should be kept the same with differences in establishment dates to rule out maturity factor for their resistance to cold injury and higher accumulation of stress response factors like proline.

The presented results provide a foundation to develop a further project for defining and testing different management practices to avoid or reduce the impact of low temperature stress on warm season turfgrasses.

## References

Croce, P., De Luca, A., Mocioni, M., Volterrani, M., Beard, J. B. (2001) Warm-season turfgrass species and cultivar characterizations for a Mediterranean climate. *International Turfgrass Society Research Journal* 9: 855–859.

Duncan, R. R. (1999) Environmental compatibility of seashore paspalum (saltwater couch) for golf courses and other recreational uses. II. Management protocols. *International Turfgrass Society Research. Journal 8*: 1216–1230.

Duncan, R. R., Carrow, R. N. (2000) Seashore Paspalum: The environmental turfgrass. John Wiley & Sons.

Gatschet, M. J., Taliaferro, C. M., Anderson, J. A., Porter, D. R., Anderson, M. P. (1994) Cold acclimation and alterations in protein synthesis in bermudagrass crowns. *Journal of the American Society for Horticultural Science 119*: 477–480.

Hanna, W. W., Anderson, W. F. (2008) Development and impact of vegetative propagation in forage and turf bermudagrasses. *Agronomy Journal* 100 (Suppl3): S–103. doi:10.2134/agronj2006.0302c.

McAuliffe, K. W. (2008) The role of performance testing and standards in the sports turf industry: a case study approach. *Acta Horticulture* 783: 391–398. https://doi.org/10.17660/ActaHortic.2008.783.41.

Morris, K. N., Shearman, R. C. (1998) NTEP turfgrass evaluation guidelines. In *NTEP turfgrass evaluation workshop,* Beltsville, MD (1–5).

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# APPENDICES

## Appendix 3.1 Statistical significance of differences in quality and texture of warm season turfgrasses (March-July) under glasshouse and field conditions

| | P-77 | YK | LP | AD | Lg | SA | WG | ZN | SS | RS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | **Glasshouse** | | | | | |
| **Quality (March)** | **abc** | **abc** | **ab** | **e** | **bcd** | **bcd** | **bcd** | **abcd** | **de** | **a** |
| | | | | | | | | | | **F (9, 20) = 6.642, P < 0.05** |
| **Quality (May)** | **bc** | **a** | **ab** | **e** | **cde** | **de** | **de** | **de** | **bcd** | **ab** |
| | | | | | | | | | | **F (9, 20) = 18.472, P < 0.05** |
| **Quality (July)** | **bcd** | **a** | **b** | **e** | **e** | **e** | **de** | **e** | **cde** | **bc** |
| | | | | | | | | | | **F (9, 20) = 21.316, P < 0.05** |
| | | | | | **Open Field** | | | | | |
| **Quality (March)** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** |
| | | | | | | | | | | **F (9, 20) = 1.182, P < 0.05** |
| **Quality (May)** | **b** | **a** | **b** | **b** | **b** | **b** | **b** | **a** | **b** | **b** |
| | | | | | | | | | | **F (9, 20) = 11.840, P < 0.05** |
| **Quality (July)** | **b** | **a** | **b** | **bc** | **b** | **cd** | **bc** | **a** | **b** | **d** |
| | | | | | | | | | | **F (9, 20) = 20.032, P < 0.05** |
| | | | | | **Glasshouse** | | | | | |
| **Texture (March)** | **a** | **a** | **b** | **b** | **b** | **b** | **b** | **c** | **bc** | **c** |
| | | | | | | | | | | **F (9, 20) = 49.444, P < 0.05** |
| **Texture (May)** | **cd** | **cd** | **c** | **d** | **cd** | **cd** | **cd** | **b** | **c** | **a** |
| | | | | | | | | | | **F (9, 20) = 39.762, P < 0.05** |
| **Texture (July)** | **b** | **b** | **b** | **b** | **b** | **b** | **b** | **a** | **b** | **a** |
| | | | | | | | | | | **F (9, 20) = 16.444, P < 0.05** |
| | | | | | **Open Field** | | | | | |
| **Texture (March)** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** |
| | | | | | | | | | | **F (9, 20) = 0.374, P < 0.05** |
| **Texture (May)** | **abc** | **abc** | **ab** | **c** | **bc** | **bc** | **c** | **ab** | **bc** | **a** |
| | | | | | | | | | | **F (9, 20) = 9.733, P < 0.05** |
| **Texture (July)** | **ab** | **bc** | **ab** | **c** | **ab** | **c** | **c** | **bc** | **c** | **a** |
| | | | | | | | | | | **F (9, 20) = 10.667, P < 0.05** |

In each row, varieties sharing different letters differ significantly from each other at p<0.05 (One-way ANOVA using varieties as factor; Tukey's HSD post-hoc test

## Appendix 3.2 Statistical significance of differences in density and uniformity of warm season turfgrasses (March-July) under glasshouse and field conditions

| | P-77 | YK | LP | AD | Lg | SA | WG | ZN | SS | RS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | **Glasshouse** | | | | | |
| **Density (March)** | **abc** | **abc** | **ab** | **c** | **bc** | **c** | **bc** | **abc** | **abc** | **a** |
| | | | | | | | | | | **F (9, 20) = 4.965, P < 0.05** |
| **Density (May)** | **abcd** | **a** | **ab** | **e** | **bcde** | **cde** | **bcde** | **de** | **cde** | **abc** |
| | | | | | | | | | | **F (9, 20) = 9.833, P < 0.05** |
| **Density (July)** | **abc** | **a** | **ab** | **d** | **abcd** | **abcd** | **bcd** | **d** | **cd** | **ab** |
| | | | | | | | | | | **F (9, 20) = 9.713, P < 0.05** |
| | | | | | **Open Field** | | | | | |
| **Density (March)** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** |
| | | | | | | | | | | **F (9, 20) = 1.677, P < 0.05** |
| **Density (May)** | **abc** | **a** | **abc** | **c** | **abc** | **bc** | **abc** | **ab** | **abc** | **abc** |
| | | | | | | | | | | **F (9, 20) = 4.636, P < 0.05** |
| **Density (July)** | **bc** | **a** | **bcd** | **cd** | **bcd** | **d** | **bcd** | **a** | **b** | **bcd** |
| | | | | | | | | | | **F (9, 20) = 15.444, P < 0.05** |
| | | | | | **Glasshouse** | | | | | |
| **Uniformity (March)** | **bc** | **ab** | **ab** | **cd** | **cd** | **d** | **cd** | **ab** | **bc** | **a** |
| | | | | | | | | | | **F (9, 20) = 16.444, P < 0.05** |
| **Uniformity (May)** | **a** | **b** | **bc** | **bc** | **bc** | **bc** | **bc** | **c** | **bc** | **c** |
| | | | | | | | | | | **F (9, 20) = 12.741, P < 0.05** |
| **Uniformity (July)** | **b** | **b** | **b** | **b** | **b** | **b** | **b** | **b** | **b** | **a** |
| | | | | | | | | | | **F (9, 20) = 8.896, P < 0.05** |
| | | | | | **Open Field** | | | | | |
| **Uniformity (March)** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** |
| | | | | | | | | | | **F (9, 20) = 1.144, P < 0.05** |
| **Uniformity (May)** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** |
| | | | | | | | | | | **F (9, 20) = 1.933, P < 0.05** |
| **Uniformity (July)** | **b** | **a** | **b** | **b** | **b** | **b** | **b** | **a** | **b** | **b** |
| | | | | | | | | | | **F (9, 20) = 12.667, P < 0.05** |

In each row, varieties sharing different letters differ significantly from each other at p<0.05 (One-way ANOVA using varieties as factor; Tukey’s HSD post-hoc test

**Appendix 3.3 Manuscript prepared and submitted for publication in proceedings from the oral presentation presented at International Horticulture Congress (IHC-2014) in Brisbane Australia**

# Comparative Evaluation of Visual Quality Attributes for Warm Season Turfgrasses

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**Keywords:** Visual quality, warm season turfgrasses, $C_4$, cool season, *Cynodon*, *Zoysia*, *Paspalum, Pennisetum*

## Abstract

**The New Zealand turf industry has a growing interest in warm season ($C_4$) grasses due to their water use efficiency under heat stress and summer dormancy of cool season ($C_3$) grasses, especially in the upper parts of the North Island. Ten commercially available cultivars of four warm season grass genera (*Cynodon, Zoysia, Paspalum* and *Pennisetum*) were established in the glasshouse and field at Palmerston North, New Zealand, using seeds or transplanted sprigs. Established turf plots were scored visually for density, uniformity, texture, smoothness, % cover, colour and green up on a scale (1-9) as prescribed by NTEP (National Turf Evaluation Program, USA) for one growing season during 2013. Significant changes in the quality traits of all cultivars were observed both in the field and the glasshouse. Decline in visually assessed turf quality, and browning off were observed during the cooler months in the field and during autumn in the glasshouse because of fungal attack. Seeded varieties of *Cynodon* were most susceptible in both situations. Yukon (*Cynodon dactylon*) and Zenith (*Zoysia japonica*) exhibited poor development. Vegetatively propagated material showed superior quality compared with seeded varieties. Regal Staygreen (*Pennisetum clandestinum*) retained colour best in the field in winter. AgriDark (*C. dactylon*) and Sea Spray (*Paspalum vaginatum*) were also good performers compared to other varieties tested.**

## INTRODUCTION

Turfgrass is an important and integral part of modern urban infrastructure. Turf has recreational uses, and often is an element in the landscaping of commercial, residential and public premises. Turf provides aesthetic appeal, confers environmental benefits and offers a safe playable surface for various sport activities (Ulrich, 1986; Beard & Green, 1994; Duble, 1996).

Cool season ($C_3$) grasses have historically been the mainstay of the New Zealand turf industry. A range of varieties of *Lolium, Festuca* and *Agrostis* are widely grown across the country. However cool season grasses suffer heat stress and lose their vigour and aesthetic appeal under high temperature or drought conditions. Accordingly, turf venues using these species experience problems related to summer dormancy, especially in the northern areas of the North Island (Bay of Plenty, Auckland Region, Northland and parts of Waikato). Warm season ($C_4$) grasses are gaining popularity in such situations because of their better tolerance of heat stress and drought.

Different venues have different performance requirements for turf reflecting the needs of their main users. Hence, very objective criteria can be identified for determining the grass type to be employed at any particular venue, in order to match varieties established with venue requirement, available resources and environmental and soil conditions (Carrow et. al., 2010). Continuous efforts in selection and breeding have not only widened the range of available germplasm sources but also given rise to a current market situation where a range of turfgrass species and varieties are available, specifically bred for different purposes and venues. However, in all cases there is strong user demand for a year-round green and playable surface which presents a challenge for turf researchers and practitioners, especially under high usage and unfavourable growth conditions. Warm season grasses offer a potential improvement compared to cool season grasses in summer since they possess a unique morphology, high photosynthetic efficiency and reduced water requirement (Volterrani et. al.; 1997Zhou and Abaraha, 2007), but they tend to be frost sensitive, and winter performance can be an issue. Thus, research to identify the varieties with superior performance for traits such as colour retention and uniformity during autumn, winter and early spring is likely to be very helpful to turf managers.

Given this need for objective data on winter performance of the various commercially available warm season turfgrass varieties, plots of a range of $C_4$ grasses were established with the following objectives:

4- To observe the various species and varieties through an autumn-winter-spring (cool season) cycle under temperature protected (glasshouse) and frost-exposed (open field) conditions to better understand their ecophysiology;

5- To record visual attributes for mat quality (Quality, Ground cover, Density, Texture) through the cool season cycle.

## MATERIALS AND METHODS

Nine different varieties (Table 1) of four warm season turf genera (*Cynodon, Zoysia, Pennisetum and Paspalum*) were established (1X 1m) in a glasshouse at the Plant Growth Unit (PGU), Massey University, Palmerston North and in the field at the New Zealand Sports Turf Institute (NZSTI) State Highway 57, Palmerston North using a sand carpet of 50 mm depth over the existing soil surface in both cases. For both trials, varieties were planted in a randomized complete block design (RCBD) with four replicates. Seeds or stolons were used for propagation, depending on the variety (Table 1). Temperature records were maintained for the glasshouse throughout experiment while field temperature data was obtained through NIWA database collected from nearest location at AgResearch. Highlights of collected data are provided in Table 2. After planting in Nov–Dec 2012, plots were allowed to establish for three months. Field plots for seeded varieties were covered with polypropylene germination mats to facilitate the seed germination which were removed early in January 2013 after seedling emergence. Post-establishment, plots were evaluated periodically from March 2013 until July 2013 for overall quality, ground cover, density, texture (these first four presented below), and also uniformity and colour (data not presented) using standards developed by NTEP (Morri**s**, 2003) on a visual scale of 1–9 with “9” as the best or highest value and “1” as poorest or lowest value. Results were analysed using independent sample t-tests at $P < 0.05$ level of significance to check for differences between two locations and one way

ANOVA was used at $P < 0.05$ level of significance to check for differences between varieties in respective months. Analyses were performed using Microsoft Excel 2010 and SPSS 21 (Statistical Package for Social Sciences).

## RESULTS AND DISCUSSION

Clear differences among cultivars for the various attributes scored were seen at both experimental sites (Fig. 1, 2). Plots in the glasshouse showed relatively vigorous growth and better percentage groundcover during early stages of establishment compared with open field plots where growth was slow and compact with short internodes and small leaves. Seeded varieties particularly La Paloma, Yukon and Zenith struggled for survival after germination covers were removed in first week of January 2013. Cooler night temperature around 7–8$^{o}$C during the same period may be a possible reason for this. However, performance of Princess-77 and Sea Spray was similar to that of vegetatively propagated varieties.

### Quality

At the end of summer most varieties had established good quality swards both in the field and the glasshouse, except for a lower quality score in the glasshouse for Regal Staygreen due to increased stolon elongation in the low light conditions and a lower score for Zenith in the field, relating to ongoing issues following slow establishment (Fig. 1A). Quality scores fell during May, especially for seeded varieties of Bermuda grass in the glasshouse. In general field and glasshouse quality scores ranked similarly for all varieties, but Zenith maintained significantly better quality scores in the glasshouse than in the field, while the reverse was true for La Paloma (Fig 1-B). Cold exposure during the winter months in the field caused the expected browning off and associated deterioration in turf quality but variation was recorded among varieties in the onset of the effect. Santa Ana, AgriDark and Regal Staygreen retained a better quality under field conditions (Fig 1-C), while AgriDark, Santa Ana and Sea Spray showed best results in the glasshouse. An outbreak of a fungal infestation in the glasshouse during April was the main cause of quality differences between varieties. Seeded *Cynodon* varieties were most affected.

### Ground Cover

Development of ground cover tended to be slightly better in the glasshouse than in the field and occurred earlier in vegetatively propagated varieties of *Cynodon* than in the seeded varieties. For the other species, Sea Spray (*P. vaginatum*) and Regal Staygreen (*P. clandestinum*) ground cover development was comparable with vegetative *Cynodon* varieties, while Zenith (*Z. japonica*) developed ground cover well in the glasshouse but struggled in the field (Fig. 1-D). For some varieties, especially the seeded *Cynodon* varieties, there was a reduction in ground cover during the winter months (Fig 1-E,F). The earlier attainment of ground cover in the vegetatively propagated *Cynodon* varieties and Sea Spray can be attributed to rapid, vigorous stolon formation in these varieties.

### Density

At the end of summer (March), little difference in turf density was observed between glasshouse and field plots, though exceptions were higher density of Zenith (*Z japonica*) in the glasshouse following on from better establishment, and higher density of Regal Staygreen

(*P. clandestinum*) in the field due to reduced stolon internode elongation (Fig 2-A,B,C). With falling temperatures from May to July those varieties which lost ground cover in winter also lost density (Fig 2-B,C). Clearly the ground cover and density are closely linked and in these experiments appear to provide a useful measure of cold susceptibility.

### Texture

Vegetatively produced varieties of *Cynodon* (AgriDark, Santa Ana, Windsorgreen) and Sea Spray had finer texture than the other varieties. Zenith and Regal Staygreen were coarse textured, especially under glasshouse conditions (Fig 2). Texture is largely genetically determined, but can be influenced by environmental factors. Most varieties showed generally coarser texture under glasshouse conditions except AgriDark and Santa Ana where no statistically significant texture difference between field and glasshouse was detected.

## CONCLUSIONS

In the establishment phase, the warm season turfgrasses trialled were susceptible to overnight temperatures below 10$^{o}$C which occurred in Palmerston North in the field even in January, as well as to desiccation. Seeded varieties of *Cynodon* and Zenith (*Z. japonica)* were most affected. Cold susceptibility of those varieties was reflected in slow mat development and decreased winter percentage ground cover. Vegetative *Cynodon* varieties established faster than seeded varieties and maintained good density during the winter both in the glasshouse and the field, but lost colour. Regal Staygreen had the most thickly developed mat in the field (though not under low light glasshouse conditions) but a disadvantage of this variety is the coarser leaf texture. Among the varieties tested Zenith *(Z japonica*) showed the least cold tolerance making it unsuitable for cool climates like Palmerston North's, but it is possibly suitable in warmer districts such as Northland.

## ACKNOWLEDGEMENTS

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## Literature Cited

Beard, J.B. and Green, R.L. 1994. The role of turfgrasses in environmental protection and their benefits to humans. J. Environ. Qual. 23:452-460.

Carrow, R.N., Krum, J.M., Flincroft, I. and Cline, V. 2010. Precision turfgrass management: challenges and field applications for mapping turfgrass soil and stress. Precis. Agri. 11:115-134. DOI 10.1007/s11119-009-9136-y.

Duble, R.L. 1996. Turfgrasses, their management and use in the southern zone. Texas A&M University Press, College Station, Texas.

Morris, K.N. 2003. The National Turfgrass Research Initiative. National Turfgrass Federation, Inc./ National Turfgrass Evaluation Program, Beltsville Agric. Res. Centre, Beltsville, Maryland, 20705 USA.

NIWA. 2014. The National Climate Database. http://cliflo.niwa.co.nz/

Ulrich, R.S. 1986. Human responses to vegetation and landscapes. Landscape Urban Plan. 13:29-44. http://dx.doi.org/10.1016/0169-2046(86)90005-8

Volterrani, M., Grossi, N., Pardini, G., Miele, S., Gaetani, M., and Magni, S. 1997.Warm season turfgrass adaptation in Italy. Intl. Turfgrass Soc. Res. J. 8:1344-1354.

Zhou, S. and Abaraha, A. 2007. Response to heat stress in warm season and cool season turfgrass cultivars. Scientific Res. Essays. 2:095-100.

## Tables

Table 1. Warm season turfgrass varieties, sources and establishment method

| # | Species | Variety | Supplier | Method |
|---|---|---|---|---|
| $V_1$ | ***Cynodon dactylon*** | Princess-77 | PGG Wrightson | Seed |
| $V_2$ | ***Cynodon dactylon*** | Yukon | PGG Wrightson | Seed |
| $V_3$ | ***Cynodon dactylon*** | La Paloma | PGG Wrightson | Seed |
| $V_4$ | ***C. dactylon* × *transvaalensis*** | AgriDark | Cervadon Ltd. | Vegetative |
| $V_5$ | ***C. dactylon* × *transvaalensis*** | Santa Ana | Hololio Turf Farm | Vegetative |
| $V_6$ | ***Cynodon dactylon*** | Windsorgreen | Supplier A | Vegetative |
| $V_7$ | ***Paspalum vaginatum*** | Sea Spray | PGG Wrightson | Seed |
| $V_8$ | ***Zoysia japonica*** | Zenith | PGG Wrightson | Seed |
| $V_9$ | ***Pennisetum clandestinum*** | Regal Staygreen | PGG Wrightson | Seed |

Table 2. Distribution of minimum temperature during data collection

| | | No of Days/Month | | | | |
|---|---|---|---|---|---|---|
| | | **Below 0 °C** | **0-5 °C** | **5-10 °C** | **10-15 °C** | **Above 15 °C** |
| Field | **March** | 0 | 0 | 14 | 15 | 2 |
| | **April** | 0 | 2 | 5 | 30 | 3 |
| | **May** | 2 | 10 | 13 | 6 | 0 |
| | **June** | 2 | 10 | 15 | 3 | 0 |
| | **July** | 1 | 10 | 19 | 1 | 0 |
| | | | | | Source: NIWA ( Palmerston North Aws) | |
| Glasshouse | **March** | 0 | 0 | 2 | 19 | 10 |
| | **April** | 0 | 0 | 0 | 12 | 18 |
| | **May** | 0 | 0 | 0 | 17 | 15 |
| | **June** | 0 | 0 | 0 | 9 | 21 |
| | **July** | 0 | 0 | 0 | 6 | 25 |

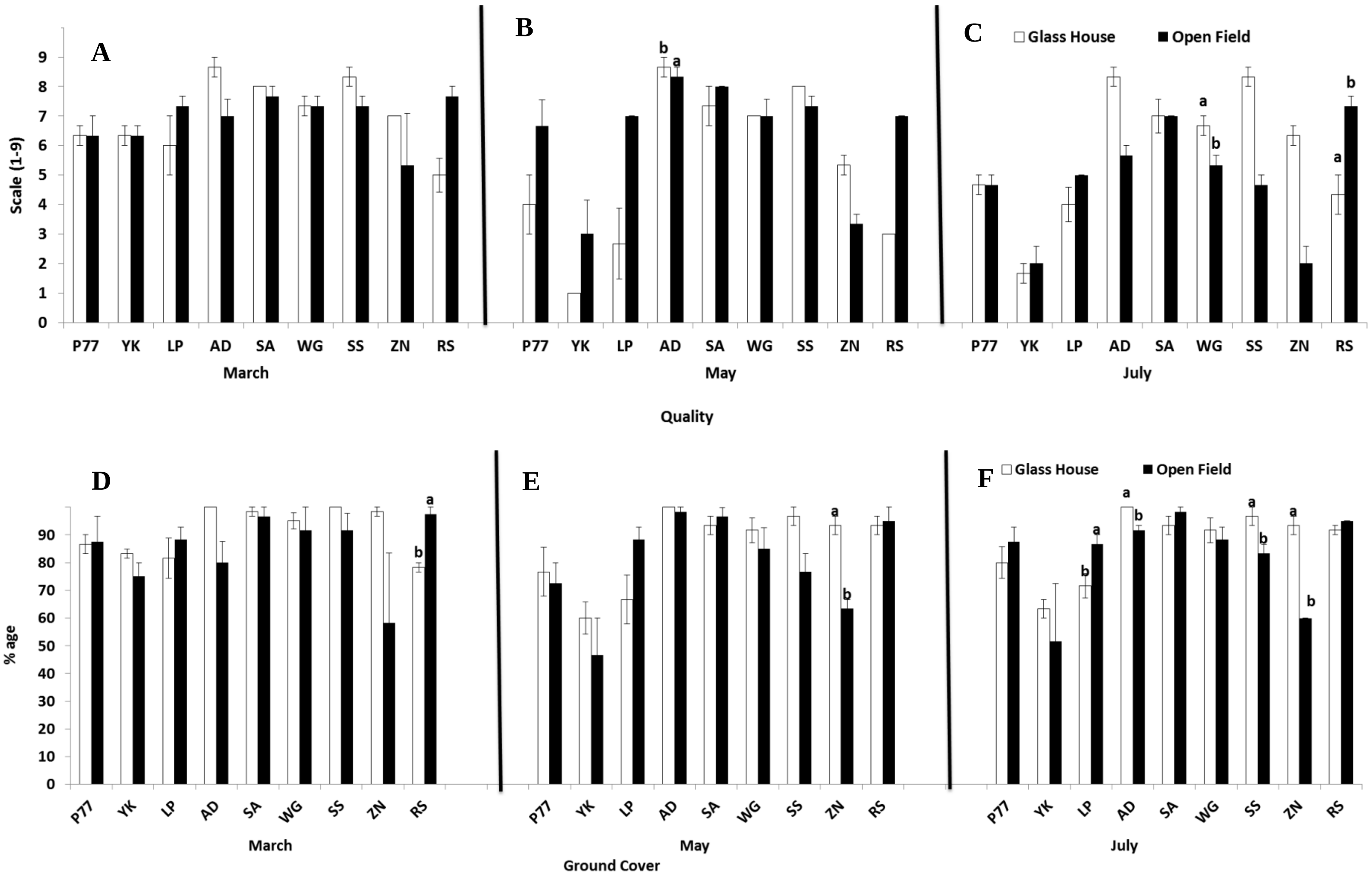

**Fig:1 Comparison of quality (overall visual appearance) and ground cover percentage of turf mat under glasshouse and open field over six months.** P-77=Princess-77, YK=Yukon, LP =La Paloma, AD =AgriDark, SA=Santa Ana, WG=Windsorgreen, SS =Sea Spray, ZN = Zenith, RS=R.Staygreen. Lettering ("a" for higher value and "b" for lower value) is used to show the comparison between glasshouse and field plots. The effect of location is significant at *P*<0.05

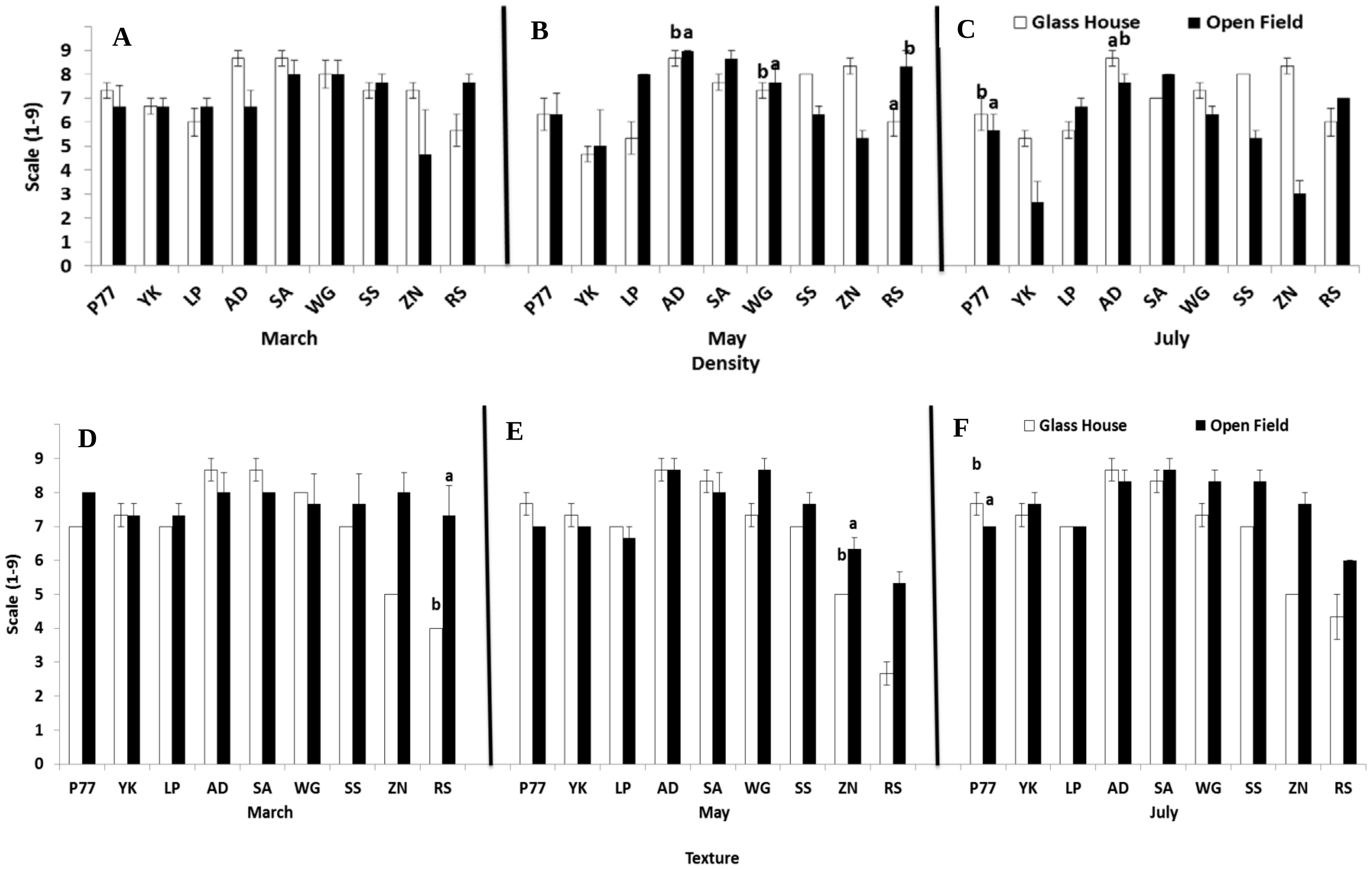

**Fig:2 Comparison of density and texture of turf mat under glasshouse and open field over six months**

P-77=Princess-77, YK=Yukon, LP =La Paloma, AD =AgriDark, SA=Santa Ana, WG=Windsorgreen, SS =Sea Spray, ZN = Zenith, RS=R.Staygreen. Lettering (“a” for higher value and “b” for lower value) is used to show the comparison between glasshouse and field plots. The effect of location is significant at *P*<0.05

## Appendix 4.1 Significant differences between sampling for glasshouse and Field plots soil for (a) Green mass (b) Dead mass and (c) Root mass.

| | Princess 77 | Yukon | La Paloma | Agridark | Santa Ana | W. Green | Sea Spray | Zenith | R. Staygreen |
|---|---|---|---|---|---|---|---|---|---|
| **(a) Green Mass** | | | | | | | | | |
| **Glasshouse** | abc | **a** | **ab** | **cd** | **bcd** | **bcd** | **d** | **bcd** | **e** |
| | F (9, 20) = 52.970, P < 0.05 | | | | | | | | |
| **Field Plots** | abc | **a** | **ab** | **ab** | **abc** | **a** | **bc** | **a** | **c** |
| | F (9, 20) = 6.678, P < 0.05 | | | | | | | | |
| **(b) Dead Mass** | | | | | | | | | |
| **Glasshouse** | abc | **ab** | **ab** | **c** | **bc** | **abc** | **a** | **abc** | **ab** |
| | F (9, 20) = 4.748, P < 0.05 | | | | | | | | |
| **Field Plots** | ab | **ab** | **ab** | **a** | **ab** | **a** | **ab** | **a** | **b** |
| | F (9, 20) = 3.515, P < 0.05 | | | | | | | | |
| **(c) Root Mass** | | | | | | | | | |
| **Glasshouse** | a | **a** | **ab** | **a** | **a** | **a** | **ab** | **b** | **ab** |
| | F (9, 20) = 4.697, P < 0.05 | | | | | | | | |
| **Field Plots** | a | **a** | **a** | **a** | **a** | **a** | **a** | **a** | **a** |
| | F (9, 20) =0.877, P < 0.05 | | | | | | | | |

In each row, varieties sharing different letters differ significantly from each other at $p<0.05$ for Green mass (a) Dead mass (b) and Root mass (c) dry weight (One-way ANOVA using varieties as factor; Tukey's HSD post-hoc test). Mean values of Glasshouse (upper row) and Field plots (lower row) Green mass, Dead mass and Root mass values have already been presented in Fig. 4.1

**Appendix 4.2 Statistical significance of differences in total stolon length and total rhizome length**

| | Total Stolon length | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Princess 77** | **Yukon** | **La Paloma** | **Agridark** | **Legend** | **Santa Ana** | **W. Green** | **Sea Spray** | **Zenith** | **R. Staygreen** |
| **GH** | abc | ab | a | d | bcd | bcd | abc | cd | abc | abc |
| | F (9, 20) = 7.781, P < 0.05 | | | | | | | | | |
| **F** | ab | a | ab | c | abc | abc | bc | bc | a | abc |
| | F (9, 20) = 6.435, P < 0.05 | | | | | | | | | |

| | Rhizome length | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Princess 77** | **Yukon** | **La Paloma** | **Agridark** | **Legend** | **Santa Ana** | **W. Green** | **Sea Spray** | **Zenith** | **R. Staygreen** |
| **GH** | a | a | a | b | a | a | a | a | a | a |
| | F (9, 20) = 6.827, P < 0.05 | | | | | | | | | |
| **F** | a | a | a | d | ab | cd | bc | a | a | a |
| | F (9, 20) = 23.178, P < 0.05 | | | | | | | | | |

In each row, varieties sharing different letters differ significantly from each other at p<0.05
(One-way ANOVA using varieties as factor; Tukey's HSD post-hoc test)

## Appendix 4.3 Comparative dry mass of actual leaf, vertical branches, stem/stolon, root, rhizome in Glasshouse (PGU) and Field plots (NZSTI)

| Var. | AL (GH) | AL (OF) | VB (GH) | VB (OF) | SS (GH) | SS (OF) | R (GH) | R (OF) | RH (GH) | RH (OF) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Princess 77** | 79.18 ±8.788 | 58.98 ±10.10 | 42.19 ±8.29 | 30.96 ±12.39 | 59.11 ±7.50 | 161.82 ±49.46 | 24.68 ±1.75 | 125.16 ±43.41 | 0 ±0 | 0 ±0 |
| **Yukon** | 22.13 ±2.289 | 15.87 ±1.97 | 11.07 ±1.07 **(b)** | 36.76 ±2.76 **(a)** | 31.99 ±9.73 **(b)** | 178.21 ±23.08 **(a)** | 16.09 ±6.38 | 65.60 ±19.41 | 0 ±0 | 0 ±0 |
| **La Paloma** | 81.37 ±6.20 **(a)** | 40.96 ±2.18 **(b)** | 19.58 ±2.17 | 26.95 ±5.23 | 33.01 ±7.22 **(b)** | 112.86 ±11.29 **(a)** | 33.35 ±1.36 **(b)** | 95.27 ±14.20 **(a)** | 0 ±0 | 0 ±0 |
| **Agridark** | 130.33 ±16.99 **(a)** | 45.21 ±15.68 **(b)** | 67.93 ±8.35 **(a)** | 25.37 ±3.46 **(b)** | 196.93 ±44.66 | 310.46 ±9.47 | 24.25 ±2.24 **(b)** | 129.64 ±6.55 **(a)** | 13.58 ±3.58 **(b)** | 303.59 ±44.44 **(a)** |
| **Santa Ana** | 88.23 ±6.88 | 73.47 ±2.56 | 72.28 ±4.20 **(a)** | 28.74 ±8.80 **(b)** | 145.82 ±23.97 | 257.37 ±38.21 | 19.70 ±2.29 **(b)** | 126.44 ±24.69 **(a)** | 0 ±0 **(b)** | 144.02 ±3.92 **(a)** |
| **W.Green** | 96.29 ±4.57 **(a)** | 44.60 ±2.41 **(b)** | 49.13 ±7.69 **(a)** | 13.97 ±1.62 **(b)** | 188.15 ±35.63 | 236.51 ±27.98 | 25.59 ±8.47 **(b)** | 119.99 ±6.03 **(a)** | 0 ±0 **(b)** | 94.36 ±6.79 **(a)** |
| **Sea Spray** | 110.01 ±4.99 **(a)** | 79.91 ±5.39 **(b)** | 104.79 ±6.08 **(a)** | 54.45 ±5.78 **(b)** | 156.71 ±24.96 | 205.88 ±23.65 | 31.35 ±5.15 | 122.11 ±34.60 | 0 ±0 | 0 ±0 |
| **Zenith** | 86.71 ±6.60 **(a)** | 15.58 ±1.26 **(b)** | 37.70 ±4.49 **(a)** | 10.89 ±3.01 **(b)** | 96.99 ±25.09 | 87.92 ±26.26 | 57.27 ±10.55 | 79.80 ±20.30 | 0 ±0 | 0 ±0 |
| **R.Staygreen** | 569.03 ±24.77 **(a)** | 116.86 ±6.15 **(b)** | 146.90 ±22.54 **(a)** | 66.59 ±14.27 **(b)** | 160.73 ±6.23 | 182.03 ±11.67 | 36.66 ±0.57 **(b)** | 107.14 ±21.00 **(a)** | 0 ±0 | 0 ±0 |

Results are means of 3 replicates. Where pairs of bars have different letters (higher significantly different value is represented by (a) and lower value by (b)), the effect of location is significant at $P \leq 0.05$.

## Appendix 4.4 Statistical significance of differences structural component weights (actual leaf, vertical branches, stem/stolon, root, rhizome) for glasshouse and field plots

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| AL | | | | | | | | | |
| (a) | | | | | | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| b | a | b | b | b | b | b | b | b | c |
| F (9,20) = 198.387, P<0.05. | | | | | | | | | |
| (b) | | | | | | | | | |
| bc | a | ab | ab | bc | bc | ab | c | a | d |
| F (9,20) = 20.029, P<0.05. | | | | | | | | | |
| VB | | | | | | | | | |
| (a) | | | | | | | | | |
| ab | a | a | bc | ab | bc | ab | cd | ab | d |
| F (9,20) = 20.915, P<0.05. | | | | | | | | | |
| (b) | | | | | | | | | |
| abc | abc | ab | ab | abc | abc | a | bc | a | bc |
| F (9,20) = 5.046, P<0.05. | | | | | | | | | |
| SS | | | | | | | | | |
| (a) | | | | | | | | | |
| ab | a | a | cd | d | abcd | cd | bcd | abc | bcd |
| F (9,20) = 9.130, P<0.05. | | | | | | | | | |
| (b) | | | | | | | | | |
| abc | abcd | ab | d | cd | cd | bcd | abcd | a | abcd |
| F (9,20) = 6.780, P<0.05. | | | | | | | | | |
| R | | | | | | | | | |
| (a) | | | | | | | | | |
| a | a | ab | a | a | a | a | ab | b | ab |
| F (9,20) = 4.697, P<0.05. | | | | | | | | | |
| (b) | | | | | | | | | |
| a | a | a | a | a | a | a | a | a | a |
| F (9,20) = 0.877, P<0.05. | | | | | | | | | |
| RH | | | | | | | | | |
| (a) | | | | | | | | | |
| a | a | a | b | ab | a | a | a | a | a |
| F (9,20) = 3.121, P<0.05. | | | | | | | | | |
| (b) | | | | | | | | | |
| a | a | a | c | ab | ab | ab | a | a | a |
| F (9,20) = 34.523, P<0.05. | | | | | | | | | |

In each row, varieties sharing different letters differ significantly from each other at $p<0.05$

(One-way ANOVA using varieties as factor; Tukey's HSD post-hoc test)

## Appendix 5.1 Comparison of 12 successive internode distances in glasshouse (PGU) and field plots (NZSTI)

| | P77 | | YK | | LP | | AD | | Lg | | SA | | WG | | ZN | | SS | | RS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Var | GH | OF | GH | OF | GH | OF | GH | OF | GH | OF | GH | OF | GH | OF | GH | OF | GH | OF | GH | OF |
| ID1 | 0.038 ± .022 | 0.079 ± .046 | 0.032 ± .019 | 0.085 ± .049 | 0.066 ± .038 | 0.042 ± .024 | 0.026 ± .015 | 0.046 ± .027 | 0.243 ± .140 | 0.023 ± .013 | 0.055 ± .032 | 0.055 ± .032 | 0.023 ± .013 | 0.136 ± .078 | 0.229 ± .132 | 0.043 ± .025 | 1.240 ± .716 | 2.839 ± 1.639 | 0.115 ± .067 | 0.243 ± .140 |
| ID2 | 0.025 ± .015 | 0.046 ± .026 | 0.017 ± .010 | 0.017 ± .010 | 0.031 ± .018 | 0.140 ± .081 | 0.012 ± .007 | 0.025 ± .015 | 0.061 ± .035 | 0.125 ± .072 | 0.045 ± .026 | 0.265 ± .153 | 0.031 ± .018 | 0.135 ± .078 | 0.123 ± .071 | 0.050 ± .029 | 0.951 ± .549 | 3.375 ± 1.949 | 0.138 ± .080 | 0.704 ± .407 |
| ID3 | 0.450 ± .260 | 1.616 ± .933 | 0.676 ± .390 | 0.762 ± .440 | 3.715 ± 2.145 | 2.779 ± 1.605 | 0.665 ± .384 | 1.209 ± .698 | 1.573 ± .908 | 0.392 ± .227 | 0.298 ± .172 | 3.565 ± 2.059 | 3.261 ± 1.883 | 1.565 ± .903 | 1.395 ± .805 | 0.945 ± .546 | 5.993 ± 3.460 | 0.719 ± .415 | 1.829 ± 1.056 | 0.616 ± .356 |
| ID4 | 0.046 ± .026 | 0.131 ± .075 | 0.023 ± .013 | 0.098 ± .057 | 0.042 ± .024 | 0.050 ± .029 | 0.059 ± .034 | 0.072 ± .042 | 0.110 ± .063 | 0.072 ± .042 | 0.05 ± .029 | 0.075 ± .043 | 0.053 ± .031 | 0.123 ± .071 | 0.327 ± .189 | 0.056 ± .032 | 3.959 ± 2.286 | 5.730 ± 3.309 | 1.401 ± .809 | 0.884 ± .510 |
| ID5 | 0.171 ± .098 | 0.104 ± .060 | 0.042 ± .024 | 0.084 ± .048 | 0.117 ± .067 | 0.044 ± .025 | 0.122 ± .070 | 0.031 ± .018 | 0.059 ± .034 | 0.067 ± .038 | 0.047 ± .027 | 0.051 ± .030 | 0.032 ± .019 | 0.042 ± .024 | 0.266 ± .153 | 0.084 ± .048 | 3.422 ± 1.976 | 4.183 ± 2.415 | 2.691 ± 1.554 | 0.997 ± .576 |
| ID6 | 2.393 ± 1.382 **(a)** | 1.555 ± .898 **(b)** | 2.751 ± 1.589 | 1.129 ± .652 | 5.255 ± 3.034 | 1.262 ± .728 | 0.596 ± .344 | 0.488 ± .282 | 1.961 ± 1.132 | 1.151 ± .664 | 2.741 ± 1.583 | 1.327 ± .766 | 3.402 ± 1.965 | 1.865 ± 1.077 | 2.708 ± 1.564 | 1.381 ± .798 | 3.662 ± 2.114 | 3.529 ± 2.037 | 9.203 ± 5.313 | 1.263 ± .729 |
| ID7 | 0.199 ± .115 | 0.087 ± .050 | 0.068 ± .039 | 0.052 ± .031 | 0.053 ± .031 | 0.021 ± .012 | 0.060 ± .035 | 0.053 ± .031 | 0.146 ± .084 | 0.066 ± .038 | 0.052 ± .030 | 0.044 ± .025 | 0.089 ± .051 | 0.072 ± .042 | 0.469 ± .271 | 0.093 ± .054 | 4.010 ± 2.315 | 4.695 ± 2.711 | 10.938 ± 6.315 | 3.738 ± 2.158 |
| ID8 | 0.104 ± .060 | 0.071 ± .041 | 0.046 ± .026 | 0.029 ± .017 | 0.015 ± .009 | 0.145 ± .084 | 0.142 ± .082 | 0.074 ± .043 | 0.057 ± .033 | 0.058 ± .033 | 0.168 ± .097 | 0.056 ± .032 | 0.057 ± .033 | 0.107 ± .062 | 0.342 ± .198 | 0.064 ± .037 | 3.445 ± 1.989 | 4.717 ± 2.723 | 9.239 ± 5.334 | 1.790 ± 1.034 |
| ID9 | 3.947 ± 2.279 | 1.730 ± .999 | 1.851 ± 1.069 | 1.570 ± .907 | 3.076 + 1.776 | 2.517 ± 1.453 | 0.541 ± .312 | 1.355 ± .782 | 1.785 ± 1.031 | 0.327 ± .189 | 1.69 ± .976 | 0.643 ± .371 | 1.685 ± .973 | 2.181 ± 1.259 | 4.119 ± 2.378 | 1.061 ± .613 | 2.713 ± 1.566 | 2.54 ± 1.466 | 7.567 ± 4.368 | 1.460 ± .843 |
| ID10 | 0.017 ± .010 | 0.026 ± .015 | 0.047 ± .027 | 0.118 ± .068 | 0.093 ± .054 | 0.032 ± .019 | 0.096 ± .056 | 0.017 ± .010 | 0.029 ± .017 | 0.081 ± .047 | 0.082 ± .047 | 0.02 ± .012 | 0.146 ± .084 | 0.121 ± .070 | 0.530 ± .306 | 0.093 ± .054 | 2.317 ± 1.338 | 1.273 ± .735 | 4.661 ± 2.691 | 0.950 ± .549 |
| ID11 | 0.036 ± .021 | 0.056 ± .032 | 0.035 ± .020 | 0.139 ± .080 | 0.035 ± .020 | 0.031 ± .018 | 0.159 ± .092 | 0.03 ± .017 | 0.05 ± .029 | 0.032 ± .019 | 0.03 ± .017 | 0.071 ± .041 | 0.075 ± .043 | 0.130 ± .075 | 0.320 ± .185 | 0.175 ± .101 | 2.109 ± 1.218 | 2.739 ± 1.581 | 5.676 ± 3.277 | 1.013 ± .585 |
| 1D12 | 1.04 ± .60034 | 1.448 ± .83587 | 13.01 0.584 ± | 1.958 ± 1.130 | 2.833 ± 1.635 | 0.886 ± .511 | 8.507 ± .4960 | 10.937 ± .24906 | 2.329 ± 1.34 | 2.353 ± 1.358 | 1.022 ± .590 | 3.540 ± 2.043 | 1.542 ± .890 | 3.516 ± 2.02 | 6.766 ± 3.906 | 1.133 ± .65423 | 1.862 ± 1.07 | 2.273 ± 1.312 | 3.311 ± 1.911 | 1.260 ± .727 |

Results are means of 3 replicates. Where pairs of bars have different letters (higher significantly different value is represented by (**a**) and lower value by (**b**))**,** the effect of location is significant at $P < 0.05$.

## Appendix 5.2 Statistical significance of differences of Twelve Successive internodes for glasshouse and field plots

| | P-77 | YK | LP | AD | Lg | SA | WG | ZN | SS | RS |
|---|---|---|---|---|---|---|---|---|---|---|
| ID1 (a) | a | a | a | a | a | a | a | a | b | a |
| | F (9, 20) = 17.584, P < 0.05 | | | | | | | | | |
| ID1 (b) | ab | ab | ab | a | ab | ab | ab | ab | b | ab |
| | F (9, 20) =2.335, P < 0.05 | | | | | | | | | |
| ID2 (a) | a | a | a | a | a | a | a | a | b | a |
| | F (9, 20) = 205.592, P < 0.05 | | | | | | | | | |
| ID2 (b) | a | a | a | a | a | a | a | a | b | a |
| | F (9, 20) = 9.576, P < 0.05 | | | | | | | | | |
| ID3 (a) | b | ab | b | ab | ab | ab | b | ab | b | a |
| | F (9, 20) = 3.293, P < 0.05 | | | | | | | | | |
| ID3 (b) | cd | cd | d | ab | ab | bc | ab | ab | bcd | a |
| | F (9, 20) = 13.557, P < 0.05 | | | | | | | | | |
| ID4 (a) | a | a | a | a | a | a | a | a | c | b |
| | F (9, 20) = 27.833, P < 0.05 | | | | | | | | | |
| ID4 (b) | a | a | a | a | a | a | a | a | b | b |
| | F (9, 20) = 23.826, P < 0.05 | | | | | | | | | |
| ID5 (a) | a | a | a | a | a | a | a | a | c | b |
| | F (9, 20) = 44.155, P < 0.05 | | | | | | | | | |
| ID5 (b) | a | a | a | a | a | a | a | a | b | b |
| | F (9, 20) = 49.482, P < 0.05 | | | | | | | | | |
| ID6 (a) | a | a | a | a | a | a | a | a | a | a |
| | F (9, 20) = 1.366, P < 0.05 | | | | | | | | | |
| ID6 (b) | e | bcde | de | ab | a | cde | abc | abcd | abc | cde |
| | F (9, 20) = 11.624, P < 0.05 | | | | | | | | | |
| ID7 (a) | a | a | a | a | a | a | a | a | b | b |
| | F (9, 20) = 10.969, P < 0.05 | | | | | | | | | |
| ID7 (b) | a | a | a | a | a | a | a | a | b | b |
| | F (9, 20) = 21.668, P < 0.05 | | | | | | | | | |
| ID8 (a) | a | a | a | a | a | a | a | a | b | b |
| | F (9, 20) = 12.959, P < 0.05 | | | | | | | | | |
| ID8 (b) | a | a | a | a | a | a | a | a | b | b |
| | F (9, 20) = 23.413, P < 0.05 | | | | | | | | | |
| ID9 (a) | ab | ab | ab | a | ab | ab | ab | b | ab | ab |
| | F (9, 20) = 2.471, P < 0.05 | | | | | | | | | |
| ID9 (b) | bc | c | c | ab | a | c | ab | a | ab | a |
| | F (9, 20) = 15.004, P < 0.05 | | | | | | | | | |
| ID10 (a) | a | a | a | a | a | a | a | a | b | b |
| | F (9, 20) = 26.720, P < 0.05 | | | | | | | | | |
| ID10 (b) | a | a | a | a | a | a | a | a | c | b |
| | F (9, 20) = 163.149, P < 0.05 | | | | | | | | | |
| ID11 (a) | a | a | a | a | a | a | a | a | b | b |
| | F (9, 20) = 15.697, P < 0.05 | | | | | | | | | |
| ID11 (b) | a | a | a | a | a | a | a | a | b | b |
| | F (9, 20) = 46.688, P < 0.05 | | | | | | | | | |
| ID12 (a) | cd | abc | abc | a | ab | ab | abc | bc | ab | a |
| | F (9, 20) = 6.115, P < 0.05 | | | | | | | | | |
| ID12 (b) | ab | ab | b | ab | ab | ab | ab | a | ab | a |
| | F (9, 20) = 3.717, P < 0.05 | | | | | | | | | |

In each row, varieties sharing different letters differ significantly from each other at p<0.05 (One-way ANOVA using varieties as factor; Tukey’s HSD post-hoc test

**Appendix 5.3 Statistical significance of differences of leaf attributes (blade length, blade width and sheath length) for glasshouse and field plots**

| | P-77 | YK | LP | AD | Lg | SA | WG | ZN | SS | RS |
|---|---|---|---|---|---|---|---|---|---|---|
| Blade Length (a) | ab | b | b | ab | ab | ab | ab | a | ab | c |
| | F (9, 20) = 19.393, P < 0.05 | | | | | | | | | |
| Blade Length (b) | d | c | d | b | bc | b | b | a | c | e |
| | F (9, 20) = 162.919, P < 0.05 | | | | | | | | | |
| Sheath Length (a) | abc | bc | c | ab | abc | abc | abc | ab | a | d |
| | F (9, 20) = 24.004, P < 0.05 | | | | | | | | | |
| Sheath Length (b) | cd | c | cd | a | ab | ab | ab | b | ab | d |
| | F (9, 20) = 29.687, P < 0.05 | | | | | | | | | |
| Blade Width (a) | cde | ef | def | b | f | cd | bc | a | cdef | g |
| | F (9, 20) = 321.736, P < 0.05 | | | | | | | | | |
| Blade Width (b) | c | c | c | b | c | b | b | a | c | d |
| | F (9, 20) = 215.419, P < 0.05 | | | | | | | | | |

Where a= glasshouse and b = field plots.

In each row, varieties sharing different letters differ significantly from each other at p<0.05 (One-way ANOVA using varieties as factor; Tukey’s HSD post-hoc test

## Appendix 6.1 Analysis of Variance for Proline

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| **ExposT** | 1 | 2.69781 | 2.69781 | 2.69781 | 213.90 | 0.000 |
| **Recover** | 1 | 12.69141 | 12.69141 | 12.69141 | 1006.26 | 0.000 |
| **Tempre** | 3 | 0.75332 | 0.75332 | 0.25111 | 19.91 | 0.000 |
| **Specz** | 3 | 1.15576 | 1.15576 | 0.38525 | 30.55 | 0.000 |
| **ExposT*Recover** | 1 | 1.82250 | 1.82250 | 1.82250 | 144.50 | 0.000 |
| **ExposT*Tempre** | 3 | 1.20172 | 1.20172 | 0.40057 | 31.76 | 0.000 |
| **ExposT*Specz** | 3 | 0.51026 | 0.51026 | 0.17009 | 13.49 | 0.001 |
| **Recover*Tempre** | 3 | 1.20547 | 1.20547 | 0.40182 | 31.86 | 0.000 |
| **Recover*Specz** | 3 | 0.27381 | 0.27381 | 0.09127 | 7.24 | 0.009 |
| **Tempre*Specz** | 9 | 0.66564 | 0.66564 | 0.07396 | 5.86 | 0.007 |
| **ExposT*Recover*Tempre** | 3 | 0.95370 | 0.95370 | 0.31790 | 25.21 | 0.000 |
| **ExposT*Recover*Specz** | 3 | 0.32404 | 0.32404 | 0.10801 | 8.56 | 0.005 |
| **ExposT*Tempre*Specz** | 9 | 0.63737 | 0.63737 | 0.07082 | 5.61 | 0.008 |
| **Recover*Tempre*Specz** | 9 | 0.16947 | 0.16947 | 0.01883 | 1.49 | 0.280 |
| **Error** | 9 | 0.11351 | 0.11351 | 0.01261 | | |
| **Total** | 63 | 25.17578 | | | | |

**ExposT**= Exposure length(Sudden/Gradual); **Recover=** After two weeks of green up; **Tempre**= Temperature effect; **Specz**= Species effect

## Appendix 6.2 Analysis of Variance for MDAz

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| **ExposT** | 1 | 573.67 | 573.67 | 573.67 | 87.83 | 0.000 |
| **Recover** | 1 | 1583.99 | 1583.99 | 1583.99 | 242.51 | 0.000 |
| **Tempre** | 3 | 5303.31 | 5303.31 | 1767.77 | 270.65 | 0.000 |
| **Specz** | 3 | 1381.71 | 1381.71 | 460.57 | 70.51 | 0.000 |
| **ExposT*Recover** | 1 | 31.82 | 31.82 | 31.82 | 4.87 | 0.055 |
| **ExposT*Tempre** | 3 | 5633.61 | 5633.61 | 1877.87 | 287.50 | 0.000 |
| **ExposT*Specz** | 3 | 1083.86 | 1083.86 | 361.29 | 55.31 | 0.000 |
| **Recover*Tempre** | 3 | 23.78 | 23.78 | 7.93 | 1.21 | 0.360 |
| **Recover*Specz** | 3 | 17.01 | 17.01 | 5.67 | 0.87 | 0.493 |
| **Tempre*Specz** | 9 | 669.52 | 669.52 | 4.39 | 11.39 | 0.001 |
| **ExposT*Recover*Tempre** | 3 | 332.90 | 332.90 | 110.97 | 16.99 | 0.000 |
| **ExposT*Recover*Specz** | 3 | 108.19 | 108.19 | 36.06 | 5.52 | 0.020 |
| **ExposT*Tempre*Specz** | 9 | 156.38 | 156.38 | 17.38 | 2.66 | 0.081 |
| **Recover*Tempre*Specz** | 9 | 162.83 | 162.83 | 18.09 | 2.77 | 0.073 |
| **Error** | 9 | 58.79 | 58.79 | 6.53 | | |
| **Total** | 63 | 17121.36 | | | | |

**ExposT**= Exposure length(Sudden/Gradual); **Recover=** After two weeks of green up; **Tempre**= Temperature effect; **Specz**= Species effect

## Appendix 6.3 Analysis of Variance for LMWSug

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| **ExposT** | 1 | 47212 | 47212 | 47212 | 2.83 | 0.127 |
| **Recover** | 1 | 3875194 | 3875194 | 3875194 | 232.66 | 0.000 |
| **Tempre** | 3 | 334993 | 334993 | 111664 | 6.70 | 0.011 |
| **Specz** | 3 | 347784 | 347784 | 115928 | 6.96 | 0.010 |
| **ExposT*Recover** | 1 | 7821 | 7821 | 7821 | 0.47 | 0.510 |
| **ExposT*Tempre** | 3 | 33361 | 33361 | 11120 | 0.67 | 0.593 |
| **ExposT*Specz** | 3 | 117716 | 117716 | 39239 | 2.36 | 0.140 |
| **Recover*Tempre** | 3 | 679311 | 679311 | 226437 | 13.59 | 0.001 |
| **Recover*Specz** | 3 | 367422 | 367422 | 122474 | 7.35 | 0.009 |
| **Tempre*Specz** | 9 | 106411 | 106411 | 11823 | 0.71 | 0.691 |
| **ExposT*Recover*Tempre** | 3 | 8665 | 8665 | 2888 | 0.17 | 0.912 |
| **ExposT*Recover*Specz** | 3 | 341789 | 341789 | 113930 | 6.84 | 0.011 |
| **ExposT*Tempre*Specz** | 9 | 116126 | 116126 | 12903 | 0.77 | 0.645 |
| **Recover*Tempre*Specz** | 9 | 67943 | 67943 | 7549 | 0.45 | 0.873 |
| **Error** | 9 | 149907 | 149907 | 16656 | | |
| **Total** | 63 | 6601655 | | | | |

**ExposT**= Exposure length(Sudden/Gradual); **Recover=** After two weeks of green up; **Tempre**= Temperature effect; **Specz**= Species effect

**Appendix 6.4 Analysis of Variance for HMWSug**

| Source | **DF** | **Seq SS** | **Adj SS** | **Adj MS** | **F** | **P** |
|---|---|---|---|---|---|---|
| **ExposT** | 1 | 165.251 | 165.251 | 165.251 | 16.94 | 0.003 |
| **Recover** | 1 | 955.892 | 955.892 | 955.892 | 98.01 | 0.000 |
| **Tempre** | 3 | 606.620 | 606.620 | 202.206 | 20.73 | 0.000 |
| **Specz** | 3 | 70.792 | 70.792 | 23.597 | 2.42 | 0.133 |
| **ExposT*Recover** | 1 | 55.279 | 55.279 | 55.279 | 5.67 | 0.041 |
| **ExposT*Tempre** | 3 | 1034.302 | 1034.302 | 344.767 | 35.35 | 0.000 |
| **ExposT*Specz** | 3 | 259.823 | 259.823 | 86.608 | 8.88 | 0.005 |
| **Recover*Tempre** | 3 | 19.259 | 19.259 | 6.420 | 0.66 | 0.598 |
| **Recover*Specz** | 3 | 248.771 | 248.771 | 82.924 | 8.50 | 0.005 |
| **Tempre*Specz** | 9 | 286.740 | 286.740 | 31.860 | 3.27 | 0.046 |
| **ExposT*Recover*Tempre** | 3 | 65.032 | 65.032 | 21.677 | 2.22 | 0.155 |
| **ExposT*Recover*Specz** | 3 | 110.942 | 110.942 | 36.981 | 3.79 | 0.052 |
| **ExposT*Tempre*Specz** | 9 | 414.156 | 414.156 | 46.017 | 4.72 | 0.015 |
| **Recover*Tempre*Specz** | 9 | 169.834 | 169.834 | 18.870 | 1.93 | 0.170 |
| **Error** | 9 | 87.773 | 87.773 | 9.753 | | |
| **Total** | 63 | 4550.465 | | | | |

**ExposT**= Exposure length(Sudden/Gradual); **Recover=** After two weeks of green up; **Tempre**= Temperature effect; **Specz**= Species effect

## Appendix 6.5 Analysis of Variance for Discoloration

| **Source** | **DF** | **Seq SS** | **Adj SS** | **Adj MS** | **F** | **P** |
|---|---|---|---|---|---|---|
| **ExposT** | 1 | 3122.02 | 3122.02 | 3122.02 | 631.20 | 0.000 |
| **Recover** | 1 | 2058.89 | 2058.89 | 2058.89 | 416.26 | 0.000 |
| **Tempre** | 3 | 1610.80 | 1610.80 | 536.93 | 108.55 | 0.000 |
| **Specz** | 3 | 3208.67 | 3208.67 | 1069.56 | 216.24 | 0.000 |
| **ExposT*Recover** | 1 | 833.77 | 833.77 | 833.77 | 168.57 | 0.000 |
| **ExposT*Tempre** | 3 | 1112.42 | 1112.42 | 370.81 | 74.97 | 0.000 |
| **ExposT*Specz** | 3 | 1826.05 | 1826.05 | 608.68 | 123.06 | 0.000 |
| **Recover*Tempre** | 3 | 169.55 | 169.55 | 56.52 | 11.43 | 0.002 |
| **Recover*Specz** | 3 | 467.42 | 467.42 | 155.81 | 31.50 | 0.000 |
| **Tempre*Specz** | 9 | 565.64 | 565.64 | 62.85 | 12.71 | 0.000 |
| **ExposT*Recover*Tempre** | 3 | 145.67 | 145.67 | 48.56 | 9.82 | 0.003 |
| **ExposT*Recover*Specz** | 3 | 204.80 | 204.80 | 68.27 | 13.80 | 0.001 |
| **ExposT*Tempre*Specz** | 9 | 546.27 | 546.27 | 60.70 | 12.27 | 0.000 |
| **Recover*Tempre*Specz** | 9 | 5.89 | 55.89 | 6.21 | 1.26 | 0.370 |
| **Error** | 9 | 44.52 | 44.52 | 4.95 | | |
| **Total** | 63 | 15972.36 | | | | |

**ExposT**= Exposure length(Sudden/Gradual); **Recover=** After two weeks of green up; **Tempre**= Temperature effect; **Specz**= Species effect