Scuola Superiore Sant'Anna
di Studi Universitari e di Perfezionamento

DOTTORATO DI RICERCA IN
"SCIENZA DELLE PRODUZIONI VEGETALI"

XXII° CICLO
Settore scientifico disciplinare: AGR/02

TESI DI DOTTORATO

Tissue biomechanical strength, wear resistance and recovery in C4 turfgrass species: physiological and morphological factors and innovative evaluation techniques.

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ANNO ACCADEMICO 2010-2011

To my beloved Valeria and Leandro.

For my father and mother dearest.

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INTRODUCTION

Further to aesthetic value, turfgrass canopies are often required to supply a high level of resistance to severe wear. This is particularly true of sports installations (football, golf, rugby, baseball, horse racing, tennis, etc.), but ornamental and functional turfgrass canopies are not exempt from having to withstand wear. As such, one of the main aspects of turfgrass variety selection is indeed selection for wear resistance (and recovery), and one of the main goals of turfgrass maintenance is minimizing wear effects and maximising recovery.

Due to these particular requirements of turfgrass in terms of wear resistance and recovery, and given the very high maintenance interventions required to maximize these traits, any further knowledge on the physiological and biological mechanisms underlying wear resistance and recovery could lead to a decrease or in an optimization of turfgrass maintenance. In terms, this would have considerable positive financial repercussions on the annual budgets necessary to maintain high-profile turfgrass surfaces, and would also increase the environmental sustainability of turfgrass surfaces due to a decrease in maintenance operations and in agronomic inputs (fertilizers, irrigation water, pesticides, etc.).

Despite benefiting from a Mediterranean (or “transition”) climate, due to the lack of a true Mediterranean turfgrass culture, countries of the Mediterranean basin have until now favoured the use of C3 turfgrass species more suited to the northern European climatic areas. These species generally

exhibit a scarce adaptation to climates below the 44°N parallel, with very high irrigation water requirements, high susceptibility to pests and generally low heat stress tolerance. Furthermore, these species have always been associated with a much lower wear resistance and recovery, compared to C4 turfgrass species.

In recent years, thanks to the influx of American turfgrass culture spreading to southern Europe, and thanks to an intense turfgrass research activity by southern European Universities, C4 warm-season grass subfamilies (*Chloroideae* and *Panicoideae*) have witnessed a widespread of use for the establishment of sports, ornamental and functional turfgrass. These species, though subject to a varying degree of winter dormancy in Mediterranean areas, exhibit a much higher drought, heat and pest stress tolerance, coupled with a much higher wear resistance and recovery (thanks also to their stoloniferous and rhizomatous growth habit). These fundamental traits of C4 turfgrass species make for a much improved adaptation to Mediterranean areas, and consequently a much easier and environmentally friendly establishment and maintenance of C4 turfgrass surfaces.

Turfgrass wear effects are considered to be the sum of soil compaction and tissue wounding. The first member of this equation (soil compaction) can be successfully addressed during turfgrass establishment (with the adoption of sandy rootzone layers and drainage) and during maintenance (with the use of specific soil decompaction machines). The second member of the equation (tissue wounding) still needs much research in order to effectively comprehend the mechanisms that underlie turfgrass tissue wear resistance.

Recovery in C4 turfgrass species is facilitated by the frequent presence of stolons and rhizomes, which supply numerous regrowth points

(nodes) from which turfgrass can recuperate. However, many differences in recovery are present among C4 turfgrass species and these also need to be further investigated.

The present doctorate thesis consists of the *summa* of two experimental trials in which the author **aimed to evaluate C4 turfgrass species' mechanical resistance and recovery, and their physiological and biological basis, devoid of soil compaction effects**.

Study One consisted of breaking down turfgrass wear resistance by evaluating the tensile strength of various plant organs (leaves, stolons and rhizomes). Tensile strength was measured in general terms (thus giving a feedback on species' strength) and per unit area of tested tissue (thus giving a feedback on the species' tissue strength, and hence on its' constituents). These results were plotted against laboratory investigation on main tissue constituents.

Study Two consisted of evaluating turfgrass wear resistance as tested with a numerical control wear simulating machine used by FIFA to measure artificial turf wear resistance. Wear resistance and recovery results (in terms of shoot density lost and recovered, both in absolute and percentage terms) were plotted against laboratory investigation on main tissue constituents.

The two experimental trials are presented as *in litteris* manuscripts that are due for submission to scientific journals, and as such they can be read and evaluated independently from one another.

STUDY ONE

Physiological and morphological factors influencing leaf, rhizome and stolon tensile strength in C4 turfgrass species.

Introduction

Warm-season grass subfamilies (*Chloroideae* and *Panicoideae*) are the most widely used turfgrass species for the creation of sports, ornamental and functional lawns in most areas between the 40°N and 40°S parallels. Among these, *Cynodon dactylon* [L.] Pers., *Zoysia japonica* Steudel and *Zoysia matrella* (L.) Merrill. are the most wear tolerant species (Youngner 1961; Turgeon 2005). These species share a great adaptability to wide pH and salinity ranges (Marcum, 1999), a high colonization and recuperation capability deriving from their stoloniferous and\or rhizomatous growth habit, high temperature and drought resistance, but differ markedly in their wear resistance, as it has been so far measured in field trials (Beard *et al*, 1981; Trenholm *et al* 1999; Shearman *et al* 2001).

Turfgrass wear is known to be the sum of two main effects: soil compaction and plant tissue wounding (Beard 1973; Carrow and Petrovic 1992). Compaction affects root system growth, nutrient uptake and gas exchange capability of the

root system, and water circulation within the soil, with results often considered akin to anoxia (Feldman 1984; Unger and Kaspar 1994; Dest *et al.* 2009). Plant tissue injury is generalized, with all plant tissues suffering wounds and death from wear and, although the meristematic apex ("crown") is the vital part of the turfgrass plant (Beard 1973) that if damaged leads directly its death, severe damage to other tissues (shoots, stolons, rhizomes, roots) can also cause plant withering and general decrease in turfgrass density. Therefore, plant tissue mechanical resistance is to be considered a primary factor in determining turfgrass wear resistance.

Sanson (2006) stated that plant leaves' resistance is achieved by a number of mechanisms, which operate at the molecular, cell, tissue, and structural levels, with monocotyledon and dicotyledon differing in venation patterns and cell wall volume fractions.

Several studies have tackled crop and forage monocotyledon species' tissue strength: Sun and Liddle (1993) suggested that leaf tensile strength in *Cynodon dactylon* increases with age, while MacAdam and Mayland (2003) found that in tall fescue leaf tensile strength per unit area was negatively correlated with both leaf blade width and thickness, because of the greater distance between veins and therefore more mesophyll tissue volume in wider leaves, and hence a higher proportion of cell contents to fiber. Balsamo and Orkwiszewski (2008) found that tensile strength in leaves of *Zea mays* is associated with percent tissue lignification and the appearance of leaf architectural characters that are associated with the transition from the juvenile to the adult phase. Wright and Illius (1995) found that schlerenchima and fibre content positively influenced the

fracture properties of certain forage grasses, and that fracture usually occurs at a zone of weakness at the intercalary meristem.

Given the predominant role of cell wall constituents in imparting plant tissue mechanical strength (Doblin *et al.* 2010), turfgrass wear resistance has often been correlated with several biological and physiological parameters such as total cell wall (Shearman and Beard 1975), modified acid detergent fibre (Canaway 1981), sucrose phosphate synthase and sucrose synthase activity, stem and leaf moisture (Trenholm *et al.* 2000), leaf flexibility (Sun and Liddle 1993), evapotranspiration rate, leaf chlorophyll concentration, membrane permeability, leaf peroxidase activity, spectral reflectance, tissue potassium (Shearman and Beard 1973; Carrol and Petrovic 1991) and silica concentration (Trenholm *et al.* 2001).

As indicated by FIFA, traction measurements are routinely carried out in laboratory environment on artificial turf fibres and tufts of fibres (FIFA 2008).

The present research was aimed at evaluating the mechanical resistance of the plant organs that make up a mature sward of three select C4 turfgrass species, via traction tests carried out in a controlled laboratory environment and with FIFA-approved numerical control machinery, and correlating these results with laboratory analysis of main plant tissue constituents, in order to increase the understanding of the biological factors that effectively contribute to the strength of turfgrass plant tissues.

Materials and methods

Plant material and growth conditions

In March 02 2009 three 50 cm^2 plugs per species were harvested from mature swards of *Cynodon dactylon* x *Cynodon transvaalensis* cv. Tifway 419 (Cdxt), *Zoysia matrella* (L.) Merr. Cv. Zeon(Zm) and *Paspalum vaginatum* Sw. cv. Salam (Pv) grown on a silt loam soil at the research station of the Centre for Research on Turfgrass for Environment and Sports - University of Pisa (43° 40' N, 10° 19' E; 6 m a.s.l.). Plugs were washed clean from native soil and transferred to 5000 ml pots containing peat (Brill Type 3 Special: 50% white peat – 50% black peat; pH: 5.5-6.0; salt content: 0.7-1.2 g l^{-1}; N: 100-160 mg l^{-1}; P_2O_5: 120-200 mg l^{-1}; K_2O: 140-240 mg l^{-1}). The nine pots (three per species) were arranged in a completely randomized block with three replications in a greenhouse environment and allowed to grow as stolon donor plants (plants suspended 1.5 m above ground) for 209 days (max T° 35±4 °C; min T° 25±3 °C; 80±5 % relative humidity). After initial rooting in the pots, only one mowing at 1 cm height was carried during the trial (April 10 2009) in order to uniform vegetation. During the 209 days of greenhouse growing the identical management practices for all species were: daily irrigation with 5 mm of water; four fertilizations (April 10, May 11, June 8 and July 13) each with 5 g m^{-2} of N, 1 g m^{-2} of P_2O_5 and 2 g m^{-2} of K_2O; no fungicide or weed control application; no trimming of resulting stolons.

Traction tests

All tests were carried out from September 28 2009 to October 01 2009 at Labosport Italia laboratories (Cernusco Lombardone – Italy). Laboratory conditions were maintained constant at 23°C temperature and 50% relative humidity. The device used to carry out the tests was an "MTS Insight 1" single-

column electromechanical dynamometer with 1 kN load cell. Traction speed was set at 500 mm min^{-1} and distance between vices was 25 mm for leaves and 15 mm for stolons and rhizomes. Rubber inserts were glued on the dynamometer vices in order to minimize plant tissue damage from tightening of the vices. The output of the MTS dynamometer for each tested sample was traction peak value (N).

Leaves rhizomes and stolons were randomly collected from the pot. Stolon and rhizome samples were prepared for testing including 1 node and 1 cm of internode fore and aft of the node.

From stolons, the 3^{rd}, 7^{th} and 14^{th} nodes from the tip of the stolon were selected for investigation.

The total number of samples was:

- Stolons: 3 nodes (3^{rd}, 7^{th} and 14^{th}) x 3 species x 3 replicates (pot) x 3 samples = 81 nodes tested.
- Leaves: 10 leaves x 3 species x 3 replicates (pot) = 90 total leaves tested.
- Rhizomes: 3 nodes x 3 species x 3 replicates (pot) = 27 total nodes tested.

The following equation, deriving from plant tissue biometrics (see following paragraph) and from peak load measurements, was used for all species and plant organs to calculate traction peak load per unit area (N mm^{-2}):

Traction peak load per unit area (N mm^{-2}) = Traction peak load (N) / section (mm^2) [1]

The calculation of traction peak load per unit area was aimed at removing the effect of species-specific plant tissue dimensions from traction data, and introducing the parameter of absolute plant tissue traction resistance.

Plant tissue biometrics

All measurements were carried out from September 28 2009 to October 01 2009 at Labosport Italia laboratories. Stolon diameter (mm), rhizome diameter (mm) and leaf blade thickness were measured via a Mitutoyo Absolute IP54 digital feeler gauge with a 0.5 N max load and ± 2 nm error. Leaf blade width (mm) was measured via the digital ruler function of a AM413MT-Dino-Lite 20x-200x digital microscope. Leaf blade width and thickness were measured in the middle of the leaf blade's length. Stolon and rhizome diameters were measured in the middle of the apical internodes.

All measurements were carried out on 20 samples for each organ randomly collected from each pot.

Tissue sections were then calculated as follows:

$$\text{Leaf section } (mm^2) = \text{leaf width (mm)} \times \text{leaf thickness (mm)} \quad [2]$$

$$\text{Stolon or Rhizome section } (mm^2) = \pi \, (\text{stolon or rhizome diameter (mm)}/2)^2 \quad [3]$$

Tissue constituent laboratory analysis

On October 02 2009 plant tissues were sampled in the same manner as described in the 'Traction tests' paragraph and stored at -80°C. Plant tissue samples were tested for their content in the following substances: starch (mg g^{-1}

FW), glucose (nmol mg^{-1}), sucrose (nmol mg^{-1}), fructose (nmol mg^{-1}), dry matter content (%), lignin (absorbance at 335 nm g^{-1} FW) and silica (absorbance at 650 nm g^{-1} FW). All tests were carried out on rhizome, stolon (3^{rd}, 7^{th} and 14^{th} nodes from the stolon apex) and leaf (except for starch content) of each species. All the analyses were conducted in triplicate. The laboratory test methods for the determination of the above substances were as follows:

a) Starch. Samples (100 mg FW) were grounded in a mortar and resuspended in 100 mL of 10 mM KOH, and boiled for 1 min. To each sample were then added 1 ml of 1N HCl. The starch standard solution was prepared using 100 mg of potato soluble starch dissolved in 100 ml dH_2O and boiled 1 min. To both samples and standards (final volume 50 ml) was added 1 mL of fresh iodine solution (0.13% K_2 and 0.3% KI, dissolved in dH_2O) and the absorbance was read at 595 nm in few minutes. Starch concentration was expressed as mg starch/g FW.

b) Glucose, sucrose and fructose. Samples (100 mg FW) were rapidly frozen in liquid nitrogen and ground to a powder, then extracted as described by Tobias *et al.* (1992) and assayed for glucose, fructose and sucrose content through coupled enzymatic assay methods (Guglielminetti *et al.* 1995). The efficiency of the methods was tested by using known amounts of carbohydrates. Incubations of the samples and standards were carried out at 37 °C for 30 min. The reaction mixtures (1 mL) were as follows. Glucose: 100 mM Tris-HCl, pH 7.6, 3 mM $MgCl_2$, 2 mM ATP, 0.6 mM NADP, 1 unit Glc6P dehydrogenase; the A_{340} was recorded. Fructose was assayed as described for glucose plus the addiction of 2 units of PGI; the increase in A_{340} was recorded. Sucrose was first broken down using 85 units of invertase (in 15 mM Na-acetate, pH 4.6) and the

resulting glucose and fructose were assayed as described above. Recovery experiments evaluated losses taking place during the extraction procedures. Two experiments were performed for each metabolite by adding known amounts of authentic standards to the sample prior to the extraction. The concentration of the standards added were similar to those estimated to be present in the tissues in preliminary experiments. The recovery ranged between 97 and 104%.

c) Dry matter content. Samples (1 g FW) were dried in an oven at 60°C for 1 week and than the dry matter was measured.

d) Lignin. Lignin was quantified via a thioglycolic acid (TGA) precipitation method (Lee *et al.* 2001). Pigments and soluble phenolics were removed from samples (100 mg FW) by three changes of absolute methanol over 3 days. The cleared tissue was then dried in an oven at 30°C for 1 day. The dried tissue was suspended in 1 ml 10% (v/v) TGA in 2M HCl (v/v) and heated for 4 h at 100°C in a water bath. When cooled, the solution and all tissues were then transferred to a fresh tube and subjected to centrifugation at 14000xg for 15 min. The supernatant was decanted and the pellet washed with 2.0 ml dH_2O before centrifugation at 14000xg for 10 min. The supernatant was decanted and 1.0 ml 1M NaOH added and mixed to solubilize the TGA derivatives. The suspension was then incubated overnight at 4°C before centrifugation at 14000xg for 10 min. The NaOH supernatant was added to a fresh tube and the TGA derivatives precipitated by adding 0.4 ml concentrated HCl and incubating in an ice bath for 1 h. The reddish/brown precipitate was pelleted by centrifugation for 10 min at 14000xg. The supernatant was decanted and the precipitate dissolved in 1 ml 0.5 M NaOH. The absorbance (A) of each resultant orange solution was

determined at 335 nm (Graham and Graham 1991) and the concentration of wall bound TGA derivatives expressed as absorbance at 335nm g^{-1} FW.

e) Silica. Content of silica in the different plant species was determined using an autoclaved-induced digestion (Elliot and Snyder 1991). Samples (100 mg FW) were dried at 60°C for 48 h and then ground using liquid nitrogen. Samples of plant tissue were wetted with 400 µL of 50% H_2O_2 in 100-mL polyethylene tubes previously rinsed with 0.1 M NaOH and DW. To each tube was added 900 mg of 50% NaOH (W/V) at room temperature, and each tube was gently vortexed. The tubes were individually covered with loose fitting plastic caps. The rack of tubes was placed in an autoclave at 138 kPa for 1 h. After atmospheric pressure was reached, the tubes were removed and the contents brought to 50 mL with dH_2O. Silica in dilutions of samples was determined colorimetrically by the following procedure: to each sample were added with mixing 7.8 mL of 20% acetic acid, 2 mL of ammonium molybdate solution (54 g/L, pH 7.0), 1 mL of 20% tartaric acid, 0.2 mL of reducing solution (described below).

Five minutes elapsed between the addition of the ammonium molybdate and the tartaric acid. The reducing solution was made by combining solution A (2 g of Na_2SO_3 in 25mL of dH_2O plus 0.4 g of l-amino-2-naphthol-4-sulfonic acid) and solution B (25 g of $NaHSO_3$ dissolved in about 200 mL of dH_2O) and diluting to 250 mL. Following the addition of all the reagents, the samples were mixed well and allowed to stand for 30 min. Plastic ware rinsed with 0.1 M NaOH was used throughout. The colour was read at 650 nm with a 1 cm path length. The silica content was expressed as A650nm g^{-1} FW.

Statistical analysis

All statistical analysis were carried out with a COSTAT 6.400 software (CoStat 2008). Bartlett's test on homogeneity of variances was carried out on all data sets. Non normally distributed data sets were log transformed until normal distribution was obtained.

Species effect was evaluated in leaves and rhizomes data via ANOVA in a completely randomized block design, while stolons data was subject to ANOVA in a 2 way completely randomized block design (interaction of species x ordinal node effects). In all cases, Tukey's Honestly Significant Difference (HSD) for $P<0.05$ was used to detect differences between means.

All parameters for each species were then submitted to a multiple regression analysis to find the equation of the subset of 5 parameters with the highest regression coefficient (R^2) for traction peak load (N) and traction peak load per unit area (N mm^{-2}).

Results

Leaves

Highest leaf width (Table 1) was recorded by Pv (2.83 mm), while Cdxt and Zm recorded a much finer leaf texture ($P<0.005$). Again, Pv showed the thickest leaf (0.19 mm), with Zm (0.14 mm) and Cdxt (0.10 mm) showing lower thickness with successive steps in significance levels ($P<0.005$).

These two last parameters determined an average calculated leaf section of 0.53 mm^2 for Pv which is roughly two fold higher than Zm (0.24 mm^2) and threefold Cdxt (0.16 mm^2) ($P<0.005$).

Leaf traction peak load (Fig. 1) was significantly higher ($P<0.005$) for Zm (4.61 N), with Pv and Cdxt leaves showing similar values of a lower magnitude.

However, the analysis of calculated data on traction peak load per unit area (Fig. 2), while confirming the highest value of Zm leaf tissue (20.20 N mm^{-2}) followed by Cdxt (13.51 N mm^{-2}), highlights a Pv leaf tissue strength (4.82 N mm^{-2}) that is roughly five times lower than Zm ($P<0.005$).

No significant differences between species were found for glucose and fructose content (Fig. 7), while Cdxt sucrose content (60.76 nmol mg^{-1}) was roughly three times higher than Pv and Zm ($P<0.05$).

Lignin absorbance (Fig. 4) in Zm (96.79) was roughly three times higher than both Pv and Cdxt ($P<0.005$).

Silica absorbance (Fig. 5) was significantly different ($P<0.05$) with Cdxt showing the highest value of absorbance at 650 nm (12.34), followed by Zm (6.89) and Pv (5.02).

Dry matter (DM) percentage (Fig. 6) was highest ($P<0.005$) and very similar in Cdxt and Zm (43.57% and 42.44% respectively), while Pv showed much lower dry matter (29.39%).

The multiple regression equations of the subset of 5 parameters for all species with the highest determination coefficient (R^2) for peak load and peak load per unit area were:

Leaf peak load =

0.28 +0.06(Glucose) -0.04(Sucrose) +0.01(Lignin) +0.09(DM) -0.83(Silica)

(R^2=0.96)

Leaf tissue peak load per unit area =

-5.24 +1.58(Glucose) -3.01(Fructose) +0.17(Lignin) +0.19(DM) -0.29(Silica)

(R^2=0.99).

Rhizomes

Cdxt (3.00 mm^2, Table 1) and Pv (3.38 mm^2) had significantly higher rhizome sections than Zm (1.54 mm^2) (P<0.005).

Peak load (Fig. 1) (P<0.01) was highest for Zm (60.94 N), with Cdxt and Pv showing similar values (40.02 N and 41.38 N, respectively).

This trend continued in peak load per unit area (Fig. 2), but with higher (P<0.005) differences between Zm (40.88 N mm^{-2}) and Cdxt and Pv (13.84 N mm^{-2} and 12.96 N mm^{-2}, respectively).

Starch content (Fig. 3) (P<0.005) indicated a much higher concentration in Cdxt rhizomes (193.78 mg g^{-1}) compared to Zm (47.62 mg g^{-1}) and Pv (26.31 mg g^{-1}), while no significant differences were found for glucose, sucrose and fructose concentrations (Fig. 7).

Lignin absorbance (Fig. 4) (P<0.005) in Zm (295.92) was approximately threefold that of Cdxt (101.49) and six fold that of Pv (54.75), while no statistically significant differences were found for silicate (Fig. 5) content and dry matter percentage (Fig. 6).

The best multiple regression equations for peak load and peak load per unit area were:

Rhizome peak load =

2.36 +14.02(Glucose) -3.14(Sucrose) +1.07(Fructose) +0.23(Lignin) -7.07(Silica)

(R^2=0.61)

Rhizome tissue peak load per unit area =

7.54 +7.43(Glucose) -1.99(Sucrose) +0.23(Lignin) -0.20(DM) -6.24(Silica)

(R^2=0.79).

Stolons

The species x node interaction was significant in stolon section (Table 2) (P<0.05), with Pv stolon internodes consistently thicker than Cdxt and Zm stolon internodes. Pv nodes differed between them, with central nodes thicker than basal and apical ones.

No significant interaction was found for peak load, but species effect was significant (Fig. 1) (P<0.005) with Zm (35.64 N) and Pv (32.87 N) showing higher traction resistance compared to Cdxt (23.37 N). Node effect (Fig. 10) was also significant (P<0.005) with 3rd node (18.19 N) being significantly weaker than 7th (34.77 N) and 14th (38.92 N) node.

Significant species x node interaction (Fig. 8) (P<0.01) was found for traction peak load per unit area, with Zm basal nodes significantly stronger than all other nodes (over 30 N mm^{-2}), while Pv3 (7.56 N mm^{-2}), Pv7 (12.27 N mm^{-2}) and Cdxt3 (12.48 N mm^{-2}) were found to be the nodes with the weakest tissues.

Starch content (Fig. 3) was significant at species level (P<0.005) with Cdxt (160.17 mg g^{-1}) showing considerably higher levels of starch compared with Pv and Zm.

Stolon glucose (P<0.05), sucrose (P<0.01) and fructose (P<0.01) concentrations were significant at species level (Fig. 7), with Pv (14.29 nmol

mg^{-1}, 58.84 nmol mg^{-1} and 15.40 nmol mg^{-1} respective sugar content) consistently showing the highest concentrations and Zm (8.60 nmol mg^{-1}, 18.13 nmol mg^{-1} and 6.92 nmol mg^{-1} respective sugar content) consistently showing the lowest concentrations.

Species x node interaction for lignin absorbance (Fig. 9) was highly significant ($P<0.005$), with highest concentration in Zm14 (343.46), followed by Zm7 (281.06), Zm3 (167.54) and then by all the other nodes and species that do not differ significantly between them.

Silica absorbance (Fig. 5) was significant at species level only ($P<0.005$), with Cdxt returning the highest absorbance (3.16), while Zm and Pv do not differ significantly between them.

Dry matter percentage (Fig. 6) was significant at species level ($P<0.005$) with a significantly higher dry matter percentage for Cdxt (47.27%) and Zm (45.26%) compared with Pv (23.87%), while at node level (Fig. 11) ($P<0.005$) basal nodes showed a much higher dry matter percentage than the 3rd node.

The best multiple regression equations for peak load and peak load per unit area were:

Stolon peak load =

-22.48 +11.91(Section) +0.68(Glucose) +0.07(Lignin) +0.42(DM) -1.51(Silica)

(R^2=0.61)

Stolon tissue peak load per unit area =

-1.25 +0.18(Glucose) +0.18(Fructose) +0.05(Lignin) +0.24(DM) -0.76(Silica)

(R^2=0.79).

Discussion

One of the rationales behind this study is that measured traction peak load (N) indicates the strength of a species as would be witnessed in the field, while calculated traction peak load per unit area (N mm^{-2}) is a measure of tissue mechanical strength, and hence more strictly correlated with histological constituents and architecture.

The leaf width (Pv>Zm=Cdxt) findings are in accordance with the National Turfgrass Evaluation Program's results (NTEP, 2007*a*; NTEP 2007*b*; NTEP 2009). Leaf thickness and dry matter indicates Pv as the species with the most succulent leaf. This would be in accordance with the characteristics of Pv as a species originating from swampy and waterlogged areas (Barnard 1969; Colman and Wilson 1960).

Leaf tissue traction peak load data confirm Zm as the species generally indicated as most resilient, although no previous micromechanical tests were carried out on Zm and this assumption relies only on field experiments and evaluations (NTEP 2007*b*), but also rebut the general assumption of Pv as a generally "weak" species (and hence unsuitable for use in sports turf), in accordance with previous findings on Pv leaf tensile strength (Trenholm *et al*. 2000). Previous micromechanical tests on leaf tissues of bermudagrass (Alamar *et al.* 2008) produced much higher results, but with no indication of variety or leaf dimensions.

Indeed, once the section of leaf tissue subject to traction is entered into the equation (Pv>Zm>Cdxt), the specific strength of each entry's leaf tissues are highlighted - Zm emerges as the strongest and, on a leaf tissue mechanical resistance standpoint alone, the most suitable for highly-trafficked areas.

Starch levels are with the ecophysiology of the different species. The species originating from arid areas (Cdxt), and hence more adapted to the energy expenditure associated with the repeated closing and opening of stoma, accumulates more starch, while Pv (originating from waterlogged and other high humidity areas) accumulates the least starch. Zm has an intermediate climate adaptation and has an intermediate starch accumulation level.

The content of total soluble sugars (TSS) in stolons indicates how the strongest species (Zm) has consistently the lowest TSS content, and this emerges as one of the clearest “markers” for predicting tissue strength.

As such, Zm would appear to deplete TSS and starch in favour of the construction of strength-inducing cell-wall constituents (lignin, etc.). This would indicate a higher aptitude to mechanical stress (and hence wear tolerance), but it could hamper its recovery.

Younger organs (stolons) contain less starch, but in weaker species (Cdxt and Pv) this does not determine a lower TSS content. TSS levels denote a lack of biological stress, since in no species and no organ are there sugar starvation symptoms.

Leaf TSS showed no clear correlation with tissue mechanical resistance, but it can be said that, in these optimal trial conditions, Cdxt shows a higher capability of synthesizing sugars in leaves that is compatible with its ecophysiology of a species originating from arid areas.

Glucose was the TSS that was consistently found in all multiple regression equations explaining tissue strength, and as such it can be considered a marker for this trait..

From the results of this study, leaf lignin content seems to be one of the main factors determining leaf tensile strength in Zm, while the similar lignin content in Pv and Cdxt is in accordance with previous research results (Trenholm *et al.* 2000). The higher lignification of leaf cells in Zm, along with its correlation with tissue age (Akin 1989), is also in accordance with its slow growth (Patton *et al.* 2007; Volterrani *et al.* 2008), while the opposite applies to Pv and Cdxt.

Despite being present in all turfgrass tissues (Epstein 1999), leaf tissue silica content seemed to have a detrimental effect on tensile strength and generally enters multiple regression equations for leaves as a negative term.

Rhizome tensile strength seems to be unaffected by dimension (even in absolute form) and by sugar and starch content. This once again highlights a supremacy of the tissue component factor over the organ dimensional factor. The higher starch content of Cdxt is in accordance with its faster linear growth (Volterrani et al., 2008) and recuperative potential, starch being the fuelling material for these two distinctive traits. The tissue constituents that most seem to influence rhizome tensile strength are lignin (with a positive contribution) and silica with a negative contribution.

In accordance with the findings of Wright and Illius (1995), it was observed that stolons subject to tensile stress break in the area at the intercalary meristem at the apical zone in the immediate proximity of a node. Once again, dimensional factors seem to have little influence on the tensile properties of stolons, with tissue constituents and dry matter contributing in a decisive manner.

Older stolon tissues have higher strength than younger ones, and lignin (positive influence) and silica (negative influence) are the tissue constituents that most influence this trait. An in-depth investigation on schlerenchima for

these species would probably shed further light on the stolon tissue resistance mechanisms. The consistently low starch and sugars content in Zm tissues are in accordance with its slow growth.

Conclusions

Several aspects emerged from the present work that can be summarized as follows:

1. Tensile strength tests on leaf, rhizome and stolon tissues of Cdxt, Zm and Pv can supply useful information regarding these species' starch, sugars, dry matter, lignin and silica content.
2. Tensile strength was more influenced by tissue constituents than by tissue dimension.
3. The results of tensile strength tests are in accordance with these species' wear resistance as tested in previous work, with Zm stronger than Cdxt and Pv.
4. In rhizomes and stolons, tissue breakage usually occurs in the area at the intercalary meristem at the apical zone in the immediate proximity of a node.
5. Older tissues have higher tensile strength thanks to their higher lignification.
6. Starch and sugars content found in tissues is in accordance with the species' previously observed linear growth rate, with Cdxt faster than Pv and Zm.
7. Starch content is generally inversely proportional to lignin content.
8. Stolon TSS content, and glucose in general, is a clear marker of tissue mechanical strength.

9. Lignin is the principle constituent in determining tissue tensile strength, and as such it could be used as a turfgrass wear resistance predictor in the cultivar breeding stages.
10. Silica is a constituent undermining tissue tensile strength.
11. Leaves are the plant organs with the highest silicization and the lowest lignification of tissues.

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STUDY ONE

TABLES

Table 1. Cdxt, Zm, Pv: leaf width, leaf thickness, leaf section, rhizome section. Species effect. Means followed by the same letter in a column do not differ significantly at P ≤ 0.05. *, **, *** significant for 0.05, 0.01, 0.005 probability levels respectively.

	Leaf width (mm)	Leaf thickness (mm)	Leaf section (mm^2)	Rhizome section (mm^2)
Cdxt	1,68 b	0,10 c	0,16 c	3,00 a
Zm	1,66 b	0,14 b	0,24 b	1,54 b
Pv	2,83 a	0,19 a	0,53 a	3,38 a
	***	***	***	***
CV (%)	12,8	12,74	17,80	22,42

Table 2. Cdxt, Zm, Pv – stolon nodes 3, 7 and 14: section. Interaction Species x Node, Species effect, Node effect. Means followed by the same letter in a column do not differ significantly at P ≤ 0.05. *, **, *** significant for 0.05, 0.01, 0.005 probability levels respectively.

	Stolon section (mm^2)
Species x Node	
Tifway 3	1,33 c
Tifway 7	1,60 c
Tifway 14	1,58 c
Zeon 3	1,32 c
Zeon 7	1,39 c
Zeon 14	1,53 c
Salam 3	2,53 b
Salam 7	3,15 a
Salam 14	2,61 b
	*
Species	
Tifway	1,51 b
Zeon	1,41 b
Salam	2,76 a
	***
Node	
3	1,73 b
7	2,05 a
14	1,91 ab
	**
CV (%)	18,64

STUDY ONE

FIGURES

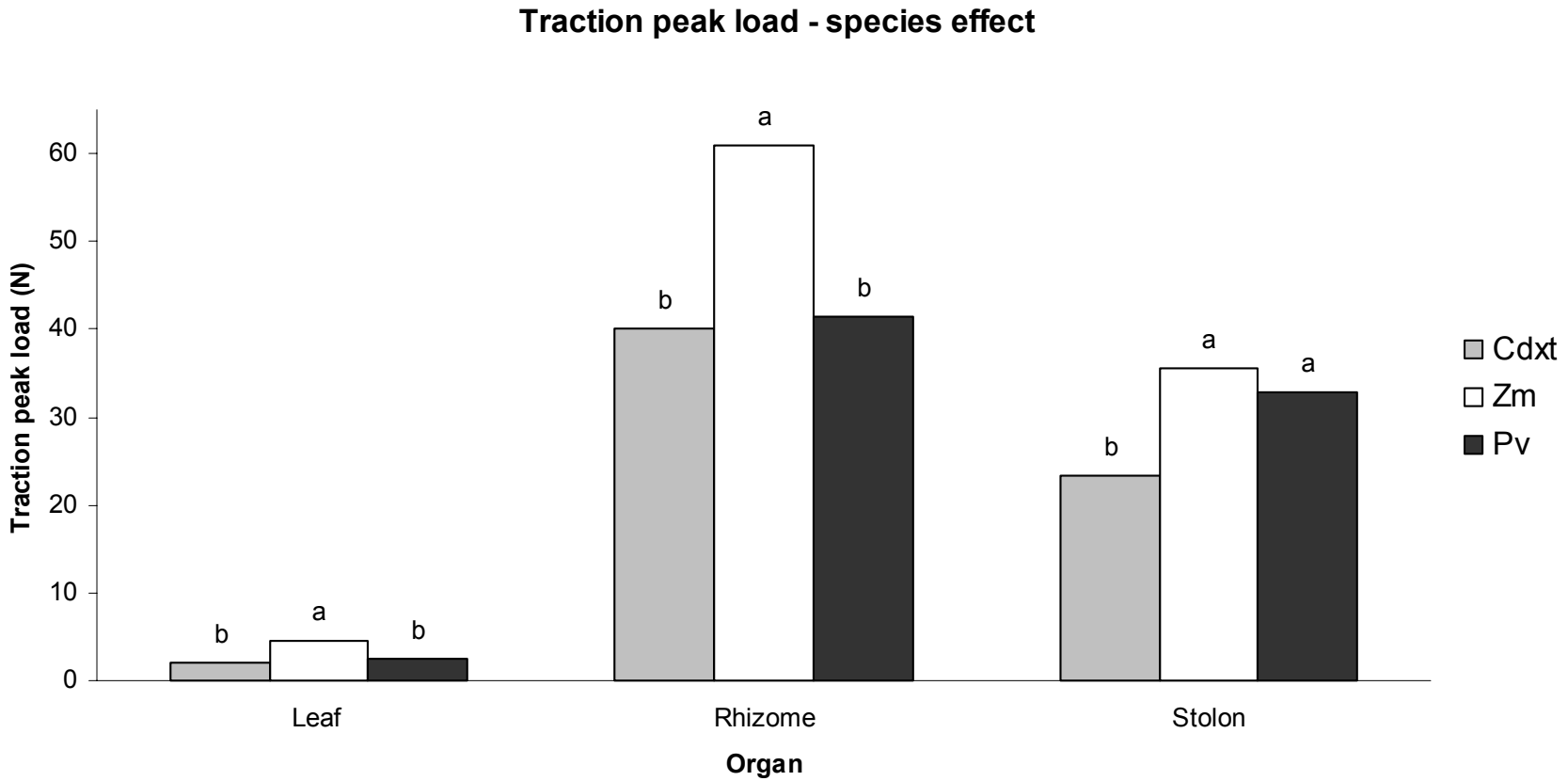

Figure 1. Cdxt, Zm, Pv – leaf, rhizome, stolon: traction peak load – species effect. Within each organ (leaf, rhizome, stolon): column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

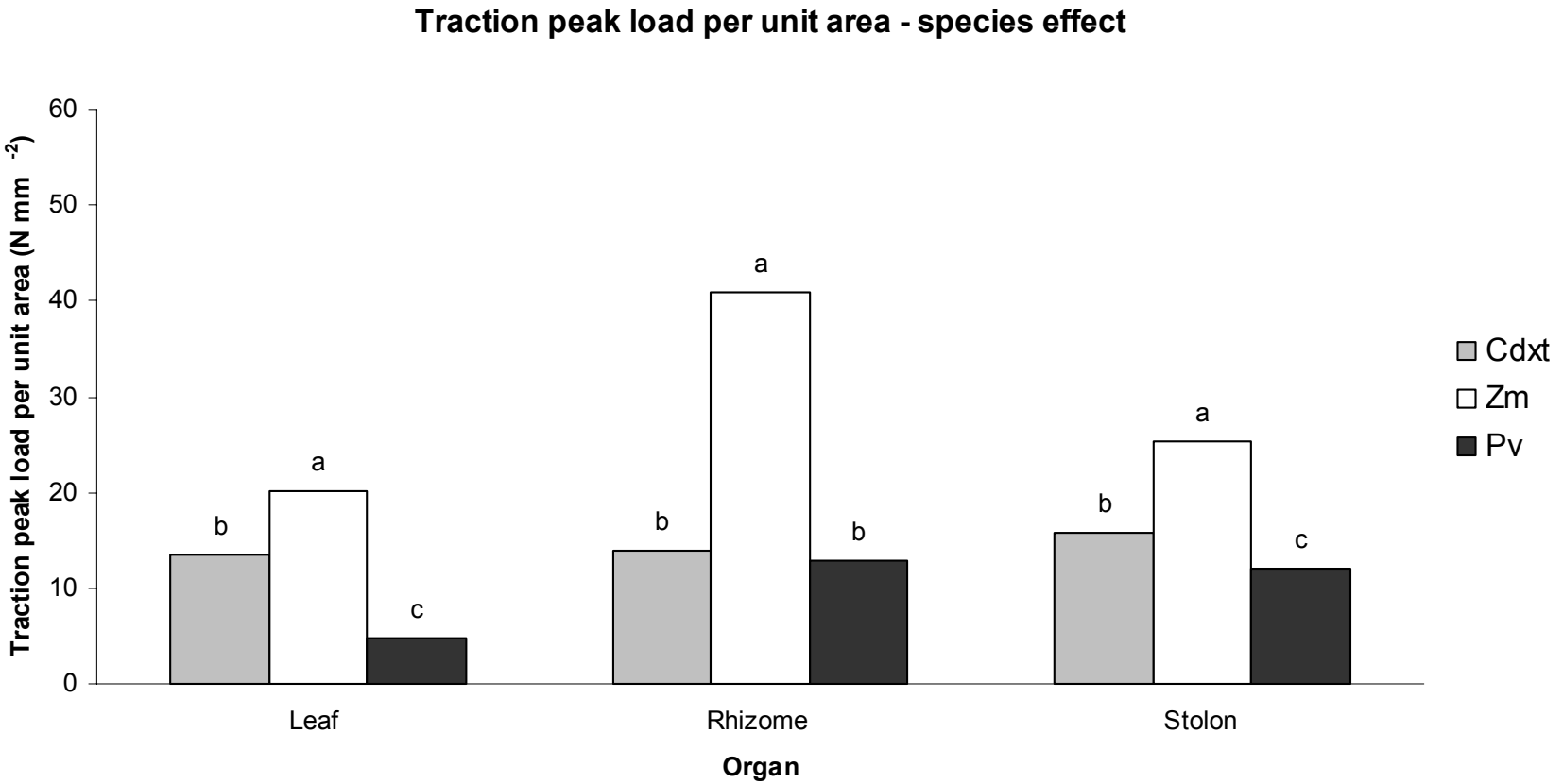

Figure 2. Cdxt, Zm, Pv – leaf, rhizome, stolon: traction peak load per unit area – species effect. Within each organ (leaf, rhizome, stolon): column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

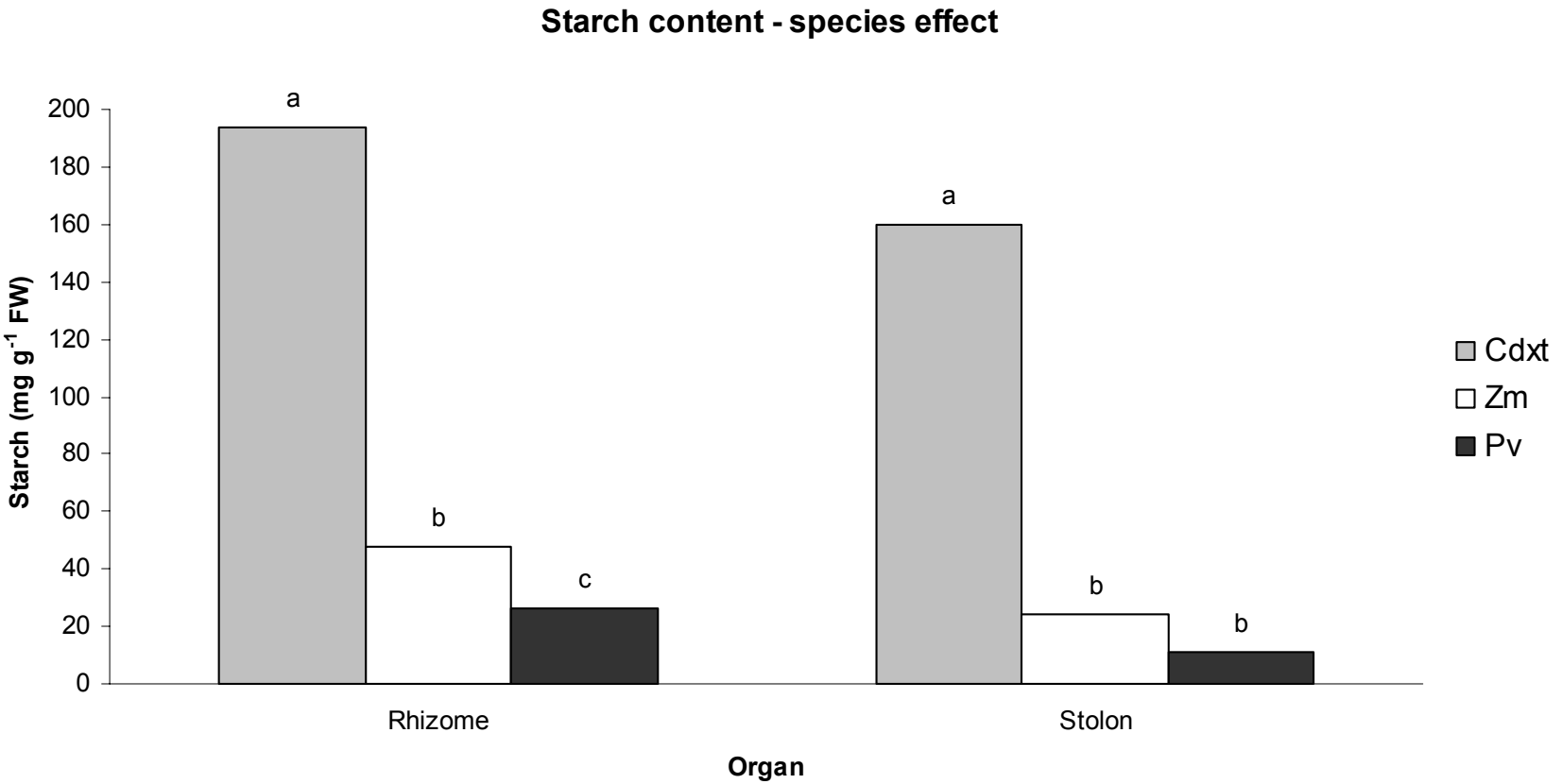

Figure 3. Cdxt, Zm, Pv – rhizome, stolon: starch content – species effect. Within each organ (leaf, rhizome, stolon): column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

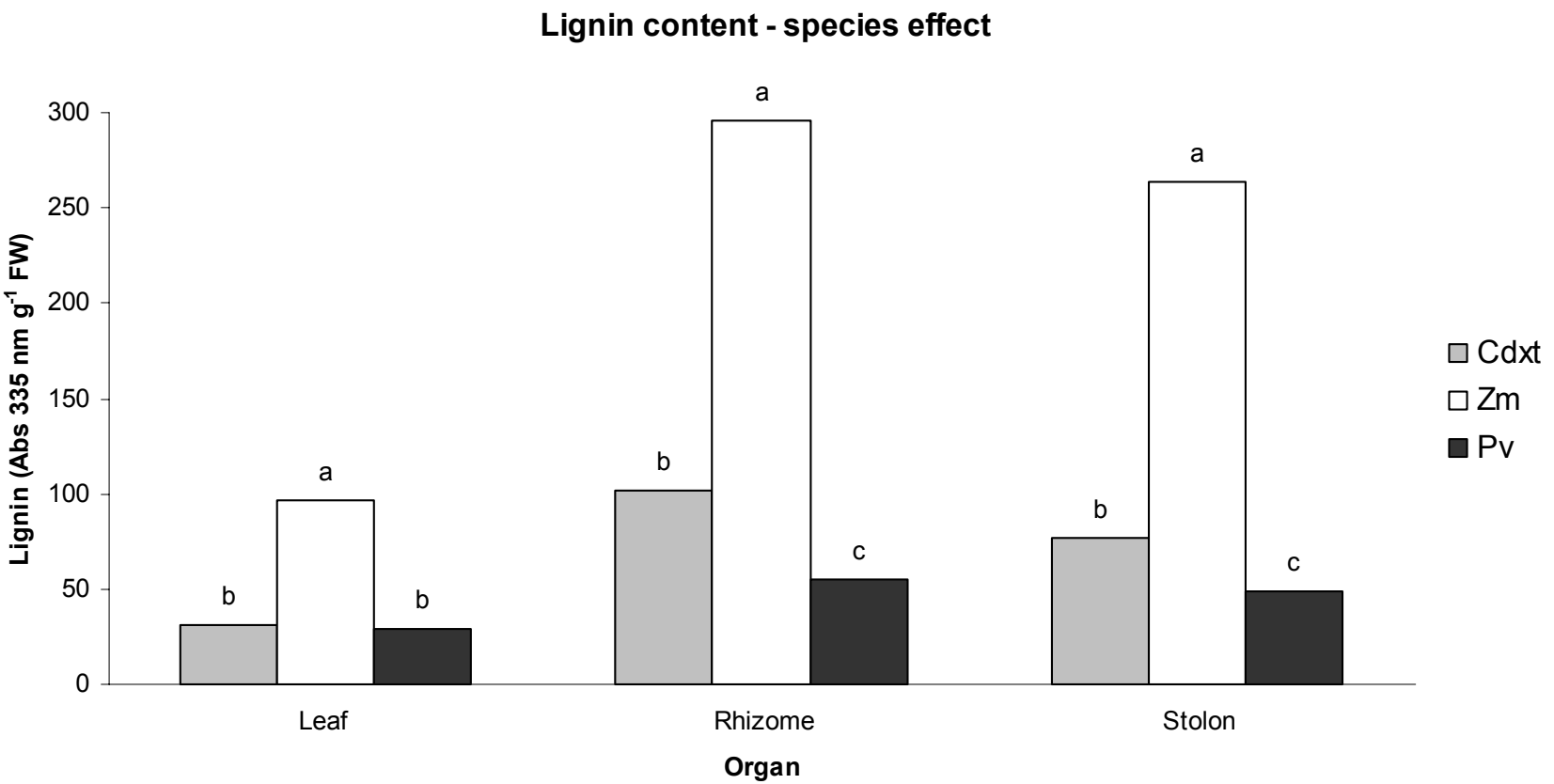

Figure 4. Cdxt, Zm, Pv – leaf, rhizome, stolon: lignin content – species effect. Within each organ (leaf, rhizome, stolon): column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

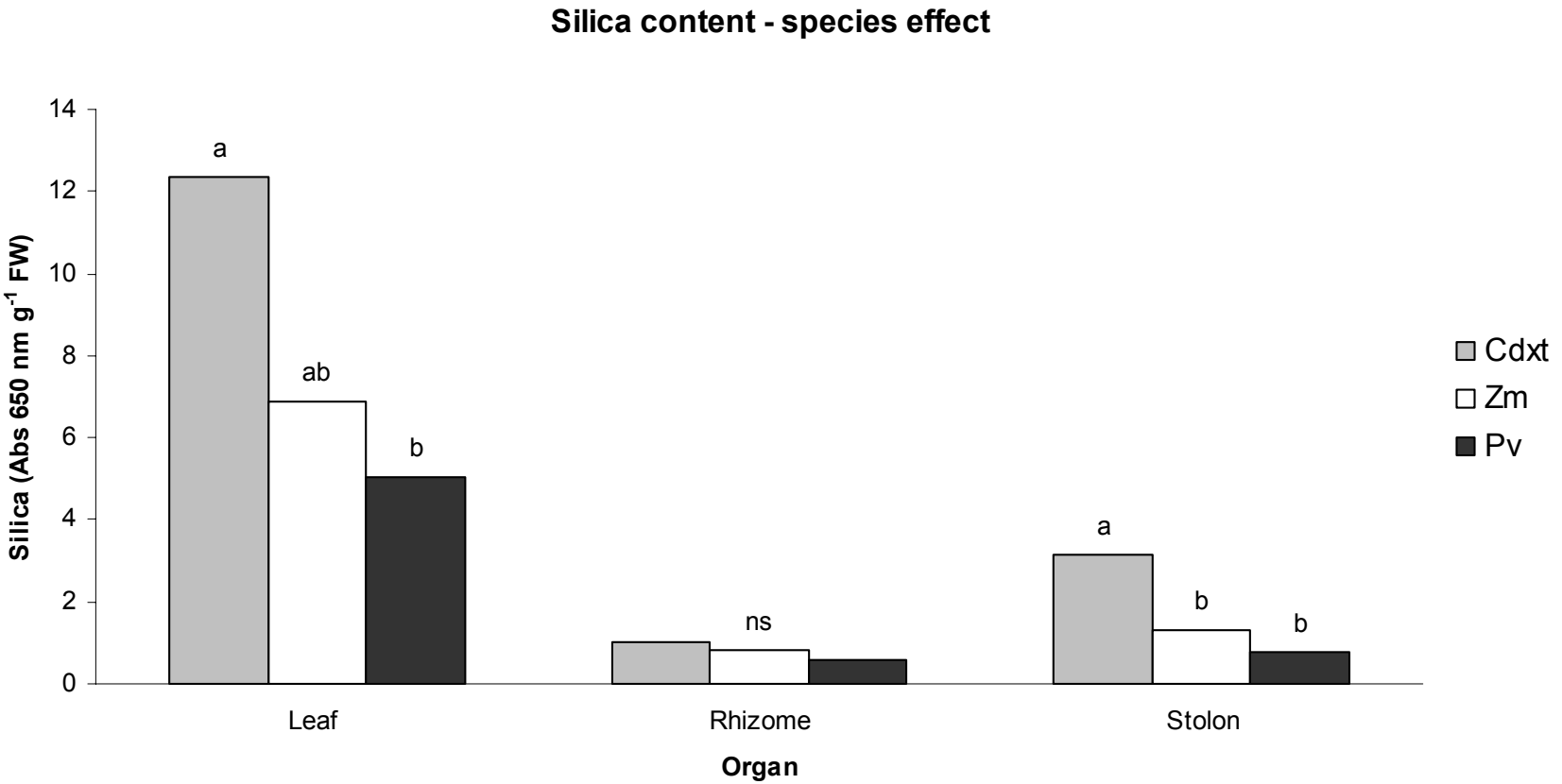

Figure 5. Cdxt, Zm, Pv – leaf, rhizome, stolon: silica content – species effect. Within each organ (leaf, rhizome, stolon): column treatments with the same letter do not differ significantly at the P≤0.05 probability level; ns = not significant.

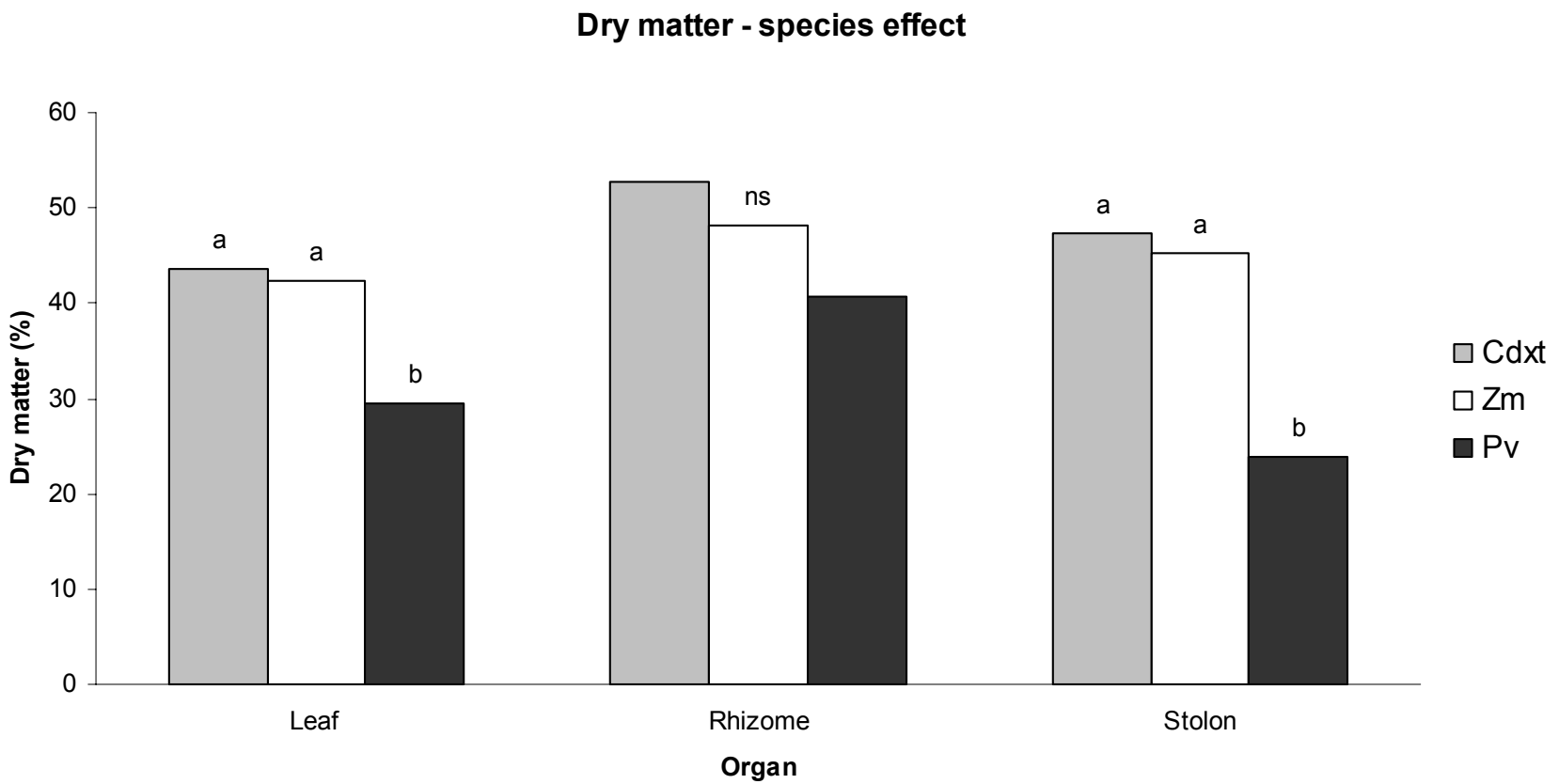

Figure 6. Cdxt, Zm, Pv – leaf, rhizome, stolon: dry matter percentage – species effect. Within each organ (leaf, rhizome, stolon): column treatments with the same letter do not differ significantly at the P≤0.05 probability level; ns = not significant.

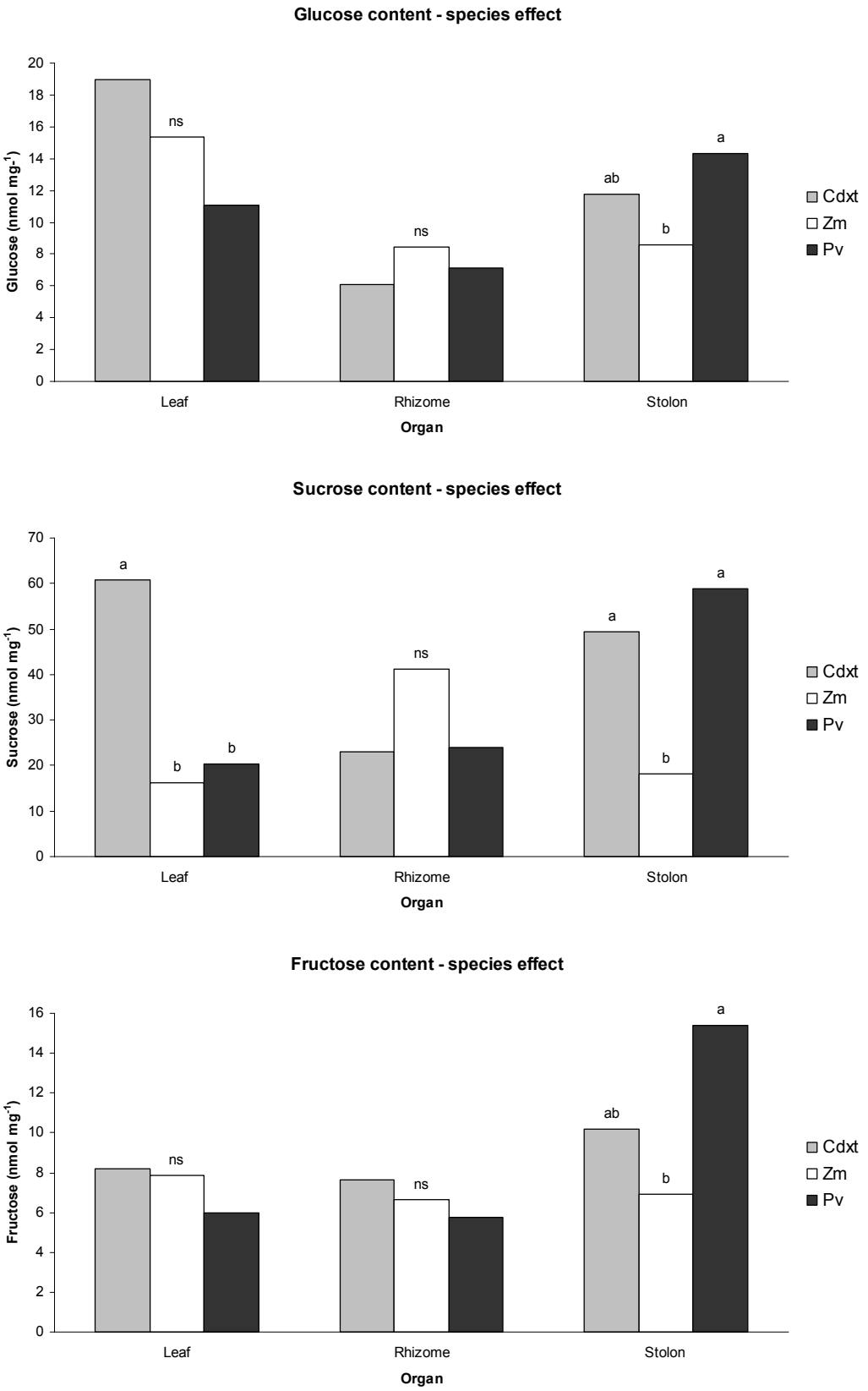

Figure 7. Cdxt, Zm, Pv – leaf, rhizome, stolon: glucose, sucrose and fructose content – species effect. Within each organ (leaf, rhizome, stolon): column treatments with the same letter do not differ significantly at the P≤0.05 probability level; ns = not significant.

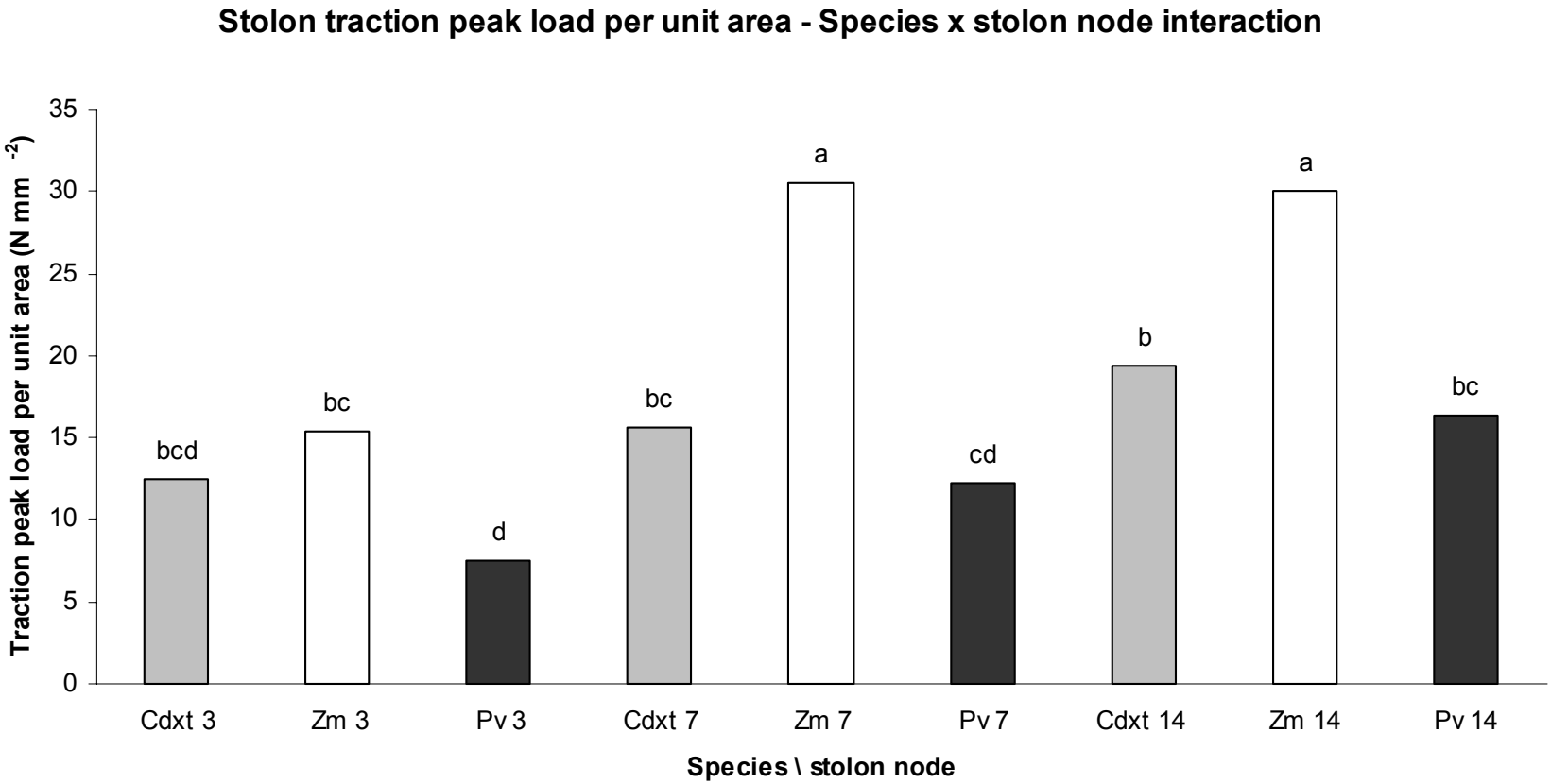

Figure 8. Cdxt, Zm, Pv – stolon nodes 3, 7 and 14: traction peak load per unit area – species x stolon node interaction. Column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

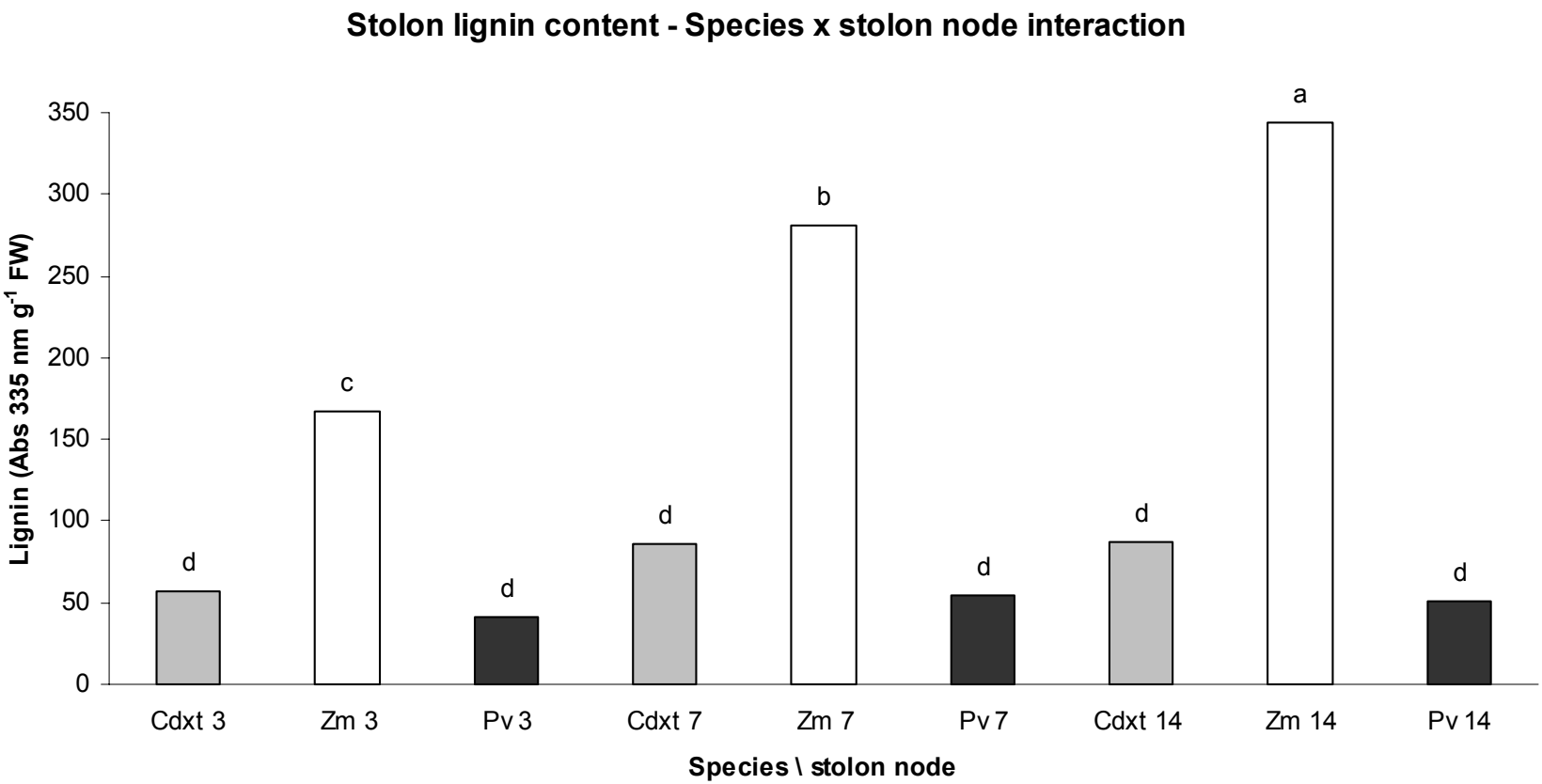

Figure 9. Cdxt, Zm, Pv – stolon nodes 3, 7 and 14: lignin content – species x stolon node interaction. Column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

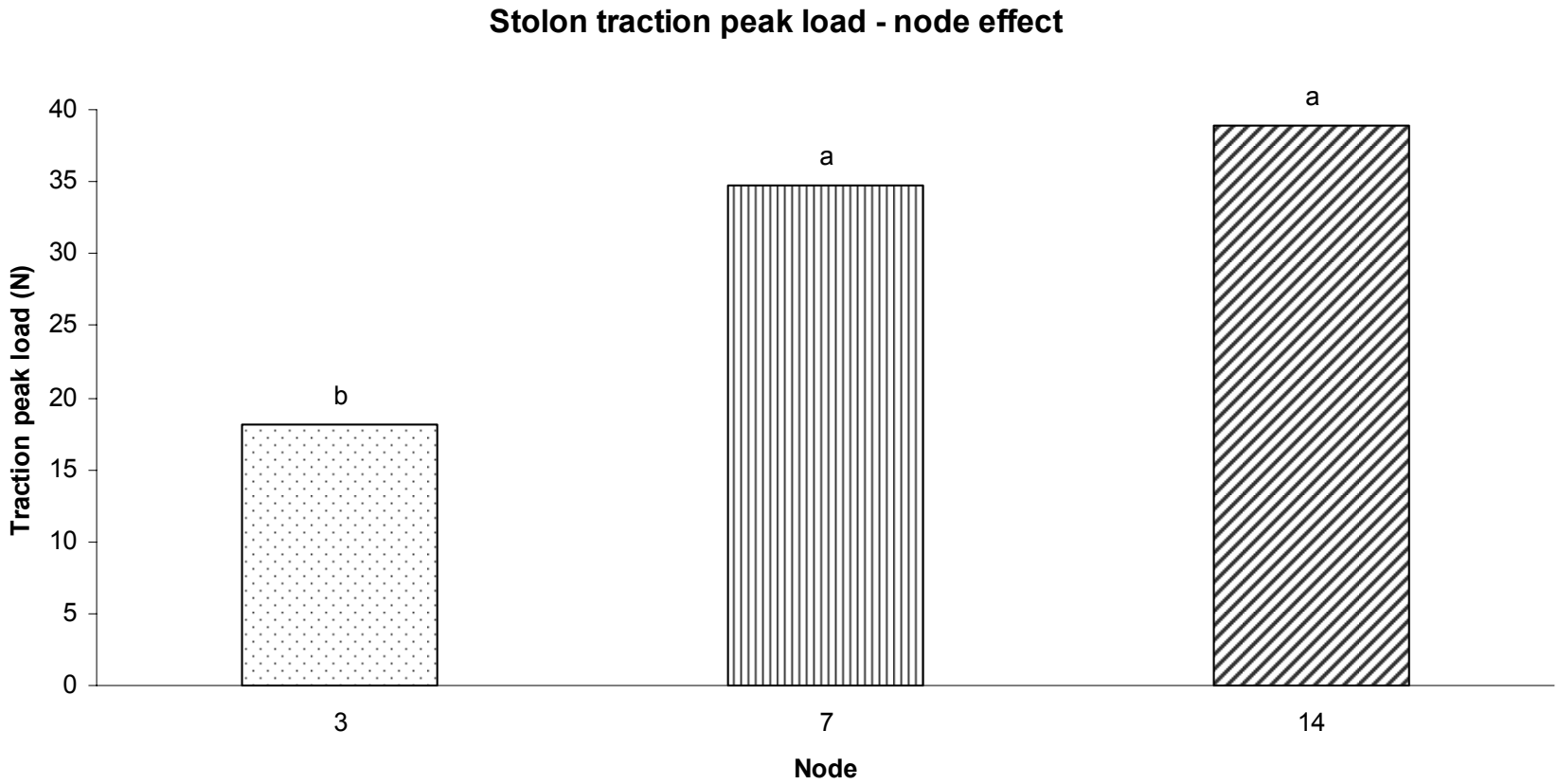

Figure 10. Cdxt, Zm, Pv – stolon nodes 3, 7 and 14: traction peak load – stolon node effect. Column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

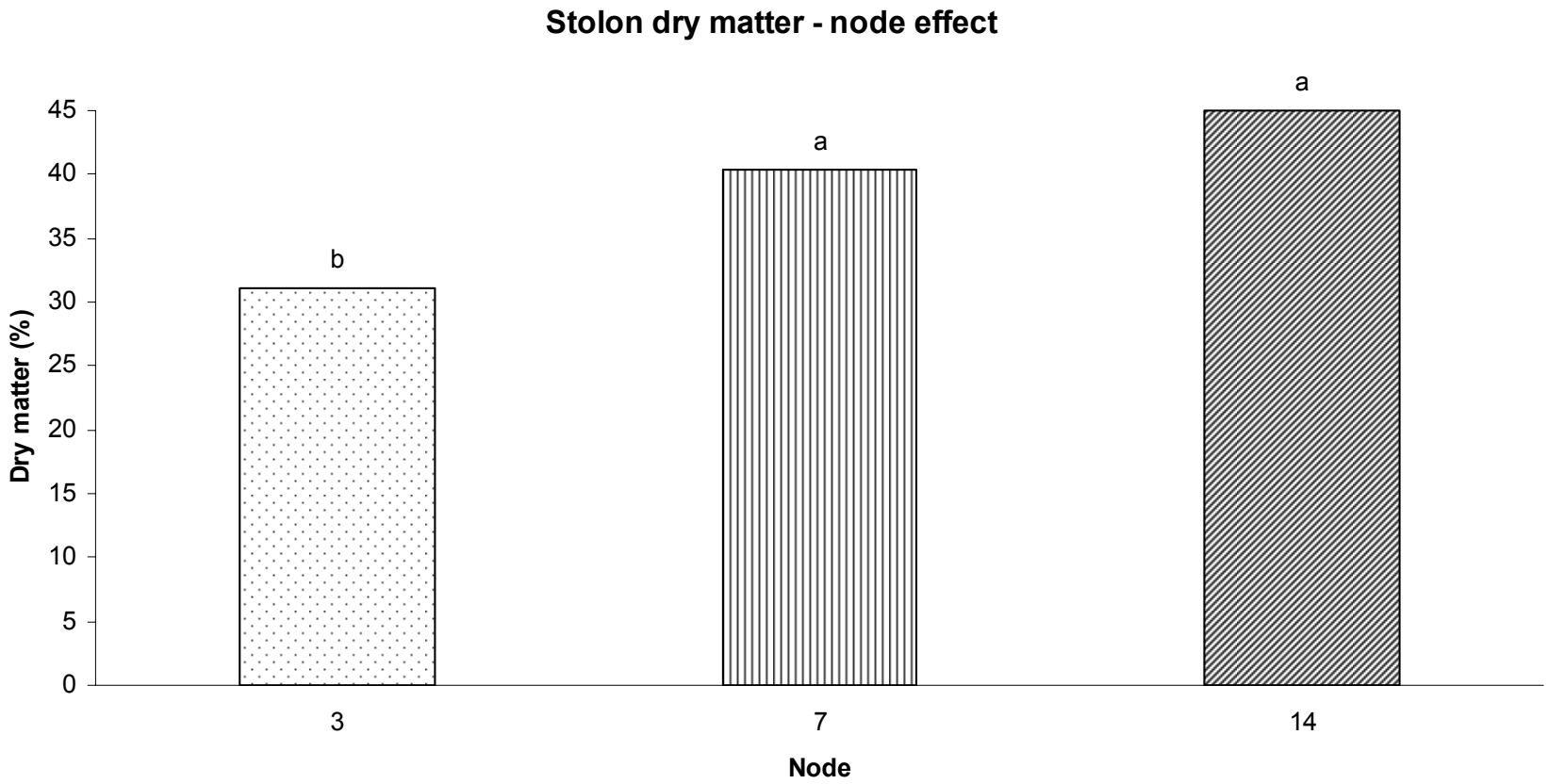

Figure 11. Cdxt, Zm, Pv – stolon nodes 3, 7 and 14: dry matter percentage – stolon node effect. Column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

STUDY TWO

Physiological and morphological factors influencing wear resistance and recovery in C3 and C4 turfgrass species.

Introduction

Wear resistance and recovery are probably the most important characteristics sought in turfgrass species, especially when these are destined to sports turf use.

In general, turfgrass wear is considered to be the sum of soil compaction and plant tissue injury effects (Beard, 1973; Carrow and Petrovic, 1992). The detrimental effects of soil compaction are generated by reduced root system growth, scarce water circulation within the soil (including a decrease in soil drainage capabilities) and a decrease of the gaseous portion of the soil triphasic system, with results similar to anoxia for turfgrass plants (Dest *et al.* 2009; Feldman 1984; Unger and Kaspar 1994). However, while little can be done to increase turfgrass plant tissue intrinsic wear resistance, soil compaction effects can be controlled with the adoption of modified rootzone layers that are more difficult to compact (i.e. silica or volcanic sands and gravels) (Baker 1990; Snow 1993; Volterrani and Magni 2004), the inclusion of synthetic fibers in native or

modified soil (Baker 1997; Henderson *et al.* 2003), the adoption of slit or pipe drainage systems (Daniel 1969; Gibbs 1988; Ward 1983) and standard soil decompaction maintenance practices (Canaway *et al.* 1986; Cockerham *et al.* 1993).

Turfgrass wear resistance has so far only been evaluated in field experiments relying on the application of wear by different traffic simulators (Bonos *et al.* 2001; Canaway 1976; Carrow *et al.* 2001; Cockeram and Brinkman 1989; Sherman *et al.* 1974; Sherman *et al.* 2001; Henderson *et al.* 2005; Vanini *et al.* 2007), or by evaluating natural wear as inflicted by athletes in training or match play (Baker et al. 1992). Field turfgrass wear application methods and evaluations are not able to segregate soil compaction from plant tissue wounding effects, and could be heavily influenced by soil and weather conditions, management practices and can be prone to human error. Furthermore, natural wear inflicted by athletes is highly differential according to the type of sport played and the area of the pitch taken into consideration.

From a plant tissue point of view, turfgrass wear resistance has been correlated with total cell wall (Shearman and Beard, 1975), modified acid detergent fiber (Canaway, 1981), sucrose phosphate synthase and sucrose synthase activity, stem and leaf moisture (Trenholm et al., 2000), evapotranspiration, leaf chlorophyll concentration, membrane permeability, leaf peroxidase activity, spectral reflectance, tissue potassium concentration (Shearman and Beard, 1973; Carrol and Petrovic, 1991) and silica (Trenholm et al., 2001a). However, all these parameters have been correlated with turfgrass wear resistance as measured in field conditions.

FIFA's regulation of the materials and methods associated with the construction of artificial turf pitches (FIFA 2008*a*; FIFA 2008*b*) has spawned the development of a series of machinery and instruments for the lab and field evaluation of artificial turf that are highly standardized and which show great potential for natural turf evaluation. One of these, the Lisport machine (EN 15306:2007), could be routinely used to evaluate turfgrass wear resistance in laboratory and devoid of most soil effects.

Turfgrass wear recovery is intended as the capability of regenerating single plant tissues, and hence regenerating whole canopies or portions of canopies, after wounding or death of plants deriving from human or animal activity. As such, single plant and canopy recovery is a fundamental characteristic in turfgrass species, especially when these are used in sports turf. Turfgrass recovery is species-specific and is correlated to growth rate and number of meristematic growing points from which new growth develops (Hoffman *et al*. 2010*a*) and sucrose phosphate synthase and sucrose synthase activity (Bayrer *et al*. 2006). Specific agronomical practices can influence turfgrass recovery, such as nitrogen fertilization and irrigation (Canaway 1985; Trenholm *et al*. 2001*b*), but also the season in which turfgrass canopies are subject to wear (Park *et al.* 2010).

The present research was aimed at evaluating the wear resistance of select C3 and C4 turfgrass species and mixes, as measured in the laboratory with a numerical control machinery and practices that minimize soil compaction effects. Turfgrass recovery was also evaluated in greenhouse after wear simulation and all the results obtained (wear and recovery) were regressed against plant organ biometric data and plant tissue constituents data.

Materials and methods

Plant material and growth conditions

In March 29 2010 nine 98 cm x 44 cm x 4 cm sods per species were machine-harvested from mature swards of *Cynodon dactylon* x *Cynodon transvaalensis* cv. Tifway 419 (Cdxt), *Zoysia matrella* (L.) Merr. Cv. Zeon (Zm), *Paspalum vaginatum* Sw. cv. Salam (Pv) and a sports-type mix of *Lolium perenne* L. (cvs. Speedster 35% and Greenway 35%) + *Poa pratensis* L. (cvs. SR2100 15% and Greenknight 15%) (C3), grown on a silt loam soil at the research station of the Center for Research on Turfgrass for Environment and Sports - University of Pisa (CeRTES) (43° 40' N, 10° 19' E; 6 m a.s.l.). Sods were pressure-washed clean from native soil and transferred to rectangular plastic containers of the same dimensions as the sods, containing a 2 cm layer (8.62 l per container) of silica sand as a growing medium (pH: 7.6; lime: 6.4 g kg^{-1}; EC: 68.7 µS cm^{-1}; CEC: 6.82 meq 100 g^{-1}). The 36 sods (nine per species) were arranged in a completely randomized block with three replications in a greenhouse environment and grown for 100 days to remove climatic and soil effects on sward characteristics (max T° 34±5 °C; min T° 25±4 °C; 85±8 % relative humidity). Mowing with clippings removal was carried out weekly at 3.0 cm with a bench-mounted reel mower. Irrigation was carried out daily for the first 4 weeks, and subsequently three times a week (5.0 l m^{-2} for all irrigations). Foliar fertilizations were carried out on April 21, May 5 and June 3 with 5 g m^{-2} of N, 1.7 g m^{-2} of P_2O_5 and 1.7 g m^{-2} of K_2O. Fungicide applications were carried out on March 29, April 28, May 19 and July 05 with 20 cm^3 of commercial product containing azoxystrobin (23.2%) in 2 l for all sods. No weed control was carried out.

After wear simulation (July 06-07) sods were transferred back to the greenhouse to evaluate turfgrass recovery for 61 days (until September 06). During recovery mowing with clippings removal was carried out weekly at 3.0 cm with a bench-mounted reel mower. Irrigation was carried out three times a week with 5.0 l m^{-2} per irrigation. Foliar fertilizations were carried out on July 13 and August 10 with 5 g m^{-2} of N, 1.7 g m^{-2} of P_2O_5 and 1.7 g m^{-2} of K_2O. Fungicide applications were carried out on July 20 and July 27 with 20 cm^3 of commercial product containing azoxystrobin (23.2%) in 2 l for all sods. No weed control was carried out.

Wear simulation tests

All tests were carried out in July 06-07 2010 at Labosport Italia laboratories (Cernusco Lombardone – Italy). Laboratory conditions were maintained constant at 23°C temperature and 50% relative humidity. The device used to carry out the tests was a FIFA-approved "Lisport" machine (FIFA 2008, EN 15306:2007). This numerical control machine consists of two (118 ± 5 mm Ø x 300 mm) studded cylinders with 145 ± 5 studs and weighing 31 ± 0.5 kg (including the axle). Football studs are standard 13 ± 0.5 mm cylindrical nylon studs with a Shore A hardness of 96 ± 2. and are randomly bolted onto the cylinder's surface. Cylinders speed across the turf is 0.25 ± 0.05 m s^{-1} and, in order to maximize the randomization studs contact with the tested sample, the test sample tray is also oscillated transversely by 20 ± 1 mm at a speed of 0.015 ± 0.005 m s^{-1}. The forward cylinder rolls by contact with the tested sample, while the hind roll is moved by a chain linkage at a ratio of 1:2.5 in order to impart a "ripping" effect on the tested specimen. The cylinders are

floating and therefore no vertical adjustment was necessary with the samples' turfgrass heights.

Prior to testing, the sods were manually trimmed down to the Lisport test tray dimensions (81.5 x 42 cm). The uniform surface of abraded turfgrass was 500 x 300 mm.

Three wear levels were evaluated for each species: 0 Lisport cycles, 20 continuous Lisport cycles and 40 continuous Lisport cycles, where each Lisport cycle consists of one outward and one return path on the tested sample.

Plant tissue biometrics

All measurements were carried out on July 02 2010, before wear simulation (= T0) at CeRTES. Stolon diameter (mm), rhizome diameter (mm) and leaf blade thickness were measured via a Mitutoyo Absolute IP54 digital feeler gauge with a 0.5 N max load and ± 2 nm error. Leaf blade width (mm) and internode length were measured via the digital ruler function of a AM413MT-Dino-Lite 20x-200x digital microscope. Leaf blade width and thickness were measured in the middle of the leaf blade's length. Stolon and rhizome diameters were measured in the middle of the apical internodes. All measurements were carried out on 20 samples for each organ randomly collected from the sods.

Tissue sections were then calculated and expressed as follows:

Leaf section = leaf width x leaf thickness. [1]

Stolon or Rhizome section = π (stolon or rhizome diameter/2)^2 [2]

Shoot and horizontal stem density measurements

All measurements were carried out at CeRTES on July 02 2010 (before wear simulation = T0), on July 14 2010 (one week after wear simulation = T1) and on September 06 2010 (after recovery = T2). From each sod a 66.44 cm^2 plug was harvested and the number of live shoots was manually counted. Data is calculated and expressed as follows:

T0, T1, T2 Shoot density = T0, T1, T2 live shoots number / 66.44 cm^2 [3]

T1 shoot retention after wear (%) =

100 – [(T0 shoot density-T1 shoot density)/ T0 shoot density] * 100 [4]

T2 shoot recovery (%) =

100 – [(T1 shoot density-T2 shoot density)/ T1 shoot density] * 100 [5]

On the same T0 samples (plugs of 66.44 cm^2) for each species, the horizontal stem density (HSD) was manually evaluated by measuring and summing the length of all the rhizome and stolon fractions found in C3 plugs. Data was calculated and is expressed as follows:

T0 HSD (cm cm^{-2}) = total rhizome and stolon fraction length / 66.44 cm^2 [6]

For C3 species only, further calculated data deriving from measured biometrics was T0 nodes cm^{-2} (T0 HSND), that supplied an indication of the potential recuperative points in each species, and T0 horizontal stem volume (T0 HSV),

that supplied an indication of sugars and starch available for recuperation (Volterrani *et al*. 2009). Data was calculated and is expressed as follows:

T0 HSND = [6] / internode length [7]

T0 HSV = [6] * [2] [8]

Tissue constituent laboratory analysis

On July 02 2010 (T0) plant organs were sampled from the sods and stored at -80°C. The organs were sampled as follows:

- leaves: four fully expanded leaves x 3 samples x sod (replicate) x species
- rhizomes (Cdxt, Zm and Pv only): three 2 cm portions x 3 samples x sod (replicate) x species
- stolons (Cdxt, Zm and Pv only): three 2 cm portions x 3 samples x sod (replicate) x species
- stems (C3 only): three 2 cm portions x 3 samples x sod (replicate) x species

Plant tissue samples were tested for their content in the following substances: starch (mg g^{-1} FW), glucose (nmol mg^{-1}), sucrose (nmol mg^{-1}), fructose (nmol mg^{-1}), dry matter content (%), lignin (absorbance at 335 nm g^{-1} FW) and silica (absorbance at 650 nm g^{-1} FW). Starch content investigations were not carried out on leaf samples. All the analyses were conducted in triplicate. The laboratory test methods for the determination of the above substances were as follows:

a) Starch. Samples (100 mg FW) were grounded in a mortar and resuspended in 100 mL of 10 mM KOH, and boiled for 1 min. To each sample were then added 1 ml of 1N HCl. The starch standard solution was prepared using 100 mg of potato soluble starch dissolved in 100 ml dH_2O and boiled 1 min. To both samples and standards (final volume 50 ml) was added 1 mL of fresh iodine solution (0.13% K_2 and 0.3% KI, dissolved in dH_2O) and the absorbance was read at 595 nm in few minutes. Starch concentration was expressed as mg starch/g FW.

b) Glucose, sucrose and fructose. Samples (100 mg FW) were rapidly frozen in liquid nitrogen and ground to a powder, then extracted as described by Tobias *et al.* (1992) and assayed for glucose, fructose and sucrose content through coupled enzymatic assay methods (Guglielminetti *et al.* 1995). The efficiency of the methods was tested by using known amounts of carbohydrates. Incubations of the samples and standards were carried out at 37 °C for 30 min. The reaction mixtures (1 mL) were as follows. Glucose: 100 mM Tris-HCl, pH 7.6, 3 mM $MgCl_2$, 2 mM ATP, 0.6 mM NADP, 1 unit Glc6P dehydrogenase; the A_{340} was recorded. Fructose was assayed as described for glucose plus the addiction of 2 units of PGI; the increase in A_{340} was recorded. Sucrose was first broken down using 85 units of invertase (in 15 mM Na-acetate, pH 4.6) and the resulting glucose and fructose were assayed as described above. Recovery experiments evaluated losses taking place during the extraction procedures. Two experiments were performed for each metabolite by adding known amounts of authentic standards to the sample prior to the extraction. The concentration of the standards added were similar to those estimated to be

present in the tissues in preliminary experiments. The recovery ranged between 97 and 104%.

c) Dry matter content. Samples (1 g FW) were dried in an oven at 60°C for 1 week and than the dry matter was measured.

d) Lignin. Lignin was quantified via a thioglycolic acid (TGA) precipitation method (Lee *et al.* 2001). Pigments and soluble phenolics were removed from samples (100 mg FW) by three changes of absolute methanol over 3 days. The cleared tissue was then dried in an oven at 30°C for 1 day. The dried tissue was suspended in 1 ml 10% (v/v) TGA in 2M HCl (v/v) and heated for 4 h at 100°C in a water bath. When cooled, the solution and all tissues were then transferred to a fresh tube and subjected to centrifugation at 14000xg for 15 min. The supernatant was decanted and the pellet washed with 2.0 ml dH_2O before centrifugation at 14000xg for 10 min. The supernatant was decanted and 1.0 ml 1M NaOH added and mixed to solubilize the TGA derivatives. The suspension was then incubated overnight at 4°C before centrifugation at 14000xg for 10 min. The NaOH supernatant was added to a fresh tube and the TGA derivatives precipitated by adding 0.4 ml concentrated HCl and incubating in an ice bath for 1 h. The reddish/brown precipitate was pelleted by centrifugation for 10 min at 14000xg. The supernatant was decanted and the precipitate dissolved in 1 ml 0.5 M NaOH. The absorbance (A) of each resultant orange solution was determined at 335 nm (Graham and Graham 1991) and the concentration of wall bound TGA derivatives expressed as absorbance at 335nm g^{-1} FW.

e) Silica. Content of silica in the different plant species was determined using an autoclaved-induced digestion (Elliot and Snyder 1991). Samples (100 mg FW) were dried at 60°C for 48 h and then ground using liquid nitrogen. Samples

of plant tissue were wetted with 400 µL of 50% H_2O_2 in 100-mL polyethylene tubes previously rinsed with 0.1 M NaOH and DW. To each tube was added 900 mg of 50% NaOH (W/V) at room temperature, and each tube was gently vortexed. The tubes were individually covered with loosefitting plastic caps. The rack of tubes was placed in an autoclave at 138 kPa for 1 h. After atmospheric pressure was reached, the tubes were removed and the contents brought to 50 mL with dH_2O. Silica in dilutions of samples was determined colorimetrically by the following procedure: to each sample were added with mixing 7.8 mL of 20% acetic acid, 2 mL of ammonium molybdate solution (54 g/L, pH 7.0), 1 mL of 20% tartaric acid, 0.2 mL of reducing solution (described below). Five minutes elapsed between the addition of the ammonium molybdate and the tartaric acid. The reducing solution was made by combining solution A (2 g of Na_2SO_3 in 25mL of dH_2O plus 0.4 g of l-amino-2-naphthol-4-sulfonic acid) and solution B (25 g of $NaHSO_3$ dissolved in about 200 mL of dH_2O) and diluting to 250 mL. Following the addition of all the reagents, the samples were mixed well and allowed to stand for 30 min. Plastic ware rinsed with 0.1 M NaOH was used throughout. The colour was read at 650 nm with a 1 cm path length. The silica content was expressed as A650nm g^{-1} FW.

Statistical analysis

All statistical analysis were carried out with a COSTAT 6.400 software (CoStat 2008). Bartlett's test on homogeneity of variances was carried out on all data sets. Non normally distributed data sets were log transformed until normal distribution was obtained.

Species effect was evaluated in leaves, stolons, rhizomes and stems via ANOVA in a completely randomized block design.

The interaction (species x wear level effect) for shoot density and live shoot retention and recovery was analyzed in a 2 way completely randomized block design.

In all cases, Tukey's Honestly Significant Difference (HSD) for $P<0.05$ was used to detect differences between means.

All parameters for C3 species were then submitted to a multiple regression analysis to find the equation of the subset of 5 parameters with the highest regression coefficient (R^2) for T0 shoot density, T1 live shoot retention (20 and 40 Lisport cycles) and for T2 shoot recovery (20 and 40 Lisport cycles).

Results

Horizontal stem and leaves biometrics

Cdxt and Pv showed the thickest horizontal stems (3.12 and 2.62 mm^2 respectively – Table 1), with Zm returning much lower thickness (1.49) ($P\leq0.005$).

The internode length on Pv (1.23 mm) was significantly shorter than Cdxt and Zm (1.57 and 1.71 respectively) ($P\leq0.01$).

Zm was the species that produced the highest amount of horizontal stems, with a HSD of 15.84 cm cm^{-2} (Table 1), compared to Cdxt and Pv (5.77 and 5.48 respectively) ($P\leq0.005$).

Zm was also the species with the highest HSND and HSV ($P\leq0.005$) (9.57 nodes cm^{-2} and 2.37 cm^3 cm^{-2}) while, for both these parameters, Pv and Cdxt attained lower values than Zm.

Highest leaf width (Table 2) was recorded by C3 mix (2.59 mm), followed by Pv (2.20), while Cdxt and Zm recorded a much finer leaf texture ($P<0.005$).

Again C3 and Pv showed the thickest leaves (0.21 and 0.19 mm respectively), with Zm and Cdxt (0.14 and 0.13 respectively) showing thinner leaves ($P<0.005$).

Leaf thickness and width determined an average calculated leaf section of 0.53 mm^2 for C3, followed by Pv (0.42) and Zm and Cdxt (0.20 and 0.18 respectively) ($P<0.005$).

Rhizomes

Starch content ($P<0.005$ – Figure 5) was much higher in Cdxt rhizomes (135.52 mg g^{-1}) compared to Zm and Pv (41.91 and 24.60 respectively).

Rhizome glucose content (Figure 7) was highest in Cdxt and Pv (92.20and 79.24 nmol mg^{-1} respectively), while Zm had a roughly threefold lower content (31.03) ($P\leq0.05$).

Sucrose content was highest in Cdxt and Zm (121.40 and 130.65 respectively) and lowest in Pv (73.69) ($P\leq0.05$). No significant differences were found for fructose concentration.

Lignin concentration in Zm (450.41) was considerably higher than Cdxt and Pv (171.24 and 96.27 respectively) ($P<0.01$ – Figure 6).

Zm was the species with the highest silica content in rhizomes (4.53 – $P<0.05$ – Figure 8), followed by Pv (2.31) and by Cdxt (1.63).

Dry matter was highest in Zm (54.97 % - $P<0.05$ – Figure 9), followed by Cdxt (46.36 %) and by Pv (29,06 %).

Stolons

Stolon (Cdxt, Zm, Pv) and stem (C3) starch content (Figure 5) was much higher in Cdxt (205.23 mg g^{-1}), while Zm, Pv and C3 exhibited a significantly lower content (40.44, 16.48 and 15.59 respectively) ($P<0.005$).

Glucose content (Figure 7) was highest in Cdxt, Zm and Pv (31.28, 29.39 and 30.07 respectively), with C3 stems showing a threefold lower content (10.07) ($P<0.05$).

This result is inverted for fructose ($P<0.005$) and sucrose ($P<0.005$), with Cdxt, Zm and Pv stolons showing significantly lower levels of these soluble sugars compared to C3 stems.

Lignin content ($P<0.005$ – Figure 6) was considerably higher in Zm stolons (473.71), followed by Cdxt stolons (196.87), then by Pv stolons (90.57) and by C3 stems (11.65).

Cdxt stolons showed the highest degree of tissue silicization (4.53 – $P<0.005$ – Figure 8), followed by Zm (3.23) and Pv (1.47). C3 attained a silicization level intermediate between Zm and Pv (2.10).

Zm showed the highest dry matter percentage (58.11 % - $P<0.005$ – Figure 9), followed by Cdxt (45,72 %) and by Pv and C3 (32.72 % and 28,61 %, respectively).

Leaves

Zm leaves had the highest glucose content (33.10 nmol mg^{-1} – Figure 7), while Cdxt and C3 showed lower intermediate levels (22.84 and 27.01 respectively) and Pv returned the lowest glucose content (6.89) ($P\leq0.05$).

Both for fructose and sucrose, C3 leaves had the highest content (35.23 for fructose and 84.99 for sucrose), with Cdxt and Zm leaves showing lower intermediate contents (6.61 and 18.56 for Cdxt, 6.34 and 16.40 for Zm), while Pv had a largely inferior content (1.10 and 2.58) ($P \leq 0.05$).

Lignin content was highest in Zm leaves (143.57 – Figure 6) and approximately twofold that of Cdxt and Pv (72.34 and 58.53 respectively) and approximately sevenfold that of C3 species (20.06) ($P \leq 0.05$).

Cdxt showed the highest leaf tissue silica content (12.94 – $P<0.05$ – Figure 8), followed by Zm (6.61) and by Pv and C3 (4.09 and 3.12, respectively).

The C4 species with the highest dry matter percentage was Zm (42.30 % - $P<0.005$), followed by Cdxt (34.61 %) and by Pv (25.68 %). C3 showed an intermediate value between Cdxt and Pv (29.53 %).

T0, T1 and T2 shoot density

T0 shoot density (species effect – Figure 2) was significantly higher in Zm (7.10 shoots cm^{-2}), followed by Cdxt (5.05) and by Pv and C3 attaining lower levels of density (3.39 and 3.08 respectively) ($P<0.005$).

The species x wear intensity interaction on T1 (after wear) shoot density (Figure 3) was significant ($P<0.005$), with Zm 20 and Zm 40 treatments showing the highest density (4.22 and 1.55) while Cdxt 20, Cdxt 40, Pv 20 and Pv 40 treatments obtained a lower value (1.37, 1.09, 1.40 and 1.23 respectively) and lower still for C3 20 and C3 40 (0.67 and 0.43 respectively).

The species x wear intensity interaction on T2 (after recovery) shoot density was significant ($P<0.005$), with Zm 20 showing the highest density (4.60), while

Cdxt 20, Cdxt 40, Zm 40, Pv 20 and Pv 40 obtained a lower density (1.88, 2.12, 2.38, 1.59 and 2.01 respectively), followed by C3 20 (0.67) and C3 40 (0.43).

The multiple regression equation of the subset of 5 parameters for C4 species with the highest adjusted determination coefficient (R^2) for T0 shoot density was:

T0 shoot density (C4) =

1.48 +0.38(HSD) -0.34(HSND) +0.001(Stolon starch) -0.005(Stolon sucrose) +0.06(Rhizome dry matter)

(R^2=0.99)

T1 shoot retention after wear and T2 shoot recovery

The species x wear intensity interaction on T1 shoot retention percentage (Figure 4) was significant (P<0.005), with Zm 20 retaining the highest percentage of shoots (59.49%), followed by Pv 20 (41.26), followed by Cdxt 20, Cdxt 40, Zm 40, Pv 40 and C3 20 (27.34, 22.19, 22.08, 36.33, 21.68 respectively) and by C3 40 (13.87).

The same interaction on T1 shoot recovery was significant (P<0.005), with Cdxt 40 showing the highest shoot recovery percentage (93.34), followed by Cdxt 20, Zm 40 and Pv 40 (37.07, 59.29 and 62.23 respectively) and by Zm 20, Pv 20, C3 20 and C3 40 (8.95, 14.06, 6.04 and 8.03 respectively).

The multiple regression equations of the subset of 5 parameters for C4 species with the highest adjusted determination coefficient (R^2) for T1(20) shoot retention, T1 (40) shoot retention, T2(20) shoot recovery and T2 (40) shoot recovery were:

T1 (20) shoot retention (C4) =

71.28-15.78(HSV) -0.15(Stolon starch) +0.09(Stolon lignin) +0.13(Stolon glucose) -0.54(Stolon fructose)

(R^2=0.99)

T1 (40) shoot retention (C4) =

101.7-13.07(HSV) -0.20(Stolon starch) +6.01(Stolon silica) -1.67(Rhizome fructose) -0.18(Rhizome DM)

(R^2=0.99)

T2 (20) shoot recovery (C4) =

37.51 -0.22(HSD) -6.08(leaf width) +0.16(Stolon starch) -1.83(Stolon silica) -0.30(Rhizome DM)

(R^2=0.99)

T2 (20) shoot recovery (C4) =

140.38 -15.97(HSV)+3.20(Stolon glucose) -0.60(Stolon sucrose) +4.15(Leaf glucose) -4.68(Leaf DM)

(R^2=0.99)

Discussion

The longer internodes of Zm and Cdxt compared to Pv are not in accordance with previous research, that however was focused on new turfgrass establishment of these species and not on fully-established turf (Volterrani *et al*. 2008).

The thinner horizontal stem of Zm is in accordance with its extremely fine texture and higher dry mass percentage. HSD in Zm is approximately threefold that of Cdxt and Pv, which indicates this specie's capacity of creating an extremely dense weave of horizontal stems.

The highest Horizontal Stem Node Density scored by Zm would indicate a higher recuperation potential by this species. However, given the observed lower recuperation capacity of Zm, tissue constituents appear to play a more important role in recuperation than mere node density.

The same can be said of Horizontal Stem Volume, where the higher HSV found in Zm (which could be interpreted as an indication of the volume of starch and soluble sugars available for recuperation) does not determine a faster recuperation.

The wider and thicker leaves of C3 do not determine a higher wear resistance, as indeed DM results indicate that most of the fresh weight of leaves is made up of water.

In general, no clear pattern can be established between leaf biometrics and C4 wear resistance, with the thinner-leaved Zm and Cdxt exhibiting a significantly different wear resistance and the coarser-leaved Pv being more akin to Cdxt than to Zm in terms of wear resistance. The same absence of patterns was detected for leaf biometrics and wear recovery.

In general, this would indicate a higher influence of tissue constituents on wear resistance and recovery, compared to mere biometrics.

Starch content showed a consistent negative correlation with shoot retention after light wear (20 cycles) and as such low starch is can be considered a reliable marker of wear resistance. Inversely, starch seems to determine best recovery in C4 species, and it can be considered a reliable marker for species recuperative potential.

Total soluble sugar (TSS) content did not show appreciable differences within C4 species, while the very high sucrose and fructose content in C3 is probably derived from fructans and starch (Damiani *et al.* 2004). This explainable by the fact that these two soluble sugars are the starting constituent (sucrose) and the final hydrolysis product (fructose) of fructans. At the same time, glucose is very low in C3 as it does not enter directly in fructan metabolism.

The higher initial shoot density of Zm is determined by its high HSD and HSND. High HSV in Zm is also determined by high HSD and HSND, that overcome the low section (HSS) component of Zm horizontal stems.

Thanks to this very high initial density, and thanks to its intrinsically stronger tissues, Zm emerges as the species with the highest wear resistance, both in absolute and shoot retention percentage terms. Cdxt and Pv emerge as very similar species in terms of absolute wear resistance, but tissue-wise loses a lower percentage of its shoots, indicating a higher resilience of its tissues and shoots compared with Cdxt. As expected and as indicated in previous studies (Gaussoin *et al.* 2001), C3 species offer a much lower wear resistance and a virtually nil recuperative potential compared with C4 species. This is due to their lower initial density, lower lignin content and the absence of vegetative propagation organs.

Despite the fact of having the highest number of potential recuperative points (HSND), Zm is the slowest recuperating species (and the opposite is true for Cdxt and Pv), indicating how the C4 species' physiology and biochemistry is a more powerful asset than mere node density when it comes to recuperation.

All C4 species recuperated a higher percentage of shoots after mere severe wear (40 cycles) and this different behavior in recovery from 20 and 40 cycles deserves further study. This would help to better comprehend whether this is due to better usage of light, space and nutrients dictated by the decrease in canopy density associated with wear, or if there are precise physiological and enzymatic reactions associated with severe wear that induce a more prompt and intense recovery.

Silica is frequently studied as a factor influencing turfgrass wear resistance, and in the present study stolon silica content positively influenced shoot retention after severe wear (40 cycles). This is probably due to a higher shear resistance imparted by silica to organ tissues, although this would have to be confirmed by further research.

Dry matter was a frequent marker entering multiple regression analysis of wear resistance and recovery. This is in accordance with findings on the lower mechanical resistance of turfgrass tissues with high water content (Trenholm *et al.* 2000; Hoffman *et al.* 2010*b*), and would also indicate that a higher dry matter content corresponds to a higher availability of TSS and starch to fuel recovery from wear.

In general, lignin showed to be the more clear and consistent marker for wear resistance, thanks to the mechanical resistance properties it imparts to organ tissues.

Conclusions

Several aspects emerged from the present work that can be summarized as follows:

1. The Lisport machine can be successfully used in an effective and reproducible way to fathom out natural turfgrass wear resistance, devoid of soil compaction effects.
2. Wear resistance for C4 species as observed in the field does not necessarily coincide with the relative tissue intrinsic resistance, but rather with the initial canopy density.
3. C4 species show a wear resistance that is much higher than C3 species.

4. C3 species show a virtually nil recuperative capability (mainly due to the lack of vegetative propagation organs).
5. The species with a very high intrinsic (tissue) wear resistance are also the species with the slowest recuperation potential. This seems to be due to lower levels of starch and TSS available for recovery.
6. Starch was a clear marker of wear resistance (negatively correlated) and recovery (positively correlated).
7. Silica was a marker positively correlated with wear resistance.
8. Lignin was the clearest marker found to be positively correlated with wear resistance.
9. A more severe wear induces a higher percentage of shoot recovery, and this particular aspects deserves further investigation.

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STUDY TWO

TABLES

Table 1. T0 Cdxt, Zm, Pv: horizontal stem internode length, calculated Horizontal Stem Section (HSS), Horizontal Stem Density (HSD), calculated horizontal stem node density (HSND), calculated horizontal stem volume (HSV).
*, **, *** significant for 0.05, 0.01, 0.005 probability levels respectively. Means followed by the same letter in a column do not differ significantly at the P≤0.05 probability level.

	Internode length (cm)	HSS (mm^2)	HSD ($cm\ cm^{-2}$)	HSND ($nodes\ cm^{-2}$)	HSV ($cm^3\ cm^{-2}$)
Cdxt	1,57 a	3,12 a	5,77 b	3.79 b	1.54 b
Zm	1,71 a	1,49 b	15,84 a	9.57 a	2.37 a
Pv	1,23 b	2,62 a	5,48 b	4.52 b	1.43 b
	**	***	***	***	***
CV (%)	**17,96**	**23,31**	**7,99**	**23.42**	**22.09**

Table 2. T0 Cdxt, Zm, Pv and C3: leaf width, leaf thickness, calculated leaf section.
*, **, *** significant for 0.05, 0.01, 0.005 probability levels respectively. Means followed by the same letter in a column do not differ significantly at the P≤0.05 probability level.

	Leaf width (mm)	Leaf thickness (mm)	Leaf section (mm^2)
Cdxt	1,49 c	0,13 b	0,18 c
Zm	1,48 c	0,14 b	0,20 c
Pv	2,20 b	0,19 a	0,42 b
C3	2,59 a	0,21 a	0,53 a
	***	***	***
CV (%)	**12,19**	**19,31**	**14,67**

STUDY TWO

FIGURES

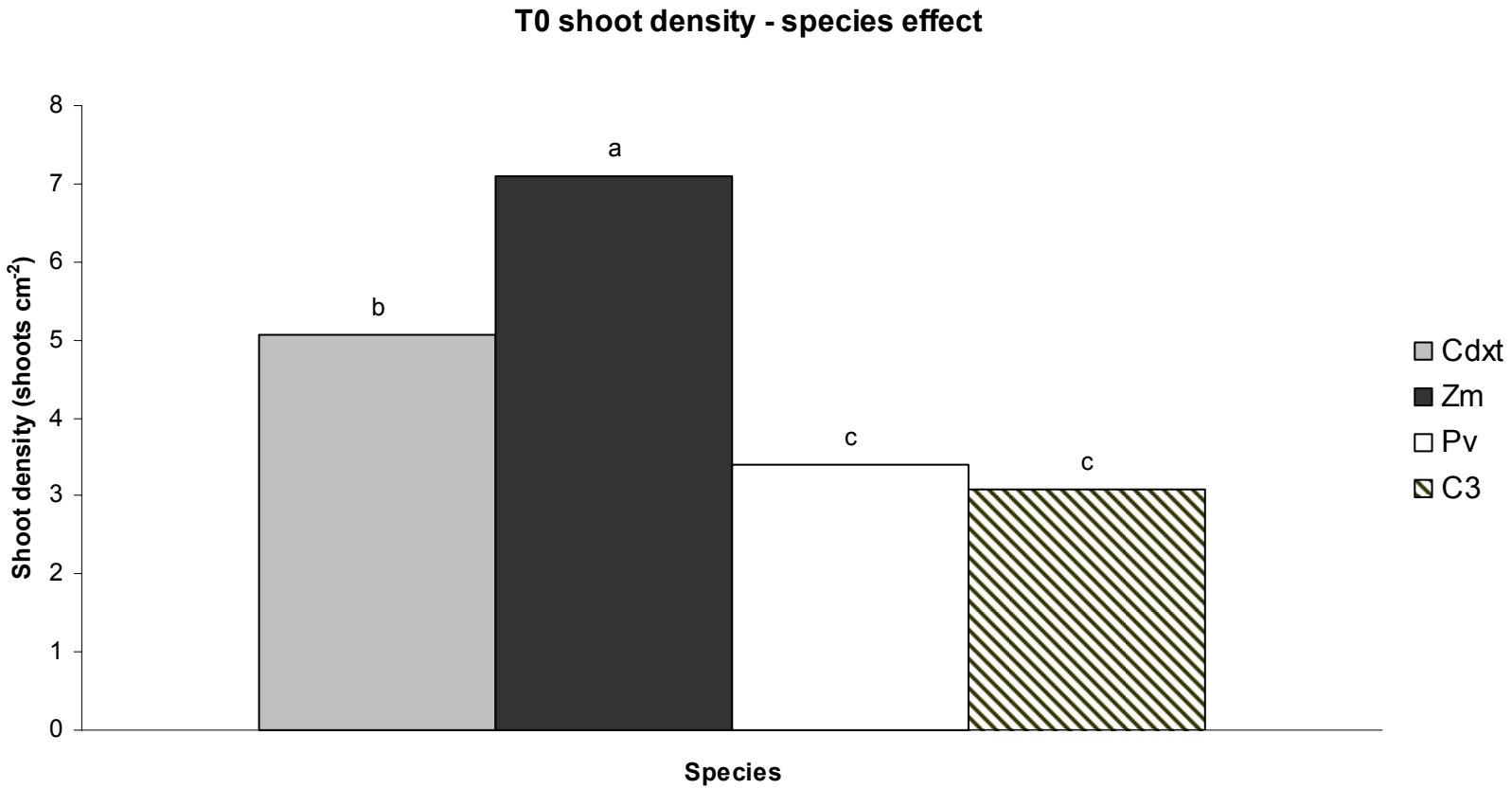

Figure 1. Cdxt, Zm, Pv and C3 – T0 shoot density – species effect. Column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

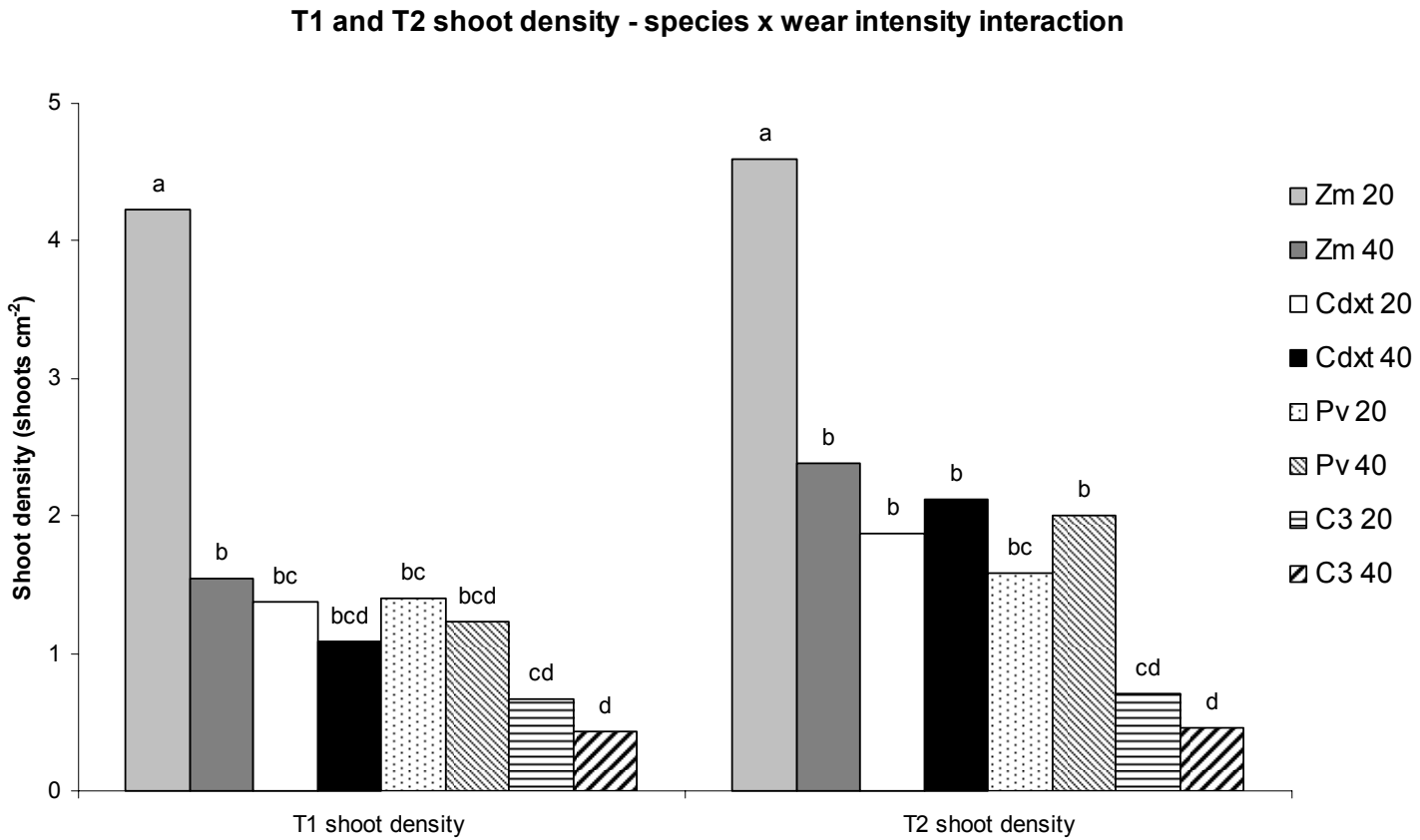

Figure 2. Cdxt, Zm, Pv and C3 – T1 and T2 shoot density – species x wear intensity interaction. Column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

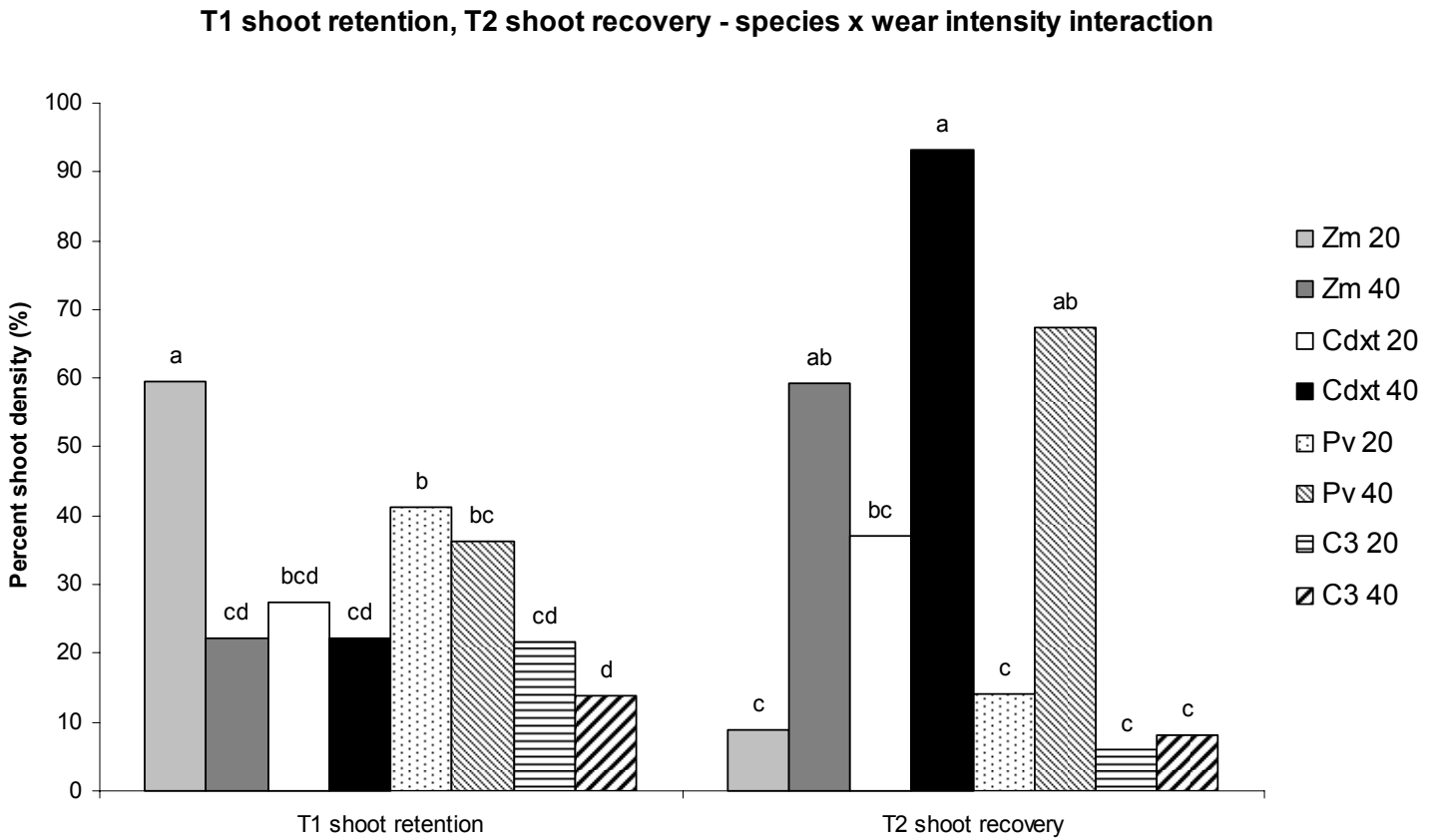

Figure 3. Cdxt, Zm, Pv and C3 – T1 shoot retention and T2 shoot recovery – species x wear intensity interaction. Column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

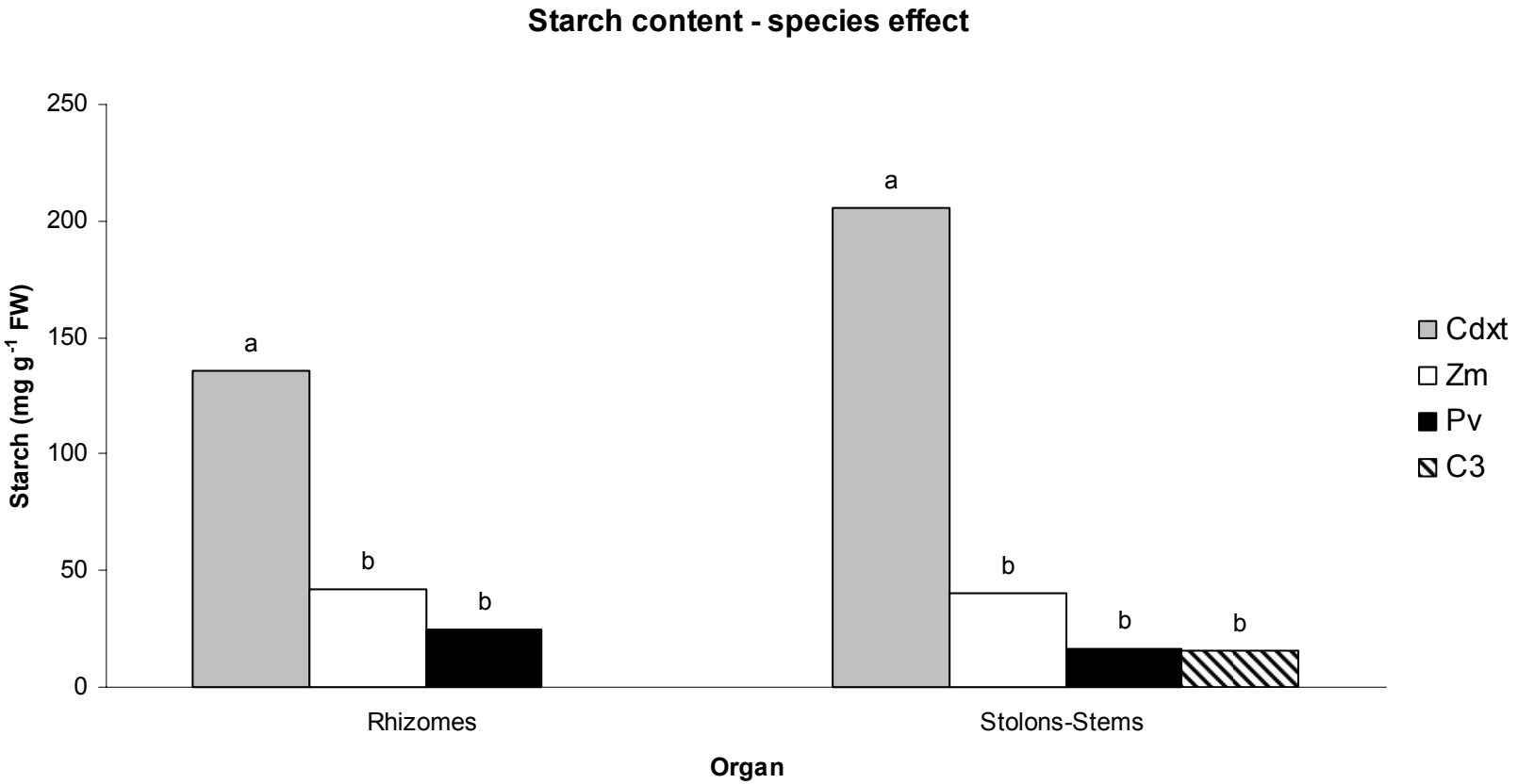

Figure 4. Cdxt, Zm, Pv and C3 – rhizomes, stolons-stems: T0 starch content – species effect. Within each organ (rhizome, stolon) column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

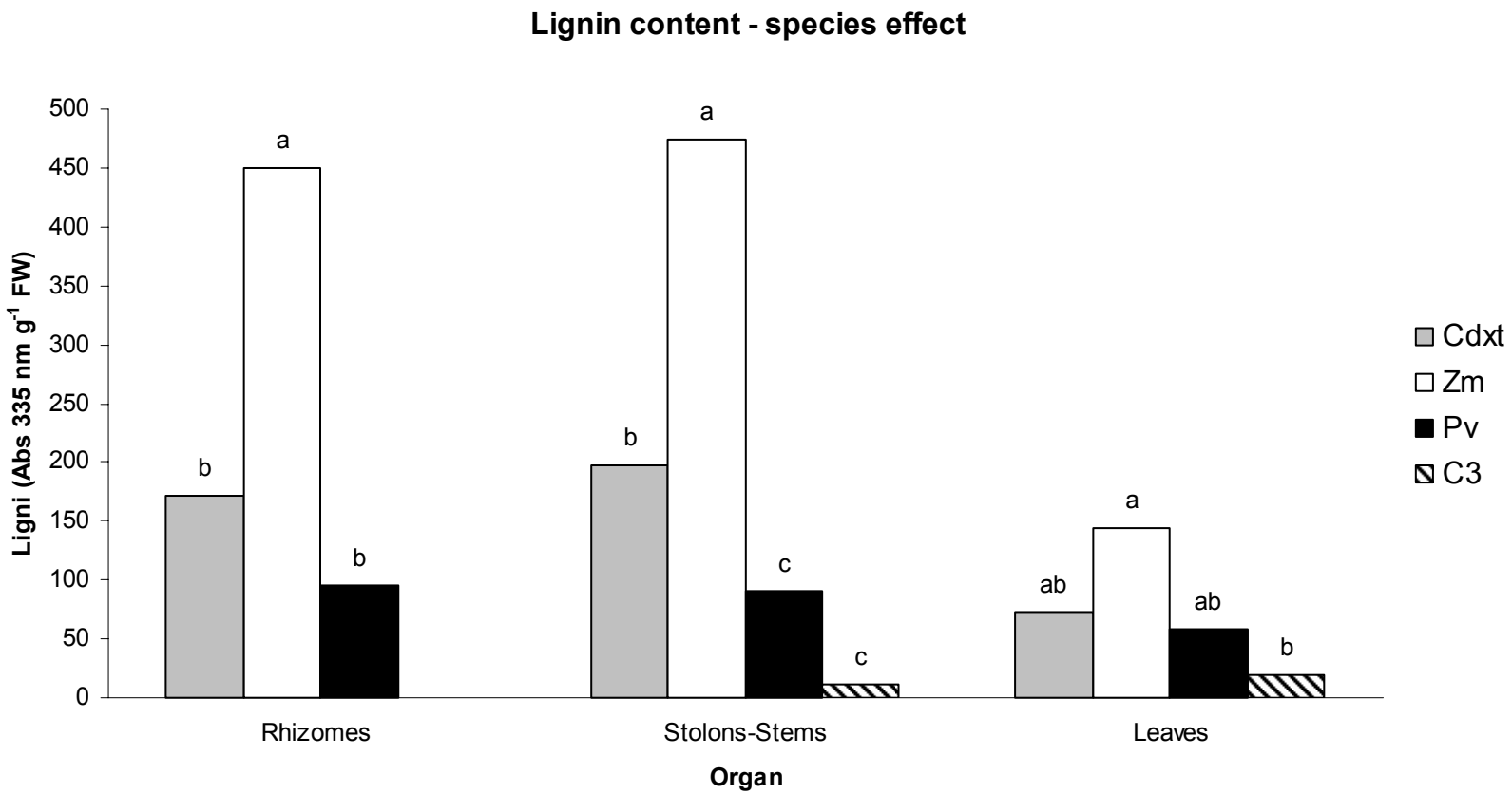

Figure 5. Cdxt, Zm, Pv and C3 – rhizomes, stolons-stems, leaves: T0 lignin content – species effect. Within each organ (rhizomes, stolons-stems, leaves) column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

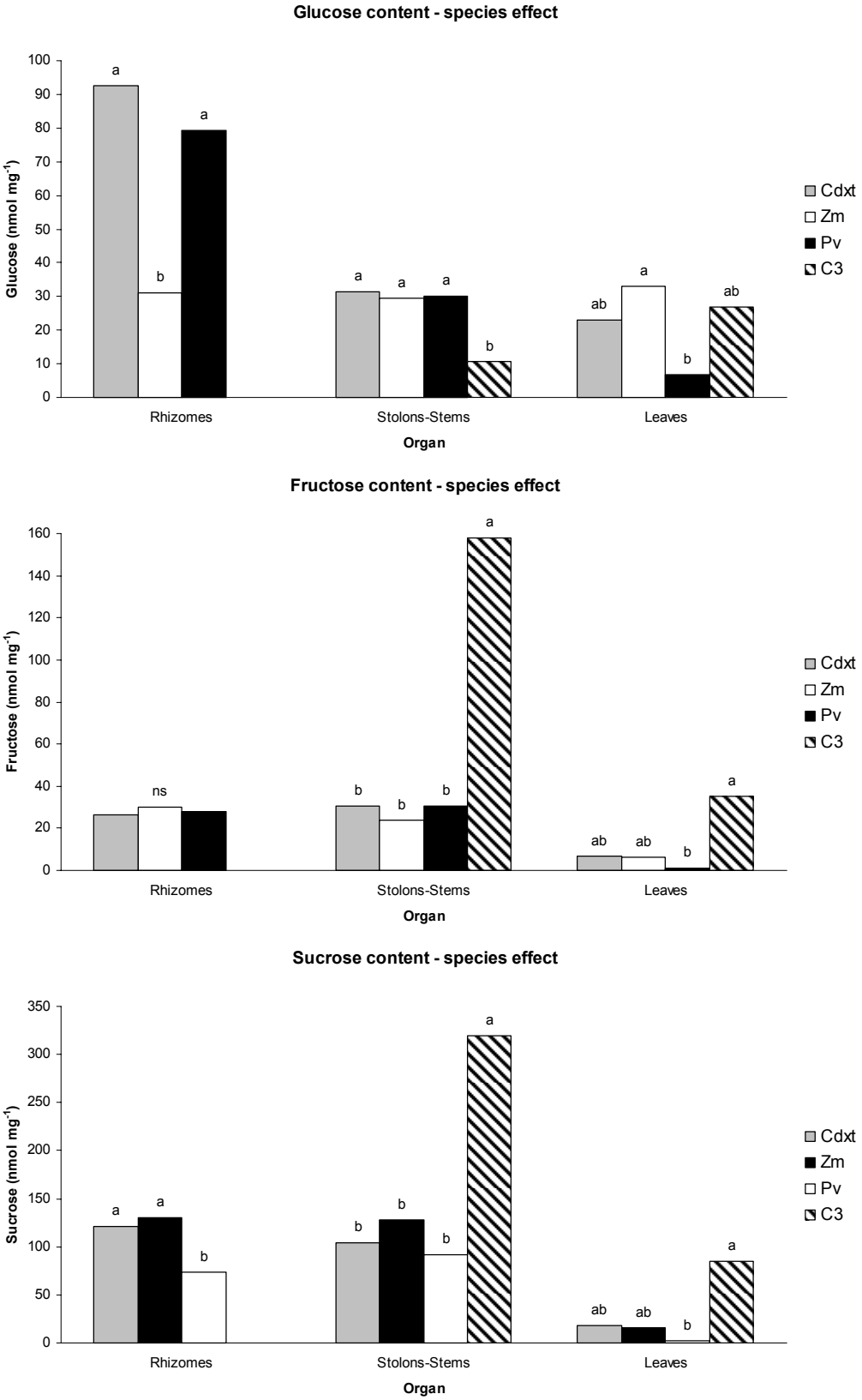

Figure 6. Cdxt, Zm, Pv and C3 – rhizomes, stolons-stems, leaves: T0 glucose, sucrose and fructose content – species effect. Within each organ (rhizomes, stolons-stems, leaves) column treatments with the same letter do not differ significantly at the P≤0.05 probability level; ns = not significant.

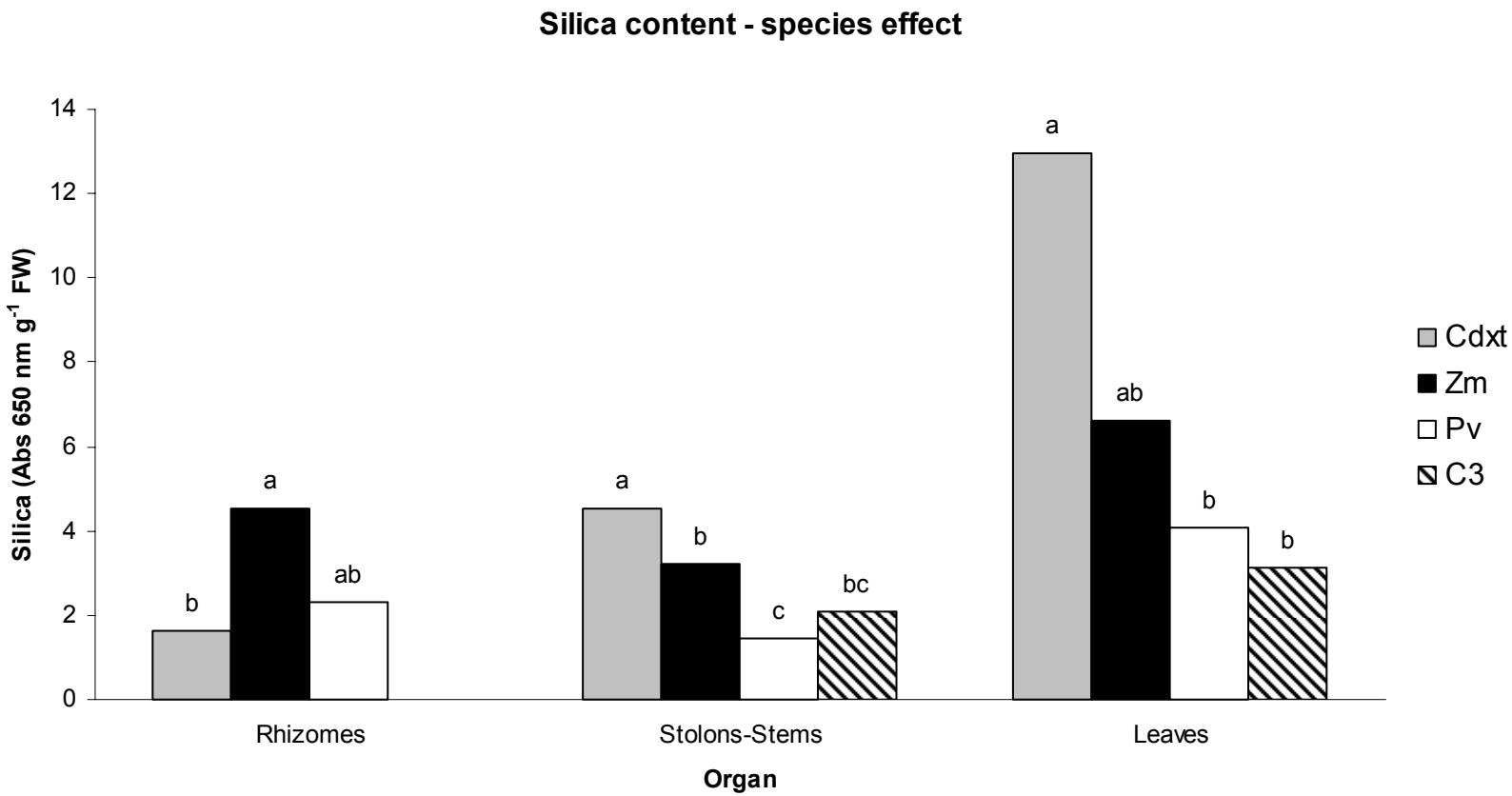

Figure 7. Cdxt, Zm, Pv and C3 – rhizomes, stolons-stems, leaves: T0 silica content – species effect. Within each organ (rhizomes, stolons-stems, leaves) column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

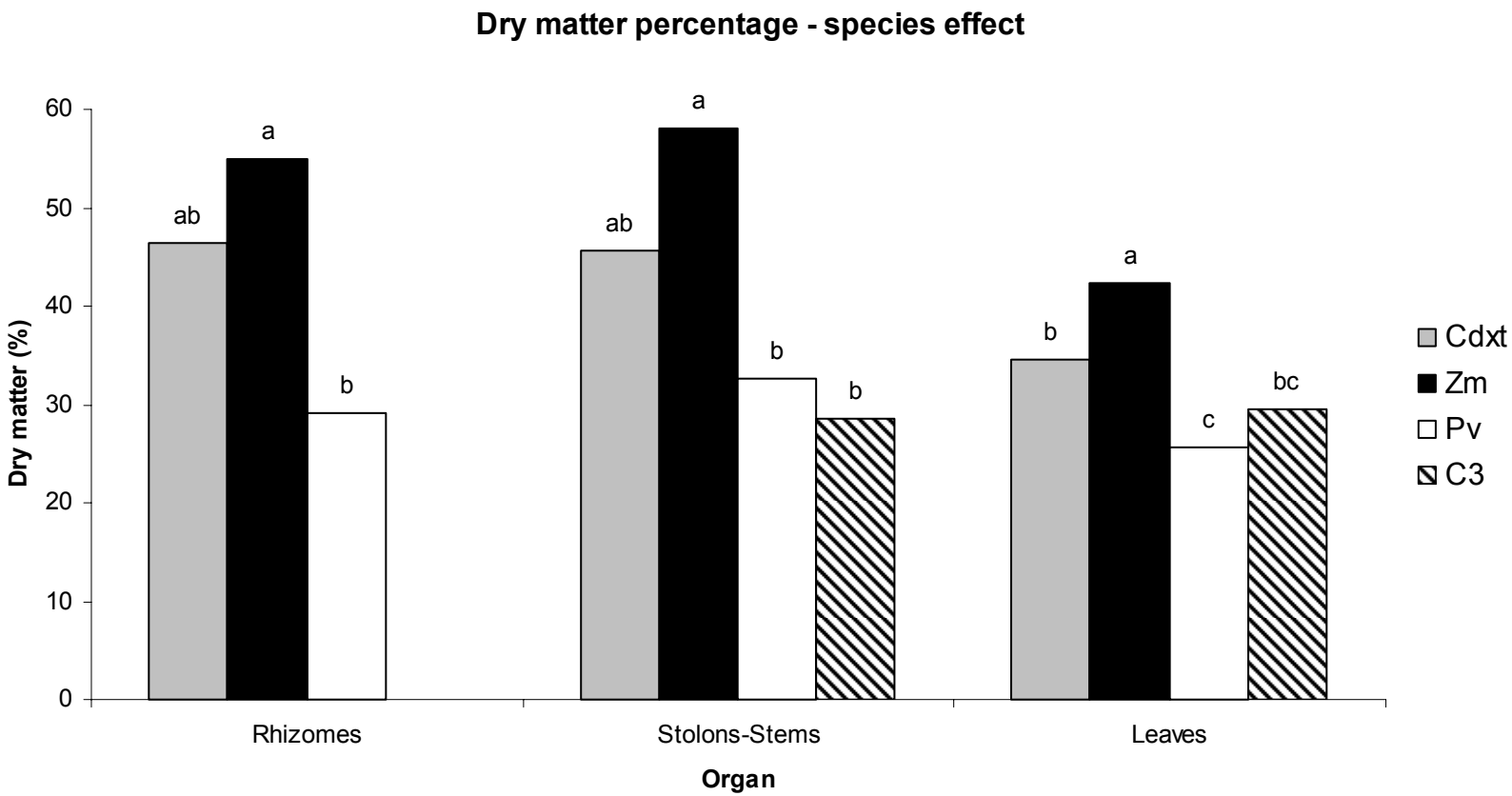

Figure 8. Cdxt, Zm, Pv and C3 – rhizomes, stolons-stems, leaves: T0 dry matter percentage – species effect. Within each organ (rhizomes, stolons-stems, leaves) column treatments with the same letter do not differ significantly at the P≤0.05 probability level.

CONCLUSIONS

The present research has shown that it is possible to successfully evaluate C4 turfgrass species tissue mechanical resistance, wear resistance and recovery devoid of soil compaction effects. This has been made possible by the careful standardization of test samples and areas (through a period of greenhouse acclimation in which differences originating from soil, fertilization, climate and management practices have been annulled or minimized), and by the in-laboratory use of highly automatized testing machinery that effectively removes differences originating from climate or operator factors.

The results have shown that differences between species in terms of tissue mechanical resistance, wear resistance and recovery can be traced down to specific tissue constituents that effectively contribute to tissue strength and recuperative energy, while other constituents – although not directly contributing to tissue resistance or plant recovery – can be considered as biological or physiological markers for these traits.

Another interesting finding of the present research has been to partially clarify the effective implications of biometrics in the dynamics of tissue mechanical resistance, wear resistance and recovery. On this subject, the introduction of strength per tested tissue unit area and (Study One) and the adoption of percentage shoot loss or recovery (Study Two) have successfully managed to distinguish between a species' characteristics as observed in the field and the effective strength of a specie's tissues. By depurating results from the dimensional factor, the true differences between different species' tissues have been clarified.

Further to the above points, the research has shown that turfgrass density (both in terms of shoot density and node density) is not in itself an indicator of a turfgrass canopy's wear resistance, nor of its recovery capability. Indeed, one of the fundamental findings emerging throughout the present research is that tissue constituents are far more influential on tissue mechanical

resistance, wear resistance and recovery compared to mere biometrics and density.

Lastly, the use of highly specialized and virtually unmanned laboratory machinery has proved a factor that can drastically increase the precision of this type of turfgrass investigations. Compared to traditional machinery (i.e. traditional field wear simulators), this type of machinery can effectively remove most operator-derived errors.

The author believes that the very specific results originating from this work could be a particularly useful tool for:

a) turfgrass genetics and selectors in order to improve the efficacy of the selection and genetic improvement for the traits of wear resistance and recovery;

b) chemical companies that can target the development of new chemical products at increasing (or decreasing) certain tissue constituents associated with these traits (i.e. lignin).

According to the figure shown on the right, the positive outcome of improved turfgrass genetics and the arrival on the market of specific chemicals could determine a reduction of the time consuming and financially draining management practices aimed at maximising wear resistance and recovery. This would not only decrease the social impact of high-profile turfgrass species but also, thanks to a decrease in the use of various agronomical input factors, it would determine a welcome decrease in the environmental impact of these otherwise extremely useful, aesthetically pleasing and widely enjoyed surfaces.

ACKNOWLEDGEMENTS

The Author wishes to thank the following scientists and Companies:

Dr. Marco Volterrani (CeRTES-UNIPI) for inspiring and supporting this research.
Dr. Lorenzo Guglielminetti (DBPA-UNIPI) for supplying invaluable support and advice in the physiology lab.
Dr. Nicola Grossi (CeRTES-UNIPI) for his continuing collaboration in this project.
Dr. Sara Stefanini for her support in sampling and laboratory analysis.
Drs. Simone Magni and Monica Gaetani (CeRTES-UNIPI) for their support and advice.
Labosport Italia Srl for the use of their dynamometer and Lisport machinery, and hospitality within their laboratory.
Azienda Agricola Pacini for the use of their greenhouses and other facilities.

ATTACHMENT 1

STUDY ONE PHOTO REPORT

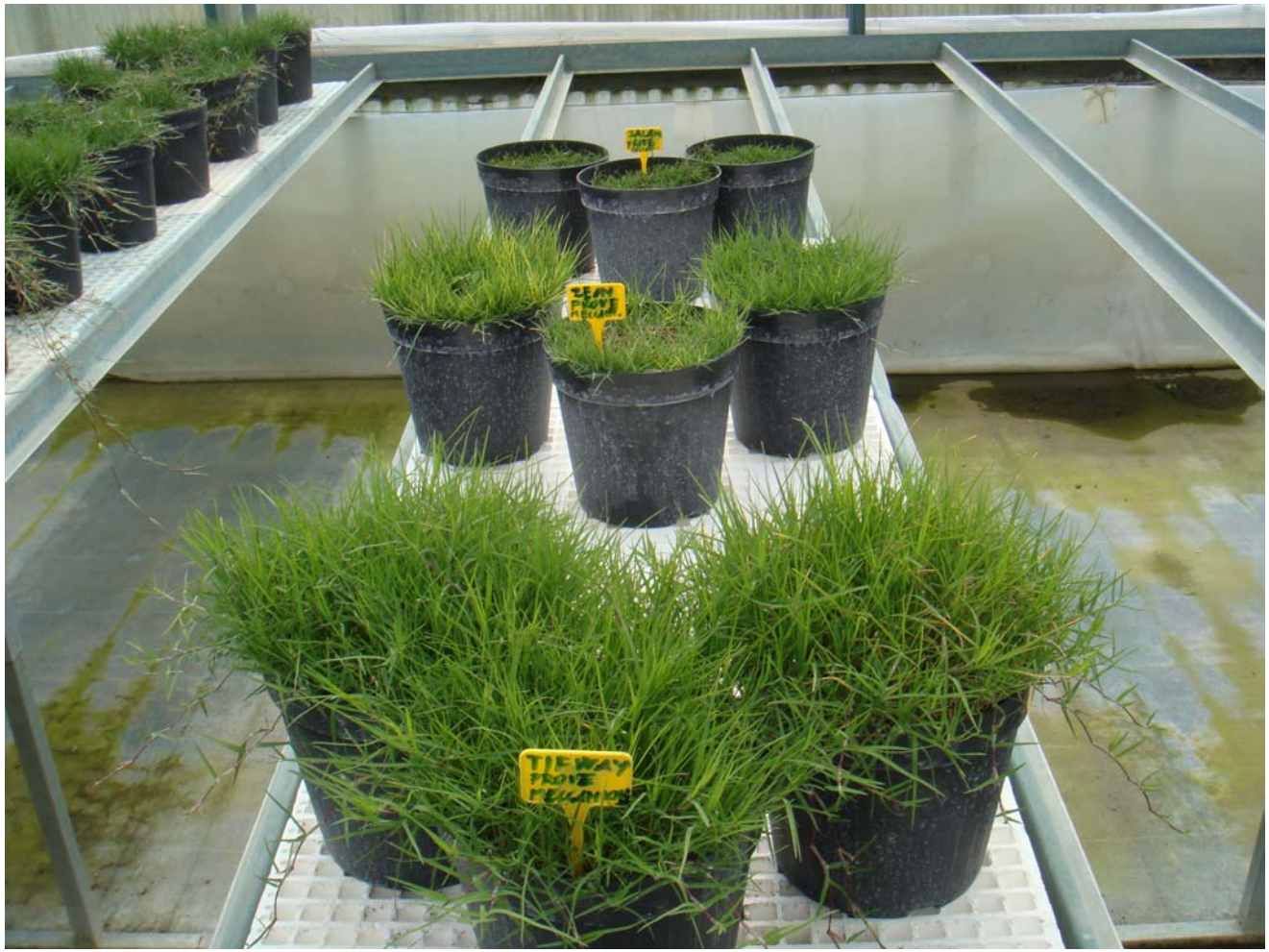

Photo 1. Cdxt, Pv and Zm plugs freshly brought into greenhouse from the field, to be raised as donor plants. May 2009.

Photo 2. Cdxt, Pv and Zm donor plants ready for explant of organs to be submitted to trials. September 2009.

Photo 3. Cdxt, Pv and Zm donor plants at the Labosport Italia laboratory. September 2009.

Photo 4. The MTS Insight Dynamometer used for tensile strength tests. September 2009.

Photo 5. Details of the MTS Insight Dynamometer used for tensile strength tests. September 2009.

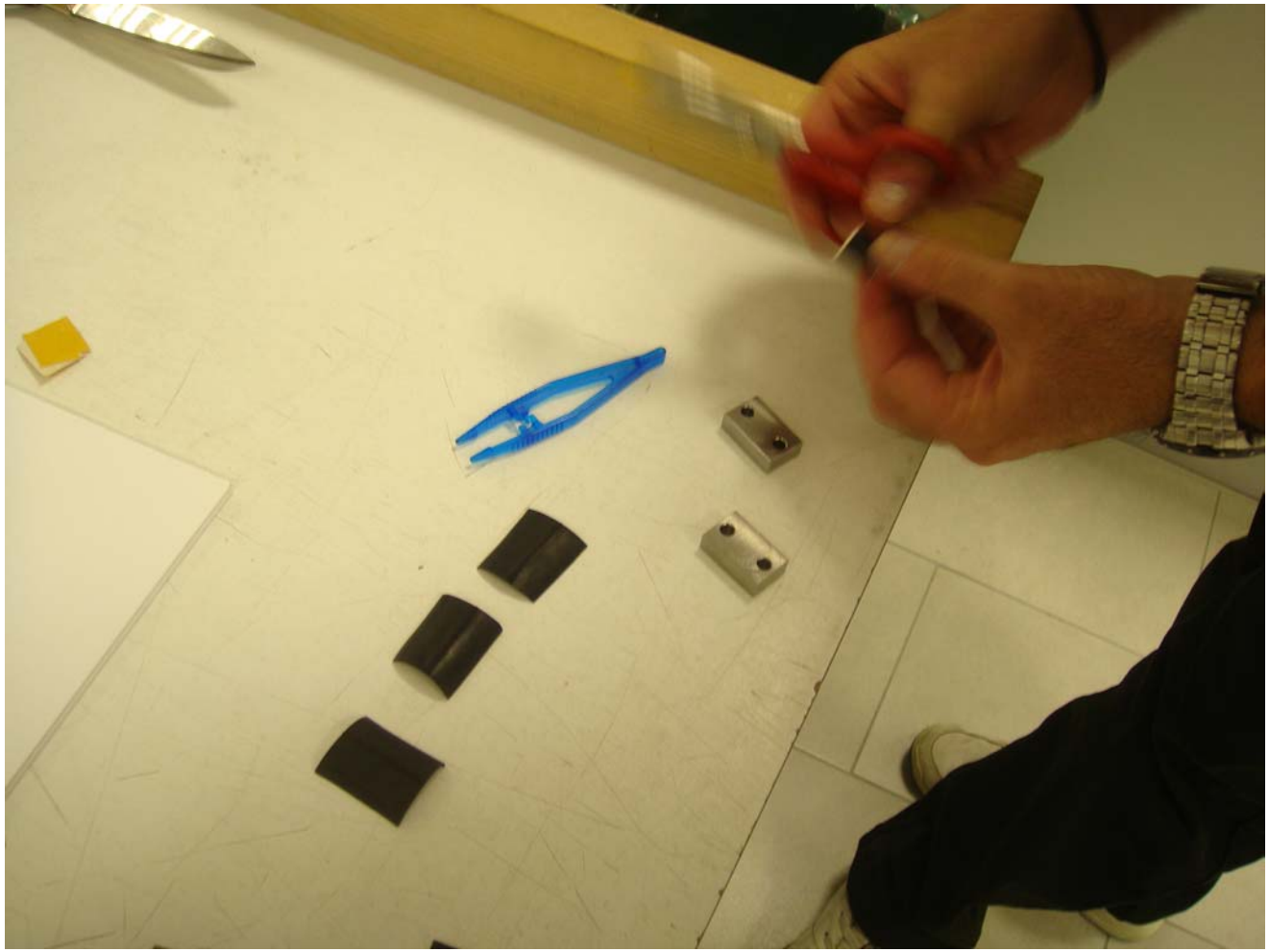

Photo 6. Fitting rubber inserts to the dynamometer's vices to avoid tissue damage. September 2009.

Photo 7. Mitutoyo digital feeler gauge used to measure organ biometrics. September 2009.

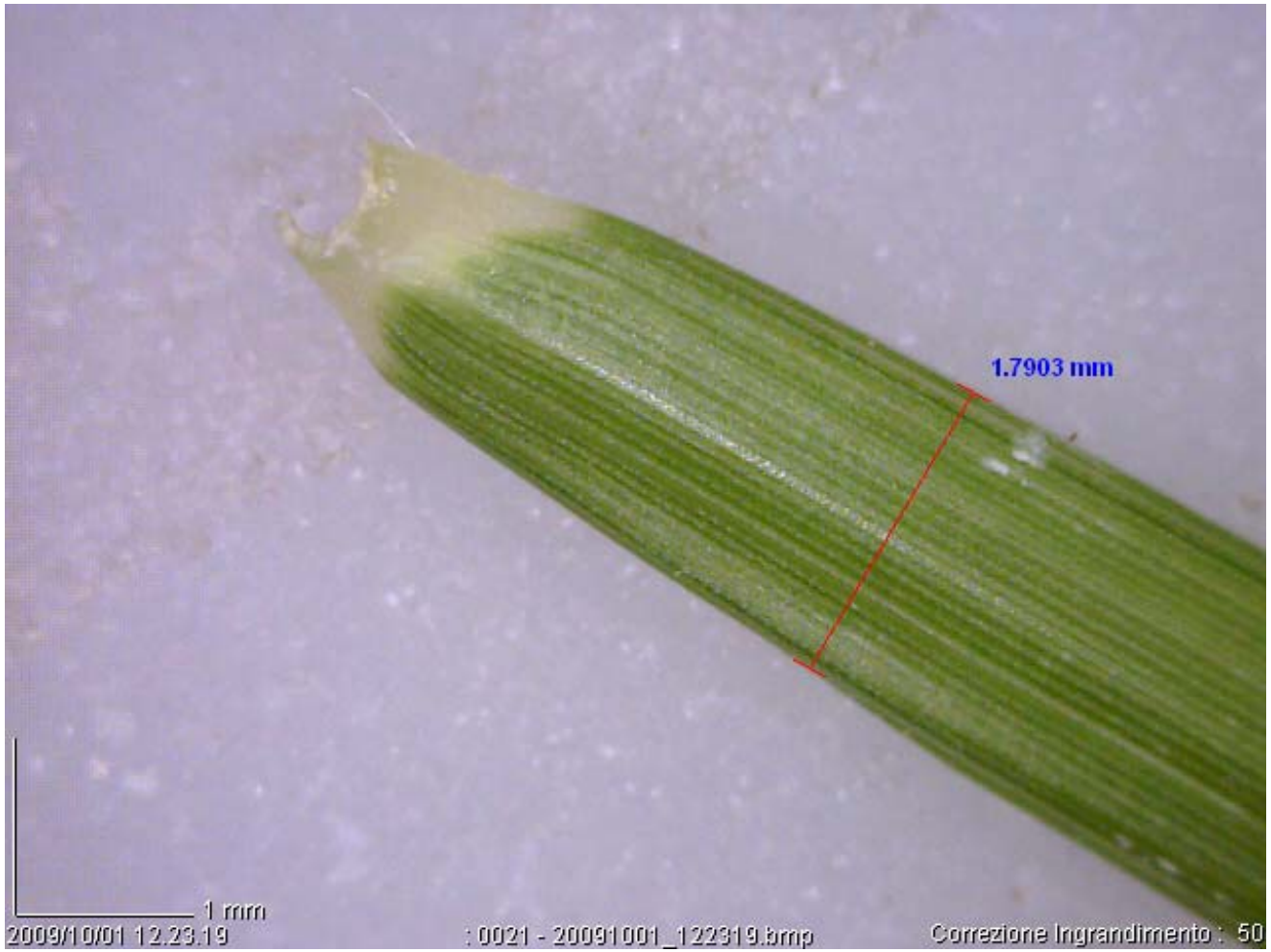

Photo 8. Measuring Zm leaf width with the digital ruler function of a Dyno-Lite digital microscope. September 2009.

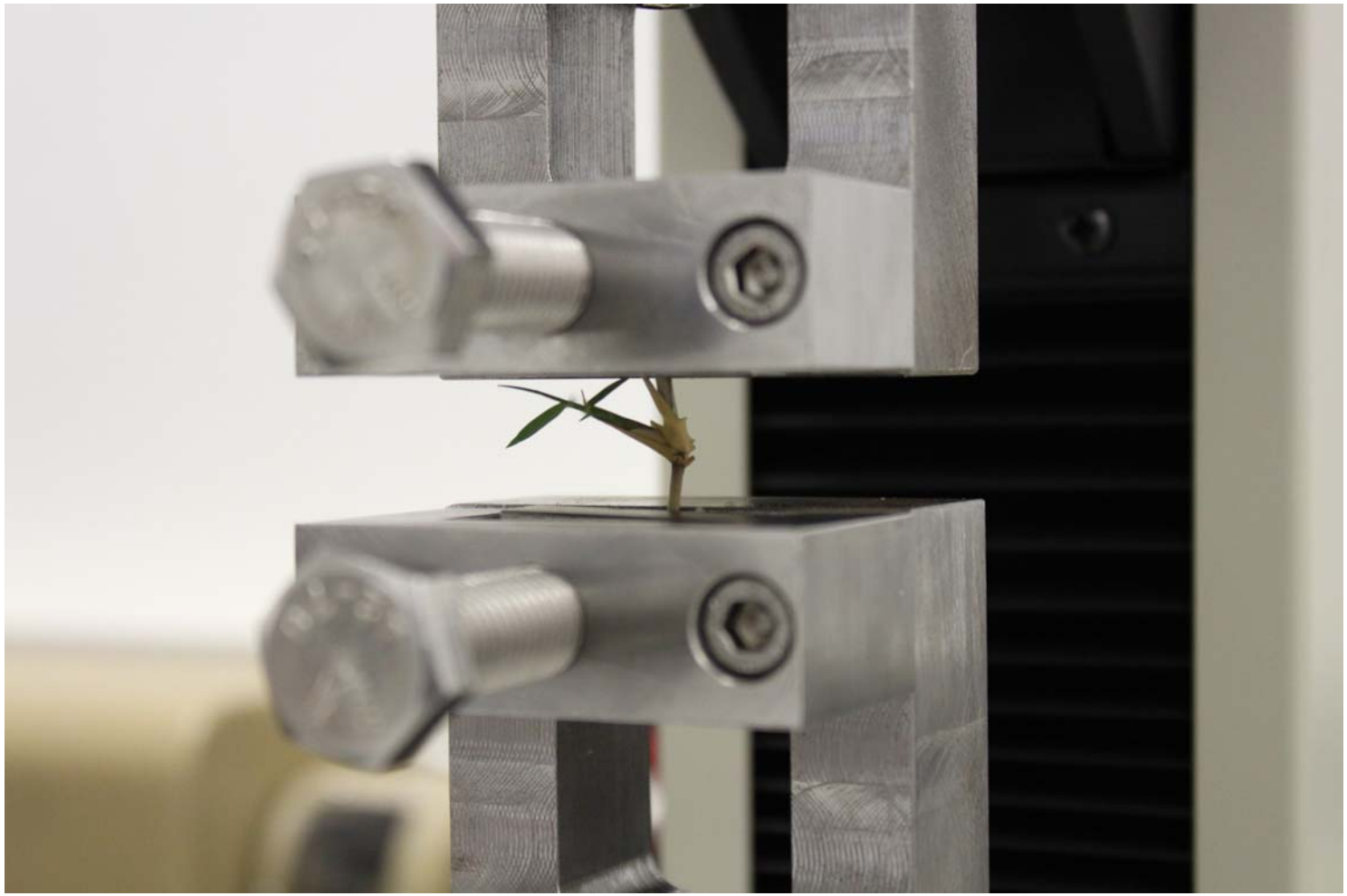

Photo 9. Measuring Cdxt stolon tensile strength. September 2009.

Photo 10. Measuring Cdxt stolon tensile strength – typical above-node meristem breakage point. September 2009.

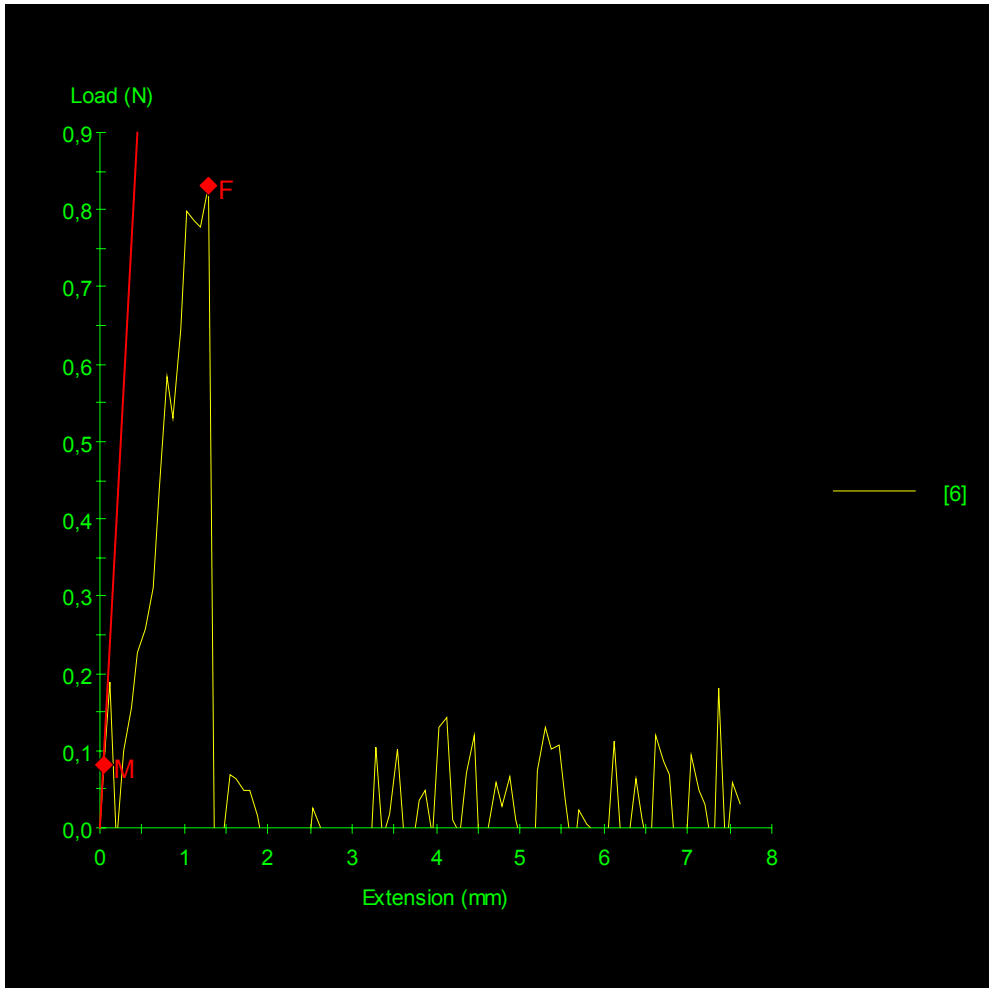

Photo 11. Typical dynamometer output for Cdxt leaf tensile strength test. September 2009.

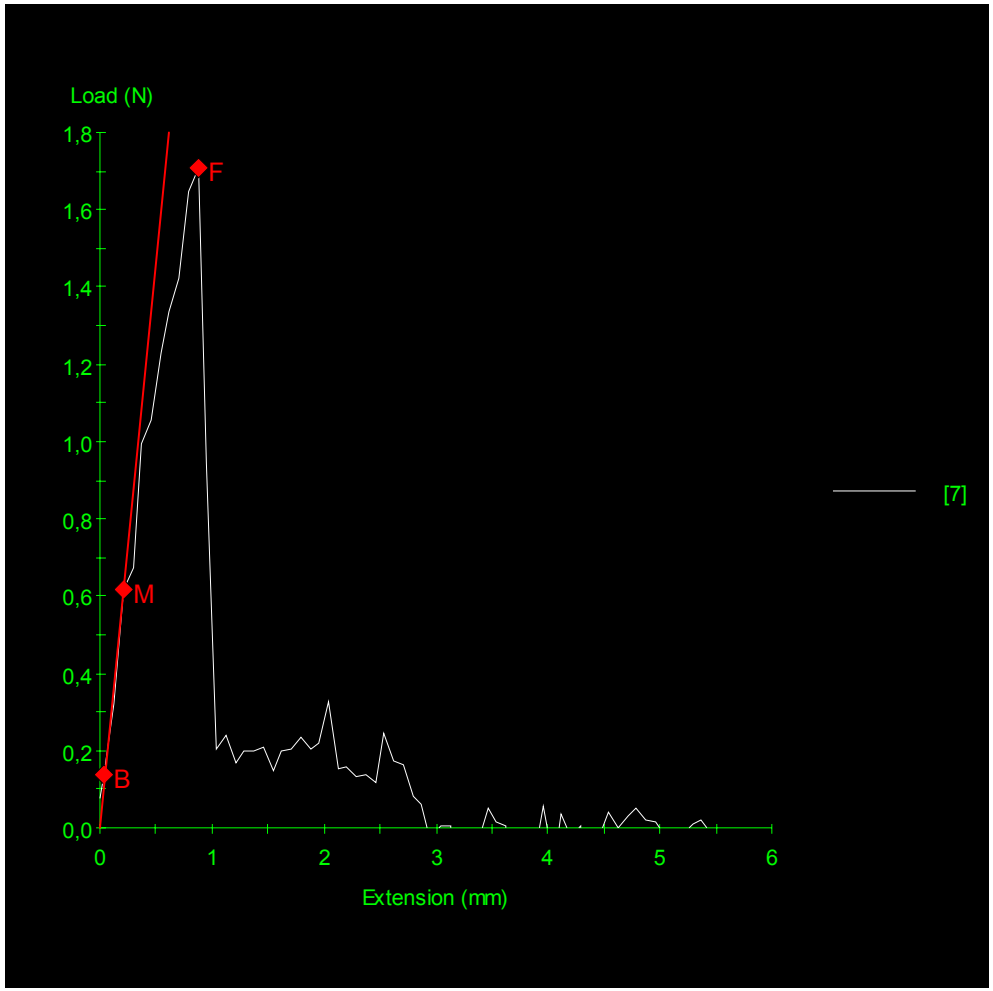

Photo 12. Typical dynamometer output for Zm leaf tensile strength test. September 2009.

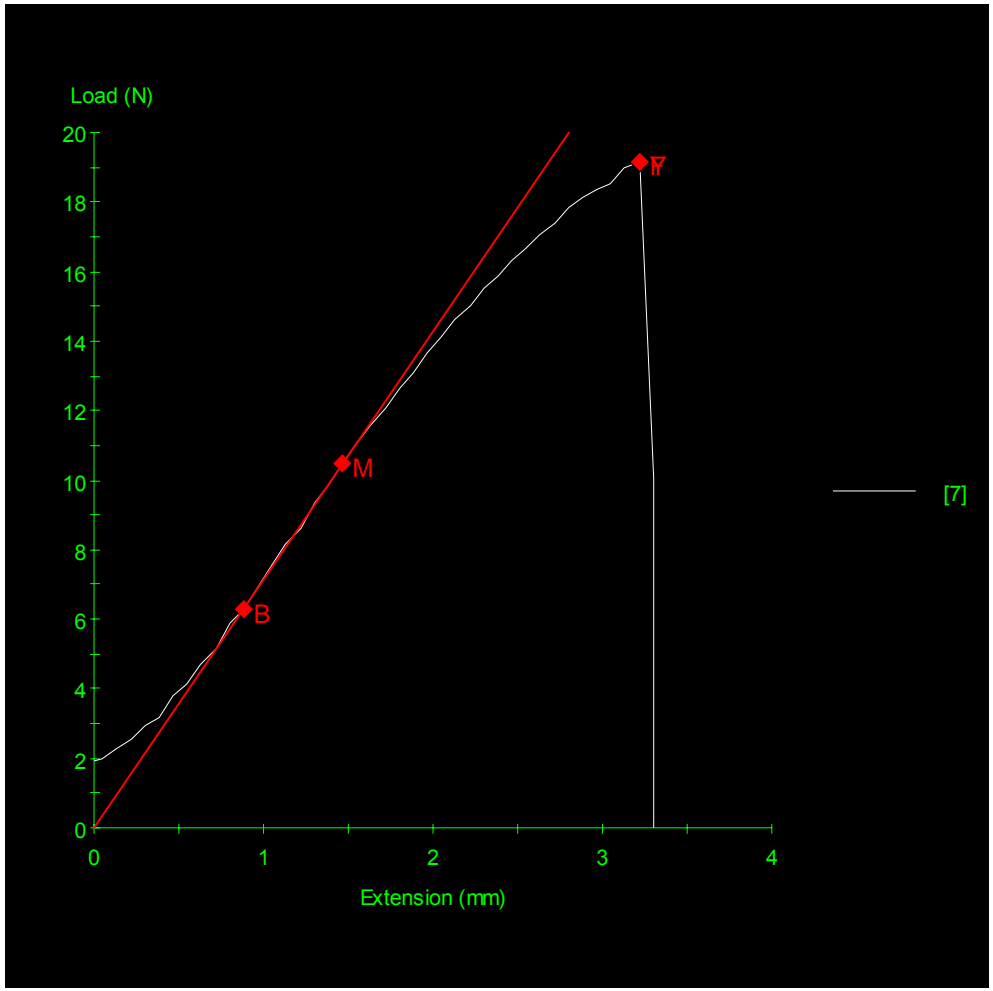

Photo 13. Typical dynamometer output for Cdxt stolon tensile strength test. September 2009.

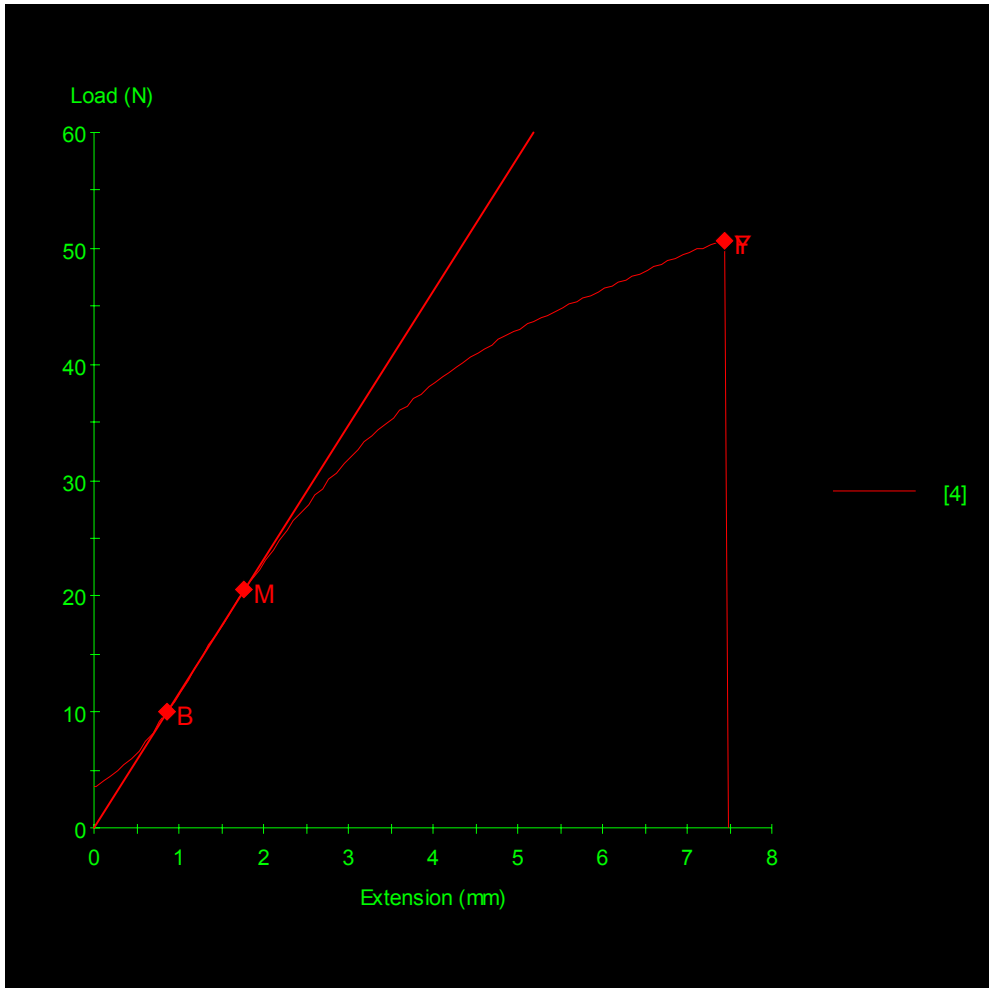

Photo 14. Typical dynamometer output for Zm stolon tensile strength test. September 2009.

ATTACHMENT 2

STUDY TWO PHOTO REPORT

Photo 1. Turfgrass sod rolls freshly harvested from the field. April 2010.

Photo 2. Washing soil off turfgrass sod rolls. April 2010.

Photo 3. Washed sod exposing roots and rhizomes. April 2010.

Photo 4. Turfgrass slabs in greenhouse ready for acclimation. April 2010.

Photo 5. Turfgrass slabs in greenhouse ready for transport to Labosport Italia laboratories. July 2010.

Photo 6. Turfgrass slabs testing in the Lisport machine (1). July 2010.

Photo 7. Turfgrass slabs testing in the Lisport machine (2). July 2010.

Photo 8. Turfgrass slabs testing in the Lisport machine (3). July 2010.

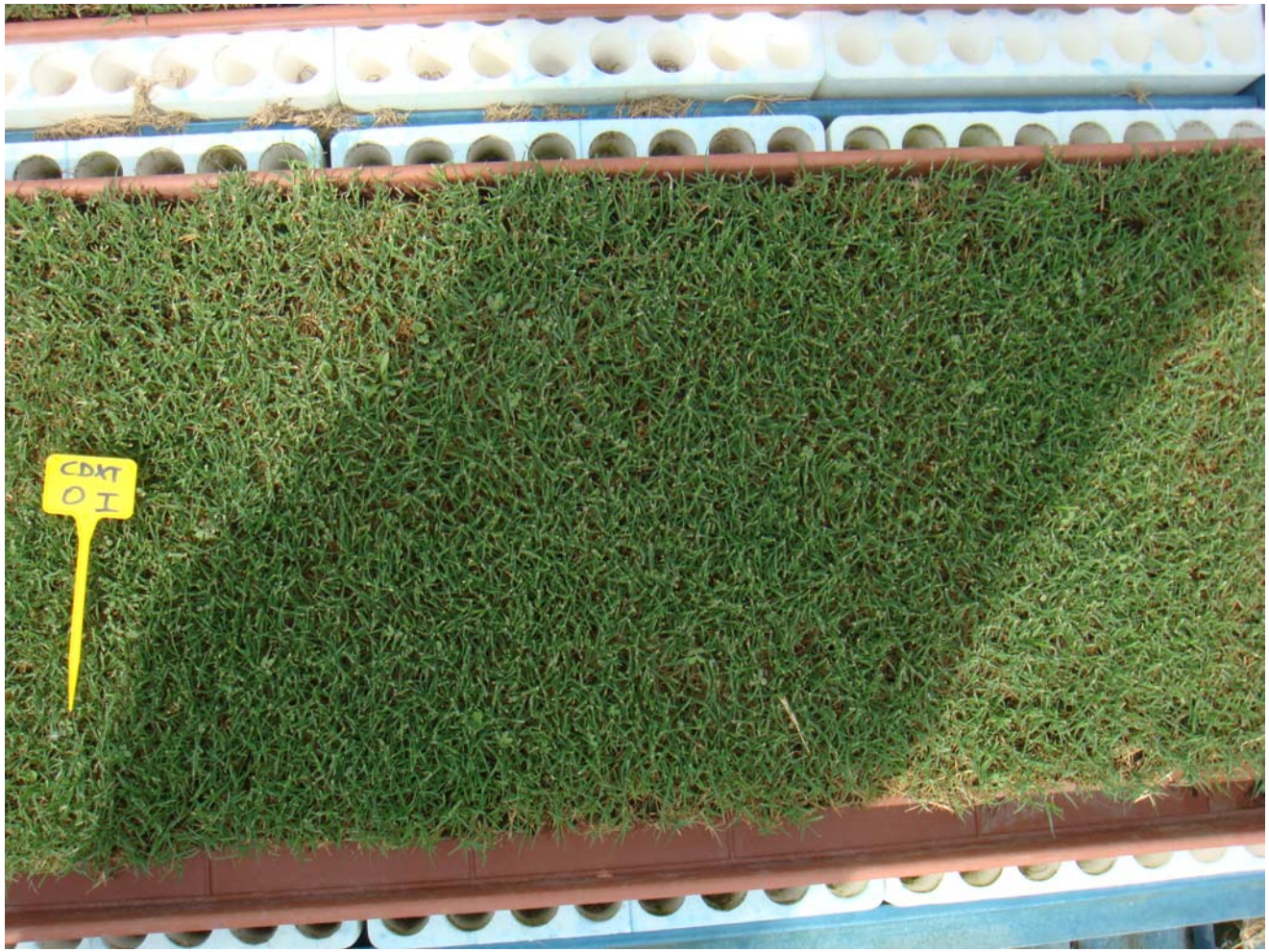

Photo 9. T_0: typical unworn Cdxt. July 2010.

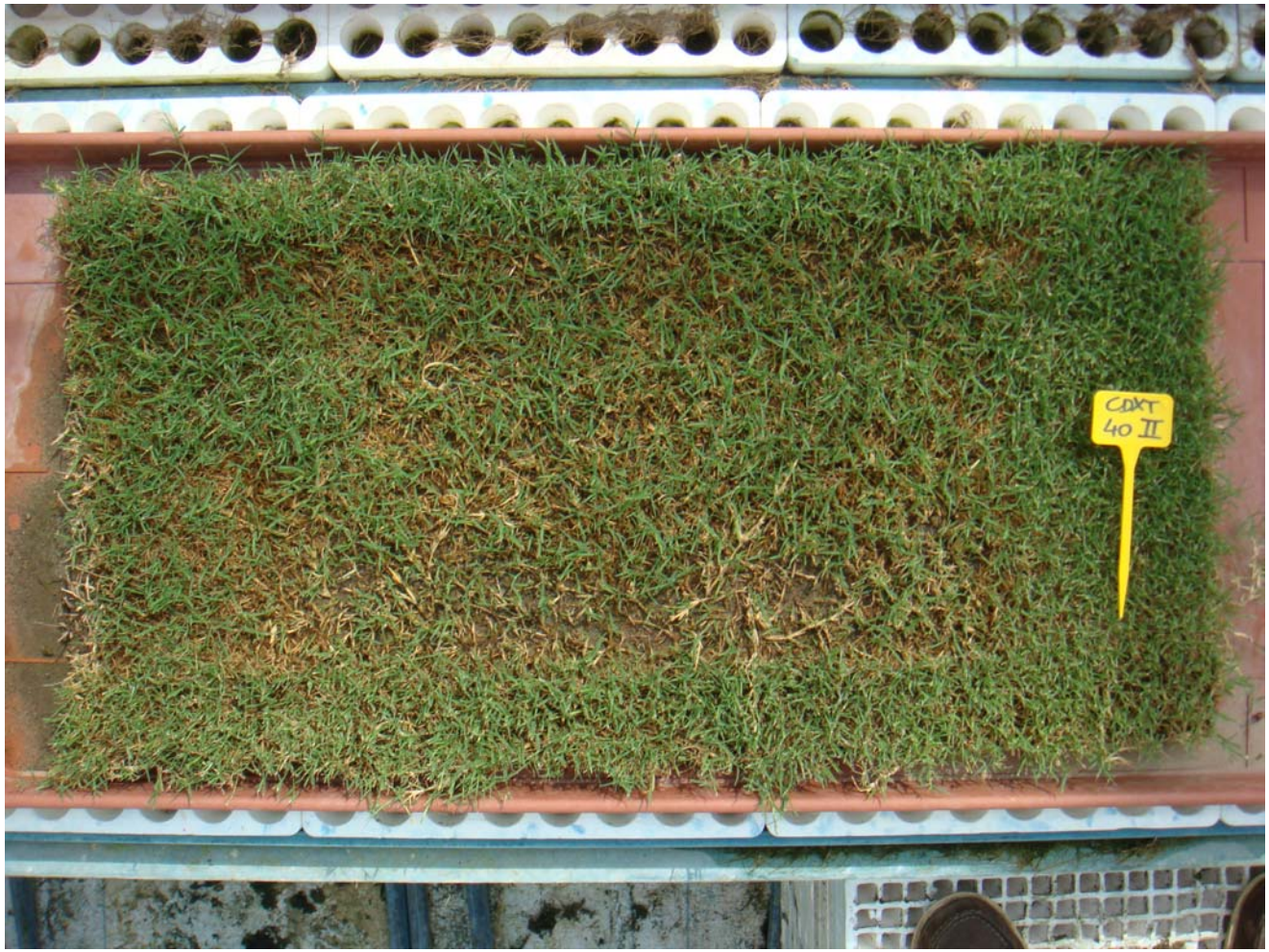

Photo 10. T_1:typical worn Cdxt (40 Lisport cycles). July 2010.

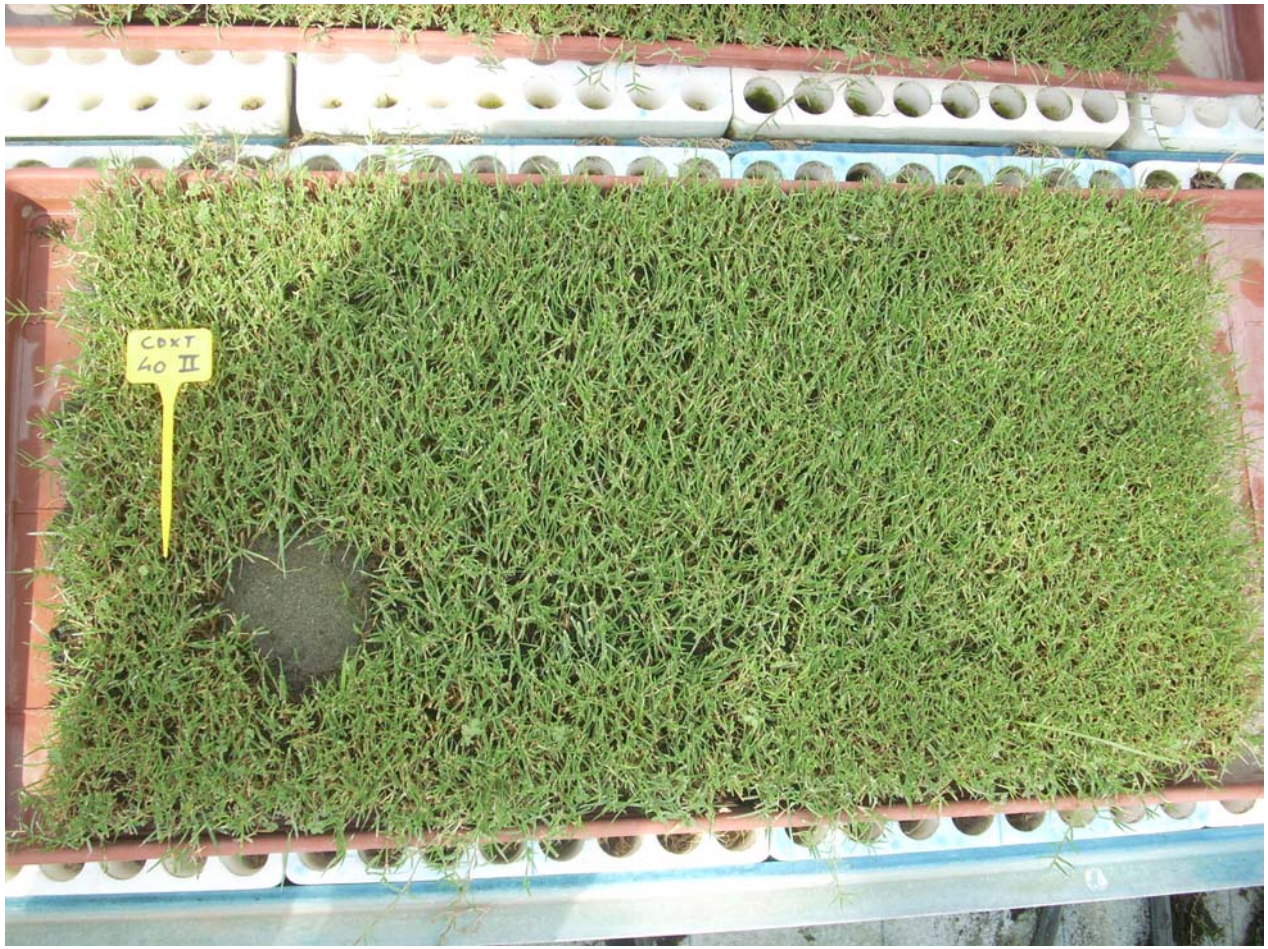

Photo 11. T_2:Typical worn Cdxt (40 Lisport cycles) after 30 dd recuperation; grey area is the plug hole to count shoots. August 2010.