CHAPTER ONE

Minirhizotrons for Measuring Creeping Bentgrass Root Development

ABSTRACT

The minirhizotron method has received limited study as a method for *in situ* evaluation of turfgrass root systems. Forty-five minirhizotrons were installed April 1986 to evaluate the rooting of a 'Penncross' creeping bentgrass (*Agrostis palustris* Huds.) putting green and to compare minirhizotron observations to destructive core sampling. Video recording of the minirhizotron tubes and soil sampling were performed late-May and mid-October 1987. Root weight density (RWD) and root length density (RLD) at individual depth zones were significantly correlated at five of six depth zones in May, but only two depth zones in October. No significant correlations were found between RWD and minirhizotron root observations (MRO), and one significant correlation was observed between RLD and MRO at individual depth zones for the two sampling dates. Spatial variation in soil conditions and rooting between the minirhizotrons and soil sampling sites was thought to be the reason for these poor relationships at the small depth intervals examined. When data was combined across all depth zones correlations between RWD and RLD, RWD and MRO, and RLD and MRO were all highly or very highly significant. Seasonal changes in slope indicated that no single relationship between RLD and MRO can be expected. MRO and RWD

measurements showed root quantities were greater in May than October. The lack of change in RLD measurements between May and October suggested that RLD may not be sensitive to temporal fluctuations in "active" root quantity. RLD measurements were thought to be affected by the presence of senescent roots which were not easily distinguished from the active roots during the counting procedure. RWD measurements, although including the same senescent root material, may better reflect changes in active root quantity because the senescent material should lose weight as it slowly decays. Minirhizotron observations offer the advantage of viewing the senescence of roots over time based on discoloration of the roots. Therefore RWD and MRO should be better indices of the active root quantity and distribution. The data indicated that the minirhizotron method provided an acceptable and much less expensive means to characterize the profile distribution of a creeping bentgrass root system.

INTRODUCTION

Most methods used to study root growth are quite labor intensive and require at least partial destruction of the experimental site (Bohm, 1979). The destruction of field space renders that area useless until reestablished and eliminates the ability for repeated sampling at the same site. To overcome these disadvantages, glass wall methods were developed to observe root growth *in situ*. McMichael and Taylor (1987) provided a historical overview of the development of the various "glass wall" methods.

Several rhizotron facilities have been constructed to examine turfgrass root systems (Karnok and Kucharski, 1982; DiPoala et al., 1982; Shearman and Barber, 1987). Rhizotrons are very costly to construct and adequate replication of treatments may be limited by the number of viewing compartments (McMichael and Taylor, 1987). The minirhizotron method has received minimal attention as a method for *in situ* evaluation of turfgrass root systems (Branham et al., 1986; Hendricks and Branham, 1987). Minirhizotrons offer the advantages of reduced costs and destruction of the experimental site, and greater portability and replication (McMichael and Taylor, 1987).

This study evaluated the minirhizotron method as a means of measuring rooting in a 'Penncross' creeping bentgrass (*Agrostis palustris* Huds.) putting green and compared the minirhizotron method to destructive soil core sampling.

MATERIALS AND METHODS

The study was initiated in April 1986 on a 5-year old 'Penncross' creeping bentgrass green (*Agrostis palustris* Huds.) grown on a modified loamy sand soil (original soil was a fine-loamy, mixed, mesic, Typic Hapludalf). On 26 and 27 April, 1986 three butyrate tubes of a 0.91 m length and 51 mm i.d. were centrally installed within 3.7 by 4.6 m plots. Fifteen plots were used for a total of 45 minirhizotrons. An installation angle of thirty degrees from horizontal was used to minimize preferential root growth along minirhizotrons. The entry ports of the minirhizotrons were installed facing north and just below the turf surface to minimize exposure to thermal radiation and to facilitate mowing, respectively. Pilot holes were bored with a Giddings probe and lightly brushed to alleviate soil smearing along the wall of the pilot hole. The plot area received 3 passes of 0.68 t water-filled rollers to smooth the surface and ensure soil/tube contact at the surface.

Traffic was simulated with compaction treatments performed June through September in 1986 totaling 70 passes and June through August for 54 passes in 1987 utilizing the water-filled rollers. Nitrogen was applied at 9.8 and 14.7 g m^{-2} in 1986 and 1987, respectively. Phosphorus and potassium were applied to meet soil test recommendations. The green was maintained at a 6 mm cutting height. Pesticides were used as needed to control insects, weeds, and diseases.

Minirhizotron root observations (MRO) were made 27 May and 12 Oct., 1987 using the minirhizotron video recording system described by Ferguson and Smucker (1989). The number of active roots in each viewing frame was counted and the frames corresponding closest to the appropriate depth interval (4 frames / 24 mm) was summed for a total number of roots observed. Active roots were considered to be those roots white in color and having sharp edges. As roots aged they became darker in color and began to lose definition as the edges became blurred. Roots of dark color and having blurred edges were considered inactive and not counted.

Three soil samples per plot were taken within 3 days of video recording for root length and weight determinations. Each 20 cm^2 by 300 mm deep subsample was sectioned into 25 mm intervals over the first 75 mm of depth and then 75 mm intervals over the remaining 225 mm. Roots were separated from soil with the hydropneumatic elutriation system (Smucker et al., 1982) and stored in a 4 degree C cooler until length and weight measurements could be performed. Unfortunately, the 0 to 25, 25 to 50, and six of the 50 to 75 mm samples from the late-May sampling were improperly stored. This improper storage resulted in a marked reduction in root weight density (RWD). Root length density (RLD) was determined using the line-intersect method (Newman, 1966; Tennant, 1975). RLD and RWD represent root length and weight determinations on a soil volume basis.

RESULTS AND DISCUSSION

The correlations of root weight density (RWD), root length density (RLD), and minirhizotron root observations (MRO) at the various depth zones are presented in Tables 1.1 and 1.2 for the May and October sampling dates, respectively. Generally, correlation of RWD and RLD were very highly significant at individual depth zones for the May sampling. However, the October sampling showed only two depth zones with a significant correlation between RWD and RLD. This could result from a more uniform root morphology and age early in the growing season (May) compared to later in the season (October). Visual observations

Table 1.1. Correlation of root weight density (RWD), root length density (RLD), and minirhizotron root observations (MRO) at various depth zones; sampled late-May 1987.

Depth zone (mm)	Intercept	Slope	Correlation
RWD (mg cm^{-3}) vs RLD (m cm^{-3})			
0 to 25[z]	1.08	0.75	0.829 ***
25 to 50[z]	0.31	0.82	0.882 ***
50 to 75[y]	0.51	0.24	0.555 NS
75 to 150	0.17	0.08	0.595 *
150 to 225	0.07	0.10	0.792 ***
225 to 300	0.02	0.32	0.869 ***
0 to 75[x]	0.07	0.97	0.944 ***
50 to 300[w]	-0.07	0.39	0.954 ***
RWD (mg cm^{-3}) vs. MRO (# cm^{-2})			
0 to 25[z]	23.90	-0.93	-0.339 NS
25 to 50[z]	16.55	-1.56	-0.198 NS
50 to 75[y]	9.57	-1.33	-0.246 NS
75 to 150	4.17	-1.61	-0.344 NS
150 to 225	0.26	0.28	0.121 NS
225 to 300	0.13	0.28	0.155 NS
0 to 75[x]	9.32	2.17	0.509 **
50 to 300[w]	-0.17	1.64	0.816 ***
RLD (m cm^{-3}) vs. MRO (# cm^{-2})			
0 to 25	24.17	-0.98	-0.323 NS
25 to 50	20.03	-3.31	-0.392 NS
50 to 75	10.28	-3.76	-0.278 NS
75 to 150	6.27	-14.88	-0.456 NS
150 to 225	0.11	-2.43	0.139 NS
225 to 300	0.08	1.39	0.287 NS
0 to 300	0.99	4.49	0.847 ***

*, ** and *** represent significance at the 0.05, 0.01, and 0.001 probability level, respectively.

NS designates not significant.

z, note root weights reduced during storage.

y, n=12

x, n=33 (improperly stored samples)

w, n=57 (properly stored samples)

Table 1.2. Correlation of root weight density (RWD), root length density (RLD), and minirhizotron root observations (MRO) at various depth zones; sampled mid-October 1987.

Depth zone (mm)	Intercept	Slope	Correlation
RWD (mg cm^{-3}) vs RLD (m cm^{-3})			
0 to 25	4.44	0.04	0.120 NS
25 to 50	1.89	0.17	0.314 NS
50 to 75	0.24	0.56	0.720 **
75 to 150	0.16	0.21	0.689 **
150 to 225	0.09	0.11	0.443 NS
225 to 300	0.04	0.14	0.198 NS
0 to 300	0.13	0.53	0.977 ***
RWD (mg cm^{-3}) vs. MRO (# cm^{-2})			
0 to 25	12.95	-0.17	-0.072 NS
25 to 50	0.43	1.52	0.294 NS
50 to 75	-0.11	1.64	0.442 NS
75 to 150	3.55	-2.30	-0.461 NS
150 to 225	0.80	-0.39	-0.069 NS
225 to 300	0.45	-2.13	-0.205 NS
0 to 300	0.74	1.20	0.918 ***
RLD (m cm^{-3}) vs. MRO (# cm^{-2})			
0 to 25	14.80	-0.71	-0.088 NS
25 to 50	-2.40	3.37	0.353 NS
50 to 75	1.87	0.98	0.206 NS
75 to 150	2.43	-1.92	-0.116 NS
150 to 225	-0.61	11.68	0.526 *
225 to 300	0.50	-3.88	-0.263 NS
0 to 300	0.48	2.26	0.928 ***

*, ** and *** represent significance at the 0.05, 0.01, and 0.001 probability level, respectively.
NS designates not significant.

found a large number of white, slender, actively growing roots in May while roots appeared darker, thicker and more contorted in October.

The root system of creeping bentgrass is reported to replace itself annually (Beard, 1973). During the growing season, roots are subjected to numerous stresses which can influence root diameter, branching, and mortality, thereby altering root weight and length relationships (Russell, 1977). Spring measurements may reflect a more homogeneously developed root system before climatic and soil stresses act to vary root system weight and length relationships. Hendricks (1988) found similar RLD values under a creeping bentgrass fairway turf. Regression analysis of his data showed RLD was significantly correlated with MRO in the first three 25 mm depth zones, but the 0 to 25 mm zone was negatively correlated. No correlations were found at depths below 75 mm in his data. Combining the various depth zones in this study demonstrated a good relationship between RWD and RLD for both sampling dates (Tables 1.1 & 1.2; Figure 1.1).

Only one depth zone in the October sampling showed a significant correlation between RLD and MRO, while no significant correlations were observed between RWD and MRO at individual depth zones for both sampling dates. Most likely, the spatial variation in soil conditions and rooting between the minirhizotrons and actual soil sampling sites was too large for the small depth intervals examined in this study. When root measurements were combined over all depth intervals, RWD and RLD were both significantly correlated with MRO for both the May and October sampling dates (Tables 1.1 & 1.2; Figures 1.2 & 1.3). The greater slope for the correlation of RWD and MRO in May (Table 1.1) compared to the October correlation (Table 1.2) suggested a greater

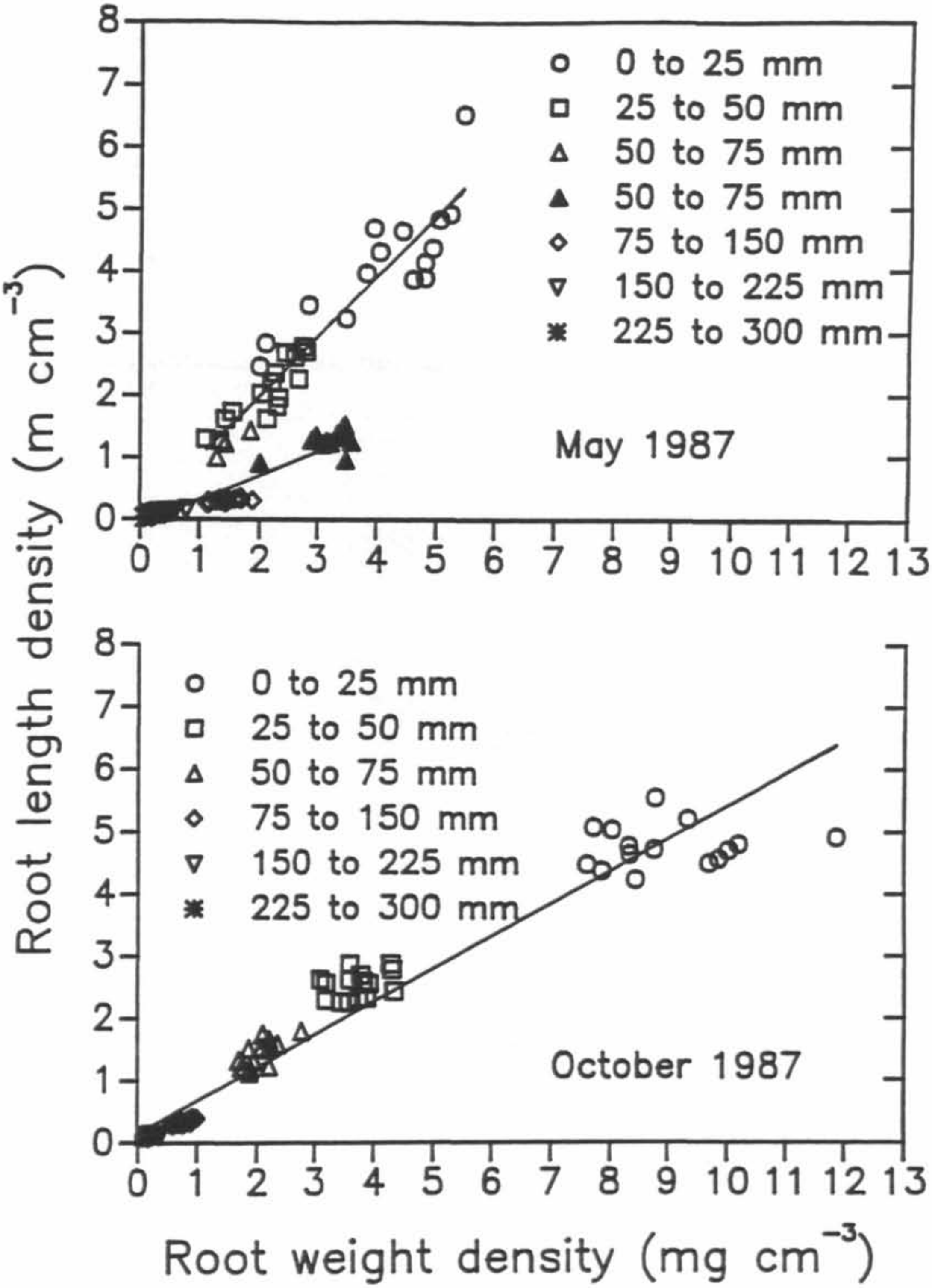

Figure 1.1. The relationship of root weight density and root length density of a creeping bentgrass green in late-May and in mid-October 1987. Note: Open circles, squares, and triangles denote data which lost weight due to improper storage of late-May samples.

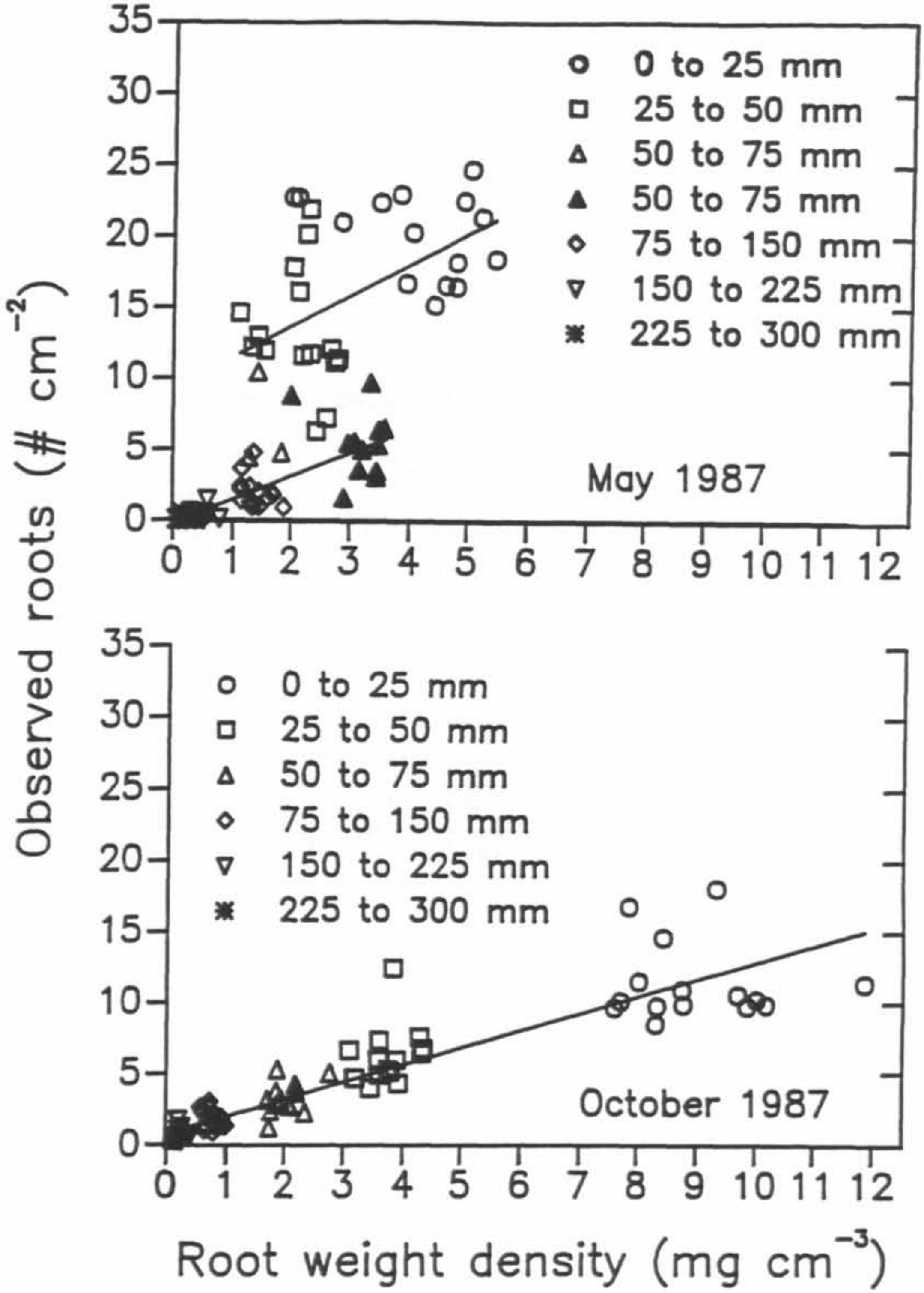

Figure 1.2. The relationship of root weight density and minirhizotron observations of a creeping bentgrass green in late-May and mid-October 1987. Note: Open circles, squares, and triangles denote data which lost weight due to improper storage of late-May samples.

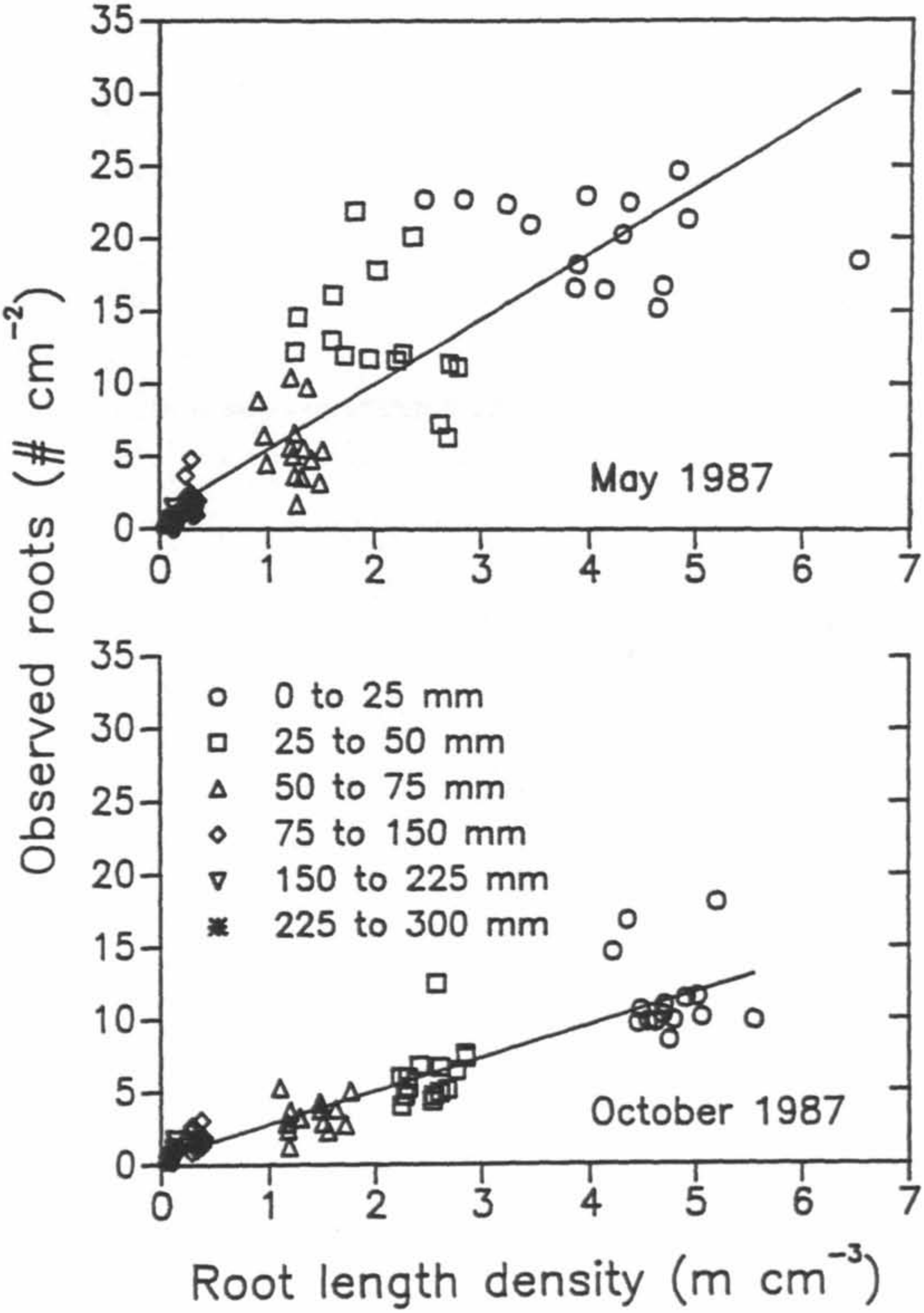

Figure 1.3. The relationship of root length density and minirhizotron observations of a creeping bentgrass green in late-May and mid-October 1987.

number of roots per unit weight of roots in the spring (Figure 1.2). This would be expected when a root system is in its early stages of annual development and roots have not reached their maximum volume and weight for the season. The slope for the RLD and MRO correlation (Figure 1.3) was considerably larger in May (Table 1.1) than October (Table 1.2) indicating that a single relationship between the two methods does not exist throughout an entire growing season.

Figure 1.4 displays graphically the grand means of all three root measurements at the various depth zones for the May and October sampling dates. RWD for the 0 to 25 and 25 to 50 mm depth zones were not plotted because of the confounding weight loss during storage of the May root samples. The data show that MRO and RWD were greater in May than October, while RLD did not indicate a decrease in October root quantity. Using a rhizotron, Koski (1983) observed that the seasonal development of root length under a creeping bentgrass turf had two peak periods of activity. The first and greatest period occurred March through June. The second peak of activity occurred during October but was smaller in magnitude. Garwood (1967) observed similar seasonal fluctuations in active root length of several cool-season grasses. In this study, RLD from soil samples would appear to be insensitive to changing active root quantities, while MRO and RWD appeared to be better indicators of active root quantity throughout the growing season. In a perennial turfgrass system, RLD may be a poor index of active roots because it is difficult to distinguish between active and senescent roots during counting. Senescent roots along minirhizotron tubes are not readily visible because their darker color blends into the background color of the soil reducing the chance of being counted.

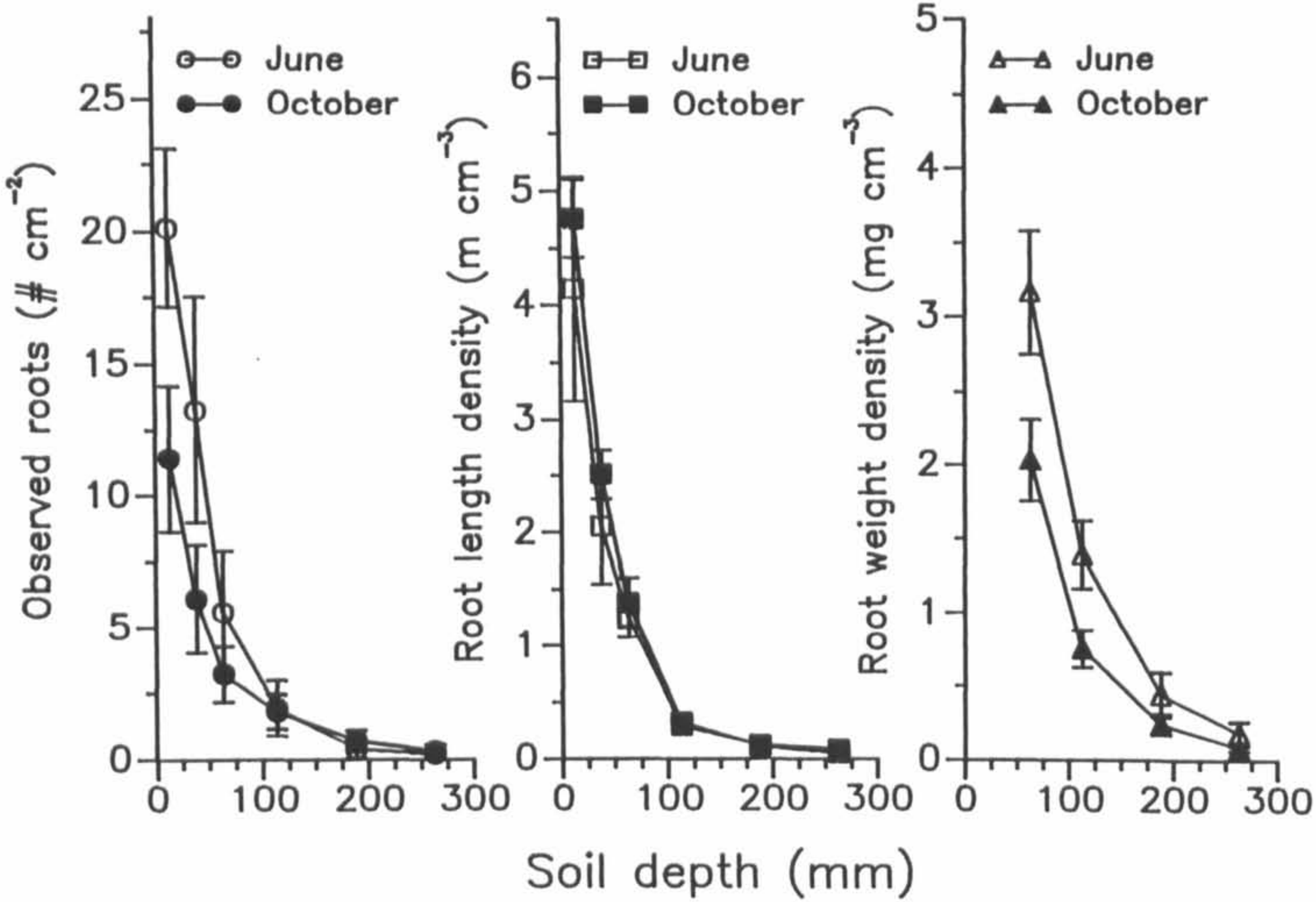

Figure 1.4. The grand means of minirhizotron observations, root length density, and root weight density at individual depth zones in late-May and mid-October 1987.

However, RWD may reflect changes in active root quantities because senescent roots lose weight as cell tissue decays and sloughs off. Minirhizotrons and other "glass wall" methods are considered to be the most suitable method for studying root phenology (Bohm, 1979).

SUMMARY

Root weight density (RWD) and root length density (RLD) at individual depth zones were significantly correlated in May but not in October. Reasons for the lack of correlation in October might have been due to greater heterogeneity in root morphology and age later in the growing season.

Few significant correlations were found between RWD and minirhizotron root observations (MRO) and no correlations were observed between RLD and MRO at individual depth zones. Spatial variation in soil conditions and rooting between the minirhizotrons and soil sampling sites was thought to be the reason for these poor relationships at the small depth intervals examined.

When data was combined across all depth zones, correlations between RWD and RLD, RWD and MRO, and RLD and MRO were all highly significant. These results indicated that the minirhizotron method provided an acceptable means to characterize the profile distribution of the root system of a creeping bentgrass green. The minirhizotron method offers the advantage of repeated sampling at the same site, enabling the researcher to observe more easily the seasonal growth dynamics of root systems. These types of observations can assist in evaluating management programs which may affect rooting and subsequently, water use efficiency.

Seasonal changes in slope indicated that no single relationship between RLD and MRO can be expected. The lack of change in RLD measurements between May and October suggested that RLD may be insensitive to temporal fluctuations in "active" root quantity. MRO and RWD demonstrated greater root quantities in May than October and therefore should be better indices of root quantity.

The minirhizotron method provides many advantages for root growth research. However, as with all methods there exist limitations. Long term observation of this creeping bentgrass root system found a large accumulation of roots along minirhizotrons at the 0 to 48 mm soil depth zone. Early in the spring it was impossible to determine the total number of roots present because the entire viewing area was full of entwined roots. It was also observed that operator consistency in counting the roots on video was poor between operators, particularly for images containing a large number of roots. Therefore, it is best to have one person for processing video images.

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