

ROOT SEGREGATION OF C3 AND C4 SPECIES USING CARBON ISOTOPE COMPOSITION

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Abstract

Partitioning roots for studying cropping systems containing more than one species is important since root growth interaction could influence system performance. The study objective was to test a method for segregating plant species roots from soil samples taken in a mixed stand of corn (*Zea mays* L.), a C4, and kura clover (*Trifolium ambiguum* M. Bieb.), a C3 plant. Soil cores containing both corn and kura clover roots were obtained at three distances from the corn row and at two depths in a Rozetta silt loam soil (moderately well drained, fine-silty, mixed, superactive, mesic Typic Hapludalf). Root composition of these C4 and C3 species was based on $^{13}\text{C}/^{12}\text{C}$ ratios expressed as $\delta^{13}\text{C}$. A significant linear relationship ($r^2 = 0.99$) was found between the $\delta^{13}\text{C}$ and the percentage of corn roots in samples containing known ratios of corn and kura clover roots. This relationship was used to determine corn and kura clover root percentages in field samples. Ratios of $^{13}\text{C}/^{12}\text{C}$ effectively segregated corn and kura clover root materials obtained from soil samples and seem to be a powerful tool for partitioning roots of C3 and C4 plants in similar studies.

PARTITIONING ROOTS for studying cropping systems containing more than one species is important since root growth interaction could influence system performance. Root growth pattern studies have addressed a variety of soil and crop management related issues (Barber, 1971; Vepraskas and Waggoner, 1990; Dwyer et al., 1995); however, none of these studies separated different plant species concurrently growing in the same area. While this is not a problem with root studies involving monoculture-cropping systems, it does pose a significant dilemma when studying cropping systems containing more than one species. This is especially true if rooting pattern information for the individual crops is desired. Differences in stable carbon isotope ratios ($^{13}\text{C}/^{12}\text{C}$) between photosynthetic types can be used to quantify the proportion of C3 and C4 species contributing to a mixture of plant material (Ludlow et al., 1976; Ode et al., 1980). During photosynthesis, carbon dioxide fixation of C3 species discriminates against the heavier isotope (^{13}C) more than do C4 species. Stable carbon isotope composition ($\delta^{13}\text{C}$), that is, the ratio of $^{13}\text{C}/^{12}\text{C}$ relative to that found in the PDB (Pee Dee belemnite) standard,

is measured by mass spectrometry. The standard is a fossil carbonate deposit, which has a $^{13}\text{C}/^{12}\text{C}$ ratio of 0.0112372.

$$\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \quad [1]$$

where $R = ^{13}\text{C}/^{12}\text{C}$.

As a result of this fractionation of stable isotopes during photosynthesis, values of terrestrial plants fall into two discrete ranges; values of C3 plants are between -23 and -34‰ , whereas C4 plants range from -9 to -17‰ (Bender, 1968; Smith and Epstein, 1971; O'Leary, 1988). Negative values of $\delta^{13}\text{C}$ indicate that the plant material is depleted in ^{13}C compared with the PDB standard.

The stable carbon isotope composition ($\delta^{13}\text{C}$) method has been used as a tool to study the relative proportions of cool season (C3) and warm season (C4) species in diet selection by mammalian herbivores (Ludlow et al., 1976; Tieszen et al., 1979), grassland insects (Boutton et al., 1983), and collembolan species (Briones et al., 1999); to study C3 and C4 plant community composition changes through soil organic matter composition assessment (Dzurec et al., 1985); to follow C dynamics in the soil (Balesdent and Mariotti, 1996; Harris et al., 2001); to evaluate plant contribution of C3 and C4 species to aboveground biomass (Ludlow et al., 1976; Ode et al., 1980) and belowground root mass (Ludlow et al., 1976; Svejcar and Boutton, 1985; Dzurec et al., 1985; Wong and Osmond, 1991; Polley et al., 1992); rooting dynamics (Svejcar et al., 1988); and rooting patterns (Mordelet et al., 1997) in species mixtures. This latter could be particularly relevant for a cover crop system of C3 and C4 species where root measurements of stable carbon isotope composition ($\delta^{13}\text{C}$) could allow root mass partitioning between these species types.

A cropping system for which carbon isotope composition may be useful for root segregation is corn (*Zea mays* L.) with a living mulch of kura clover (*Trifolium ambiguum* M. Bieb.). Kura clover has a C3 photosynthetic pathway, whereas corn is a C4 plant. Kura clover is a persistent perennial rhizomatous forage with deep rooting (Speer and Allinson, 1985; Taylor and Smith, 1998). This system, when appropriately managed to selectively suppress the kura clover living mulch, has potential to produce favorable corn yields and maintain constant ground cover favorable to soil conservation (Zemenchik et al., 2000; Affeldt et al., 2004).

The objective of this study was to test a method for segregation of root material from soil samples taken from a living mulch system of C3 (kura clover) and C4 (corn) species.

Materials and Methods

Plot Management

The living mulch plots of corn and kura clover used to test the method were located at the University of Wisconsin, Lancaster Agricultural Research Station ($42^{\circ}50' \text{ N}$; $90^{\circ}47' \text{ W}$; elev., 325 m) on a Rozetta silt loam soil. Mean annual precipitation at this location is 762 mm and mean annual temperature is 7.7°C .

Liberty Link {glufosinate [2-amino-4-(hydroxymethyl)phos-

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Published in Crop Sci. 45:879–882 (2005).

doi:10.2135/cropsci2004.0170

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phanyl) butanoic acid]-resistant} corn (Pioneer hybrid 36H75) was sown on 5 May 2000 into a stand of 'Rhizo' kura clover that had been established in 1994. Kura clover received two different herbicide suppression treatments. Glyphosate [N-(phosphonomethyl)glycine] and dicamba [3,6-dichloro-2-methoxybenzoic acid] applications were made immediately before planting, band applications with dicamba and clopyralid [3,6-dichloro-2-pyridinecarboxylic acid] were made immediately after planting, and glufosinate applications were made when corn reached the V3 stage (Ritchie et al., 1993). Affeldt et al. (2004) describe details regarding herbicide application rates. Plots contained four 8-m long corn rows with 0.76-m spacing.

The first treatment (band suppression) resulted in an approximately 99% desiccation of the kura clover shoots in a 0.25-m wide band centered over the corn row. Percentage kill estimates were based on visual evaluation. This strip provided spatial separation between the emerging corn and the untreated kura clover. The second herbicide treatment (broadcast suppression) resulted in approximate 99% desiccation of the kura clover shoots throughout the plot. Total precipitation in May and June was approximately twice the 30-yr mean providing sufficient moisture for the kura clover to recover from herbicide suppression and grow vigorously in the summer (Affeldt et al., 2004).

Root Sampling and Processing

Soil cores were obtained with an Uhland core sampler in late July 2000 when corn was approximately 2.20 to 2.40 m tall and silking, at the R1 stage of development (Ritchie et al., 1993). Cylindrical soil cores were 7.6 cm in diam. by 7.6 cm in length (0.35-L volume), and were obtained between plants within the corn row and at 0.19- and 0.38-m distances from the row. At each of these three positions relative to the corn row, samples were obtained at two depths, 0.025 to 0.100 m and 0.125 to 0.200 m. Overall, 240 samples were taken to conduct the experiment. For each combination of position relative to the row and depth, five randomly located subsamples were taken along the corn row that were individually analyzed for stable carbon isotope composition. If random subsampling location within the row corresponded with a corn plant, the subsampling location was moved to the side of the plant and remained within the row.

The bulk density and water content of each core was measured. A subsample was taken from each core, weighed, oven dried at 105°C, and reweighed to determine the water content. The soil water content of the core was assumed to equal that in the subsample and the bulk density was calculated from the water content and known volume of the core.

The remaining portion of the cores was stored at 4°C. Roots were separated from soil using a hydropneumatic root elutriator (Smucker et al., 1982) fit with a 0.930-mm screen. Live roots were manually sorted from nonroot and dead-root materials based on the color of the cortex and other visual evidence (Tufekcioglu et al., 1999). Live roots were whitish- or pale-colored, elastic, and were free of decay. Dead roots were brown or black, brittle, and were in various stages of decay. All living sampled material was considered root material, recognizing a portion of the kura clover sample may be rhizomes. The live root material from each sample was oven dried at 65°C in a paper coin envelope and, after desiccation, weighed on an analytical balance with precision of 1.0×10^{-4} g. Each sample was then ground with an Intermediated Wiley Mill (A.H. Thomas Co., Philadelphia, PA) and passed through a 1-mm screen. One representative subsample from each ground root sample was placed in a tin capsule; the capsules were crimped and sent for stable carbon isotope (^{13}C) analysis to

UC Davis Stable Isotope Facility, Davis, CA. The analysis was done with Europa Scientific Integra, a continuous flow Isotope Ratio Mass Spectrometer (IRMS) integrated with on-line sample combustion (PDZ Europe, Cheshire, England). The $\delta^{13}\text{C}$ values were expressed relative to the international Pee Dee Belemnite (PDB) standard using per mil units (Craig, 1957).

To determine the percentage of corn roots in each field sample, the relationship between $\delta^{13}\text{C}$ and percentage of corn roots in a given sample containing corn and kura roots was required. Corn and kura clover roots were dug from within the plot area and root systems were carefully washed. Roots that remained attached to kura clover or corn plants were harvested and processed separately (as described above for the field samples). After grinding, seven mixtures of known percentages of corn and kura clover roots were made. Gravimetric mixture ratios were (corn/kura clover) 0:100, 10:90, 30:70, 50:50, 70:30, 90:10, and 100:0. There was one analysis for each mixture. These samples were processed for carbon isotope analysis as were the field samples described above. A regression equation was developed for $\delta^{13}\text{C}$ vs. corn root percentage obtained from these mixtures of corn and kura clover root material. This regression equation was the basis for determining corn root percentage in each of the plot-obtained soil cores.

Statistical Analysis

The living mulch experimental plots were arranged in a randomized split plot design with four replications. The different kura clover herbicide suppression treatments (main plots) were split by sampling positions relative from the corn row. An ANOVA was performed on SAS version 8.2 (SAS Institute, 2001) using PROC MIXED due to multiple random effects of the model. Treatment, distance, depth, and their interactions were the fixed effects used in the model. There were three random effects in the model due to the split plot design of the experiment. The random effects were replication (treatment), replication \times distance (treatment), and residual error. For the analysis of corn and kura clover root mass, the response variables were log transformed to improve the normality of the residuals. Differences in treatments were considered significant at a probability level of 0.05.

Results and Discussion

Because C3 species discriminate more than C4 species against the heavier isotope, we anticipate a functional relationship between the proportion of C4 material and the amount of ^{13}C found in the mixture similar to that observed by (Ludlow et al., 1976). The linear regression between $\delta^{13}\text{C}$ and corn root percentages is shown in Fig. 1. A strong linear relationship ($r^2 = 0.99$) between $\delta^{13}\text{C}$ and percentage corn roots in the known mixtures is evident. The relationships of root materials between C3 and C4 species are similar to that observed by (Svejar and Boutton, 1985). Additionally, the least squares fit in these relationships across studies have been very good, $r^2 = 0.97$ or greater. This well-defined relationship demonstrates that carbon isotopes can be used to quantify the proportions of roots from a mixed culture of C4 and C3 species. The practicality of using carbon isotope composition to monitor root distribution in a mixed corn and kura clover system was further examined.

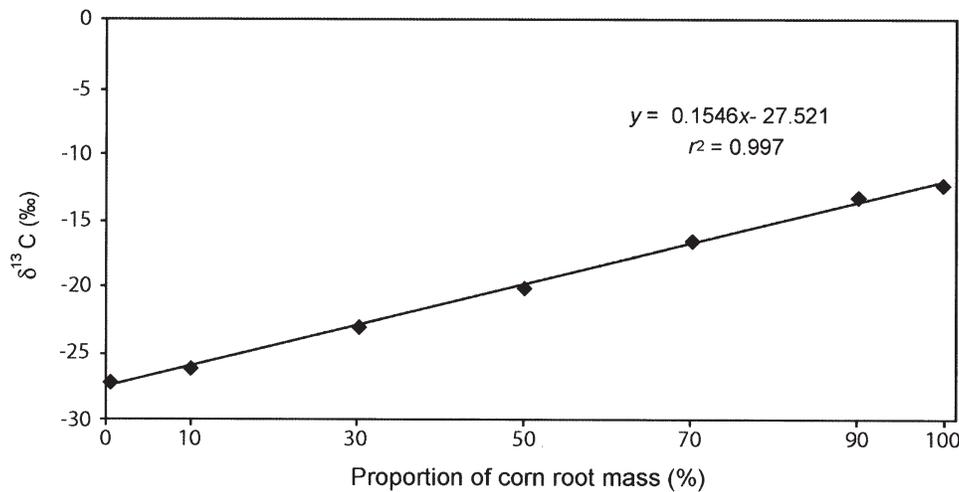


Fig. 1. Relationship between $\delta^{13}\text{C}$ and the percentage of corn mass in samples containing known amounts of corn and kura clover root material.

Bulk Density

Soil core bulk density means were of 1.25 Mg m^{-3} for shallower and 1.29 Mg m^{-3} for the deeper depth ($P < 0.05$). Position relative to the corn row was not significant ($P < 0.05$), suggesting that there were no wheel track effects on bulk density in the sampled area. Increased soil bulk density from wheel traffic can alter corn root growth patterns (Chaudhary and Prihar, 1974; Bauder et al., 1985); therefore, it was important to determine if wheel tracks affected root growth in this study.

Corn Root Mass

For the broadcast herbicide treatment, the $\delta^{13}\text{C}$ values ranged from -18.05 to -28.87% for the shallower and from -16.90 to -21.57% for the deeper depth. For the band herbicide treatment, the $\delta^{13}\text{C}$ values ranged from -18.00 to -20.13% for the shallower and from -18.37 to -19.58% for the deeper depth.

Maximum root mass occurred in the row position and root mass decreased with depth and distance from the row (Table 1), consistent with published data (Barber, 1971). Corn root growth typically decreases with depth and distance from the plant (Barber, 1971). Root growth patterns with distance from the row, based on data in Table 1, become more evident when potential root

Table 1. Corn and kura clover root mass with distance from the corn row and depth as affected by suppression of kura clover (herbicide treatment).

Crop	Herbicide treatment	Depth	Distance from row, m		
			0	0.19	0.38
— $\text{g} \times 10^{-3} \text{ cm}^{-3}\ddagger$ —					
Corn	broadcast	0.025–0.100	1.40 \ddagger	0.29	0.28
		0.125–0.200	0.17	0.25	0.18
	band	0.025–0.100	0.35	0.26	0.27
		0.125–0.200	0.27	0.06	0.17
Kura clover	broadcast	0.025–0.100	1.03	1.61	1.53
		0.125–0.200	0.12	0.38	0.24
	band	0.025–0.100	0.24	0.38	0.59
		0.125–0.200	0.28	0.06	0.20

\ddagger Multiply the reported numbers by this to obtain the actual numbers.

\ddagger Values are averages of 20 cores from subsampling locations for each position across four replications.

growth from the adjacent rows is considered. The samples obtained 0.38 m from the row are equidistant from two adjacent rows. Thus, these samples are composed of corn roots from both rows; that is, the mass reported for the 0.38-m distance from the row is approximately twice the expected value if only one row existed. While the adjacent corn row could also affect the amount of corn root material observed in the sample obtained at the 0.19-m position, the distance from the adjacent corn row, 0.57 m, strongly suggests this effect would be considerably less than at the 0.38-m position. Both depth and distance effects were statistically significant, while the kura clover suppression treatment effect was not (Table 2).

Kura Clover Root Mass

As with corn, kura clover root mass was significantly affected by depth (Table 2). Averaged across suppression managements, greater kura clover root mass existed in the 0.025- to 0.100-m depth increment than at the deeper depth (Table 1). Because of the spreading growth pattern of kura clover and its spatially uniform coverage (before herbicide application treatments), the relationship between kura clover root mass and row position is more difficult to predict. Past research documents that herbicide rates used in these cropping systems only temporarily restrict top growth (Affeldt et al., 2004). This study suggests application rates used were not sufficient to dramatically affect belowground growth, probably because these plant materials were protected from di-

Table 2. Statistical analysis for corn and kura clover root mass as affected by suppression of kura clover, distance from the corn row and depth from the soil surface.

Effect	df	Corn		Kura clover	
		F value	P > F	F value	P > F
Treatment	1	0.89	0.3798	3.36	0.1157
Distance	2	3.71	0.0354	0.19	0.8276
Treatment \times distance	2	0.96	0.3935	3.08	0.0599
Depth	1	9.80	0.0038	10.66	0.0027
Treatment \times depth	1	0.02	0.8980	0.97	0.3317
Distance \times depth	2	0.53	0.5952	0.06	0.9453
Treatment \times distance \times depth	2	2.47	0.1012	0.80	0.4587

rect herbicide contact. This may help explain the relatively quick regrowth experienced with this band-suppressed kura clover living mulch system. A significant kura clover management treatment effect on kura clover root growth did not exist, a reasonable finding for a living mulch cropping system and for a cover crop with rhizomes and known vigorous recovery after suppression (Zemenchik et al., 2000).

Conclusions

Carbon isotope ratios seemed well suited to segregate corn and kura clover roots obtained from soil in which both crops were growing concurrently. Samples with known amounts of C₄ and C₃ root materials produced a well-defined relationship between $\delta^{13}\text{C}$ and known proportions of C₄ root material. This relationship, coupled with field sampling and published root separation methods, detected rooting patterns consistent with those found in the literature. The method detected expected root growth patterns, that is, reduced corn root mass with depth and distance from the plant, while also detecting varying amounts of kura clover roots with depth. This method is recommended for evaluating the root mass proportion of C₃ and C₄ species existing in the same soil volume.

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