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Loss of dry matter and cell contents from fibrous roots of sugar beet due to sampling, storage and washing*

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Abstract

To obtain correction factors for estimating root dry weight from washed samples and to test the efficiency of various procedures for storing root samples, dry matter losses were determined by simulating root washing methods with roots obtained from a nutrient culture. For sugar beet dry matter losses were higher than values previously found for wheat and ryegrass: about 30% for the procedure normally used and about 40% for samples pretreated with sodium pyrophosphate. The largest share of water-soluble sugars was lost from root samples within one day of storing roots. The N content of roots expressed on the basis of remaining dry matter rose first during handling of the root samples and decreased in samples stored for a longer period. In most cases no cell wall material (cellulose and lignin) is lost from the root samples; expressed on the basis of remaining dry weight the contents consequently rose.

Introduction

For the calculation of shoot/root ratios of plants and of the carbon balance of agro-ecosystems (Brussaard *et al.*, 1988) reliable estimates of root dry weight in the field are required. Bias due to the loss of rootlets during washing of root samples is often recognized as a problem; by using fine-meshed sieves such losses can be kept to a minimum (Schuurman and Goedewaagen, 1971). Without loss of rootlets, a considerable loss of root dry weight is still possible due to loss of part of the cortical and epidermal cells, including root hairs, root caps and associated mucilage and/or by loss of cell contents from all remaining root cells. Dry matter losses due to standard techniques for manipulation of root samples at our laboratory have been investigated for wheat (Van Noordwijk and Floris, 1979), ryegrass (Floris and De Jager, 1981), cucumber and tomato. As the diameter of ryegrass roots did not change during simulated washing and storage methods (Floris and De Jager, 1981), loss of cortex and epidermis probably is not a major factor responsible for loss of dry matter. In this article losses from fibrous roots of sugar beet and the possible nature of these losses will be discussed.

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Methods

Sugar beet, cv Regina, were sown in paper pots (diameter 2 cm, height 13 cm, filled with a sandy soil); after six weeks the young plants were transferred first to 1-l and later to 10-l pots filled with an aerated nutrient solution, containing the following concentrations of ions (m*M*/l): 10 NO_3, 1.2 H_2PO_4, 2.4 SO_4, 4.5 K, 3.5 Ca, 2.4 Mg and Fe, Mn, Zn, B, Cu and Mo as trace elements; the solution was replaced twice weekly. Growth of the plants was satisfactory (Table 1) and a normal taproot was formed; after 100 days the fibrous roots turned brown and started to disintegrate, so the experiment was terminated. At 39, 81 and 102 days after

Table 1. General characteristics of sugar beet growth; average and standard deviation per plant

Plant part	Days after start nutrient culture		
	39	81	102
Fresh weight, g plant $^{-1}$ (av. ± st. dev.)			
Leaves	80.3 ± 12.6	802 ± 153	1009 ± 186
Tap root	32.0 ± 8.7	971 ± 173	1923 ± 373
Fibrous roots	19.6 ± 3.9	99 ± 24	97 ± 34
Dry matter, %			
Leaves	9.5	9.0	10.7
Tap root	14.8	13.4	12.1
Fibrous roots	6.9	7.6	7.9
Rel. growth rate, g (g day) $^{-1}$			
Leaves	0.05	0.01	
Tap root	0.08	0.03	

the start of the nutrient culture a number of plants was harvested. Roots of the harvested plants were subjected to simulated washing and storage methods as used for pinboard and auger samples from the field; treatments included a pyrophosphate pre-treatment as used for washing roots from clay (Schuurman and Goedewaagen, 1971).

At each harvest root samples of approximately equal size were collected (at the first harvest consisting of a complete, at the second and third of half a root system) and the fresh weight was determined, after 30 s in a household centrifuge to remove excess water. Control samples were dried directly (48 h at 70°C); other samples were subjected to various treatments as listed in Table 2, before measuring a second 'fresh weight' and drying. The loss of dry matter due to treatments was calculated from sample fresh weight, sample dry weight and dry matter percentage of the control samples. All treatments were done in duplicate, the control in four replicates.

The chemical composition of the root samples was determined as regards water-soluble sugars (anthrone), total N content, and cellulose and lignin (Goering and Van Soest, 1970).

Results

Dry matter loss due to various treatments is shown in Table 3 and Fig. 1. Cutting the roots and storing them for 1 day at 20°C resulted in a dry matter loss of 5–11% (treatments AS ----- and PS -----). Subsequent washing increased losses only slightly; the pyrophosphate pre-treatment increased dry matter loss to about 20% before and 25% after washing. Storage of root samples for a considerable period in a freezer (treatment AS 4 - W - -) apparently does not lead to additional dry matter losses when the roots are thawed and washed. Thymol, a bactericide, when added to roots after washing and prior to storage at 20°C for two weeks, did not prevent the loss of a further 20% of dry matter (compare treatments PS - - W a - with PS - - W a c). Dry matter losses for 'auger' sample roots were generally smaller than those for 'pinboard' sample roots when the same steps in the procedure are compared. With various combinations of treatments as actually used for pinboard or

Table 2. Treatments in various stages

Stage	Symbol	Treatment
Sampling	**P**	**'Pinboard': cutting approximately 1/3 of the main axes**
	A	**'auger': cutting all roots to 5-cm pieces**
	S	**storing for 24 h at 20°C in a moist environment**
Storage	1	1 week at 20°C
before washing	2	2 weeks at 4°C
	3	drying at 20°C and rewetting in sodium pyrophosphate
	4	indeterminate period in a freezer
Pre-treatment	P	vacuum pretreatment overnight in 2.7 g l^{-1} sodium pyrophosphate ($Na_4P_2O_7$)
Washing	W	running tap water for 0.5 h ('auger') or 3 h ('pinboard')
Storage	a	1 day at 20°C
after washing	b	2 days in running tap water
	c	2 weeks in water of 20°C with thymol

Table 3. Mean loss of dry weight, as a percentage of initial dry weight, for various treatments; treatment codes as in Table 2

Treatment	Days after start culture			Average ± st. dev. of mean
	39	81	102	
PS - - - - -	10	10	12	10.7 ± 0.7
PS - - W - -	14	15	13	13.9 ± 0.7
PS 1 - W - -	45	44	35	41.5 ± 2.0
PS 2 - W - -	26	21	7	17.8 ± 3.2
PS 4 - W b -	30	36	29	31.8 ± 1.6
PS - - W a -	13	15	20	16.2 ± 1.8
PS - - W b -	23	31	21	24.8 ± 1.9
PS - - W a c	35	49	19	34.2 ± 5.2
PS - p - - -	25	24	10	19.8 ± 3.4
PS - p W - -	30	35	10	24.9 ± 4.0
PS - p W a c	39	41	46	42.2 ± 1.2
PS 1 p W - -	53	62	33	49.3 ± 4.7
PS 2 p W - -	33	34	27	31.2 ± 2.5
PS 4 p W a c	52	39	36	42.2 ± 3.4
AS - - - - -	7	16	−7	5.3 ± 3.9
AS - - W - -	1	16	0	5.7 ± 3.2
AS 3 - W - -	30	22	21	24.5 ± 1.8
AS 4 - W - -	16	7	10	11.3 ± 1.2
AS - - W a c	39	42	24	35.2 ± 3.2
AS - p W - -	24	27	15	21.9 ± 2.7
AS 3 p W a c	43	45	11	33.0 ± 6.9
AS 4 p W - -	33	37	37	35.7 ± 0.9

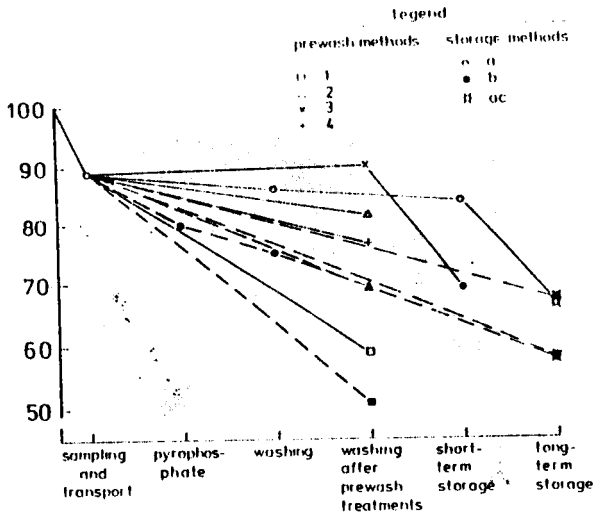

Fig. 1. Dry matter content of roots after various combinations of treatments, as a percentage of the control; *broken* lines and *closed* symbols refer to treatments that include pyrophosphate.

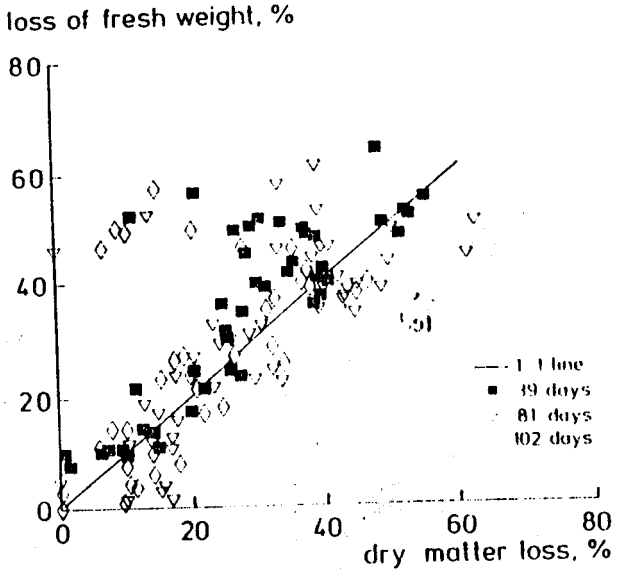

Fig. 2. Relation between loss of fresh weight and loss of dry weight from individual samples.

auger samples, dry matter losses of up to 50% were found. Dry matter losses at the first and second harvest were higher than those at the third harvest.

As Fig. 2 shows, relative loss of fresh weight from the samples was approximately equal to relative loss of dry weight; treatments that include storage in a freezer before washing resulted in a large loss of fresh weight and relatively small loss of dry matter (points in the upper left-hand corner of the graph). The apparent dry matter content of roots obtained with most of the treatments (final dry matter weight as a fraction of final 'fresh weight') is close to the original dry matter content, even if half the dry matter and half the fresh weight is lost. Somehow the loss of turgor and cell water is proportional to the loss of cell components. Treatments that include deep-freezing are an exception.

Table 4 shows the contents of several cell components. Water-soluble sugars were lost almost completely from samples stored for 1 day; in all other treatments losses were complete. N content (as a percentage of dry matter remaining) rose in several treatments by up to 10% of the original value; losses of N in these treatments were smaller than losses of dry matter. In treatments with a large loss of dry matter, the loss of nitrogen as a percentage of the initial amount present (dry weight times N content of control) was usually more than proportional to the loss of dry weight, and consequently the final N content was lower than the control value. Losses from cell wall material were

Table 4. Content of some components of fibrous root dry matter, after various treatments, expressed as a percentage of initial content and relative losses as a percentage of initial amount present

Days	Treatment	Total DM	Water-soluble sugars		N-total		Cellulose	
		%-loss	%	%-loss	%	%-loss	%	%-change
39	Control	0	3.28	0.0	4.70	0.0	14.5	0.0
	PS - - - - -	10	0.56	85	5.11	2.1	17.1	+5.8
	PS 2 - W - -	26	0.0	100	4.43	30	20.4	+4.5
	PS 1 - W	45	0.0	100	3.70	57	24.4	−7.8
	AS - W a c	40	0.0	100	4.02	49	23.1	−4.8
	PS - p W a c	39	0.0	100	4.06	48	24.8	+3.9
81	Control	0	1.02	0.0	5.24	0.0	17.7	0.0
	PS - - - - -	10	0.32	72	5.16	11	19.1	−2.6
	PS 2 - W - -	20	0.0	100	5.30	19	19.1	−14
	PS 1 - W - -	44	0.0	100	4.75	50	25.4	−19
	AS - - W a c	32	0.0	100	4.24	39	25.7	−4.2
	PS - p W a c	41	0.0	100	4.56	43	26.2	−13
102	Control	0	3.95	0.0	4.58	0.0	18.9	0.0
	PS - - - - -	12	0.0	100	4.31	17	22.8	+6.1
	PS 2 - W - -	7	0.0	100	4.64	5.9	21.7	+6.7
	PS 1 - W - -	35	0.0	100	4.87	31	23.3	−20
	AS - - W a c	24	0.0	100	5.04	16	21.5	−13
	PS - p W a c	46	0.0	100	4.69	45	25.4	−27

small. Cellulose contents as a percentage of dry matter remaining increased in all treatments; losses of cellulose occurred only in some treatments with a heavy dry matter loss. Lignin contents (not shown) initially were 1–4% for the three sample dates and rose in all treatments which led to a loss of dry matter; often the increase was higher than can be accounted for by the loss of dry matter. As 'lignin' contents are determined as the remaining fraction after various extractions, some doubts exist about the validity of the data.

Discussion

Part of the loss of root dry weight is doubtless due to root respiration during the first day after sampling. Barber and Martin (1976) reported dry matter losses of 7–13% in one day due to root respiration under sterile conditions. In the sugar beet experiment nearly all water-soluble sugar was lost in one day at 20°C. Based on normal respiration rates, a dry matter loss of around 10% in one day can be expected (Floris and De Jager, 1981). In an unpublished experiment with cucumber roots, however, a respiration inhibitor (0.1 m*M* KCN + 25m*M* salicyl-hydroxamate at pH 6.5) did not reduce dry matter loss during storage of roots for one day.

Initially no nitrogenous compounds were lost, and N contents consequently rose. The standard procedure for obtaining roots for chemical analysis, comparable to treatment PS--W-- with reduced intensity of washing, may thus lead to slight overestimates of N contents; at least no ground exists for the fear that N contents of roots will be underestimated by washing roots. Cellulose was lost only in the treatment involving two weeks' storage at 20°C with thymol added, and in the third sampling period when roots had started to decay. As in most treatments the loss of cell wall material was limited, we may conclude that by far the largest share of dry matter losses is made up of cell contents, not of root tissues such as epidermis or cortex. This conclusion is in line with the lack of change in root diameter due to washing and storage reported by Floris and De Jager (1981). Increase of lignin content as a percentage of dry matter during decomposition of organic residues is normal (Jawson and Elliot, 1986; Parr and Papendick, 1978), but the increase in absolute amount reported here cannot easily be explained.

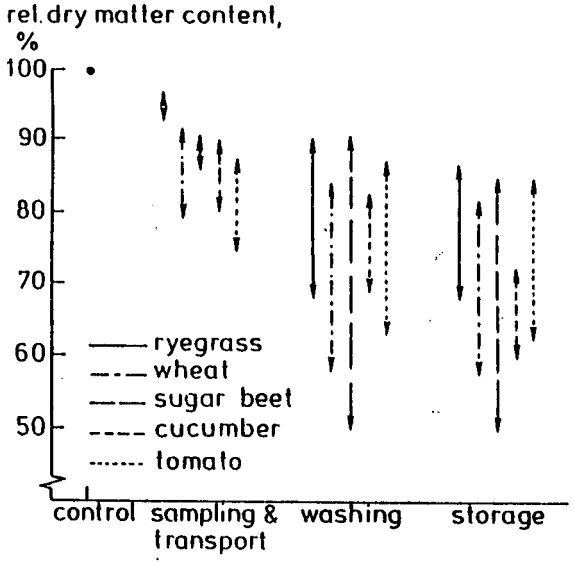

Fig. 3. Relative dry matter content of roots in various stages of the handling of root samples of five crop species; the range is given for various treatments.

Fig. 3 compares the range of dry matter losses due to various treatments in three stages of the handling of root samples from five crops. The sugar beet losses reported here are relatively high and vary widely. Therefore details of the washing and storage technique used should be known before appropriate correction factors can be chosen for the calculation of the carbon inputs into the soil. When estimates of root turnover are available, washed root samples can be used for estimating total root production. Combined with estimates of root exudates per unit root weight carbon input into the soil ecosystem during a growing season can be calculated (Van Noordwijk, 1987). For the estimates of root biomass present at a reference date, correction factors of 1.43 to 2.00 should be used to account for dry matter losses of 30 to 50% due to handling root samples.

The validity of applying such correction factors, obtained from roots grown on a nutrient solution, to roots obtained from the field may be questioned. As roots in soil may have a different structure and chemical composition, washing and storage methods may have a different effect. Because roots grown in the soil cannot be obtained in sufficient quantity without some storage, washing and handling of the samples, no adequate reference value of the dry matter content can be obtained for directly estimating the effect of such treatments. Indirectly, the relative weight of structural (cell wall) material and cell contents can be used as a criterion, as losses are largely made up of the latter fraction. Dry matter contents of field-grown roots may be considerably higher than the values reported here when water or nutrient uptake limit plant growth and sugars and non-structural carbohydrates accumulate in the root. Under such conditions we may expect relative dry matter losses to be higher than the values reported here.

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