PEPPERMINT RHIZOME STUDIES

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Abstract

Once established, peppermint (Mentha piperita, L) *grows anew from rhizomes each spring. Thus, it would be useful to develop methods to measure the energy stored in the peppermint rhizomes for regrowth. Etiolated growth measurements of non-structural biomass (NSB) have been shown to be effective for alfalfa taproots. Our objective was to compare NSB by this method with measurements of carbohydrate. Rhizomes were sampled monthly from four locations throughout a growing season. NSB was correlated (r=0.74) with the total nonstructural carbohydrate (TNC), and either method could be used to study energy storage, although the NSB was more sensitive to seasonal changes, and less technically demanding. Both NSB and TNC decreased in summer and increased in the fall as the plants stored carbohydrate for winter survival and regrowth.*

Introduction

Peppermint spring regrowth and subsequent yield are presumably dependent on the health of the rhizomes that survive winter, as observed previously (Mitchell, 1994). In this respect, peppermint is analogous to alfalfa *(Medicago sativa,* L), and considerable research has established the storage of non-structural carbohydrates as an important parameter in stand longevity and productivity (Heichel et al., 1988; Sheaffer et al., 1988). As in alfalfa, methods to evaluate non-structural carbohydrates of peppermint rhizomes would be useful for studying the effect of harvest timing and other agronomic practices. Because peppermint is planted from rhizomes, a test for regrowth potential would be useful for comparing rhizomes from different locations or treatments. Standard methods of sugar and total nonstructural carbohydrate (TNC) analysis may be helpful, and newer methods should be considered, such as NIR. spectroscopy and etiolated growth.

Etiolated growth of alfalfa has been proposed as a simple, inexpensive way to measure the non-structural biomass of alfalfa taproots (Rechel, 1993), that may be useful for peppermint rhizomes. Burton and Jackson (1962) used etiolated growth to measure the NSB of sod, and later updated the method to take direct measurements in the field (Burton, 1995). The measurement of NSB by etiolated growth has advantages of simplicity as well as cost, because it requires only a balance, a dark room, and containers.

The objectives of this study were to develop methods for measuring etiolated growth as a means of estimating non-structural biomass of peppermint rhizomes, and to compare NSB to measurements to laboratory measurements of 'INC for the growing season.

Materials and Methods

On 13 April 1995, four plots (1 m by 10 m) were chosen from an established field of 'Murray Mitcham' peppermint that had been planted in March 1994. The field had never been harvested. Plots were selected by visual inspection for uniform stand density. Sampling was repeated 25 May, 20 June, 20 July, 16 August, 14 September, and 12 October. Peppermint harvest occurred on 13 August.

Samples were dug from randomly selected areas of each replication. Each sample was dug from a 300 by 800-nun rectangle to a depth of 100 mm, and included rhizomes that were at least 200-mm long. Soil was washed from the samples, then eight rhizomes of at least 200-nun length were chosen from the sample. Fine roots and green stems were removed from all rhizomes. Four additional rhizomes (50 to 150-mm long) were also taken from the sample and freeze-dried for later analysis.

To estimate moisture content of each of the rhizomes, a 30-mm segment was removed, weighed fresh, dried in an oven at 75 °C overnight, and weighed again. The remaining rhizome was treated with fungicide by placing in a 1-L beaker of 6.0 g/L of pentachloronitrobenzene (PCNB, Terraclor 75 percent wettable powder, Uniroyal) for five minutes. Rhizomes were then planted 80 mm deep in trays of moist vermiculite, and placed in the dark at room temperature.

Etiolated rhizomes were observed periodically for an endpoint for growth, usually manifest by a blackening of the tip of the etiolated stem after four to five weeks. The plants were then separated into rhizomes or etiolated growth (comprising the newly developed shoots and roots), and the NSB (g/kg) was calculated as the difference between the initial and final dry mass divided by the initial dry mass. Etiolated growth (g/kg) was calculated as the dry mass of the

Table 1. **Correlation matrix for etiolated growth, sugars, extracted sugars, TNC, nitrogen, and NSB collected at Madras, OR, in 1995. Values presented are means of all replications for a single date.**

	Etiolated Growth	TNC	Soluble Sugar	Extracted Sugar	Nitrogen
NSB	0.62	0.74	0.51	0.45	0.72
Etiolated Growth	1	0.37	-0.05 (0.92)[1]	0.84	0.91
TNC	...	1	0.88	0.21	0.53
Soluble Sugar	...	...	1	-0.28	0.13
Extracted Sugar	...	...	...	1	0.79

[1]the number in parentheses refers to correlation excluding the October data—see text.

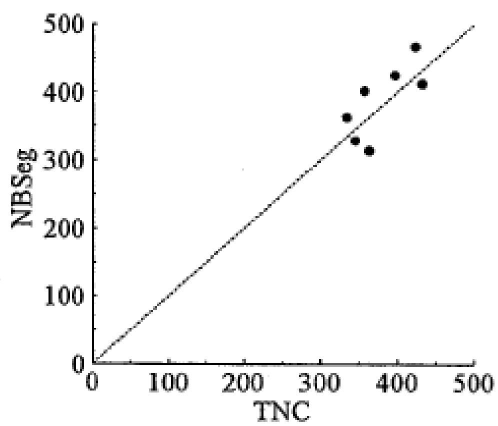

Figure 1. NSB vs. TNC.

growth divided by the initial mass. There may have been a problem in the data for the October rhizomes, which were inadvertently neglected towards the end of the growth cycle, and dried out before sampling. Some etiolated roots were difficult to remove from the vermiculite aggregates, possibly resulting in underestimates of mass etiolated growth. The associated rhizome sampling, and the NSB data which is not based on the direct measurement of mass etiolated growth, however, were not affected.

The four freeze-dried rhizomes of each replication were ground on a fine sieve prior to lab analysis. Soluble sugar content was determined by the method of Candolfi-Vasconcelos and Koblet (1990). TNC was measured after extraction with 1 M perchloric acid. The additional sugars that were detectable after extraction normally represents starch in most plants, but peppermint stores carbohydrate as fructan and in other forms. Here, this fraction will be referred to as "extracted sugars."

Correlation analysis was perfomed on Minitab (Minitab, Inc., State College, PA) according to the Pearson product moment.

Results and Discussion

Our hypothesis that NSB was related to measurements of TNC was supported by a high correlation coefficient (r=0.74) between the measurements (Table 1). Furthermore, the absolute value of NSB and TNC are very similar, as shown in the comparison in Figure 1. The NSB measurements were more sensitive than the TNC values in that they were slightly higher than TNC for the greatest values, and slightly lower for the lowest.

The correlation between other measurements provide interesting insights. The TNC values were well correlated to soluble sugar (r=0.88), which may be expected for a component of the whole. TNC was not as well correlated with extracted sugar (r=0.21), suggesting that soluble sugars provided most of the variability in TNC in this study.

The N content of the rhizomes were well correlated with parameters of NSB (r=0.72), etiolated growth (r=0.91), and extracted sugars (r=0.79). In spite of these high correlation coefficients, we should not conclude that rhizome N content was the cause of the NSB or etiolated growth; such a correlation only shows that rhizome N level and other measurements varied synchronously. However, the high correlation between N and etiolated growth suggests that more experiments of rhizome regrowth with N as a treatment variable may provide useful information.

The NSB and TNC behaved in a similar fashion throughout the season (Figure 2), with high values in the spring followed by a decline in the summer, and an increase in the fall. This pattern is similar to the carbon assimilation of alfalfa and many crops, wherein the plant directs carbohydrate toward growth in the spring and toward storage in the fall (Heichel et al., 1988). Unlike alfalfa, which responds to harvests by

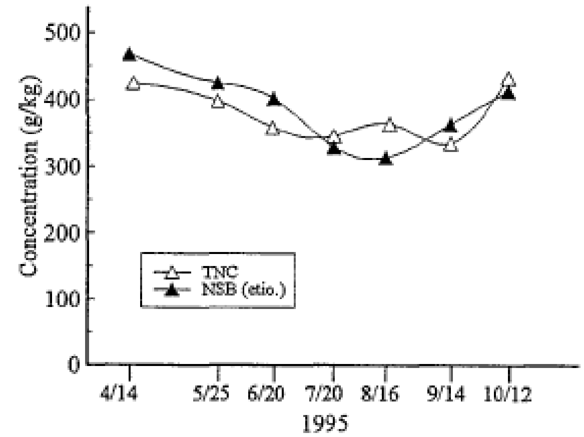

Figure 2. NSB compared to TNC throughout 1995, Madras, OR.

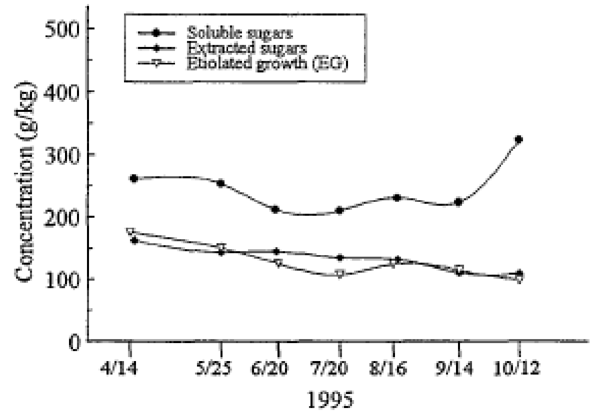

Figure 3. Soluble sugars and extracted sugars throughout 1995, Madras, OR.

initiating the storage phase of its carbohydrate cycle, peppermint NSB decreased throughout the summer even though it was not harvested until August.

The harvest that occurred just prior to sampling on 16 August produced some interesting effects on soluble sugars and etiolated growth (Figure 3). Both etiolated growth and soluble sugars increased from July to August, but then declined again in September. In contrast to soluble sugars, extracted sugars seemed unaffected by harvest and barely increased in October (Figure 3.) It is possible that the rhizome soluble sugar concentration declined in September due to the harvest that necessitated regrowth, only to increase drastically in October after the plant had grown to the point where it could replenish soluble sugar in the rhizomes. Etiolated growth also increased briefly in August, then declined in September. The measurement error in October, mentioned earlier, could have given an artificially low number for etiolated growth, which presumably would have increased drastically in a manner like the NSB data. If we delete the October data, the soluble sugar to EG correlation becomes 0.92! Evidently, both the etiolated growth and soluble sugar change similarly throughout the growing season.

In conclusion, the method of using etiolated growth was developed with some modifications from an alfalfa-NSB method. NSB of peppermint rhizomes was similar to TNC measured throughout a growing season. The measurement of NSB was technically less demanding than TNC in that it required only simple measurements and equipment (a balance, trays with vermiculite, and a dark room.)

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