Scientia Horticulturae, 46 (1991) 137–146
Elsevier Science Publishers B.V., Amsterdam

Seasonal changes in carbohydrate/nitrogen levels during field rooting of *Rosa multiflora* 'Brooks 56' hardwood cuttings*

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(Accepted for publication 9 July 1990)

ABSTRACT

Hambrick, C.E., III, Davies, F.T., Jr. and Pemberton, H.B., 1991. Seasonal changes in carbohydrate/nitrogen levels during field rooting of *Rosa multiflora* 'Brooks 56' hardwood cuttings. *Scientia Hortic.*, 46: 137–146.

Texas field rose plant producers sometimes experience poor field rooting of *Rosa multiflora* cultivar 'Brooks 56' hardwood cuttings, which are used as rootstock. To establish the best time of year for rose propagation, leafless hardwood cuttings were harvested from field-grown stock plants and propagated in raised field soil beds at intervals of 2–4 weeks from November to February. At the initiation of each propagation date, basal, medial and apical cuttings were analyzed for seasonal fluctuation of nitrogen, starch and soluble carbohydrate content to determine if rooting of *R. multiflora* cuttings was correlated with these parameters. Cutting position had no effect on percent rooting, however basal cuttings had the lowest root number. Starch content was positively correlated and nitrogen negatively correlated to rooting. In the carbohydrate–nitrogen component, the starch/N and total carbohydrate (soluble and insoluble carbohydrates)/N ratios of cuttings were more highly correlated to rooting than soluble carbohydrate/N. Maximum rooting of cuttings for field propagation was from 15 November to 15 December, which also corresponded to a low N and higher starch content in propagules harvested from stock plants. Arginine was one of the most prevalent amino acids in cuttings under both high and low rooting periods.

Keywords: adventitious root formation; arginine; C/N ratio; propagation; rootstock; rose; starch.

Abbreviations: C=carbohydrate; C/N=carbohydrate–nitrogen component (ratio); KI=potassium iodide.

INTRODUCTION

Texas is the second largest producer of field-grown rose plants in the U.S.A. with an industry valued at >$20 million annually (B. Brent, personal com-

*Contribution from the Texas Agricultural Experiment Station, College Station, TX, U.S.A., No. TA-22940.

munication, 1981). Inefficient production results in <65% of *Rosa multiflora* hardwood cuttings initially planted for rootstocks developing into marketable No. 1 grade, budded (grafted) 2-year-old rose plants (Fann et al., 1983). Uniformity of rooting of hardwood cuttings differs by field location and time of propagation. Poor rooting has been attributed to seasonal timing of cutting propagation and marginal field soil conditions (Davies, 1985). The greatest labor demands are in the fall when 2-year-old rose plants are dug and processed, and rootstock is propagated. To take advantage of available labor, particularly when rains delay digging, nursery producers will propagate understock during mid-fall under less than physiologically optimum conditions in raised field beds without irrigation (dry land farming). Cuttings are also propagated through February. Optimum times for collecting propagules and sticking cuttings in the field have not been determined. Information for critical propagation dates, based on the physiological conditions of cuttings, is needed to help industry make management decisions for initiating and terminating propagation.

Soluble carbohydrates and starch (Cameron and Rook, 1974; Cheffins and Howards, 1982; Treeby and Considine, 1982; Haissig, 1984), nitrogen (Stoltz and Hess, 1966; Basu and Ghosh, 1974; Welander, 1978), carbohydrate/nitrogen (C/N) ratios (Struve, 1981), amino acids (Suzuki and Kohno, 1983) and cutting position (Marini, 1983) have been reported to influence adventitious rooting of plant species. Starch determination of propagules prior to sticking rose cuttings with potassium iodide (KI) has not been a reliable technique for knowing when to initiate or terminate the field propagation of rootstock. The objective of this research was to determine the relationship of seasonal fluctuation in C/N levels and amino acids of *R. multiflora* cultivar 'Brooks 56' with the rooting potential of basal, medial and apical hardwood cuttings under outdoor field propagation conditions. Information gained from this study is necessary for rose rooting research that could be used as a basis for implementing physiological screening tests for industry propagation decisions.

MATERIALS AND METHODS

Cutting material. – Cuttings and sample material of *R. multiflora* 'Brooks 56' for laboratory analysis were taken from 1-year-old field-grown stock plants. Stock plants were the standard age from which rose cuttings are harvested by commercial rose producers. Cuttings and sample material were taken on 15 and 30 November, 15 and 30 December 1984, and 15 January and 15 February 1985. The experiment was repeated another season, but only the 1984–1985 data are presented.

Rose canes were cut and divided into three sections: basal, medial and apical cuttings. Hardwood leafless cuttings for all rooting experiments were

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Field rooting study. – Cutti
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Soluble carbohydrates a
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TABLE 1

Average climatic conditions for *R.*

Field conditions	Nov
Thermoperiod (°C)	22/
Soil thermoperiod (°C)	18/
Precipitation (mm)	90

[1]Average maximum/minimum ten

ults in <65% of *Rosa multi-*
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trimmed to a 20-cm length with all but the two distal buds removed to eliminate potential suckering. Basal cuttings were composed of the proximal portion of the cane – normally the first 22 cm with the first 2 cm removed from the base of the cane. The medial and apical cuttings were from the next two concurrent portions of the cane. The diameter of canes was not a reliable criterion for separating the three cutting types. Root-promoting compounds were not applied because they are not used in the industry, and Davies (1985) reported that various rooting compounds did not significantly increase the rooting of *R. multiflora* 'Brooks 56'.

Field rooting study. – Cuttings were propagated in the field in raised soil beds on 100-cm centers. Each cutting was placed in the soil to a depth of 18 cm on 10-cm spacings and was left undisturbed for a period of 10 weeks (Table 1). There were four replicates with a total of 40 cuttings per treatment in a randomized complete block design. After 10 weeks, cuttings were dug and soil washed from the roots. Rooting percentage and root number were determined.

Laboratory analyses. – Four separate samples of basal, medial and apical stem sections were taken at the initiation of each propagation date. Plant material was washed immediately with 0.01 N HCl followed by 0.1% Liqi-nox soap (Alconox Inc., New York), then rinsed three times with double-distilled water to remove any foreign material on the stem surfaces. Sample material was dried in a forced-air oven at 65–70°C for 48 h. The dried material was then ground in a Wiley mill through a 20-mesh screen and ground material was stored in sealed containers until analyzed.

Soluble carbohydrates and starch were determined using the anthrone method (Zipf and Waldo, 1952; Pasternack and Danbury, 1968; Wood and McMeans, 1981) as modified by Dowler and King (1966) for peach. Absorption was measured at 625 nm on a Bausch and Lomb Model 21 Spectrophotometer. Nitrogen levels were determined using the micro-Kjeldahl method as described by Stein (1981) with a Technicon autoanalyzer (Technicon Industrial Systems, Tarrytown, NY). Amino acids were determined using a Beckman amino acid analyzer as described by Moore et al. (1958).

TABLE 1

Average climatic conditions for *R. multiflora* 'Brooks 56' propagules on raised field propagation beds

Field conditions	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Thermoperiod (°C)	22/10[1]	20/9	14/1	15/4	23/12	26/15
Soil thermoperiod (°C)	18/15	16/14	10/8	12/9	18/16	22/19
Precipitation (mm)	90	124	124	112	93	60

[1]Average maximum/minimum temperature.

Statistical analysis. – Standard analysis of variance procedures were used for data analysis. Differences among stem position were determined by Student–Newman–Keuls multiple range test (Steel and Torrie, 1980). Linear correlation coefficients were used for the statistical analysis of soluble carbohydrate, starch and nitrogen data, and related to rooting responses. Variables that were percent values were transformed using $\sin^{-1}(x+0.025)^{\frac{1}{2}}$ before analysis.

RESULTS AND DISCUSSION

Seasonal rooting response. – There was a significant relationship between percent rooting, root number and propagation date. The highest percent rooting and root number occurred from 15 November to 15 December (Table 2). Cuttings taken during cooler periods (30 December–15 January) had decreased rooting (42%), while low rooting in February (37%) was attributed to the competing sink of axillary bud growth which had begun on stock plants by this time (axillary bud growth was observed, but not quantified). This concurs with a field study carried out at another location (Pemberton et al., 1986b). Root number was highest on cuttings propagated from 15 November to 15 December, which may also be attributable to a more moderate temperature and sufficient precipitation levels for non-irrigated field propagation (Table 1).

TABLE 2

Seasonal rooting of field-propagated *R. multiflora* 'Brooks 56' hardwood cuttings, $n=120$

	Percent rooting	No. of roots per cutting
Propagation date		
Nov. 15	72.5	7.9
Nov. 30	85.8	7.1
Dec. 15	80.8	5.6
Dec. 30	39.2	1.8
Jan. 15	44.2	2.3
Feb. 15	37.5	2.2
Cutting position		
Apical	58.3a	4.5ab
Medical	65.8a	5.4a
Basal	55.8a	3.5b
Significance		
Month (date)	**	**
Position	NS	**
Month × position	NS	NS

Mean separation within columns by Student–Newman–Keuls test, 5% level, $n=240$.
*, **, NS are significant at the 5% or 1% levels, or non-significant, respectively.

Cutting position. – There \
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est root number (Table 2).
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Prunus persica have been re
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TABLE 3

Soluble carbohydrate, starch and N
cuttings prior to sticking, $n=12$

	P ca
Propagation date	
Nov. 15	2.
Nov. 30	2.
Dec. 15	2.
Dec. 30	2.
Jan. 15	2.
Feb. 15	1.
Cutting position	
Apical	2.
Medial	2.
Basal	1.
Significance	
Month (date)	*
Position	*
Month × position	N

Mean separation within columns b
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ıt, respectively.

Cutting position. – There was no difference in cutting position for percent rooting of field-propagated *R. multiflora*; however, basal cuttings had the lowest root number (Table 2). This agrees with unpublished rooting studies with *Rosa indica* (M. Raviv, personal communication, 1989). Apical cuttings of *Prunus persica* have been reported to root better than basal cuttings (Marini, 1983).

Chemical composition, C/N levels and rooting. – The percent soluble carbohydrate and nitrogen were highest in apical and lowest in basal cuttings, while starch content exhibited no difference among cutting types (Table 3). Starch levels decreased and N levels increased in propagules harvested from stock plants toward the end of the propagation season, which corresponded with poorer rooting capacity. In addition, high starch content was positively correlated and high N negatively correlated with rooting (Table 4). Brandon (1939), using non-quantitative KI histological staining techniques, was unable to find a correlation between the starch content and ease of adventitious root formation of selected *Rosa* species. This contrasts with the positive correlation of high starch accumulation and rooting of our research, and may explain why KI histological observation has not been a widely used test for the commercial determination of cutting fitness to root. Stored forms of carbohydrates, such as starch, are needed for the rooting of hardwood cuttings

TABLE 3

Soluble carbohydrate, starch and N content of field-propagated *R. multiflora* 'Brooks 56' hardwood cuttings prior to sticking, $n=12$

	Percent soluble carbohydrate	Percent starch	Percent nitrogen
Propagation date			
Nov. 15	2.12	8.76	0.81
Nov. 30	2.97	9.89	0.87
Dec. 15	2.77	7.26	0.87
Dec. 30	2.53	5.32	0.99
Jan. 15	2.58	6.20	1.04
Feb. 15	1.91	5.35	1.06
Cutting position			
Apical	2.95a	6.88a	1.06a
Medial	2.51a	7.23a	0.98a
Basal	1.99b	7.18a	0.78b
Significance			
Month (date)	**	**	**
Position	**	NS	**
Month × position	NS	NS	*

Mean separation within columns by Student–Newman–Keuls test, 5% level, $n=24$.
*, **, NS are significant at the 5 or 1% levels, or non-significant, respectively.

TABLE 4

Correlation of soluble carbohydrates, starch, N and C/N ratios with field propagation rooting of *R. multiflora* 'Brooks 56' apical, medial and basal hardwood cuttings. Correlation coefficients are above *P* values

	Soluble carbohydrates			Starch			Nitrogen		
	Apical	Medial	Basal	Apical	Medial	Basal	Apical	Medial	Basal
Percent rooting	0.593 NS	0.502 NS	0.413 NS	0.788 NS	0.917 **	0.911 **	−0.805 *	−0.828 *	−0.829 *
No. of roots	0.200 NS	0.344 NS	0.985 *	0.925 **	0.956 **	0.877 *	−0.959 **	−0.944 **	−0.666 NS
	Soluble carbohydrate : N ratio			Starch : N ratio			Total carbohydrate : N ratio		
	Apical	Medial	Basal	Apical	Medial	Basal	Apical	Medial	Basal
Percent rooting	0.876 *	0.854 *	0.662 NS	0.785 NS	0.883 *	0.957 **	0.830 *	0.896 *	0.954 **
No. of roots	0.670 NS	0.797 NS	0.279 NS	0.956 **	0.970 **	0.890 *	0.954 **	0.960 **	0.832 *

*, **, NS are significant at the 5 or 1% levels, or non-significant, respectively.

which are leafless and unable to photosynthesize (Hartmann et al., 1990). During highest field rooting (15 November–15 December), all three cutting types averaged 0.85% N, and during low rooting (30 December–15 February) N increased to a combined average of 1.03% (Table 3). Optimum and supraoptimum levels of N have been reported to play a role in the rooting of *Vitis* (Treeby and Considine, 1982).

The soluble carbohydrate content, averaged over all collection dates, was not significantly correlated to rooting (Table 4). Raviv (personal communication, 1989) was also unable to find any relationship between soluble carbohydrates and the apical to basal gradient in rooting capacity of *R. indica* shoots. The levels of soluble carbohydrates, starch and N recorded in this seasonal rooting experiment are in the range reported by Tukey and Green (1934) for study on the nutritional composition of rose shoots, which did not include rooting or seasonal responses. They also observed a gradient of N from the basal to apical portions of rose shoots.

For the carbohydrate–nitrogen component (C/N), starch/N and total carbohydrates/N were more highly correlated to rooting than soluble carbohydrates/N (Table 4). During high field rooting periods, propagules had a starch:N average range of 11–8:1 and 15–12:1 total carbohydrate:N (Table 5). During low rooting periods, propagules had a starch:N average range of 6–5:1 and 8–7:1 total carbohydrates:N (Table 5). Struve (1981) reported that high C/N ratios in cutting tissue promote rooting, but did not accurately predict the degree of rooting response. He suggested that differences in C/N ratios were due mainly to differences in N. The present data suggest that in *R. multiflora* C/N ratios can predict rooting and that C/N ratios are due mainly to differences in N and starch content, but not soluble carbohydrates.

Amino acid determination. – During low rooting periods, N and the amino acids arginine, aspartic acid, glutamic acid and leucine increased (Table 6). Particularly high levels of arginine have been reported in *Rosa* compared to other species (E.Khayat, personal communication, 1989), and the principal

TABLE 5

Comparison of levels of starch:N ratio and total carbohydrate:N ratio with field propagation of *R. multiflora* 'Brooks 56' hardwood cuttings

Date	Field rooting	Starch:N ratio	Total C:N ratio
Nov. 15	High	11:1	13:1
Nov. 30	High	11:1	15:1
Dec. 15	High	8:1	12:1
Dec. 30	Low	5:1	8:1
Jan. 15	Low	6:1	8:1
Feb. 15	Low	5:1	7:1

*, **, NS are significant at the 5 or 1% levels, or non-significant, respectively.

TABLE 6

Amino acid levels of stem sections of *R. multiflora* 'Brooks 56' hardwood cuttings at the time cuttings were stuck for propagation[1], $n=4$

	Dec. 15	Jan. 15	Significance[2]
Percent nitrogen	0.86	1.02	*
Percent rooting	80.8 (high)	44.2 (low)	*
Lysine	0.17	0.21	NS
Histidine	0.10	0.14	NS
Arginine	0.44	0.55	*
Aspartic acid	0.36	0.46	*
Threonine	0.16	0.22	NS
Serine	0.18	0.22	NS
Glutamic acid	0.37	0.48	*
Proline	0.26	0.25	NS
Glycine	0.20	0.28	NS
Alanine	0.20	0.26	NS
Valine	0.20	0.26	NS
Isoleucine	0.16	0.20	NS
Leucine	0.28	0.36	*
Phenylalanine	0.15	0.22	NS

[1]Nitrogen and amino acid data expressed in percent dry weight.
[2]Paired-*t* test: *, **, NS are significant at the 5 or 1% levels, or non-significant, respectively.

nitrogen reserve component of the bark and wood in apple trees (Rosaceae) is a protein enriched in arginine (O'Kennedy and Titus, 1979). In *Morus alba*, asparagine is suggested to be the main N transport compound during the initiation of cutting roots (Suzuki and Kohno, 1983). Of interest for future study would be to investigate if arginine is correlated to rooting in *R. multiflora* and if the activity of arginase, which hydrolyzes arginine, fluctuates with seasonal rooting response.

CONCLUSIONS AND RECOMMENDATIONS

Mid-November through mid-December are the optimum periods for propagating *R. multiflora* 'Brooks 56' hardwood cuttings. This roughly corresponds to the time of planting presently used by many Texas rose producers, even though growers will plant as early as 30 October and as late as 15 February to use available labor. Successful early propagation dates will most likely depend on the prevailing climatic conditions, which adequate rainfall and cool temperatures being advantageous. Planting after 30 December would not be advisable based upon these data and another seasonal study (Pemberton et al., 1986b). Collection of cuttings and field planting is advisable at temperatures $\geqslant 5°C$ as cuttings in this and another study (Hambrick et al., unpublished data, 1984) planted under colder conditions failed to give satisfactory

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ACKNOWLEDGMENTS

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1.02	*
4.2 (low)	*
0.21	NS
0.14	NS
0.55	*
0.46	*
0.22	NS
0.22	NS
0.48	*
0.25	NS
).28	NS
).26	NS
).26	NS
).20	NS
).36	*
).22	NS

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C/N ratios appear to be important for optimum periods to harvest propagules from stock plants. An 11–8:1 Starch:N and 15–12:1 total carbohydrate:N were desirable levels for optimum rooting, and as C/N decreased below these levels, so did rooting (Table 5). N levels also appeared to be an important predictor of rooting potential. When all three cutting types averaged 0–0.85% N, high rooting occurred vs. 1.03% N when rooting was low (Table 3). The importance of N in rooting underscores the potential for stock plant manipulation through fertilization practices, since low to moderate N fertility will help increase starch levels, C/N ratios and rooting success. Currently, rootstock plants of *R. multiflora* are not fertilized during the first year of commercial production, after which cuttings are taken for the next crop rotation. However, phosphorus fertilization during the first year of production has been shown to increase the subsequent propagation efficiency of hardwood cuttings (Pemberton et al., 1986a). Future research implementing simplified screening tests for starch and N in determining C/N ratios could improve industry propagation efficiency for field rose production.

ACKNOWLEDGMENTS

The authors wish to acknowledge Mark Walters, Inc. of Tyler, TX, U.S.A., for supplying rose plant material.

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Scientia Horticulturae, 46 (1991)
Elsevier Science Publishers B.V.,

Effects of sty
pollinated pistils

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ABSTRACT

Amaki, W. and Higuchi, H., 1991. I
tube growth in *Lilium longifloru*

The effects of stylar exudates on
rum cultivar 'Hinomoto' and 'Geo
florum by flushing with distilled wa
injected into the stylar canals of un
patible (cross) or incompatible (s
The exudates collected at 48 h a
lination with 'Georgia' promoted
pollination showed a slight inhibi
they were injected into the pistils c
These results suggest that stylar
creted after self-pollination in thei
time after pollination.

Keywords: *Lililum longiflorum*; p

INTRODUCTION

Lilium longiflorum sho
patible (self) pollen norm
pollen tubes is arrested w
longiflorum, cells lining t
(Rosen and Thomas, 19
changes in the tissues of t
lar canal was filled with
The exudate was utilized
synthesis (Kroh et al., 19

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