Propagation of Rosa dumetorumThuill.`laxa` and R. rugosaThunb. from root cuttings.

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الخلاصة

ان انتاج النموات الخضرية من على العقل الجذرية لل Rosa rugosaThunb. قد اظهرت تباينا فصليا، فكان اقلها في شهر نيسان ومايس. ان معاملة كامل العقلة الجذرية بال BAبتركيزات 1 و 10 ملغم\ليتر ادت الى زيادة عدد البراعم الخضرية عليها. وايضا، قد ازداد عدد النموات الخضرية نتيجة لمعاملة طرف العقلة، الاقرب لاتصالها بالجذر الرئيسي او اتصال الجذر بالساق proximal end، بال BA بتركيز 500 ملغم\ليتر ومن ثم معاملة الطرف الابعد بال IBA بتركيز 100 ملغم\ليتر. ان العقل الجذرية ذات القطر 0.3 – 1.5 سم وبطول 15 سم قد انتجت نموات خضرية اكثر من تلك الاقصر طولا، ولكن بقيت عدد النموات الخضرية لكل سم من طول العقلة متساوية. ان العقل الجذرية للR. rugosa قد استجابت للمعاملات، بشكل عام، افضل من ال R. dumetorumThuill.`laxa`.

SUMMARY

Shoot production on roots of *R. rugosa*Thunb. showed some seasonal variation, fewest being produced in April and May. The application of 1 and 10 mg litre^{-1} BA to the whole segment of root cutting increased the number of shoot-buds. Increased shoot production also resulted from the application of 1500 mg litre^{-1} BA and 100 mg litre^{-1} IBA to the proximal and distal ends respectively. Root segments 0.3-1.5 cm thick and 15 cm long produced more shoots than shorter segments butthe number of shoots cm^{-1} was similar. Isolated roots of R. rugosa responded better to treatments than did those of R. dumetorumThuill. *'l*axa'.

Key-Words: Rosa dumetorum, R. rugosa, root cuttings

INTRODUCTION

Relativelylittle information is available in the literature on the use of root cuttings (pieces of roots and not shoots derived from roots) for the propagation of woody plants, although (18) reviewed the available methods with special reference to apple (*Malus* spp.). Although this type of vegetative propagation has received increasing attention in the last few years, it still remains one of the least used methods of propagation in today's modern nurseries. (6) has suggested two principal reasons for this. First, the plant species for which it is the best propagation technique are infrequently grown in the average nursery. Second, there are practical difficulties in obtaining cuttings, since either whole plantmust be lifted or the soil must be excavated around stock plant to expose the root sections from which cuttings are taken. Both methods are labour intensive although they are usually carried out during the autumn and winter months, when other demands on labour are low.

This technique is generally used only for species which are difficult or impossible to raise from seeds or by shoot cuttings. (6) identified some species (e.g. *Ailanthus glandulosa*Desf.) which may be propagated more easily or better by root cuttings than by other methods.

The diameter and length of root cuttings may influence their ability to regenerate shoots. (26) showed that there was a reduction in the vigour of shoots produced from segments of apple roots when sections were shorter than 5 cm. (17) working with the cultivar 'Kainth' of *Pyruspashia*Buch.

reported that root segments 15 cm in length produced shoot buds more readily than did 10 or 5 cm long segments of the same thickness. The placement of root segments in the medium may also be an important factor in regeneration. (22) working with *Pyruscommunis* L. preferred the vertical insertion of root cuttings with the proximal end exposed. (13), working with roses, observed that a higher number of shoots emerged from root cuttings inserted horizontally than from those inserted vertically.(1) foundthat exposure of *Convolvulus arvensis* L. roots to light caused an increase in shoot extension growth, although shoot number was unaffected.

The production of shoots from root cuttings may show seasonal variation, with regenerative ability being higher in winter than in early and late summer. (10, 11) found a distinct periodicity of shoot production in raspberry (*Rubusidaeus* L.), with an "on-period" from November to March when 60% of root cuttings produced shoots dropping to an average of 5% during the "off-period" from May to September. No regeneration was obtained in June or July.(21) found the highest rates of shoot production from root cuttings of blackberry (*Rubusprocerus* P.J. Muell.) during the winter months. (17)reported that a higher percentage of root cuttings of *Pyruspashia*produced shoots in August than in other months. In contrast, horseradish (*Cochleariaintybus* L.) and dandelion (*Taraxacumofficinale* Weber) roots were capable of producing substantial numbers of shoots throughout the year (11; 27).

Exogenous applications of growth substances may have an important role to play in the regeneration ofroot cuttings. (16) observed bud suppression at the proximal end of seakale (*Crambemaritima* L.) roots treated with 200 mg litre $^{-1}$NAA, and more new roots were stimulated at the distal end. Similar findings were reported by (25), (14) and (3) using various concentrations of differentauxins. It has generally been shown that cytokinin applications increase bud number, stimulate cell activity in the bud producing regions and antagonise auxin suppression, and that distal application inhibits root formation at that end (e.g. 2; 15; 23). In contrast, (21) claimed that kinetin at a concentration of 0.1-10 mg litre^{-1} had no effect on the regeneration of blackberry from root cuttings. Robinson and Schwabe (19, 20) stated that, in general, auxins stimulate root development and inhibit shoot bud primordia, while cytokinins have the opposite effect. Gibberellins are reported to have much less effect on shoot production than auxins or cytokinins (14).

This study describes some experimental investigations into the vegetative propagation of*Rosa dumetorum* and R. rugosausing root cuttings. The effects of several factors which might influence shoot production were examined.

MATERIALS AND METHODS

Roots of R. dumetorum and R. rugosa were removed from established plants growing at Pen-y-ffridd Field Station, U.C.N.W., Bangor, Gwynedd by excavating the soil around them. The root pieces were washed and, unless otherwise stated, cut into 50 mm lengths. Undamaged and healthy root segments 3 to 15 mm in diameter were selected and soaked for 10 seconds in fungicide (captan 50% active ingredient) using 2 g litre^{-1}before being treated as appropriate and planted in standard sand/peat medium (50:50 by volume) in plastic seed trays (19.2 cm x 14.4 cm x 4.8 cm) or in 8.4 cm diameter plastic pots. When the containers were placed in a heated glasshouse, the temperature varied between 16 and 18`C and the average humidity was 60%. The photoperiod was a minimum of 16 hours maintained by supplementing natural daylight with 400-watt mercury vapour lamps. In the unheated unlit glasshouse, the temperature varied between 10 and 15`C. Recordings of the number of newly emerging shoots were made 2 weeks after insertion and repeated weekly for 8 weeks. The newly emerged adventitious shoots were marked by ringing with small wire loops. Complete randomized designs were used and analyses of variance were carried out on the data when necessary. In many experiments a square root + 1 transformation was used prior to statistical analysis.

Experiment 1

Regeneration of Rosa dumetorum and R. rugosafrom root cuttingstaken at different times of the year.

Five root segments of each species were inserted horizontally to a depth of 1 cm in sand in individual plastic seed trays. Three such trays for each species were placed in a heated glasshouse. The first cuttings were taken on 23 September 1982 and further sets were taken at monthly intervals for a year. The number of shoots produced was recorded 8 weeks later.

Experiment 2

Effect of the length of root segment on shoot production of R.dumetorum and R. rugosa.

Ten replicates of root segments each measuring 5, 10 or 15 cm in length, of each species, were prepared on 21 October 1983 and inserted vertically into the standard propagation medium (equal quantities of sand and peat) in black polyethylene pots (30 cm x 10 cm). The pots were then placed in a heated glasshouse for 8 weeks when the numbers of shoots produced by the individual segments were recorded.

Experiment 3

The regeneration of R. dumetorum and R. rugosa from small lengthsof root segment.

On 8 February 1984, 10 root segments each of 0.25, 0.5, 1, 2 or 4 cm in length were planted vertically in the standard medium in plastic pots. The proximal ends were level with the soil surface and the pots were placed in a heated glasshouse. Theexperiment was started on 8 February 1984 and the numbers of shoots produced from the individual root segments were recorded 8 weeks later.

Experiment 4

Effects of medium, environment and benzyladenine (BA) on shootproduction from root segments of R. dumetorum and R. rugosa.

On 28 October 1982, the proximal ends of three replicates each of five individual root segments of each species were dipped in distilled water, 100 or 500 mg litre^{-1} BA in alcohol for 10 seconds. Root segments were then placed horizontally in seed trays and covered to a depth of 1 cm with either sand, peat or John Innes compost no. 1. Trays of each treatment were placed either in a heated glasshouse, unheated glasshouse or outdoors. The total number of shoots produced in each tray placed in the heated glasshouse was recorded 8 weeks later and the final results for the other environments were collected in June 1983.

Experiment 5

Effects of benzyladenine (BA) and indolebutyric acid (IBA) on shoot production from root cuttings of R.dumetorum and R. rugosa. On 8 February 1984, root cuttings were prepared and 10 segments allocated to each of the following treatments: 1) dipped in distilled water, 2) 2 to 3 mm of the proximal end dipped in 500 or 1500 mg litre^{-1} BA solution, 3) 2 to 3 mm of the distal end dipped in 100 or 500 mg litre^{-1} IBA and 4) the proximal end dipped in BA then the distal end dipped in IBA for 10 seconds each using the same doses as that for the single treatments. Treated cuttings were inserted in plastic pots with 4 mm of the proximal end protruding above the standard propagation medium. The pots were placed in a heated glasshouse for 8 weeks, when the numbers of visible shoots per root segment were recorded.

Experiment 6

Regeneration of R. dumetorum and R. rugosa from root cuttings fully exposed to light and treated with benzyladenine (BA) and naphthalene acetic acid (NAA). This experiment was started on 12 April 1984. Root segments were placed horizontally on tissue paper in 12 cm diameter plastic trays. The trays were watered as required with: distilled water, 1, 10 or 100 mg litre^{-1} BA, 1, 10 or 100 mg litre^{-1} NAA, 100 mg litre^{-1} BA plus 1 mg litre^{-1} NAA or 1 mg litre^{-1} BA plus 100 mg litre^{-1} NAA. The nine treatments were replicated three times with three segments in each replicate, and trays were placed in a heated glasshouse and covered with clear polyethylene sheet. The final observations of the numbers of emerged shoots and visible buds per root segment were made 8 weeks later.

RESULTS

Experiment 1

The number of emerged shoots was greater for R. rugosa than for R.dumetorum. Some of the root segments of R. dumetorum collected in October and November 1982 produced shoots but for the rest of the year root cuttings failed to produce shoots except for occasional ones in February and April 1983. The pattern for R. rugosa was much more irregular with high values in October 1982, and January,February, March and June 1983 although in every monthly sample some cuttings produced shoots (Table 1).

Experiment 2

In general, shoot production was higher on root cuttings of R.rugosa than of R.dumetorumexcept for cuttings 5 cm in length. Segments of R. rugosa 15 cm in length showed considerable success, but there was a very limited number of shoots produced on cuttings ofR. dumetorum 10 cm in length. The highest number of root segments, which produced two to three shoots, was found in cuttings of R. rugosa15 cm in length and in those of R. dumetorum 5 cm in length (Table 2).

Experiment 3

All root cuttings of R. dumetorum failed to produce shoots as did cuttings of R.rugosa which were 0.25 or 0.5 cm in length. One cutting of 1 cm length and two cuttings of 2 cm length produced shoot-budsfor R. rugosa. Five of the 10 root cuttings which were 4 cm in length produced shoots and two of these bore two shoots (Table 3).

Experiment 4

Although, root materials of R. rugosa treated with 100 mg litre^{-1}BA and inserted into John Innes compost no. 1 medium produced few shoots, considerable success in shoot production was found from root cuttings treated with 500 mg litre^{-1} BA and inserted in sand. In general, cuttings treated with the highest concentration of BA and those inserted into sand or peat medium showed the highest numbers of shoots from root cuttings (Table 4). Shoot production on root cuttings of R. dumetorumwas not significantly increased in any treatment, although a few shoots did emerge. The results for cuttings of R. rugosawhich were placed in different environments did not differ significantly.

Experiment 5

Most of the root segments of R. rugosa produced at least one shoot and none of the growth substance treatments significantly increased the number of shoots. The numerically best treatment (nine out of 10 cuttings with one or more shoots) was the one where a combination of 1500 and 100 mg $litre^{-1}$ of BA and IBA respectively was given. Conversely only four out of 10 cuttings produced shoots after treatment with 500 BA and 500 IBA in combination (Table 5). Shoot production on root cuttings of R. dumetorum was not significantly increasedabove control values by any treatment, although some root cuttings produced shoots.

Experiment 6

All root cuttings of R. rugosa which were treated with 10 $mglitre^{-1}$ BA produced more than one adventitious shoot-bud, and this treatment also gave the highest number of shoot-buds per root cutting. Application of BA did, generally, promote the production of adventitious shoot-buds on root segments, while NAA application inhibited them. Shoot-bud production was also enhanced when a mixture of 100 mg $litre^{-1}$ BA + 1 mg $litre^{-1}$ NAA was applied (Table 6). Root segments of R. dumetorum failed to produce shoots.

DISCUSSION

R. dumetorum is known to produce few root suckers, whileR.rugosa tends to sucker freely from established root systems (8). Similar conclusions can be drawn from the investigations reported in this study (Experiments 1 and 6). Isolated roots of R. rugosa formed shoot buds much more frequently than did isolated roots of R.dumetorum (e.g. Tables 1 and 2). In some species, shoot buds are never found on roots, in others, for example, raspberry, buds occur naturally as well as on roots after they have been isolated or injured (10, 11). In a third type of species, for example, many of the aquatic members of the Podostemonaceae, one of the main functions of the root system appears to be the production of root buds (7). The differences in adventitious bud production on roots between R. dumetorum and R. rugosa must be related to inherent differences between these species.

Some root cuttings of R. rugosa collected at monthly intervals and planted under standard conditions, gave rise to adventitious shoots in every test. The period from June to March was characterized by approximately 62 per cent (average of 3.1 shoots/five root cuttings) of roots producing shoots, compared with an average of only 10 per cent(0.5 shoot/five root cuttings) in April and May (Table 1). These observations allow the suggestion that a depletion of the soluble carbohydrate in roots, associated with the onset of growth in April and May, led to a low level of regeneration. Once established, the growth of the new season might produce substance(s), which are transported downward to roots and which restore the ability to produce shoots. (10; 11) and (12), working with raspberry roots, found an "on period" from November to March and an "off period" from May to September, and related this to the aerial growth and to a positive correlation between starch content and the ability of root cuttings to produce shoots. It is likely that the results of the present study can be interpreted in similar terms. (5) proposed that good regeneration from *Populustremula* root cuttings in autumn was the result of a combination of high carbohydrate and low auxin levels at that time.The environment after root segments was planted did not seem to be critical (Experiment 4), although propagation within a glasshouse environment was more rapid than it was outdoors.

The existence of naturally occurringauxins and cytokinins has been demonstrated conclusively in roots (e.g. 4; 18; 9). Generally, BA application to the proximal ends of the root cutting (Table 5) or to whole cuttings (Tables 4 and 6) ofR. rugosa enhanced the regeneration of shoots. Shoot production was considerably increased when 1 or 10 mg litre^{-1} of BA were applied (Table 6). This could indicate that cytokinin (BA), even at low concentrations, was able to increase shoot bud numbers. (15), (18) and (9) achieved similar results. Experiment 5 showed that auxin treatment (IBA) to the distal end of root cuttings of R. rugosa did not suppress shoot growth at theproximal end (Table 5), a result which would be expected if auxin moves only from the base towards the apex of the root. The combination treatment of BA (1500 mg litre^{-1}) and IBA (100 mg litre^{-1}) to the proximal and distal ends respectively led to nine out 10 of the root segments producing one or more shoots (Table 5). These results verified (18) and (9) claims that, in roots, cytokininsmay interact with auxins in the expression of regeneration phenomena. Evidence of inhibition caused by NAA was found in Experiment 6 (Table 6). The presence of NAA at a concentration of 10 or 100 mg litre^{-1}reduced the number of cuttings which produced one or more shoot buds. The identification of the optimum auxin/cytokinin balance for shoot production in root segments of R.rugosa would merit further study.

The results furnished in Table 2 show that 15 cm long rootsegments of R. rugosa, produced a higher number of shoots than did the shorter segments of the same thickness although the numbers of shoots cm^{-1} were similar. Similar results, i.e. the longer the root segments the higher the numbers of shoots, were reported with *Pyruspashia* roots(17) and with apple (25; 24; 19). (24)reasonedthat short roots had lower total amounts of reserves but the constancy of bud frequency per unit length of root leads to the conclusion that reserves per_se are not determining the process. It is possible that when a bud arises it inhibits the development of other bud initials for some distance from it.

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Table 1: Effect of time of taking root cuttings of R. dumetorum and R. rugosa on the number of shoots produced.

Month when roots were dug and cuttings inserted		Species	
		R. dumetorum	R. rugosa
September	1982	0.0	2.7(1.9) abc
October	1982	2.7(1.9) a	3.3(2.1) abc
November	1982	1.3(1.5) a	2.7(1.9) abc
December	1982	0.0	1.0(1.4) abc
January	1983	0.0	4.0(2.1) abc
February	1983	0.7(1.2) a	3.0(2.0) abc
March	1983	0.0	5.0(2.4) a
April	1983	0.7(1.3) a	0.7(1.2) bc
May	1983	0.0	0.3(1.1) c
June	1983	0.0	4.0(2.2) ab
July	1983	0.0	2.3(1.8) abc
August	1983	0.0	2.7(1.9) abc
Means		1.3(1.5)	2.6(1.8)

Data are means of 3 replicates each of 5 root segments.

Means in brackets and a column are transformed data using square root + 1 transformation; when followed by the same letter they are not significantly different at $p<0.05$.

Zero values were not included in the analyses.

Table 2: Effect of segment length on the number of emerged shoots from root cuttings of R. dumetorum and R. rugosa.

Replicate	Segment length (cm) of roots R. dumetorum			R. rugosa		
	5	10	15	5	10	15
1	2	0	1	1	0	1
2	3	0	1	3	0	3
3	1	0	0	1	1	1
4	1	0	3	0	1	1
5	0	0	0	0	1	1
6	3	2	0	0	0	1
7	2	0	0	0	2	2
8	0	0	0	0	1	2
9	0	0	2	0	1	3
10	1	0	0	1	1	0
Means*	1.3 (1.5) ab	0.2 (1.1) b	0.7 (1.3) ab	0.6 (1.2) ab	0.8 (1.3) ab	1.5 (1.6) a
Numbers**	7	1	4	4	7	9
Means***	1.9	2.0	1.8	1.5	1.1	1.7

* mean number of shoots produced per root segment.
** total number of root segments producing one or more shoots.
*** mean number of shoots produced per successful root segment.

Means in brackets are transformed data using square root + 1 transformation; when followed by the same letter they are not significantly different at $p<0.05$.

Table 3: Effect of segment length on the number of shoots produced from root cuttings of R. rugosa.

Replicate root	Segment length (cm) 0.25	0.5	1	2	4
1	0	0	0	0	0
2	0	0	0	0	1
3	0	0	0	0	2
4	0	0	0	0	0
5	0	0	0	1	0
6	0	0	0	0	2
7	0	0	0	0	0
8	0	0	1	0	1
9	0	0	0	1	0
10	0	0	0	0	1
Means*	0	0	0.1	0.2	0.7
Numbers**	0	0	1	2	5
Means***	0	0	1.0	1.0	1.4

* mean number of shoots produced per root segment.

** total number of root segments producing one or more shoots.

*** mean number of shoots produced per successful root segment.

Data were not subjected to analysis.

Table 4: Effects of BA treatments and propagation media on the number of emerged shoots from root cuttings of *R. rugosa*.

Propagation medium	BA treatments (mg $litre^{-1}$) 0	100	500	Means*
Sand	2.7(1.9) ab	2.3(1.8) ab	3.6(2.1) a	2.9(1.9)
Peat	2.4(1.8) ab	1.6(1.5) ab	2.4(1.8) ab	2.2(1.7)
J. I. no. 1	1.2(1.5) ab	0.7(1.2) b	1.7(1.6) ab	1.2(1.4)
Means*	2.1(1.7)	1.5(1.5)	2.6(1.8)	

Data are means of 3 replicates, each of 5 root segments.

Means in brackets are transformed data using square root + 1 transformation; when followed by the same letter they are not significantly different at $p<0.05$.

Tukey's test 'honestly significant difference-H.S.D.' was followed as a multiple comparison method.

* at $p = 0.05$; H.S.D = 0.30.

Table 5: Effects of BA and IBA treatments on the number of shoots produced from root cuttings of *R. rugosa*. Benzyladenine (BA) was given to the proximal end of the cutting and IBA to the distal end.

Replicate root	Growth substance treatments (mg $litre^{-1}$)								
BA	0	500	1500	0	0	500	500	1500	1500
IBA	0	0	0	100	500	100	500	100	500
1	2	1	1	2	2	0	0	2	1
2	0	2	0	2	2	2	0	3	1
3	0	0	2	1	1	3	0	1	1
4	2	1	0	1	0	3	1	0	0
5	1	2	1	1	0	1	0	1	1
6	0	1	2	2	3	0	1	2	1
7	2	0	0	0	1	1	0	1	0
8	2	1	2	0	1	0	1	1	0
9	1	2	2	0	1	1	3	1	0
10	1	3	0	2	2	0	0	1	0
Means*	1.1 (1.4)a	1.3 (1.5)a	1.0 (1.4)a	1.1 (1.4)a	1.3 (1.5)a	1.1 (1.4)a	0.6 (1.2)a	1.3 (1.5)a	0.5 (1.2)a
Numbers**	7	8	6	7	8	6	4	9	5
Means***	1.6	1.6	1.7	1.6	1.6	1.8	1.5	1.4	1.0

* mean number of shoots produced per root segment.

** total number of root segments producing one or more shoots.

*** mean number of shoots produced per successful root segment.

Means in brackets are transformed data using square root + 1 transformation; when followed by the same letter they are not signicantly different at $p<0.05$.

Table 6: Effects of BA and NAA treatments on the number of visible shoot-buds on fully exposed root cuttings of R. rugosa.

Replicate	root cutting No. of	Growth substance treatments (mg $litre^{-1}$)								
	BA	0	1	10	100	0	0	0	100	1
	NAA	0	0	0	0	1	10	100	1	100
	1	2	5	4	1	2	0	0	0	0
1	2	3	3	4	4	0	0	0	7	0
	3	2	2	3	1	2	0	0	1	0
	1	3	3	4	3	3	0	1	1	0
2	2	2	3	3	2	2	0	0	3	1
	3	3	4	2	4	0	0	0	3	0
	1	3	0	5	0	0	0	0	2	0
3	2	1	2	4	0	0	0	0	3	0
	3	3	5	2	0	2	0	0	2	0
Means*		2.4ab	3.0a	3.4ab	1.7ab	1.2b	0	0.1	2.4ab	0.1
Numbers**		9	8	9	6	5	0	1	8	1
Means***		2.4	3.4	2.9	2.5	2.2	0	1.0	2.8	1.0

Data are means of 3 replicates, each of 3 root segments.

* mean number of shoots produced per root segment.

** total number of root segments producing one or more shoots.

*** mean number of shoots produced per successful root segment.

Means followed by the same letter are not significantly different at $p<0.05$.

The results of cuttings treated with 10, 100 mg $litre^{-1}$ NAA and 100 mg $litre^{-1}$ NAA + 1 mg $litre^{-1}$ BA were not included in the analyses or means.