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Plant Growth Regulators Increase the Rooting of Apple Rootstocks Grown in Stoolbeds

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Abstract

Poor rooting is a common problem of many dwarfing apple rootstock genotypes when propagated by layering. Four field experiments were performed in 2008 and 2009 to improve rooting of dwarfing rootstocks. Locations included Ephrata, Washington; Angers, France; and Canby, Oregon. The experiments compared the application of various plant growth regulators at various times of the year on B.9, M.9-T337, M.9-EMLA and G.41 rootstock stool beds. The chemicals used were prohexadione Ca (250 ppm or 1,000 ppm), indole-butyric acid (IBA; 1,000 ppm or 10,000 ppm) and naphthaleneacetic acid (NAA; 50 ppm or 200 ppm). IBA sprayed early in the season before mounding with sawdust consistently increased the rooting percentage; however, the treatment was expensive. Foliar applications of prohexadione Ca in the summer also increased the rooting percentage and reduced the number of spines with variations in response due to dose, location and timing. NAA applied foliarly in the summer did not increase rooting percentage.

Apple rootstocks are propagated sexually via seeds or asexually (clonally) via cuttings, micropropagation, layering or stooling (Ferree and Carlson 1987; Wertheim 1998). In the last 60 years almost all apple rootstocks have been propagated clonally via layerbeds or stoolbeds although in last 20 years micropropagation has become popular with difficult to root genotypes (Webster and Wertheim 2003). Layering techniques were first used as an alternative propagation method for fruit crops which were hard-to-root by cuttings (Knight et al. 1927). The process involves the induction of adventitious roots on shoots that are still attached to a mother plant. Mother plants are planted in the spring in an upright position (stooling) or angled (layering). In the case of layering, the mother plants are pinned or tied down horizontally at the end of the first year. In both stooling and layering the mother plants are mounded with soil or sawdust during the growing season as new shoots grow from the mother plant to induce rooting on the new shoots. The shoots are harvested in the fall as rooted shoots (liners) leaving the mother plant in the ground. The original plants are referred to as mother plants because they remain in the ground for many years and produce new shoots each year which will be induced to root and then harvested as rootstocks liners. These rootstock production fields are termed stoolbeds or layer beds. Layering is also used because of its adaptability to mechanization, high quality of the produced rooted liner, relative low cost and ease of maintenance (Anderson and Elliott 1983). Stoolbeds have the added benefit of being a reliable and constant production system. A typical stoolbed can be used for up to 10-20 years before productivity declines and it must be replaced.

Stooling and layering continue to be the most common forms of apple rootstock

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propagation; however, some rootstock genotypes do not produce many roots in a stoolbed. Rootstock breeders primarily have focus on rootstock characteristics of dwarfing, precocity, yield efficiency, diseases resistance, reduced number of spines, cold tolerance, fruit quality, anchorage, and suckering (Cummins and Aldwinckle 1974; 1983). A major selection trait that characterizes newer rootstocks, such as Geneva® G.41and G.935, is the absence of burrknots and low suckering. Both these traits are excellent in the orchard but result in few roots in a stoolbed production system (Robinson et al. 2007). Unfortunately, the rootstock genotypes that are easy to propagate generally do not have the dwarfing, yield efficiency or disease resistance that modern orchards require. Thus, improved propagation methods for dwarfing rootstocks are needed. Little has been done to improve the propagation methods involved in layering since they were described over 100 years ago. Several plant growth regulators are known to affect rooting. Indole-butyric acid has shown significant improvement of rooting with apple cuttings (Alvarez et al. 1989; Delargy and Wright 1979; Sun and Bassuk 1991). Auxins have also been used to improve rooting in micropropagation systems (Zimmerman 1984). Work with other plant growth regulators has proven to be inconclusive or genotype dependent (Webster 1995).

The goal of this research was to improve the propagability of highly desirable dwarfing apple rootstocks, but which have poor propagation characteristics in layering system by the application of plant growth regulating chemicals (auxins or vegetative growth inhibitors).

Materials and Methods

Plant Materials and Treatments. In 2008 and 2009, experiments on the effect of plant growth regulators (PGR) on rooting efficiency of dwarfing rootstocks in stoolbeds were conducted at Willow Drive Nursery in Ephrata, Washington, USA, DL Nursery in Angers, France and in 2009 at Willamette Nursery in Canby, Oregon, USA.

At Willow Drive Nursery previously established stool beds of B.9 and M.9-T337 were selected for plant growth regulator treatments. The selected beds of B.9 and M.9-T337 ran parallel to each other and were planted in 2001 on Timmerman coarse sandy loam (Natural Resources Conservation Service, https://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx). A randomized complete block design with a split-plot treatment design and 4 replications was used. The main plot was rootstock genotype, and the subplot was plant growth regulator treatment. Each subplot consists of a 3 m long section of stoolbed. The treatments were:

1. Untreated control
2. Indole-butyric acid (IBA) 10,000 ppm
3. IBA 1,000 ppm
4. Prohexadione Ca (Apogee) 250 ppm
5. Prohexadione Ca 1,000 ppm
6. Naphthaleneacetic acid (NAA) 50 ppm
7. NAA 200 ppm

The IBA sprays were applied on June 9, 2008, to the young shoots before mounding with sawdust. Sprays were applied with a pressurized sprayer with a wand. Shoots were sprayed to runoff. The stoolbed plots were then mounded a few days later. The prohexadione Ca and NAA sprays were applied on August 25, 2008, long after mounding had already occurred. Control plots received no chemical treatment. All treatments received typical management through the season including sawdust mounding, irrigation, pest control, and cultural practices.

At DL Nursery in Angers, France, previously established stoolbeds of G.41 and M.9-EMLA stoolbeds planted in 2005 were used. The stoolbed rows of each rootstock ran parallel in the field. A randomized complete block design with a split-plot treatment design and 4 replications was used. The main plot was rootstock genotype, and the subplot was plant growth regulator treatment. Each subplot consisted of a 3 m long section of stoolbed. Sprays were applied with a pressurized sprayer with a wand. Shoots were sprayed to drip. The treatments were:

1. Untreated control
2. Prohexadione Ca (Regalis) 250 ppm
3. Prohexadione Ca (Regalis) 1,000 ppm
4. NAA 50 ppm

Both of the chemicals were sprayed on the stoolbed shoots on August 11, 2008, after they had been previously mounded with soil in June (DL Nursery used soil not sawdust for mounding).

In the second year (2009) after preliminary data analysis of the data from year one, changes were made in the timing of chemical applications. At Willow Drive Nursery in Washington the 2009 treatments and their corresponding dates on both B.9 and M.9-T337 were:

1. Untreated control
2. IBA 1,000 ppm on June 17, 2009, for B.9 and June 24, 2009, for M.9-T337 (just before mounding)
3. IBA 10,000 ppm on June 17, 2009, for B.9 and June 24, 2009, for M.9-T337 (just before mounding)
4. Prohexadione Ca 250 ppm on August 3, 2009
5. Prohexadione Ca 1,000 ppm on June 25, 2009 (after mounding)
6. Prohexadione Ca 1,000 ppm on July 23, 2009
7. Prohexadione Ca 1,000 ppm on August 3, 2009
8. Prohexadione Ca 1,000 ppm on August 24, 2009
9. Prohexadione Ca 1,000 ppm on June 25 and July 23, 2009
10. NAA 50 ppm on August 3, 2009
11. NAA 200 ppm on August 3, 2009

At DL Nursery in Angers, France, treatments in 2009 were changed to focus on timing of application. Plot length was also increased to three meters. Treatments in 2009 were:

1. Untreated control
2. Prohexadione Ca (Regalis) 1,000 ppm on June 29, 2009
3. Prohexadione Ca (Regalis) 1,000 ppm on July 29, 2009
4. Prohexadione Ca (Regalis) 1,000 ppm on June 29 and July 29, 2009

At Willamette Nursery in Canby, Oregon a previously established stoolbed of G.41 with a randomized complete block design with 4 replications was used. Each plot consisted of a 3 m long section of stoolbed. Sprays were applied with a pressurized sprayer with a wand. Shoots were sprayed to runoff. The treatments and their corresponding dates were:

1. Untreated control
2. Prohexadione Ca 1,000 ppm on June 29, 2009
3. Prohexadione Ca 1,000 ppm on July 29, 2009
4. Prohexadione Ca 1,000 ppm on June 29 and July 29, 2009

Data Collection. Plots were harvested the second week of November in Washington, the end of November in France, and early January in Oregon. Rootstock shoots were taken immediately to cold storage after harvest. The rootstocks were graded in December and January. Rootstock liners from the plots were separated into “grades” by visual inspection according to typical nursery standards. (Figure 1). The four grades were: A – ≥16 roots; B – 9-15 roots; C – 1-8 roots; D – no roots.

Total number of liners of each grade from each plot was recorded. A sub-sample of 5 liners was taken at random from each grade for additional data collection. Caliper, total height, number of spines and branches, straightness of the shoot, number of roots, number of root nodes, and number of rooted nodes were recorded from each liner in the sub-sample. Caliper measurements were taken with a

Figure 1. Example of "A" grade rootstock (left), "B" grade rootstock (center), and "C" grade rootstock (right).

standard nursery gauge in inches. Caliper was measured about six inches above the root zone. Standard nursery calipers are 2/16-inch (0.318 cm), 3/16-inch (0.476 cm), ¼-inch (0.635 cm), 3/8-inch (0.953 cm) and 7/16-inch (1.111 cm) inches. Total height was measured from the bottom of the root zone to the top of the rootstock. Number of spines, branches, roots, and rooted nodes were counted, and straightness of the shoot was rated using standard nursery scale. A rootstock was considered bent if the rootstock bend was at least one inch (2.5 cm).

A partial economic analysis of the benefit of PGR sprays on profit was based on a 3 m long section of stoolbed. Liner production per 3 m was estimated to be 33 liners per meter. For A grade liners, profit was based on sale price ($0.68 for B.9, M.9-T337 and M.9-EMLA; $0.84 for G.41) minus growing costs ($0.40 for all varieties). For B grade liners profit was based on sale price (20% reduction from A grade price) minus growing costs. Cost of chemical application was an estimate of the cost of the spray product only. Profit and costs were estimated in US dollars.

Data Analysis. Data for each experiment were analyzed by analysis of variance using SAS 9.4 (SAS Institute, Cary, NC) general linear model procedure. When the F value was significant, mean separation was done by Duncan's multiple range test with $P \leq 0.05$ which uses the studentized range distribution. This method was used because different mean comparisons may vary in their significance levels.

Results

Washington 2008. In Washington, chemical treatment did not significantly affect liner caliper or proportion of straight shoots. IBA at 10,000 ppm significantly reduced the height of the rootstock liner by 3.5 cm compared to the control (Table 1). IBA at the high concentration caused more lateral growth increasing the number of spines and branches per liner by more than two compared to the control. IBA at both the low and the high concentrations increased the number of total roots compared to the control. Chemical treatment did not have any effect on the number of nodes below the sawdust line. IBA at 10,000 ppm also increased the number of rooted nodes and percent of rooted nodes compared to the control (Table 1). Caliper was not significantly different between rootstocks; however, B.9 rootstocks were taller, had fewer spines and had a higher proportion of bent shoots than M.9-T337 rootstocks. The number of roots, nodes below the sawdust line and rooted nodes was not significantly different between rootstocks while B.9 had six percent more rooted nodes than M.9-T337. IBA sprayed at 10,000 ppm resulted in crooked tips and increased branching, with the worst cases on B.9 liners (Figure 2). Prohexadione Ca sprays caused a shortening of internode length in all treatments (Figure 3). There was a significant interaction of PGR treatment and rootstock genotype on the number of spines per liner. There were no significant differences in the percentages of A or B grade liners due to plant growth regulator (PGR) treatment, while the percentages of C and D grade were significantly affected by plant growth regulator treatment (Table 2). Treatment with IBA at 1,000 ppm significantly increased the combined percent of A and B grade liners by 11% over the control. Other treatments such as prohexadione Ca at 1,000 ppm and IBA at 10,000 ppm also resulted in a numeric increase of the combined percent of A and B grade but were not significantly different from the control (Table 2). There were significant differences in liner grade out between B.9 and M.9-T337 rootstocks. B.9 had a higher percentage of A grade and a smaller percentage of C and D grade than M.9-T337. 88.2% of B.9 shoots were categorized as A and B grade rootstock liners compared to only 41.8% of M.9-T337 shoots. There was no significant interaction between PGR treatment and rootstock on liner grade out.

Figure 2. Shoot tip bending caused by foliar spray of IBA in 2008.

Figure 3. Shortened internodes caused by prohexadione Ca at 1000 ppm sprays on M.9-T337 in 2009.

Table 1. Effect of plant growth regulator sprays on rootstock liner characteristics of B.9 and M.9-T337 in Washington in 2008.

Treatment	Rootstock	Liner caliper (cm)	Liner height (cm)	# Spines per liner	Proportion of straight liners	# Roots per liner	# Root nodes below sawdust line	# Rooted nodes per liner	% Rooted nodes below sawdust
Untreated Control		0.65 a[z]	81.5 ab	0.4 c	0.799 a	9.4 c	7.6 a	3.7 a	48.5 a
Prohexadione Ca 250 ppm		0.64 a	81.5 ab	0.8 bc	0.831 a	9.6 c	7.3 a	3.6 a	48.8 a
Prohexadione Ca 1,000 ppm		0.63 a	84.3 ab	1.4 b	0.862 a	10.2 bc	7.6 a	4.0 a	52.0 a
IBA 1,000 ppm		0.64 a	81.0 bc	1.3 b	0.828 a	11.2 ab	7.6 a	3.8 a	49.7 a
IBA 10,000 ppm		0.65 a	78.0 c	2.7 a	0.728 a	12.3 a	7.7 a	4.2 a	54.8 a
NAA 50 ppm		0.64 a	84.6 a	0.7 bc	0.831 a	10.1 bc	7.3 a	4.0 a	52.5 a
NAA 200 ppm		0.64 a	82.8 ab	0.8 bc	0.847 a	9.3 c	7.4 a	3.6 a	47.9 a
PGR Treatment Significance		NS	**	**	NS	*	NS	NS	NS
	B.9	0.64 a	85.6 a	0.2 b	0.71 b	10.8 a	7.5 a	4.1 a	53.9 a
	M.9-T337	0.64 a	79.0 b	1.9 a	0.91 a	9.8 a	7.5 a	3.6 a	47.8 a
Rootstock Significance		NS	**	**	**	NS	NS	NS	NS
Significance of Interaction of PGR treatment and Rootstock		NS	NS	*	NS	NS	NS	NS	NS

[z]Means within columns with the same letter are not significantly different using Duncan multiple range test P≤ 0.05; n=175 for PGR treatment means, n=612 for rootstock means, and n=87 for interaction means.

Table 2. Effect of plant growth regulator sprays on rootstock liner grade of B.9 and M.9-T337 in Washington in 2008.

Treatment	Rootstock	Percentage of stoolbed shoots in grades A-D[z]					
		%A	%B	%C	%D	%AB	%CD
Untreated Control		35.6 a[y]	27.2 a	26.1 a	11.2 bc	62.7 b	37.3 a
Prohexadione Ca 250 ppm		37.7 a	22.6 a	24.5 ab	15.2 ab	60.3 b	39.7 a
Prohexadione Ca 1,000 ppm		40.8 a	26.1 a	16.3 c	16.7 a	67.0 ab	33.0 ab
IBA 1,000 ppm		43.5 a	30.3 a	18.2 bc	8.0 c	73.8 a	26.2 b
IBA 10,000 ppm		44.3 a	22.8 a	20.7 abc	12.2 abc	67.1 ab	32.9 a
NAA 50 ppm		34.8 a	27.7 a	25.5 a	12.0 abc	62.5 b	37.5 a
NAA 200 ppm		36.4 a	25.2 a	24.5 ab	13.9 ab	61.6 b	38.4 a
PGR Treatment Significance		NS	NS	*	*	*	*
	B.9	58.9 a	29.3 a	8.9 b	2.9 b	88.2 a	11.8 b
	M.9-T337	19.1 b	22.7 a	35.6 a	22.6 a	41.8 b	58.2 a
Rootstock Significance		**	NS	**	**	**	**
Significance of Interaction of PGR treatment and Rootstock		NS	NS	NS	NS	NS	NS

[z]A grade = 16 + roots per liner, B grade = 9-15 root per liner, C grade = 1-8 root per liner, D grade = 0 root per liner

[y]Means with columns with the same letter are not significantly different using Duncan multiple range test P ≤ 0.05; n=8 for PGR treatment means, n=28 for rootstock means, and n=4 for interaction means.

Washington 2009. In 2009, liner caliper, number of spines, proportion of straight shoots and number of nodes below the sawdust line did not differ significantly among the PGR treatments (Table 3). However, prohexadione Ca at 1,000 ppm sprayed on August 24, prohexadione Ca at 1,000 ppm sprayed on August 3, prohexadione Ca at 250 ppm sprayed on August 3 and prohexadione Ca at 1,000 ppm sprayed on July 23 resulted in liners which were significantly shorter than the control. Prohexadione Ca at 1,000 ppm sprayed on August 24 resulted in significantly more roots per liner than the controls. Prohexadione Ca 1,000 ppm sprayed on August 3 and prohexadione Ca 1,000 ppm sprayed on July 23 resulted in significantly more rooted nodes per liner than the controls. Liners which received prohexadione Ca 1,000 ppm sprayed on July 23 had the largest percentage of rooted nodes, 6.4% more than the control (Table 3). Liner caliper, proportion of straight shoots and number of nodes below the sawdust line did not differ between the rootstocks. B.9 liners were taller, had fewer spines, more roots, more rooted nodes and a higher percent of rooted nodes than M.9-T337 shoots. There was no significant interaction between PGR treatment and rootstock on liner characteristics. Prohexadione Ca at 1,000 ppm sprayed on August 24, prohexadione Ca at 1,000 ppm sprayed on August 3 and IBA at 1,000 ppm sprayed on June 17 significantly increased the percentage of A grade liners compared to the control (Table 4). Prohexadione Ca at 1,000 ppm sprayed on August 24 and IBA at 1,000 ppm sprayed on June 17 resulted in significantly lower percent B grade liners than the control. The combined percentage of A and B grade liners did not differ significantly from the control (Table 4). The differences among rootstocks followed the same trend in 2009 as in 2008. B.9 had a higher percentage of A grade liners and lower percentage of B, C and D grade than M.9-T337. With B.9, the combined percentage of A and B grade liners was 98.8% while with M.9-T337 it was only 77.9%. There was a significant interaction of PGR treatment and rootstock on the percentage of B grade liners.

France 2008. Liner caliper, straightness of shoot, number of nodes below the sawdust line and the number of rooted nodes varied by rootstock, but not by PGR treatment (Table 5). Likewise, the number of spines, number of roots and the percent of rooted nodes was not significantly different among rootstocks or treatments. There was no significant interaction between PGR treatment and rootstock on liner characteristics.

Neither chemical treatment nor rootstock affected the percentage of A, B and C grade liners at DL Nursery in France (Table 6). Likewise, the combined percentage of A and B grade was not significantly different between treatments or rootstocks. There was no significant interaction between PGR treatment and rootstock on liner grade out.

France 2009. Prohexadione Ca treatment, regardless of timing, reduced the height of the shoots (Table 7). Prohexadione Ca at 1,000 ppm sprayed on June 29 and prohexadione Ca at 1,000 ppm sprayed on June 29 and July 29 increased the proportion of straight shoots by 13-17% compared to the untreated controls. Prohexadione Ca at 1,000 ppm sprayed on June 29 and July 29 also increased the number of root nodes below the sawdust line compared to the control. Prohexadione Ca sprays did not affect liner caliper or number of spines. Liner height and total number of roots were significantly different between the two rootstocks. Liners of M.9-EMLA were shorter but had more roots than liners of G.41. In all other liner characteristics, which we measured there were no significant differences between the rootstocks. There were some significant interactions between PGR treatment and rootstock on liner caliper, number of roots per liner, number of nodes below the sawdust line and number of rooted nodes below the sawdust line.

Table 3. Effect of plant growth regulator sprays on rootstock liner characteristics of B.9 and M.9-T337 in Washington in 2009.

Treatment	Rootstock	Liner caliper (cm)	Liner height (cm)	# Spines per liner	Proportion of straight liners	# Roots per liner	# Rooted nodes below sawdust line	# Rooted nodes per liner	% Rooted nodes below sawdust
Untreated Control		0.61 a[z]	79.8 ab	0.7 a	0.748 a	16.0 a	6.1 a	4.5 bc	72.9 a
Prohexadione Ca 250 ppm Aug 3		0.59 a	72.6 cd	0.3 a	0.759 a	16.2 a	6.1 a	4.4 bc	70.2 a
Prohexadione Ca 1,000 ppm June 25		0.60 a	73.4 bcd	0.5 a	0.746 a	16.9 a	6.1 a	4.2 c	70.0 a
Prohexadione Ca 1,000 ppm July 23		0.61 a	37.1 de	0.3 a	0.800 a	17.3 a	6.4 a	5.2 a	79.3 a
Prohexadione Ca 1,000 ppm Aug 24		0.62 a	63.2 e	0.6 a	0.847 a	18.7 a	6.3 a	4.9 ab	75.2 a
Prohexadione Ca 1,000 ppm Aug 3		0.60 a	72.4 cd	0.5 a	0.704 a	17.5 a	6.8 a	5.3 a	76.1 a
Prohexadione Ca 1,000 ppm June 25 and July 23		0.60 a	79.0 abc	0.7 a	0.723 a	16.4 a	6.8 a	4.8 abc	76.4 a
IBA 1,000 ppm June 17		0.60 a	80.0 ab	0.5 a	0.795 a	17.1 a	6.3 a	4.8 abc	73.1 a
IBA 10,000 ppm June 17		0.59 a	82.6 a	0.6 a	0.639 a	16.4 a	6.2 a	4.5 bc	71.1 a
NAA 50 ppm Aug 3		0.61 a	80.3 ab	0.5 a	0.726 a	15.8 a	6.4 a	4.8 abc	73.1 a
NAA 200 ppm Aug 3		0.62 a	80.5 ab	0.7 a	0.802 a	14.9 a	6.2 a	4.4 bc	70.5 a
PGR Treatment Significance		NS	**	NS	NS	NS	NS	**	NS
	B.9	0.61 a	80.0 a	0.0 b	0.630 a	21.2 a	6.5 a	5.6 a	85.0 a
	M.9-T337	0.60 a	72.9 a	0.9 a	0.839 a	13.4 a	6.2 a	4.1 a	65.1 a
Rootstock Significance		NS	NS	**	NS	NS	NS	NS	NS
Significance of Interaction of PGR Treatment and Rootstock		NS	NS	NS	NS	NS	NS	NS	NS

[z]Means within the same column with the same letter are not significantly different using Duncan multiple range test P≤ 0.05; n=112 for PGR treatment means, n=618 for rootstock means, and n=56 for interaction means.

Table 4. Effect of plant growth regulator sprays on rootstock liner grade of B.9 and M.9-T337 in Washington in 2009.

Treatment	Rootstock	Percentage of stoolbed shoots in grades A-D[z]					
		%A	%B	%C	%D	%AB	%CD
Untreated Control		55.3 cd[y]	30.4 a	13.5 a	0.7 a	85.7 a	14.3 a
Prohexadione Ca 250 ppm Aug 3		55.5 bcd	31.1 a	12.4 a	1.0 a	86.6 a	13.4 a
Prohexadione Ca 1,000 ppm June 25		60.0 abcd	27.4 ab	11.1 a	1.5 a	87.4 a	12.6 a
Prohexadione Ca 1,000 ppm July 23		60.4 abc	30.9 a	8.6 a	0.1 a	91.3 a	8.7 a
Prohexadione Ca 1,000 ppm Aug 3		64.6 ab	26.6 ab	8.6 a	0.2 a	91.2 a	8.8 a
Prohexadione Ca 1,000 ppm Aug 24		66.6 a	24.2 b	8.8 a	0.4 a	90.8 a	9.2 a
Prohexadione Ca 1000 ppm June 25 and July 23		60.8 abc	28.7 ab	9.8 a	0.6 a	89.6 a	10.4 a
IBA 1,000 ppm June 17		65.3 a	23.9 b	9.8 a	1.0 a	89.2 a	10.8 a
IBA 10,000 ppm June 17		63.6 abc	26.1 ab	8.7 a	1.7 a	89.7 a	10.3 a
NAA 50 ppm Aug 3		55.6 bcd	31.8 a	12.1 a	0.5 a	87.4 a	12.6 a
NAA 200 ppm Aug 3		51.4 d	31.7 a	15.6 a	1.3 a	83.1 a	16.9 a
PGR Treatment Significance		**	**	NS	NS	NS	NS
	B.9	80.4 a	18.4 b	1.1 b	0.0 b	98.8 a	1.2 b
	M.9-T337	39.5 b	38.4 a	20.5 a	1.6 a	77.9 b	22.1 a
Rootstock Significance		**	**	**	*	**	**
Significance of Interaction of PGR Treatment and Rootstock		NS	*	NS	NS	NS	NS

[z]A grade = 16 + roots per liner, B grade = 9-15 root per liner, C grade = 1-8 root per liner, D grade = 0 root per liner

[y]Means within the same column with the same letter are not significantly different using Duncan multiple range test P≤ 0.05; n=8 for PGR treatment means, n=44 for rootstock means, and n=4 for interaction means.

Table 5. Effect of plant growth regulator sprays on rootstock liner characteristics of M.9-EMLA and G.41 in France in 2008.

Treatment	Rootstock	Liner caliper (cm)	Liner height (cm)	# Spines per liner	Proportion of straight liners	# Roots per liner	# Root nodes below sawdust line	# Rooted nodes per liner	% Rooted nodes below sawdust
Untreated Control		0.65 a[z]	69.1 a	0.9 a	0.847 a	10.1 a	5.8 a	4.3 a	74.6 a
NAA 50 ppm		0.66 a	72.4 a	0.7 a	0.774 a	8.9 a	5.7 a	4.2 a	85.0 a
Prohexadione Ca 250 ppm		0.65 a	69.9 a	0.8 a	0.882 a	9.8 a	5.3 a	3.6 a	67.0 a
Prohexadione Ca 1,000 ppm		0.62 a	69.9 a	0.7 a	0.832 a	9.2 a	5.7 a	4.0 a	75.7 a
PGR Treatment Significance		NS	NS	NS	NS	NS	NS	NS	NS
	M.9-EMLA	0.60 b	64.3 a	0.5 a	0.983 a	10.3 a	4.1 b	3.3 b	77.7 a
	G.41	0.69 a	75.9 a	1.0 a	0.697 b	8.8 a	7.0 a	4.7 a	72.7 a
Rootstock Significance		*	NS	NS	**	NS	*	**	NS
Significance of Interaction of PGR Treatment and Rootstock		NS	NS	NS	NS	NS	NS	NS	NS

[z]Means within the same column with the same letter are not significantly different using Duncan multiple range test P≤ 0.05; n=118 for PGR treatment means, n=235 for rootstock means, and n=59 for interaction means.

Table 6. Effect of plant growth regulator sprays on rootstock liner grade of M.9-EMLA and G.41 in France in 2008.

		Percentage of stoolbed shoots in grades A-D[z]					
Treatment	Rootstock	%A	%B	%C	%D	%AB	%CD
Untreated Control		10.0 a[y]	39.4 a	49.0 a	1.6 a	49.4 a	50.6 a
NAA 50 ppm		8.3 a	41.7 a	48.6 a	1.5 a	50.0 a	50.0 a
Prohexadione Ca 250 ppm		12.6 a	36.7 a	47.4 a	3.3 a	49.3 a	50.7 a
Prohexadione Ca 1,000 ppm		9.8 a	36.2 a	50.7 a	3.3 a	46.0 a	54.0 a
PGR Treatment Significance		NS	NS	NS	*	NS	NS
	M.9-EMLA	3.9 a	39.3 a	55.2 a	1.6 a	43.2 a	56.8 a
	G.41	16.4 a	37.7 a	42.6 a	3.3 a	54.1 a	45.9 a
Rootstock Significance		NS	NS	NS	NS	NS	NS
Significance of Interaction of PGR Treatment and Rootstock		NS	NS	NS	NS	NS	NS

[z]A grade = 16 + roots per liner, B grade = 9-15 root per liner, C grade = 1-8 root per liner, D grade = 0 root per liner.
[y]Means within the same column with the same letter are not significantly different using Duncan multiple range test P≤ 0.05; n=8 for PGR treatment means, n=16 for rootstock means, and n=4 for interaction means.

Table 7. Effect of plant growth regulator sprays on rootstock liner characteristics of M.9-EMLA and G.41 in France in 2009.

Treatment	Rootstock	Liner caliper (cm)	Liner height (cm)	# Spines per liner	Proportion of straight liners	# Roots per liner	# Rooted nodes below sawdust line	# Rooted nodes per liner	% Rooted nodes below sawdust
Untreated Control		0.62 a[z]	67.6 a	0.9 a	0.799 c	8.1 a	3.4 a	2.4 b	64.2 a
Prohexadione Ca 1,000 ppm June 29		0.63 a	55.4 c	1.0 a	0.935 ab	9.0 a	3.4 a	2.5 ab	68.1 a
Prohexadione Ca 1,000 ppm July 29		0.60 a	62.7 b	0.8 a	0.862 bc	8.9 a	3.3 a	2.3 b	64.7 a
Prohexadione Ca 1,000 ppm June 29 and July 29		0.59 a	54.4 c	1.0 a	0.967 a	9.4 a	3.6 a	2.7 a	69.7 a
PGR Treatment Significance		NS	**	NS	**	NS	NS	**	NS
	M.9-EMLA	0.59 a	58.2 b	0.1 a	0.939 a	11.2 a	3.3 a	2.3 a	65.4 a
	G.41	0.63 a	62.0 a	1.7 a	0.840 a	6.5 b	3.5 a	2.6 a	68.0 a
Rootstock Significance		*	*	NS	NS	**	NS	*	NS
Significance of Interaction of PGR Treatment and Rootstock		*	NS	NS	NS	*	*	*	NS

[z]Means with the same column with the same letter are not significantly different using Duncan multiple range test P≤ 0.05; n=155 for PGR treatment means, n=310 for rootstock means, and n=78 for interaction means.

Table 8. Effect of plant growth regulator sprays on rootstock liner grade of M.9-EMLA and G.41 in France in 2009.

Treatment	Rootstock	Percentage of stoolbed shoots in grades A-D[z]					
		%A	%B	%C	%D	%AB	%CD
Untreated Control		24.9 b[y]	25.8 a	37.5 a	11.9 a	50.7 b	49.3 a
Prohexadione Ca 1,000 ppm June 29		37.4 a	31.4 a	24.0 b	7.2 b	68.8 a	31.2 b
Prohexadione Ca 1,000 ppm July 29		34.4 a	31.3 a	27.1 b	7.3 b	65.7 a	34.3 b
Prohexadione Ca 1,000 ppm June 29 and July 29		38.6 a	30.2 a	25.9 b	5.3 b	68.8 a	31.2 b
PGR Treatment Significance		**	NS	*	**	**	**
	M.9-EMLA	54.4 a	24.4 b	16.0 b	5.2 a	78.8 a	21.2 b
	G.41	13.2 b	35.0 a	41.2 a	10.7 a	48.1 b	51.9 a
Rootstock Significance		**	*	**	NS	**	**
Significance of Interaction of PGR Treatment and Rootstock		NS	NS	NS	NS	NS	NS

[z]A grade = 16 + roots per liner, B grade = 9-15 root per liner, C grade = 1-8 root per liner, D grade = 0 root per liner

[y]Means within the same column and with the same letter are not significantly different using Duncan multiple range test P≤ 0.05; n=8 for PGR treatment means, n=16 for rootstock means, and n=4 for interaction means.

All prohexadione Ca spray treatments significantly improved the grade out of liners compared to the untreated control (Table 8). Prohexadione Ca sprays, regardless of timing, produced a higher percentage of A grade liners and a lower percentage of C grade and D grade liners than the controls. The percentage of B grade liners was not affected by prohexadione Ca sprays. The combined percentage of A and B grade liners was significantly higher in all prohexadione Ca treatments than in the control. There were significant differences between rootstocks in 2009. M.9-EMLA produced a greater percentage of A grade and a lower percentage of B and C grade liners than G.41. The combined percentage of A and B grade liners was also significantly higher with M.9-EMLA than G.41. There was no significant interaction between PGR treatment and rootstock on liner grade out.

Oregon 2009. In Oregon, only G.41 rootstocks were used for the experiment. Prohexadione Ca sprayed at 1,000 ppm on June 29 and July 29 reduced the caliper of liners and increased the proportion of straight shoots compared to the untreated control (Table 9). Prohexadione Ca sprayed at 1,000 ppm on June 29 and prohexadione Ca sprayed at 1,000 ppm on June 29 and July 29 produced liners which were much shorter than the untreated liners, up to 20-25cm shorter. The two prohexadione Ca treatments also reduce the number of spines from 12.7 per liner in the control to 3.7 and 2.5 in the June and June + July prohexadione Ca sprays. The number of roots per liner, number of rooted nodes and percentage of nodes with roots was also increased by the two prohexadione Ca treatments.

Prohexadione Ca reduced the percentage of D grade liners (Table 10). Prohexadione Ca at 1,000 ppm sprayed on June 29 and prohexadione Ca at 1,000 ppm sprayed on June 29 and July 29 had a lower percentage of D grade liners than the untreated control.

Table 9. Effect of plant growth regulator sprays on rootstock liner characteristics of G.41 in Oregon in 2009.

Treatment	Liner caliper (cm)	Liner height (cm)	# Spines per liner	Proportion of straight liners	# Roots per liner	# Root nodes below sawdust line	# Rooted nodes per liner	% Rooted nodes below sawdust
Untreated Control	0.68 a[z]	98.3 a	12.7 a	0.673 b	9.8 a	5.4 a	4.0 a	72.8 b
Prohexadione Ca 1000 ppm June 29	0.66 ab	77.7 b	3.7 b	0.817 ab	14.0 a	5.9 a	5.5 a	92.7 a
Prohexadione Ca 1000 ppm July 29	0.68 a	91.7 a	10.4 a	0.750 b	11.0 a	5.6 a	4.0 a	66.3 b
Prohexadione Ca 1000 ppm June 29 and July 29	0.59 b	73.4 b	2.5 b	0.967 a	13.7 a	5.6 a	5.4 a	94.2 a
PGR Treatment Significance	*	**	**	*	NS	*	NS	**

[z]Means within the same column and with the same letter are not significantly different using Duncan multiple range test P≤ 0.05; n=56 for PGR treatment means.

Cost analysis. A partial economic analysis of the benefit of PGR sprays on profit from a 3 m long section of stoolbed showed that the profit from A grade rootstock liners varied from $1.06 USD to $24.81 while the profit from B grade rootstock liners varied from

$1.54 to $11.70 (Table 11). The cost of applying the various PGR treatments varied from $0.06 for a low dose spray of prohexadione Ca to $7.75 USD for a high dose spray of IBA. When the cost of sprays was subtracted from the combined profit for A and B grade rootstock liners the net profit ranged from $0.31 to $28.96. Prohexadione Ca was cost effective in all locations in both years. The best performance was with G.41 in Oregon with a spray of 1,000 ppm applied in late June. The lowest net profit was from a high dose of IBA (10,000 ppm) in WA with M.9. In general, the high dose of IBA and the low dose of prohexadione Ca had lower profit than the untreated control while the high dose of prohexadione Ca had higher profit than the untreated control.

Discussion

Rootstocks behave differently across climates and from year to year. The first year of our studies (2008) was a poorer rooting year for B.9 and M.9-T337 in Washington than the second year (2009). The combined percentages of A and B grade liners in untreated control plots for B.9 went from 86% in 2008 to 99.2% in 2009 while with M.9-T337 saw a jump from 39.4% in 2008 to 72.2% in 2009. M.9-EMLA in France followed a similar pattern with 41.7% A and B grade liners in 2008 and 69.6% in 2009. However, G.41 in France did much worse in 2009 than in 2008 with 31.7% A and B grade liners in 2009 compared to 52.8% A and B grade liners in 2008. Some fluctuation between the years can be explained by environmental conditions. Washington tends to have a shorter growing season (shoots form in May and stop growing in September) compared to Oregon but has higher summer temperatures and sunlight during the summer. Warmer spring temperatures could help increase shoot production initially while warmer temperatures in September and October could give the mounded shoots more time to form roots. The soil may also have an effect on how well the rootstocks grow. The soil at Willow Drive Nursery in Ephrata, Washington is a Timmerman soil consisting of coarse sandy loam while the soil at Willamette Nursery in Canby, Oregon is a Latourell soil consisting mainly of loam. The loamy soils of Oregon hold moisture better than the sandy loam of Washington, which can contribute to rooting. Rooting percentage may also be affected by the quality of labor during the mounding process. A stoolbed that is mounded very well will have a higher chance of rooting compared to one that has lots of holes not filled with sawdust. Adequate water is also important for active root growth. There are many variables involved in maximizing rooting.

An important finding from our work is that plant growth regulator treatment can improve rooting of shoots of apple rootstocks. In Washington, where B.9 and M.9-T337 were the rootstocks for the experiment, IBA sprayed at both 1,000 ppm and 10,000 ppm consistently improved the rooting success in the stoolbeds. This is consistent with increased rooting of IBA treated cuttings (Alvarez, et al., 1989; Hartmann et al. 1997). In 2008 the spraying was done over the top of the stoolbed with the intent that the chemical would run down the shoots. This resulted in crooked tips and increased branching. In an effort to solve this problem the height of the spray wand was lowered in 2009 to be below the shoot canopy, applying the hormone as close to the root zone as possible.

Prohexadione Ca sprayed at 1,000 ppm also helped increase the rooting percentage in both years. Although not statistically significant, prohexadione Ca sprayed at 1,000 ppm on August 25, 2008, gave a numeric increase in the percentage of A grade liners of 5% compared to the untreated control. Prohexadione Ca sprayed at 1,000 ppm at various timings in 2009 also increased the percentage of A grade liners by at least 5% compared to the control. The best results in Washington were seen in the late August spray

Table 11. Cost analysis of chemical treatments in Washington, France and Oregon.

Year	Location	Rootstock	Treatment	[z]Profit ($US) from A grade liners/3 m	Profit ($US) from B grade liners/3 m	Cost ($US) of treatment/3 m	Net profit from A and B grade ($US)/3 m
2008	WA	B.9	Control	15.18	4.58	0.00	19.76
2009	WA	B.9	Control	21.48	3.25	0.00	24.73
2008	WA	M.9-T337	Control	4.73	3.24	0.00	7.97
2009	WA	M.9-T337	Control	9.52	5.52	0.00	15.04
2008	FR	M.9-EMLA	Control	1.68	5.16	0.00	6.84
2009	FR	M.9-EMLA	Control	12.40	3.64	0.00	16.05
2008	FR	G.41	Control	6.20	11.70	0.00	17.90
2009	OR	G.41	Control	15.93	7.64	0.00	23.57
2009	FR	G.41	Control	2.38	7.15	0.00	9.53
2008	WA	B.9	IBA 1000 ppm	16.91	4.61	0.78	20.75
2009	WA	B.9	IBA 1000 ppm	24.50	1.79	0.78	25.51
2008	WA	M.9-T337	IBA 1000 ppm	7.45	4.12	0.78	10.79
2009	WA	M.9-T337	IBA 1000 ppm	12.10	5.10	0.78	16.42
2008	WA	B.9	IBA 10000 ppm	20.05	3.28	7.75	15.58
2009	WA	B.9	IBA 10000 ppm	24.81	1.54	7.75	18.60
2008	WA	M.9-T337	IBA 10000 ppm	4.79	3.27	7.75	0.31
2009	WA	M.9-T337	IBA 10000 ppm	10.81	5.96	7.75	9.02
2008	WA	B.9	Prohexadione Ca 250 ppm Aug	17.25	4.05	0.06	21.23
2009	WA	B.9	Prohexadione Ca 250 ppm August 3	21.81	2.98	0.06	24.73
2008	WA	M.9-T337	Prohexadione Ca 250 ppm Aug	3.86	2.45	0.06	6.25
2009	WA	M.9-T337	Prohexadione Ca 250 ppm August 3	9.30	5.98	0.06	15.21
2008	FR	M.9-EMLA	Prohexadione Ca 250 ppm Aug	1.06	5.11	0.06	6.11
2008	FR	G.41	Prohexadione Ca 250 ppm Aug	7.00	10.04	0.06	16.97
2008	WA	B.9	Prohexadione Ca 1000 ppm Aug	16.88	4.16	0.25	20.79
2009	WA	B.9	Prohexadione Ca 1000 ppm August 3	23.63	2.12	0.25	25.49
2009	WA	B.9	Prohexadione Ca 1000 ppm July 29	22.32	2.81	0.25	24.87
2009	WA	B.9	Prohexadione Ca 1000 ppm August 24	22.43	2.66	0.25	24.84
2009	WA	B.9	Prohexadione Ca 1000 ppm June 29	22.12	2.88	0.25	24.75
2009	WA	B.9	Prohexadione Ca 1000 ppm June 29 July 29	21.90	2.98	0.51	24.37
2008	WA	M.9-T337	Prohexadione Ca 1000 ppm Aug	5.99	3.37	0.25	9.11
2009	WA	M.9-T337	Prohexadione Ca 1000 ppm August 24	14.84	4.31	0.25	18.89

Table 11 (con't).

2009	WA	M.9-T337	Prohexadione Ca 1000 ppm August 3	12.54	5.56	0.25	17.85
2009	WA	M.9-T337	Prohexadione Ca 1000 ppm July 29	11.51	6.08	0.25	17.33
2009	WA	M.9-T337	Prohexadione Ca 1000 ppm June 29 July 29	12.15	5.30	0.51	16.94
2009	WA	M.9-T337	Prohexadione Ca 1000 ppm June 29	11.51	5.01	0.25	16.27
2008	FR	M.9-EMLA	Prohexadione Ca 1000 ppm Aug	1.48	5.88	0.25	7.11
2009	FR	M.9-EMLA	Prohexadione Ca 1000 ppm June 29 July 29	17.61	3.02	0.51	20.13
2009	FR	M.9-EMLA	Prohexadione Ca 1000 ppm June 29	16.38	3.33	0.25	19.45
2009	FR	M.9-EMLA	Prohexadione Ca 1000 ppm July 29	14.53	4.08	0.25	18.35
2008	FR	G.41	Prohexadione Ca 1000 ppm Aug	8.76	8.87	0.25	17.37
2009	OR	G.41	Prohexadione Ca 1000 ppm June 29	20.02	9.19	0.25	28.96
2009	OR	G.41	Prohexadione Ca 1000 ppm June 29 July 29	18.52	10.17	0.51	28.19
2009	OR	G.41	Prohexadione Ca 1000 ppm July 29	14.17	8.49	0.25	22.40
2009	FR	G.41	Prohexadione Ca 1000 ppm June 29	7.13	10.83	0.25	17.70
2009	FR	G.41	Prohexadione Ca 1000 ppm July 29	7.39	9.36	0.25	16.49
2009	FR	G.41	Prohexadione Ca 1000 ppm June 29 July 29	6.25	10.72	0.51	16.46

[z]Liners per 3 m based on percent of 100 shoots (33 liners per meter). A grade profit based on sale price ($ 0.68 for B.9, M.9-T337 and M.9-EMLA. $ 0.84 for G.41) minus cost to grow ($ 0.40 for all varieties). B grade profit based on sale price (20% reduction from A grade) minus cost to grow. Cost and profit in US dollars.

which resulted in an 11% increase in A grade liners compared to the control. It should be noted that prohexadione Ca reduced shoot height in 2009. Prohexadione Ca is a gibberellin biosynthesis inhibitor and effectively slows shoot growth. A visible symptom of prohexadione Ca which we and others have observed is the shortening of the internodes (Green 2003).

NAA sprayed at 50 ppm and 200 ppm and prohexadione Ca at 250 ppm never increased the combined percentage of A and B grade liners by more than 2% compared to the control and in some cases actually reduced the percentage of A and B grade liners compared to the control.

In France, where M.9-EMLA and G.41 were the rootstocks used in the experiments, there were no significant results in 2008. This was probably due to short plot lengths and some vertebrate pest pressure caused by rabbits which affected some plots near the edge of the field and increased variability. This was corrected in 2009 by increasing the plot length from 2m to 4m and setting the experiment back from the edge of the field. In 2009 all prohexadione Ca sprays at 1,000 ppm, regardless of application timing, consistently increased the rooting of both M.9-EMLA and G.41 liners. As with B.9 and M.9-T337 liners in Washington, prohexadione Ca sprays reduced shoot height of both M.9-EMLA and G.41 liners in France compared to the control.

Prohexadione Ca sprays consistently increased the proportion of straight shoots in France in 2009. Similar results were seen in Oregon in 2009 where G.41 was the rootstock for the experiment. Since a straighter shoot is worth more money than a bent one this result has significant economic impact. It is also important that the early prohexadione Ca sprays (late June) reduced the total number of spines and side branches per liner in Oregon where spines are typically a problem with G.41. Prohexadione Ca sprayed at 1,000 ppm in June and June + July reduced the total number of spines from 12.7 spines per liner in the control to 3.7 spines per liner. This result would effectively reduce the labor that would be required to hand remove the spines during the season or after harvest and before sale.

Anti-gibberellins, such as prohexadione Ca, either: 1) antagonize the synthesis of gibberellins which normally inhibit rooting; or 2) reduce shoot growth which may compete with the root zone for assimilates to the detriment of root formation (Davis and Sankhla 1988; Hartmann et al. 1997). Although there has previously been very little work with anti-gibberellins on apple rootstocks, our results are consistent with those seen on other plants such as bean cuttings (Davis and Sankhla 1988). Other plant growth regulators have shown conflicting results. In Poland, tests with ethephon increased the number of rooted M.26 liners, while the growth retardant Cyclocel showed no benefit to rooting (Webster 1995).

Conclusion

Many of the best apple rootstocks are difficult to propagate. The purpose of this study was to find ways to improve the propagation of rootstocks with desirable characteristics such as dwarfing capabilities, high yield, early fruit bearing and disease and pest resistance, but have poor rooting in stoolbeds.

Plant growth regulator treatments with IBA or prohexadione Ca improved rooting but the cost of IBA sprays was high and the targeting of IBA sprays must be precise to not damage the growing shoot tip. IBA could be used on a small scale with a backpack sprayer to target the spray to the root zone without affecting the shoot tips. It might be more difficult to use IBA on a large scale with boom sprayers which would not be able to accurately spray only the root zone. High concentrations of IBA are typically not cost effective.

Prohexadione Ca consistently improved rooting of the rootstocks we evaluated. It had very few negative side effects and could be adapted by either large- or small-scale growers. It can easily be broadcast over the top of the stoolbeds. Early application in June can also help control spines on problematic rootstock varieties. Most importantly, prohexadione Ca improves the rooting success in the stoolbeds and is cost effective.

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