

showed that FeNa₂-EDDHA at 56, 113, or 226 g plus 1620 g (NH₄)₂SO₄ corrected Fe chlorosis in 'Sungold' peach trees (3). Stebbins (8) noted that the yellow color and luminous reflectance of 'Elberta' peach leaves were lowered if trees received 2.0 lb. N as ammonium sulfate plus 1/2 lb. FeNa₂-EDDHA.

Shoot growth. In 1969, FeNa₂-EDDHA at 113.4 and 226.8 g increased shoot growth when compared to no fertilizer. Neither the 2-yr combined analysis nor the 1968 data showed significant difference between treatments (Table 2).

Trunk growth. The treatments had no significant effect on trunk growth.

FeNa₂-EDDHA will correct lime-induced Fe chlorosis in 'Sungold' peach trees but its overuse induces Mn deficiency (3). This study suggests that 'July Elberta' peaches are more susceptible to FeNa₂-EDDHA induced Mn deficiency than 'Sungold'. Also, the Mn:Fe ratio changes through the growing season where FeNa₂-EDDHA is applied. Future work should determine the amount of FeNa₂-EDDHA needed to overcome Fe chlorosis in peach trees without inducing Mn deficiency and the appropriate amounts of Mn needed to overcome Mn deficiency induced by application of FeNa₂-EDDHA.

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Extension of Vase-Life of Cut Flowers by Use of Isoascorbate - Containing Preservative Solutions^{1, 2}

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Abstract. A floral preservative solution containing iso-ascorbic acid, 100 ppm, sucrose, 4%, and 8-hydroxyquinoline sulfate, 50 ppm, extended cut rose life and was equal to other preservative formulations for carnations and snapdragons. Biochemical and other changes in rose petals resulting from the use of this preservative solution are described.

The extension of cut flower vase-life and improved post harvest development and maintenance is of great economic importance. Accomplishing this depends on post harvest cut flower handling and a preservative solution ensuring an ample supply of water, metabolites, and regulatory substances to petals and leaves. Various aqueous solutions of chemical compounds have been compared (4, 7, 8, 13, 18, 22, 23, 24, 38).

Our purpose was to develop a preservative solution better than those now used. This was done and the relevant flower changes including the composition and physical properties of rose petals were compared for roses kept in this and other solutions.

Materials and Methods

Blooms of roses, *Rosa hybrida* L. 'Forever Yours', 'Better Times', 'Baccara' and 'Regal Gold', carnation, *Dianthus caryophyllus* L. 'Cardinal Sim' and 'Improved White Sim', and snapdragons, *Antirrhinum majus* L. 'Pennsylvania' were cut from greenhouse-grown plants managed according to standard cultural practices (19). 'Forever Yours' was used in most of the histological and biochemical experiments. The longevity

of cut flowers was used to compare the efficiency of each preservative solution or their components. Roses (45 cm long stem), carnations, and snapdragons were kept in the test solutions in a controlled environment room under fluorescent illumination (0.5 lux/cm²), at 27°C, and 60% relative humidity.

Longevity was the number of days from cutting, when the first 2 or 3 petals of rose were unfolding, to petal drop or when "bent neck" developed. Carnations were discarded when the blooms lost color or became "sleepy" and snapdragons when half of the florets lost their crisp appearance and color. Experiments were repeated at least 4 times during fall and early winter months, using 10 blooms per treatment each time.

The aqueous solutions of the floral preservative mixtures or their ingredients, selected from preliminary experiments, were: 50 ppm 8-hydroxyquinoline sulfate (8-HQS); 100 ppm Na iso-ascorbate (Na-isoAA); 4% sucrose; "Cornell" solution (5% sucrose + 200 ppm 8-HQS + 50 ppm silver acetate); and the complete mixture (4% sucrose + 50 ppm 8-HQS + 100 ppm Na-isoAA), designated the "Ottawa" solution. The pH of this solution was 4.8 using distilled water.

Various concn of aqueous Na-isoAA or 8-HQS were compared for water uptake and inhibition of plugging of xylem in rose. Tests were conducted by placing 15 cm long stem sections, cut 3 cm below the apical bud and each bearing a single 5-leaflet leaf, in solutions. The sections were equilibrated for 24 hr in a growth room at conditions previously described and were weighed and re-weighed after a further 24 hr. Any change in fresh wt, differing from that of the water control, was

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assumed to indicate changes in the inhibition of vessel plugging and loss or retention of water. The histological examination of blockage was made on stem sections, approximately 17 cm from the base, on cut roses with intact blooms kept in the various solutions for 6 days. Transverse 20 μ thick sections were stained with toluidine blue, pH 6.8 (27). Respiration of the petals was measured in a Warburg respirometer (36). Ammonia in the petals was determined by distillation of the MgCO₃-liberated NH₃ into boric acid and titration. The pH of petals was measured after macerating a sample of 40 g in 50 ml of deionized water in a Waring blender for 1 min.

The "free space", protein synthesis, solute uptake, and amino acid leakage were determined using *d*-mannitol-1-¹⁴C (45 mc/mM), and *l*-leucine-¹⁴C (u.l., 250 mc/mM). For these measurements discs were punched with a No. 2 cork borer from randomly selected rose petals. We used methods described previously (14, 32, 33). Radioactivity was determined with a Nuclear Chicago, Mod. 186 scaler after adjusting for self-absorption, background, and coincidence loss. Protein content was measured by the methods of Kjeldahl, and of Lowry et al. (20). Rose petal colors were determined with a Gardner color difference meter using the pink and yellow standards (11). With this instrument the value of "L" indicates lightness or grade of color (100 = white, 0 = black); "a" indicates red when the value is plus, gray when zero, and green when minus; "b" shows yellow when the value is plus, gray when zero, and blue with a minus reading. Petals were hand sectioned for photography. The respiration, chemical, physical, and color tests were performed in duplicate on 2 replicate samples of fresh cut blooms or blooms 6 days after cutting and keeping in the various solutions.

Results and Discussion

The cut-life of roses 'Forever Yours', 'Baccara' and 'Better Times' averaged 10 days by use of the "Ottawa" solution (Table 1). This complete solution extended the cut-life of these roses significantly longer than its individual components, the "Cornell" solution, or the solution buffered at pH of 4.0 and containing 3% of sucrose + 200 ppm of 8-HQS based on work by Marousky (22, 24). The average cut-life of 'Regal Gold' was about 8 days but the concn of sucrose of the "Ottawa" solution had to be decreased from 4 to 2%, to avoid the browning of petals. The cut-life of snapdragons and carnations was extended to 13 and 9.7 days, respectively, when kept in the "Ottawa" solution. This solution was a significantly better preservative for snapdragons, but the "Cornell" solution was significantly better than the "Ottawa" solution for carnations. The other preservatives tested were less effective than the "Ottawa" solution in preventing the decline of cut roses, carnations, or snapdragons.

Cut roses frequently die from a loss of turgor pressure resulting from water deficiency. This is often due to "gum"

formation and plugging of the xylem elements of the rose stem (3, 9, 22, 23, 24). Burdett (3) found that the "gum" in stem sections took up ruthenium red thus indicating the presence of pectin-like material. Our earlier experiments (29) indicated that these deposits contained a mixture of compounds: carbohydrates, pectin, lipids, and protein-like substances. Similar re-deposition of compounds, particularly pectin, was shown to occur in aging bean endocarp (33).

Rose stems in the "Ottawa" solution were virtually free from "gum" deposits, while those kept in water were "plugged" and those kept in solutions of sucrose, Na-isoAA, or 8-HQS were intermediate in this respect at 6 days after cutting (Fig. 1). The prevention of xylem blockage by 8-HQS was observed in roses and gladioli by Marousky (23). The activity of 8-HQS, as an inhibitor of microorganisms producing pectolytic enzymes, may be similar to that of tannin or rufanic acid (12, 17). 8-HQS was included in the "Ottawa" solution for these reasons and for its reported effects on closure of stomata, prevention of xylem blockage, and metal-chelating properties (7, 22, 24, 40, 42). The pH of the aqueous solutions of 50 ppm of 8-HQS, or the 100

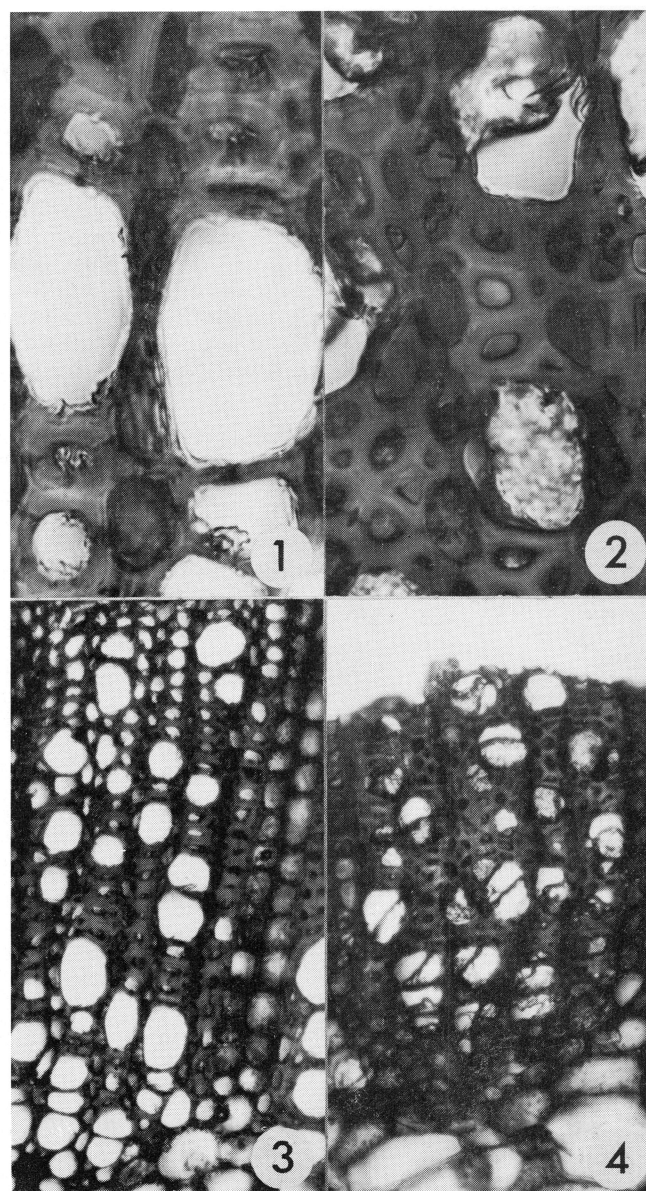


Fig. 1. Transverse sections of xylem from 6-day old excised rose stems, 'Forever Yours' comparing those kept in the "Ottawa" (1, 3) solution and water (2, 4). Stained with toluidine blue.

Table 1. Cut-life of 'Forever Yours', 'Baccara', and 'Better Times' roses, carnations, and snapdragons comparing the "Ottawa" solution with its components and other flora preservatives. Results of 4 trials of 10 flowers, each.

Formulation or solution	Cut-life, days		
	Roses	Carnations	Snapdragons
"Ottawa" sol'n.	10.0	9.7	13.0
Isoascorbic acid, (100 ppm)	6.9	7.2	8.0
8-HQS 950 ppm)	7.0	6.5	9.0
sucrose (4%, sol'n.	6.0	6.4	9.0
water	5.0	5.6	8.5
"Cornell" sol'n.	8.7	11.5	6.4
Marousky's sol'n.	7.1	7.7	8.0
LSD 5%	1.2	1.3	1.5

ppm of Na-isoAA was 3.9 and 6.0, respectively, and helped to maintain the acidity of the "Ottawa" solution near a level beneficial for water uptake by rose stems. Addition of buffer to the "Ottawa" solution caused loss of keeping quality of carnations and snapdragons.

Recent, unpublished, data indicated that roses grown in CO₂-enriched atmosphere, presumably amply supplied with photosynthetic products, had longer cut-life than similar roses grown in normal atmosphere. This finding is contradicted by Mattson and Widmer (26) who found that 'Forever Yours' roses did not benefit from CO₂-enrichment, up to 2000 ppm, of the atmosphere. Conversely, Kohl and Rundle (16) found that cut rose stems in winter have no reserves to feed the opening bud and thus a short pulse of sucrose solution prolonged and improved the vase life of 'Forever Yours' roses. The beneficial effects of sucrose on cut flowers have been enumerated by Marousky (25); in a recent review he pointed out that exhaustion of carbohydrates and the following proteolysis in cut flowers leads to "blueing" of red rose petals. Addition of sucrose to the "Ottawa" solution prevented this blueing, rise of ammonia content in petals, and change in pH of the petal extract. The pH of the petal extract of 'Forever Yours' was approximately 5.0 immediately after cutting but increased with aging: the smallest increases occurred in roses kept in sucrose-containing solutions. The solution of Na-isoAA alone produced a smaller rise in pH in the petals than either water or the 8-HQS solutions. The pH, as measured, appears to be lower than that indicated by the color of petals and it may be assumed that the contents of vacuoles were either more alkaline or that other factors, outlined by Asen et al. (1), were responsible. The color of all 4 cultivars, determined with the Gardner color difference meter, generally was closer to the original, fresh color of blooms if the cut roses were kept in the "Ottawa" solution (Table 2). The blueing, characteristic of petals of red roses kept in the sucrose-free solution, i.e. water, and particularly

Table 2. Comparisons of rose petal color values (Gardner Color Meter) of different cvs. from fresh, old-on-plant, or cut, 6-day-old blooms.

Cultivars and treatments	"L"	Color values "a"	"b"
'Forever Yours'			
Fresh cut	24.4	22.9	5.5
Old (on plant)	27.4	30.7	6.8
Water	24.6	19.9	2.2
Sucrose + 8-HQS	29.0	33.2	6.1
"Ottawa" sol'n.	28.8	33.1	6.0
LSD 5%	2.9	3.2	0.9
'Better Times'			
Fresh cut	30.0	26.9	6.1
Old (on plant)	30.0	26.9	6.1
Water	37.4	35.8	0.1
Sucrose + 8-HQS	39.8	28.0	0.4
"Ottawa" sol'n.	43.5	33.1	1.6
LSD 5%	28.8	25.8	6.1
'Baccara'			
Fresh cut	3.4	2.6	1.1
Old (on plant)	27.3	33.5	12.8
Water	32.1	40.2	11.9
Sucrose + 8-HQS	31.1	38.0	9.1
"Ottawa" sol'n.	34.8	41.5	11.4
LSD 5%	28.5	39.7	12.6
'Regal Gold'			
Fresh cut	2.5	2.0	1.2
Old (on plant)	58.9	-5.5	39.8
Water	65.4	-8.9	35.9
Sucrose + 8-HQS	63.7	-2.5	38.2
"Ottawa" sol'n.	62.8	-4.5	33.2
LSD 5%	62.3	-4.7	33.6
	2.7	1.0	2.1

"L" values are: from 100 (white) to 0 (black).

"a" values are: red (+), gray (0), green (-).

"b" values are: yellow (+), gray (0), blue (-).

noticeable with 'Better Times', was indicated by the low "b" values.

The reported promotion of growth by ascorbic acid (15) may be related to the promotion of water uptake in plant tissues and may have a direct effect on prevention of "gum" formation in rose xylem and the extension of cut-life of flowers in general. The water uptake was facilitated also by 8-HQS; however, at equimolar concn, the Na-isoAA was considerably more effective than 8-HQS in promoting the water uptake or retention of cut rose stems (Fig. 2). The maintenance of cell structure, function, and metabolism is dependent on supply of respiratory substances, energy transfer reactions, and conditions favorable for phosphorylation and formation of ATP (28, 37). Na-isoAA, being an excellent electron carrier, promotes these processes and may prevent the aging of plant tissues due to its antioxidative, regulatory, and plant growth promoting activities (5, 6, 15, 21, 30, 35). The iso-ascorbic acid is also considered to be a better antioxidant than the *l*-isomer in prevention of peroxidation of lipids (41), and, therefore, the maintenance of integrity of cell membranes. The epoxidation of violaxanthin (34, 40), a precursor of abscisic acid, may also be related to the content of ascorbic acid in the tissues. Ascorbic acid may act also as a protective agent against the detrimental effects of ammonia (31) which, due to proteolysis, may cause "blueing" of certain roses (37).

The Na salt of the iso-ascorbic acid (C₆H₇O₆Na.H₂O) was used because it oxidizes at a slower rate than the *l* or *d* acids (41) and is readily available commercially. It is assumed that, excepting the small vitamin C activity of the isoascorbate (10), the biological activities of both isomers are similar.

The effect of the "Ottawa" or its component solutions on the preservation and integrity of epidermal cells of rose petals is illustrated in Fig. 3, 1-6. The surface view of these cells from fresh cut roses (Fig. 3, 1) shows the vacuoles filled with a solution of anthocyanin, surrounded by the clear cytoplasm and the cell walls. The absence or slight traces of granular bodies in these cells was noted. The epidermal cells from roses kept in water (Fig. 3, 2), or solution of 8-HQS (Fig. 3, 3), appeared to be damaged and on the verge of disintegration, while those from roses kept in sucrose solution (Fig. 3, 5) showed the cell walls to be intact. Within these 3 treatments the pigment was distributed unevenly and tended to accumulate in intensely colored globules. The Na-isoAA treatment (Fig. 3, 4) was superior to the treatments of water, 8-HQS, or sucrose in maintaining the structure of epidermal cells. The "Ottawa" solution (Fig. 3, 6) preserved the petal epidermal cells similar to those from fresh petals, although somewhat lighter in tone. In this treatment the "flocculation" of pigment was only beginning to appear, indicating the onset of senescence, albeit much later than with the other treatments.

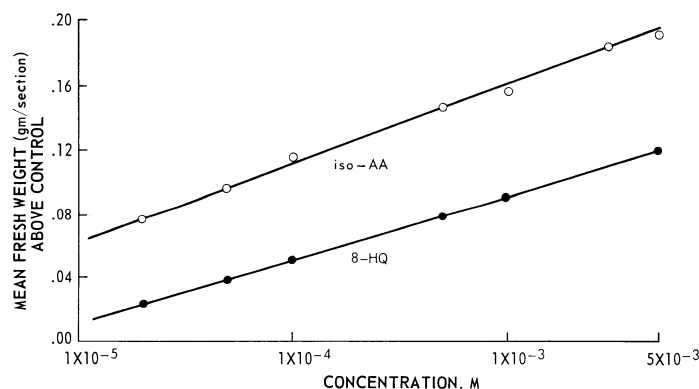


Fig. 2. Increase in fresh wt of stem sections of roses, 'Forever Yours' from aqueous solutions of sodium iso-ascorbate, and 8-hydroxyquinoline sulfate.

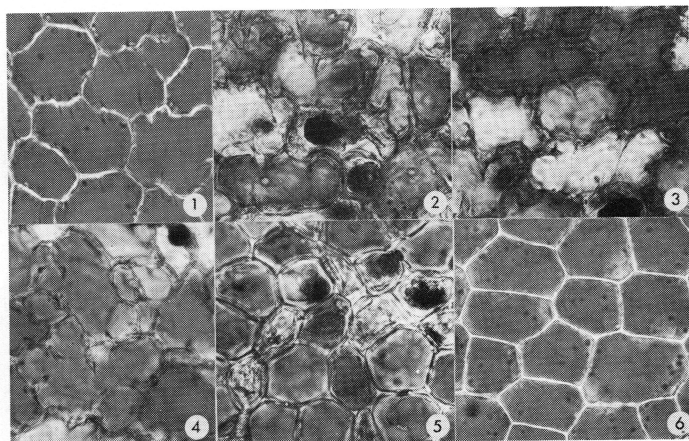


Fig. 3. Epidermal cells of petals of roses, 'Forever Yours' fresh cut or kept in the preservative or its component solutions for 6 days: 1) fresh cut, 2) water, 3) 8-hydroxyquinoline sulfate, 4) sodium iso-ascorbate, 5) 4% sucrose, 6) "Ottawa" solution.

A number of other physico-chemical measurements may also reflect on the state of aging of plant tissues (Table 3). An increase in "free space", indicating an increase in the number of cells totally permeable to solutes, with a concomitant decrease in solute uptake, usually accompanies aging (32, 33). Amino acid leakage from tissues also follows the same trend with the

production, and photosynthetic as well as oxidative phosphorylation. The free radical formed from this acid serves as an electron donor in the biosynthetic processes of nucleic acids, proteins, and other constituents of the living cell (6). The inclusion of iso-ascorbic acid into the "Ottawa" solution resulted in a novel cut flower keeping solution which was superior to the best commercial or non-commercial floral preservative mixtures tested.

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Table 3. Physical and biochemical properties of fresh and 6-day old 'Forever Yours' rose petals and blooms, kept in the "Ottawa" or its component solutions.

Treatments	"Free space" %	Solute uptake μ M/g	Amino acid incorporation, μ M/g	Solute leakage mg N/g	Respiration μ l O ₂ /90 min	Protein %	Ammonia %	pH	Fresh wt g
Fresh cut	8.0	1.16	.0126	.45	1.42	14.62	.02	6.3	11.25
Water	25.1	.39	.0058	.92	.67	9.68	.60	5.0	12.00
8-HQS, 50 ppm	25.0	.47	.0053	.54	.73	10.12	.43	5.3	12.75
Na-isoAA 100 ppm	23.7	.59	.0054	.52	.81	10.18	.25	5.4	16.20
Sucrose, 4%	20.6	.61	.0061	.48	1.09	13.25	.10	5.9	19.30
"Ottawa"	17.6	.90	.0112	.51	1.35	13.75	.09	6.2	21.0
LSD 5%	1.5	.13	.0019	.11	.26	.89	.06	—	3.80

young tissues or those maintained more or less in good condition releasing less amino acids than the aged tissues. In all cases the young, fresh petal tissues and those from roses kept in the "Ottawa" solution had less "free space", absorbed and incorporated more solutes, released less amino acids, and aged slower than the petals of roses kept in the solutions of the individual components. The protein content in petals of roses kept in water declined slower than in petals of roses kept in solutions of 8-HQS, or Na-isoAA. This may be caused by rapid aging of petals in water to the point of damage to cell membranes, possible leakage of phenolics, and subsequent inhibition of proteolytic enzymes (2).

Respiration, amino acid incorporation, and O₂ uptake was highest in the fresh tissues and lowest in the distinctly aged petals from roses kept in water; petals of roses from the "Ottawa" solution showed values close to those from the fresh roses and were followed in this respect by roses from solutions containing sucrose.

The effectiveness of the "Ottawa" solution as a floral preservative depends partly on the activity and biochemical effects of iso-ascorbic acid. These effects include participation in the activation of various enzyme systems, stimulation of ATP

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The Effect of Rootstocks on Growth and Flowering of Apple Seedlings¹

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Abstract. Flowering of seedlings was hastened on M IX and retarded on M XVI rootstocks, irrespective of the interstock and irrespective of the influence of rootstock or interstock on growth. *Malus sikkimensis* (Hook. f.) Koehne and *M. hypohensis* (Pamp.) Rehd. seedlings used as rootstocks reduced growth and retarded flowering.

Vigor and precocity of a rootstock are independent properties, in that the ability of a rootstock to induce early flowering is determined by its specific precocity only. Under optimal conditions for growth, seedlings on their own roots may flower as soon as on M IX. The latter profit less from such conditions due to the growth retarding effect of the grafting or budding operation proper. Therefore, unless orchard space is limited, there is little advantage in using a rootstock such as M IX for shortening the juvenile period of apple seedlings.

The influence of environment on the growth and on the duration of the juvenile period has been reported (11). Propagation of seedlings on rootstocks is considered as a specific environmental factor.

Notably the precocious and growth reducing rootstock M IX has been found to hasten the onset of flowering, though the extent to which flowering was promoted appeared to vary between experiments (9). Also other growth reducing rootstocks such as M 27 and M VII (5, 6, 7, 8) and apomictic seedlings of *M. sikkimensis* (1) have been observed to induce earlier flowering. Seedlings propagated on invigorating rootstocks, such as M X, M XII, or M XVI or on adult trees, did not flower sooner than on their own roots. Seemingly, there is a discrepancy between the performance of a seedling on its own

roots in which vigour plays a positive role and that on a rootstock in which the lack of vigour seems important.

Trials were set up in 1965 to gain more insight into the relation between vigour and flowering in various seedling-rootstock combinations.

Materials and Methods

Experiment 1. The performance of seedlings (1962 crosses) grafted, when 2 years old, on the dwarfing rootstock M IX and on the invigorating rootstock M XVI with or without an interstock (about 10 cm long) was compared. Seedlings grafted on their own roots (the top part providing the scions) served as a control. The rootstocks and control seedlings were transplanted from the nursery into pots and grafted or double grafted in the greenhouse during early spring (1965). Thereafter, they were brought into a cold frame and kept there for the remainder of the season. They were subsequently transferred into the nursery for another 2 seasons before being planted in the orchard (1968: 5 years from seed). This was later than usual

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