Peach/myrobalan plum graft incompatibility in the nursery

By M. A. MORENO,¹ A. MOING,¹ M. LANSAC,¹ J. P. GAUDILLERE,² and G. SALETTES¹

INRA, Centre de Bordeaux, ¹Station de Recherches Fruitières,

²Station de Physiologie Végétale, BP 81, 33883 Villenave d'Ornon cedex, France

SUMMARY

Various peach/myrobalan graft combinations were compared in nursery trials. Two peach cultivars (*Prunus persica* L. Batsch) grafted on five myrobalan × myrobalan (*Prunus cerasifera* L. Ehrh.) clones were observed during the first year of growth after budding. Graft combinations, comprising the same peach cultivars grafted on other myrobalan × myrobalan clones that had shown symptoms of 'translocated' graft incompatibility in some replicated trees during the first year, were observed during the second year of growth. The time taken for the appearance of symptoms of incompatibility was determined. The degree of incompatibility was determined from growth rates, leaf dry weight per unit area, and leaf pigment content. Root carbohydrate content was measured at leaf fall. The sanitary state of plant material was determined to explain differences in severity and/or time for symptom appearance, observed between some replicates. No relationship was found between the sanitary state of plant material and the severity or speed of expression of peach/myrobalan incompatibility. When incompatibility was expressed during the first year of growth, modifications in growth rates, leaf dry weight per unit area and leaf pigment content preceded the expression of visible symptoms on leaves by several weeks. In one peach cultivars, in which graft combinations showed symptoms of 'translocated' graft incompatibility in some replicates during the first year after budding, all replicates showed such symptoms during the second year. Therefore, the variation observed during the first year appeared to result from a delayed expression of incompatibility. Significant differences in speed and/or severity of incompatibility expression were shown between the two peach cultivars for any given rootstock.

THE sensitivity of peach (*Prunus persica* L. Batsch) to waterlogged or calcareous soils requires that it be grafted on more tolerant species such as plum and the peach × almond hybrid (Bernhard and Grasselly, 1959; Bernhard, 1970; Salettes *et al.*, 1970; Byrne *et al.*, 1990). A programme of breeding and selection is being developed at the Station de Recherches Fruitières of I.N.R.A. at Bordeaux to create new plum rootstocks for peach, with good grafting compatibility (Salettes *et al.*, 1988a). This programme includes myrobalan (*P. cerasifera* L. Ehrh.) × myrobalan crosses, as some myrobalan clones do show compatibility with most peach varieties (Grasselly, 1983), although most peach/myrobalan combinations show a

'translocated' graft incompatibility as described by Herrero (1951) and Mosse (1962).

When peach cultivars grafted on myrobalan rootstocks become incompatible, severe symptoms are usually expressed during the first year of scion growth (Herrero, 1951; Mosse, 1962). However, symptoms of incompatibility can be delayed (Herrero, 1955; Salettes *et al.*, 1988b). Moreover, in some graft combinations, symptoms of incompatibility are expressed during the first year of growth in only some of the replicated trees in spite of their clonal origin (Salettes *et al.*, 1988b). These two problems increase the difficulty in selecting suitable rootstocks for peach.

One aim of this work was to determine if the

sanitary state of plant material could explain the difference in the time of appearance of symptoms of incompatibility within replicates of a clonal scion/rootstock combination. Indeed, the failure of certain graft combinations is due to some viruses in *Prunus* (Marénaud, 1968; Mircetich and Hoy, 1981; Cummings and Gonsalves, 1986); and to mycoplasma-like organisms (MLOs) in the *Pyrus*/*Cydonia* combination (Batjer and Schneider, 1960; Hibino and Schneider, 1970). A further aim was to measure some physiological characteristics that could quantify incompatibility symptoms, i.e. growth rates, leaf dry weight per unit area, leaf pigment content, root carbohydrate content. This quantification was used to verify if incompatibility was a general response in which symptoms could be expressed with variable timing and severity.

PLANT MATERIAL

Ten rootstocks clones, resulting from myrobalan $\times$ myrobalan crosses (*Prunus cerasifera* L. Ehrh.), were budded with two peach cvs (Fuzalode and Summergrand, *Prunus persica* L. Batsch.) in August 1989 or 1990. The present work was carried out in 1991, in the first and second year of scion growth during the nursery phase. Six trees were used where possible for each graft combination in the one year old nursery as well as in the two year old nursery. Both trials were established on a slightly alkaline silt-clay soil (pH = 7.7), in south-west France.

One year old nursery trees

Both peach cultivars were grafted on the following rootstocks: (16-5 $\times$ 1079) 45 or P-45, (2032 $\times$ 2175) 6 or Q-6, (18 $\times$ 2794) 28 or R-28, (18 $\times$ 2794) 32 or R-32 and (18 $\times$ 16-5) 38 or S-38, and were observed during the first year after budding. Of the rootstock parents, clone 18 (from myrobalan B) and clones 16-5 and 1079, are incompatible with most peach cultivars. In contrast to this, clones 2032 (Grasselly, 1983), 2794 and 2175 are compatible with most peach cultivars.

Two year old nursery trees

Both peach cultivars grafted on clones (16-5 $\times$ 18) 7, (16-5 $\times$ 1079) 8, (16-5 $\times$ 2794) 5, (16-5 $\times$ 2794) 7 and (2032 $\times$ 2794) 13, were observed until the end of the second year after budding.

The graft combinations were chosen according to their behaviour during the first year of growth. Combinations with both peach cultivars grafted on clone (2032 $\times$ 2794) 13, performed well (C combinations). Combinations comprising clone (16-5 $\times$ 18) 7 showed severe symptoms of incompatibility (I combinations). The incompatibility symptoms in both peach cultivars grafted on clones (16-5 $\times$ 1079) 8, (16-5 $\times$ 2794) 5 and (16-5 $\times$ 2794) 7 developed in some replicated trees during the first year after grafting, while others remained apparently healthy. For such graft combinations ('H' combinations, for heterogeneous), where symptoms did not develop at the same time for all plants, two groups were distinguished, one comprising trees with a good performance during the first year after grafting (Hc) and the other comprising trees with severe visible symptoms of incompatibility during the first year after grafting (Hi).

METHODS

Virus and MLO detection

The sanitary state was determined in the aerial part of all nursery trees, and in the stock-plants used for hardwood cutting production of rootstocks. Enzyme-linked immunosorbent assay (ELISA) on leaves, according to Clark and Adams (1977), and indexage grafting on seedlings of peach 'GF 305' (Bernhard *et al.* 1969) were carried out to test: *Prunus* necrotic ringspot virus (PNRSV), Prune dwarf virus (PDV), American plum line pattern virus (AmLPV), European plum line pattern virus (EPLV) and Apple mosaic virus (ApMV) of the Isometric labile ringspot (ILAR) group; Cherry leaf-roll virus (CLRV), Myrobalan latent ringspot virus (MLRV), Raspberry ringspot virus (RRV), Strawberry latent ringspot virus (SLRV), Tomato black ring virus (TBRV) and Tomato ringspot virus (TomRV) of the Nepovirus group; and Apple chlorotic leaf spot virus (CLSV) of the Closterovirus group and Plum pox virus, Sharka (PPV) of the Potyvirus group.

Phloem restricted prokaryotes (MLOs) in leaf petioles (ten examined per tree) were detected by UV-light microscopy after staining petiolar sections with 4'-6-diamidino-2-phenylindole-2HCl (DAPI) (Seemüller, 1976; Dosba *et al.*, 1990).

Growth measurements

In the one year old nursery, tree growth was determined from monthly measurements of the rootstock diameter (5 cm below the graft-union) and the length of the scion main shoot. Scion diameter and number of lateral shoots were determined at the end of the first vegetative season. In addition, leaf dry weight per unit area (W/LA) determination was carried out with undamaged adult leaves of the scion, at the beginning of July and at the end of August. Ten leaves per tree were sampled and their area recorded. The samples were dried in an oven at 80°C to constant weight.

In two year old trees, growth was determined from monthly measurements of the diameter of scion and rootstock (5 cm above and below the

graft-union). For W/LA determinations, scion leaf samples were harvested at the end of May and July. They were analysed as indicated above for the samples collected in the one year old nursery.

Pigment determinations

For chlorophyll and carotenoid determination, leaf discs of known area were cut from three leaves per tree, at the same date as for W/LA determination in one year old and two year old trees. Pigments were analysed by spectrophotometry after extraction with N, N-dimethylformamide using a modification of the method of Moran and Porath (1980) described by Gaudillère *et al.* (1987).

Carbohydrate determinations

At leaf fall, two year old trees were uprooted. A randomly selected fraction of five primary roots was freeze-dried and ground to a fine powder in a laboratory mill (16 mesh). The dried powders were extracted for soluble sugars with 80°C ethanol/water (80:20, v/v) for 30 min. The supernatants were purified on ion-exchange resin (Bio-rad AG 1-X8 in the carbon form, Dowex 50W in the H⁺ form) and analysed by HPLC (Aminex HPX-87C column from Bio-rad, using water as eluant at a flow-rate of 0.6 cm³ min⁻¹ at 75°C). Total soluble sugar concentrations refers to the sum of glucose, fructose, sucrose, raffinose, stachyose, inositol and sorbitol concentrations. Starch was determined in the pellets, after soluble sugar extraction, using a hexokinase, glucose-6-phosphate linked assay (Kunst *et al.*, 1984), after hydrolysis with amyloglucosidase purchased from Merck.

FIG. 1

Growth rate changes in scion length (A) and rootstock diameter (B) of nursery grown 'Fuzalode'/myrobalan graft combinations, during the first year of growth. These data represent the means $\pm$ SD of six replicates.

TABLE I

Scion trunk diameter (mm) 5 cm above the graft-union and number of scion lateral shoots in nursery grown peach/myrobalan graft combinations, with 'Fuzalode' as scion, at leaf fall of the first year of growth. Data represent the mean $\pm$ SD of six trees

Rootstock	Scion trunk diameter	Lateral shoots
P-45	19.7 $\pm$ 5.2 ab	24.5 $\pm$ 7.2 b
Q-6	22.4 $\pm$ 5.2 a	34.7 $\pm$ 7.1 a
R-28	11.7 $\pm$ 2.2 c	6.8 $\pm$ 3.4 d
R-32	14.6 $\pm$ 3.7 c	12.0 $\pm$ 3.7 cd
S-38	15.0 $\pm$ 2.4bc	14.2 $\pm$ 3.7 c

Within each column, means with different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE II
Leaf dry weight per unit area (W/LA) (gm^{-2}) in nursery grown peach/myrobalan graft combinations, with 'Fuzalode' as scion, on two dates during the first year of growth. Data represent the mean $\pm$ SD of six trees

Rootstock	July	August
P-45	57.4 $\pm$ 3.2 e	67.0 $\pm$ 6.0 c
Q-6	60.4 $\pm$ 6.3 d	67.9 $\pm$ 9.9 c
R-28	73.9 $\pm$ 4.0 b	83.0 $\pm$ 4.4 b
R-32	71.0 $\pm$ 4.9 c	86.9 $\pm$ 8.1 ab
S-38	83.9 $\pm$ 6.6 a	88.5 $\pm$ 8.3 a

For each column, means with different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

Statistical analyses

Statistical analyses were performed using the Statistical Analysis package System program (SAS Institute Inc., 1990). The effects of factors were determined using analysis of variance according to the General Linear Models Procedure. Duncan's multiple range test was used to indicate significant differences between means at the 5% level.

RESULTS

Sanitary state

The ELISA technique and indexage by grafting on 'GF 305' indicated an absence of all the tested viruses in both nurseries and in all stockplants. Similarly, the DAPI method was unable to detect the presence of MLOs within the sieve tubes of tested nursery trees or stockplants.

Quantification of incompatibility symptoms in one year old nursery trees

External symptoms: 'Fuzalode' grafted on clones S-38, R-28 and R-32 showed severe external symptoms of 'translocated' incompatibility from the end of July. However, symptoms of curling and reddening of the leaves

were more severe in Fuzalode grafted on clone S-38. 'Summergrand' showed incompatibility symptoms for all five rootstocks: from the third week of July for clones P-45, R-28 and R-32, and a week later for clones Q-6 and S-38. The most severe symptoms in leaves were shown by 'Summergrand' grafted on clones P-45, Q-6 and S-38.

Growth: Growth rates of the scion main shoot and rootstock trunk differed significantly during the first year after budding according to the cultivar and rootstock used.

For scion height (Figure 1A), cv. Fuzalode grafted on clones R-28 R-32 and S-38, had growth rates significantly lower than those of cv. Fuzalode grafted on clones Q-6 and P-45 from the beginning of June until the end of August. Mean values for 'Fuzalode' grafted on R-28 and R-32 were already lower in May than 'Fuzalode' grafted on other clones. Graft combinations with 'Summergrand' as scion, behaved similarly but their scion growth stopped earlier than in combinations with 'Fuzalode' as scion and rootstock effects were no longer observed after the end of July (data not shown).

For rootstock diameter, in graft combinations, with 'Fuzalode' as scion (Figure 1B), growth rates were significantly lower for clones R-28, R-32 and S-38 than for clones P-45 and Q-6, from the second fortnight of June to the second fortnight of July. In graft combinations, with 'Summergrand' as scion, the same pattern was observed (data not shown).

At the end of the first vegetative season, scion trunk diameter (Table I) was significantly smaller for 'Fuzalode' grafted on clones R-28, R-32 and S-38 than for 'Fuzalode' grafted on Q-6, while 'Fuzalode' grafted on S-38 did not differ from 'Fuzalode' grafted on P-45.

TABLE III
Chlorophyll content (mg m^{-2}) and chlorophyll/carotenoid ratio in the leaves of nursery grown peach/myrobalan graft combinations, with 'Fuzalode' as scion, on two dates during the first year of growth. Data represent the mean $\pm$ SD of six trees

Rootstock	Chlorophyll		Chlorophyll/carotenoid	
	July	August	July	August
P-45	385.0 $\pm$ 34.0 a	479.8 $\pm$ 21.3 b	6.9 $\pm$ 0.7 b	9.1 $\pm$ 1.2 a
Q-6	371.4 $\pm$ 53.1 a	527.8 $\pm$ 40.5 a	8.0 $\pm$ 1.1 a	9.4 $\pm$ 1.2 a
R-28	184.4 $\pm$ 25.5 c	209.3 $\pm$ 62.0 d	4.8 $\pm$ 0.4 d	5.1 $\pm$ 0.5 c
R-32	230.9 $\pm$ 33.5 b	248.9 $\pm$ 43.4 c	5.6 $\pm$ 0.4 c	6.2 $\pm$ 0.9 b
S-38	221.4 $\pm$ 37.4 b	115.5 $\pm$ 39.1 e	5.5 $\pm$ 0.5 c	4.2 $\pm$ 0.8 d

For each column and scion cultivar, means with different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

FIG. 2

Growth rate changes in scion (A) and rootstock (B) trunk diameter in nursery grown 'Fuzalode'/myrobalan graft combinations, during the second year of growth. These data represent the means $\pm$ SD of n replicates: where for compatible combination (C), $n = 6$; for heterogeneous graft combinations, group of trees which had a good performance during the first year of growth after budding (Hc), $n = 15$; for heterogeneous graft combinations, group of trees which showed severe visible symptoms of incompatibility during the first year of growth after budding (Hi); $n = 3$; and for incompatible combination (I), $n = 5$.

Similarly, the number of lateral shoots (Table I) was significantly lower in 'Fuzalode' grafted on clones R-28, R-32 and S-38 than when grafted on clones P-45 and Q-6. Similar patterns were observed with 'Summergrand' as scion.

The scion cultivar and the rootstock had significant effects on scion leaf dry weight per unit area (W/LA) (Table II). W/LA in 'Fuzalode' grafted on clones R-28, R-32 and S-38, was already higher than grafted on clones P-45 and Q-6 at the beginning of July. Differences between compatible and incompatible graft combinations were accentuated at the end of August.

Pigments: The chlorophyll content, expressed on a per unit area basis and the chlorophyll/carotenoid ratio (Table III) were always significantly lower in 'Fuzalode' grafted on R-28, R-32 and S-38, than in 'Fuzalode' grafted on P-45 and Q-6. Differences were already displayed at the beginning of July. In graft combinations with 'Summergrand' as scion (data not shown), a similar pattern was shown at the beginning of July.

Quantification of incompatibility symptoms in two year old nursery trees

External symptoms: During the second year after budding, as during the previous year, all trees of cvs Fuzalode and Summergrand grafted on the clone (2032 $\times$ 2794) 13 performed well (C combinations). Both cultivars grafted on clone (16-5 $\times$ 18) 7 showed similar or more severe symptoms of translocated incompatibility than during the first year (I combinations). In the H combinations with cv. Summergrand which did not develop incompatibility symptoms on all trees during the first year, all the trees which did well during the first year after grafting (Hc), began to show symptoms of translocated incompatibility at the end

TABLE IV

Leaf dry weight per unit area (W/LA (gm^{-2})) in nursery grown graft combinations, with either 'Summergrand' or 'Fuzalode' as scion, on two dates during the second year of growth. Data represent the mean $\pm$ SD of ' n ' trees, the value of n is given in parentheses

Summergrand			Fuzalode		
Combination	May	July	Combination	May	July
I (4)	60.0 $\pm$ 6.2 b	79.6 $\pm$ 8.4 a	I (5)	56.0 $\pm$ 12.1 a	72.1 $\pm$ 13.2 a
Hi (11)	67.5 $\pm$ 7.0 a	67.8 $\pm$ 12.4 b	Hi (3)	55.4 $\pm$ 4.9 a	55.1 $\pm$ 4.1 c
Hc (6)	66.8 $\pm$ 7.1 a	62.7 $\pm$ 6.3 c	Hc (15)	59.0 $\pm$ 9.8 a	59.4 $\pm$ 7.9 b
C (6)	52.3 $\pm$ 6.9 c	56.9 $\pm$ 2.6 d	C (6)	51.5 $\pm$ 7.4 b	56.5 $\pm$ 5.1 bc

For each column, means with different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE V

Chlorophyll content (mg m^{-2}) in the leaves of nursery grown peach/myrobalan graft combinations, with either 'Summergrand' or 'Fuzalode' as scion, in two dates during the second year of growth. Data represent the mean $\pm$ SD of 'n' trees, the value of n is given in parentheses

Summergrand				Fuzalode			
Combination		May	July	Combination		May	July
I	(4)	148.5 $\pm$ 32.2 c	90.6 $\pm$ 28.6 d	I	(5)	181.8 $\pm$ 46.1 b	216.4 $\pm$ 92.2 b
Hi	(11)	149.2 $\pm$ 35.3 c	188.2 $\pm$ 71.2 c	Hi	(3)	214.8 $\pm$ 59.6 b	400.8 $\pm$ 112.1 a
Hc	(6)	252.2 $\pm$ 52.2 b	255.5 $\pm$ 44.9 b	Hc	(15)	365.7 $\pm$ 62.0 a	415.5 $\pm$ 57.8 a
C	(6)	380.5 $\pm$ 41.0 a	430.5 $\pm$ 41.0 a	C	(6)	373.0 $\pm$ 50.8 a	422.9 $\pm$ 53.1 a

For each column, means with different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

of the second growth season, and trees with severe symptoms of incompatibility during the first year of growth (Hi) showed even more severe symptoms during the second year. In contrast, in H combinations with 'Fuzalode' as variety, compatible trees during the first year after grafting (Hc) behaved well during the second year. Trees with incompatibility symptoms during the first year after grafting (Hi) performed poorly.

Growth: The growth rates of scion and rootstock trunk were compared during the second year after budding for the four types of graft combinations as defined above, i.e. C, Hc, Hi and I combinations. When either 'Fuzalode' (Figures 2A and 2B) or 'Summergrand' (data not shown) were used as scions, no significant differences were found between either the Hc group and C combination or the Hi group and I combination. However, at leaf fall, in graft combinations with Summergrand as scion, rootstock trunk growth rates in the Hc group were significantly lower than in the C combination, and the differences between the Hc group and those of the Hi group and I combination were not significant.

Mean values of W/LA, in graft combinations

with cv. Summergrand as scion, were significantly higher in the I combination, Hi and Hc groups than in the C combination, during May and July (Table IV). In H combinations, the Hc trees had mean values significantly higher than trees in the C combination. The Hi trees had mean values close to those of the I combination but were significantly higher in May and significantly lower in July. In graft combinations with 'Fuzalode' as scion, the behaviour of heterogeneous combinations varied according to the sampling date. They were not significantly different from either the I combination in May or the C combination in July.

Pigments: In graft combinations with cv. Summergrand as scion, the leaf chlorophyll content on an area basis (Table V) was always significantly higher in the C combination than in other combinations. The Hc trees had mean values significantly lower than trees of the C combination. In graft combinations with 'Fuzalode' as scion, the chlorophyll content (Table V) in H combinations differed according to sampling date. In May, no significant differences in chlorophyll content were found between either the Hc trees and C combination or the Hi trees and I combination. In July, mean values of chlo-

TABLE VI

Total soluble sugar and starch concentration (mg g DW^{-1}) in the primary roots of nursery grown peach/myrobalan graft combinations, with either 'Summergrand' or 'Fuzalode' as scion, at leaf fall of the second year of growth. Data represent the means $\pm$ SD of 'n' trees; the value of n is given in parentheses

Summergrand				Fuzalode			
Combination		Total soluble sugar content	Starch content	Combination		Total soluble sugar content	Starch content
I	(4)	33.9 $\pm$ 3.3 ab	2.6 $\pm$ 2.3 b	I	(5)	59.6 $\pm$ 12.0 a	6.9 $\pm$ 5.6 b
Hi	(11)	47.1 $\pm$ 17.3 a	5.7 $\pm$ 5.8 b	Hi	(3)	57.9 $\pm$ 3.8 a	51.5 $\pm$ 30.0 b
Hc	(6)	40.4 $\pm$ 8.2 a	7.5 $\pm$ 12.5 b	Hc	(15)	33.0 $\pm$ 6.9 b	143.4 $\pm$ 40.6 a
C	(6)	24.3 $\pm$ 3.2 b	142.5 $\pm$ 52.7 a	C	(6)	24.7 $\pm$ 1.4 b	135.8 $\pm$ 21.0 a

For each column, means with different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE VII
Timing of incompatibility effects as studied in peach/
myrobalan combinations during the first year of growth

Time	Modification
May-June	Reduction in the growth rate of scion shoot
mid-June	Reduction in the growth rate of rootstock trunk
early July	Increase in leaf W/LA Earlier decrease in chlorophyll Earlier decrease in chlorophyll/carotenoid ratio
late July	Severe reddening of leaves and shoots Curling of the leaf along the midrib

rophyll content for both Hc and Hi groups were not significantly different from the C combination (Table V). The chlorophyll/carotenoid ratio (data not shown) showed a quite similar pattern.

Root carbohydrate concentration: When 'Summergrand' was used as scion, the Hc and Hi groups were not significantly different from the I combination at leaf fall with respect to the root soluble sugar concentration (Table VI). With 'Fuzalode' as scion, there were no significant differences between either the Hi group and I combination, or the Hc group and C combination.

For root starch concentration (Table VI), with 'Summergrand' as scion, mean values were significantly higher in the C combination than those in I or Hi and Hc combinations. Neither the Hc nor the Hi groups were significantly different from the I combination. In graft combinations with 'Fuzalode' as scion, there were no significant differences between either the Hi group and I combination or the Hc group and C combination.

DISCUSSION

Sanitary state

No relationship was found between the sanitary state of the plant material and the early expression of peach/myrobalan incompatibility. Viruses, whether the most usual ones in fruit trees or those known to cause incompatibility in *Prunus* graft combinations (Marénaud, 1968; Mircetich and Hoy, 1981; Cummins and Gonsalves, 1986), in *Juglans* (Mircetich and Rowhani, 1984); and phloem restricted prokaryotes (MLOs) in the *Pyrus/Cydonia* combination (Batjer and Schneider, 1960; Hibino and Schneider, 1970) were not present in either the scions or the stockplants. However, the role of an unidentified or non-tested microorganism cannot be excluded.

Quantification and timing of symptoms

In one year old trees, the timing of some modifications induced by incompatibility can be summarized as indicated in Table VII. The decrease in growth rates, the increase in L/WA, the decrease in leaf chlorophyll content and chlorophyll/carotenoid ratio, preceded the expression of the typical visible symptoms of incompatibility on leaves by several weeks, indicating that the latter are secondary symptoms.

The growth rates of the scion main shoot and trunk rootstock of the incompatible combinations decrease earlier than those of the compatible ones, in agreement with previous studies on a similar type of incompatibility (Breen, 1975).

The higher values of L/WA in the incompatible combinations may be related to carbohydrate accumulation in scion leaves (Moing *et al.*, 1990). In one year old trees of incompatible combinations, at the beginning of July, the values of L/WA were higher in the combination which would develop the more severe visible symptoms of translocated incompatibility in leaves (i.e. folding upward along the midrib, yellowing, reddening) several weeks later.

The chlorophyll content in compatible combinations is similar to that usually observed in fully illuminated mature peach leaves (Kappel and Flore, 1983; Merlo *et al.*, 1991). In contrast to Moing *et al.* (1990), who worked in controlled environmental conditions, significant differences in chlorophyll content and in chlorophyll/carotenoid ratio are found here between compatible and incompatible graft combinations, during the first year after budding.

As shown previously for a similar type of graft incompatibility (Tabuenca, 1962), the starch concentration in the roots of the incompatible graft combinations at leaf fall of their second growth year was very low compared with that of the compatible ones. However, with 'Fuzalode' as scion, the mean value of total soluble sugar concentration of the primary roots of incompatible trees was twice as high as in compatible ones, mainly because of higher hexose contents. Nevertheless, the total non-structural carbohydrate concentration remained much lower in incompatible combinations than in compatible ones. The root car-

bohydrate concentrations were more modified in earlier, or more severe, development of incompatibility.

The higher W/LA in leaves and the lower carbohydrate content in roots, in incompatible graft-combinations, are in agreement with Breen's hypothesis (1975) on problems of phloem transport from the aerial parts to the roots for a similar type of graft incompatibility.

In graft combinations with 'Summergrand' as scion, where incompatibility symptoms did not develop in all plants in the first year after budding, L/WA, chlorophyll content and the chlorophyll/carotenoid ratio of the Hc trees were already significantly different from the totally compatible combination in May. In addition, at leaf fall, the rootstock trunk growth rate and the root carbohydrate content of Hc and Hi trees did not differ from I combination trees, indicating a delay in incompatibility expression in some trees. This delay has been already reported for peach grafted on myrobalan (Herrero, 1955). The different timings observed for the expression of incompatibility in replicates of the same clonal scion/rootstock combinations may result from the preparation of plant material. Insufficiently controlled factors such as hardwood cutting characteristics before and after rooting, cutting technique, grafted bud characteristics and grafting (Bernhard, pers. comm.; Herrero and Tabuenca, 1969), as well as possible environmental effects may modify the scion/rootstock balance. In breeding and selection work, this problem implies the necessity of pre-testing suitable graft combinations for several years.

Effect of peach cultivar

The cv. Summergrand grafted on myrobalan $\times$ myrobalan rootstocks shows a greater tendency to express graft-incompatibility than cv. Fuzalode grafted on the same rootstocks. During the first year after budding, 'Summergrand' shows graft-incompatibility with myrobalan genotypes that are compatible with 'Fuzalode.' In addition, in the two year old nursery, graft combinations which did not develop symptoms in all trees during the first year, showed a greater severity or speed of incompatibility expression during the second growing season with 'Summergrand' than with 'Fuzalode' as scion. The effect of the peach cultivar has been reported already for other peach/plum graft combinations (Tabuenca and Moreno, 1988). Furthermore, 'Summergrand' may be considered as a recalcitrant genotype in compatibility trials. Therefore, it is useful in selection tests of myrobalan genotypes compatible with peach cultivars.

We acknowledge Abel Bonnet for the preparation and management of plant material in the nursery. We thank Alain Bonnet and Kléber Mazy for help with the indexage grafting. We thank Bernard Lafargue for his technical assistance in the nursery sampling and measurements. We gratefully acknowledge Dr Françoise Dosba for helpful discussions. The study was partially supported by a Ministerio de Educación y Ciencia of Spain and Ministère de la Recherche et de la Technologie of France grant to the first author.

REFERENCES

- BATJER, L. P. and SCHNEIDER, H. (1960). Relation of pear decline to rootstocks and sieve tube necrosis. *Journal of the American Society for Horticultural Science*, **76**, 85–97.
- BERNHARD, R. (1970). Patrones de melocotonero. *ITEA*, **1**, 89–104.
- BERNHARD, R. and GRASELLY, C. (1959). Les pruniers porte-greffes du pêcher. *Journées Fruitières et Maraichères d'Avignon*, 1959, 75–100.
- BERNHARD, R., MARENAUD, C. and SUTIC, D. (1969). Le pêcher GF 305, indicateur polyvalent des virus des espèces à noyau. *Annales de Phytopathologie*, **1**, 603–17.
- BREEN, P. J. (1975). Effect of peach/plum graft incompatibility on seasonal carbohydrate changes. *Journal of the American Society for Horticultural Science*, **100**, 253–9.
- BYRNE, D. H., BACON, T. A. and EGILLA, J. N. A. (1990). Patrones de frutales de hueso tolerantes a suelos calizos. *ITEA*, **9**, 117–33.

- Clark, M. F. and ADAMS, A. N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology*, **34**, 475–83.
- CUMMINS, J. N. and GONSALVES, D. (1986). Constriction and decline of 'Stanley' prune associated with tomato ringspot virus. *Journal of American Society for Horticultural Science*, **111**, 315–8.
- DOSBA, F., LANSAC, M., MAZY, K., GARNIER, M. and EYQUARD, J. P. (1990). Incidence of different diseases associated with mycoplasma like organisms in different species of *Prunus*. *Acta Horticulturae*, **283**, 311–20.
- GAUDILLERE, J. P., DREVON, J. J., BERNOUD, J. P., JARDINET, F. and EUVRARD, M. (1987). Effect of periodic fluctuations of photon flux density on photosynthetic characteristics of soybean leaves. *Photosynthetic Research*, **13**, 81–9.
- GRASSELLY, C. (1983). Possibilités d'utilisation du prunier myrobolan comme porte-greffe du pêcher. *Comptes Rendus de l'Académie d'Agriculture de France*, **69**, 347–54.
- HARTMANN, H. T. and KESTER, D. E. (1975). *Plant propagation. Principles and practices*. Third edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, USA.
- HERRERO, J. (1951). Studies of compatible and incompatible graft combinations with special reference to hardy fruit trees. *Journal of Horticultural Science*, **26**, 186–237.
- HERRERO, J. (1955). Incompatibilidad entre patrón e injerto. I. Comportamiento de algunas combinaciones reciprocas. *Anales de la Estación Experimental de Aula Dei*, **4**, 149–66.
- HERRERO, J. and TABUENCA, M. C. (1969). Incompatibilidad entre patrón e injerto. X. Comportamiento de la combinación melocotonero/myrobolan injertado en estado cotiledonar. *Anales de la Estación Experimental de Aula Dei*, **10**, 937–45.
- HIBINO, H. and SCHNEIDER, H. (1970). Mycoplasmalike bodies in sieve tubes of pear trees affected with pear decline. *Phytopathology*, **60**, 499–501.
- KAPPEL, F. and FLORE, J. A. (1983). Effect of shade on photosynthesis, specific leaf weight, leaf chlorophyll content, and morphology of young peach trees. *Journal of the American Society for Horticultural Science*, **108**, 541–4.
- KUNST, A., DRAEGER, B. and ZIEGENHORN, J. (1984). Carbohydrates U. V. methods with hexokinase and glucose-6-phosphate dehydrogenase. In: *Methods of enzymatic analysis, VI, Metabolites I*. (Bergmeyer, H. U., Ed.). Third Edition Verlag Chemie, Basel, 163–72.
- MARENAUD, C. (1968). Mise en évidence, sur l'espèce abricotier, d'une incompatibilité intraspécifique due à la présence d'un virus du type chlorotic leaf spot. *Annales Epiphyties*, **19**, 225–45.
- MERLO, L., PASSERA, C. and GIULIVO, C. (1991). Seasonal, developmental and varietal effects on photosynthetic enzyme activities of peach leaves (*Prunus persica* L. Batsch). *Journal of Horticultural Science*, **66**, 27–33.
- MIRCETICH, S. M. and HOY, J. W. (1981). Brownline of prune trees, a disease associated with tomato ringspot virus infection of myrobolan and peach rootstocks. *Phytopathology*, **71**, 30–5.
- MIRCETICH, S. M. and ROWHANI, A. (1984). The relationship of CLRV and black line disease of English walnut trees. *Phytopathology*, **74**, 423–8.
- MOING, A., CARBONNE, F. and GAUDILLERE, J. P. (1990). Growth and carbon partitioning in compatible and incompatible peach/plum grafts. *Physiologia Plantarum*, **79**, 540–6.
- MORAN, R. and PORATH, D. (1980). Chlorophyll determination in intact tissues using N, N-dimethylformamide. *Plant Physiology*, **65**, 478–9.
- MOSSE, B. (1962). Graft incompatibility in fruit trees with special reference to its underlying causes. *Technical communication no. 28*. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, UK.
- SALESSES, G., SAUNIER, H. and BONNET, A. (1970). L'asphyxie radiculaire chez les arbres fruitiers. *Bulletin Technique d'Information*, **251**, 403–15.
- SALESSES, G., RENAUD, R. and BONNET, A. (1988a). Creation of plum rootstocks for peach and plum by interspecific hybridization. *Acta Horticulturae*, **224**, 339–43.

- SALESSES, G., RENAUD, R. and BONNET, A. (1988b). Création de porte-greffe par hybridation interspécifique au sein des pruniers. 8^e Colloque sur les Recherches Fruitières. (INRA-CTIFL, Eds). INRA, Bordeaux, 151–9.
- SAS INSTITUTE INC. (1990). *SAS user's guide: statistics, version 6*. Fourth edition. SAS Institute Inc., Cary, NC, USA.
- SEEMULLER, E. (1976). Investigations to demonstrate mycoplasma like organisms in diseased plants by fluorescence microscopy. *Acta Horticulturae*, **67**, 109–11.
- TABUENCA, C. (1962). Relaciones entre la composición química y el grado de compatibilidad en combinaciones de melocotonero y ciruelo. *Anales de la Estación Experimental de Aula Dei*, **7**, 1–34.
- TABUENCA, C. and MORENO, M. A. (1988). Incompatibilidad entre patrón e injerto. Comportamiento de un ciruelo como patrón de distintas especies frutales. *Anales de la Estación Experimental de Aula Dei*, **19**, 251–63.

(Accepted 8 November 1992)