

## Effects of durable inoculum pressure and high temperature on root galling, nematode numbers and survival of Myrobalan plum genotypes (*Prunus cerasifera* Ehr.) highly resistant to *Meloidogyne* spp.

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Accepted for publication 13 January 1995.

**Summary** – The effect of a durable inoculum pressure (DIP) of *Meloidogyne* spp., applied by transferring into each *Prunus* container one galled tomato plant previously inoculated under controlled conditions, was evaluated in comparison with short inoculation pressure (SIP) provided by one single direct inoculation of 10 000 J2 per plant container. Root galling, nematode numbers and plant survival were evaluated after 4 months at 25°C ± 4°C under greenhouse conditions for two highly resistant (P. 1079 and P. 2175) and one susceptible (P. 2032) micropropagated Myrobalan plum genotypes (3 month old). Three 1-year old rootstocks GF 305 and Nemaguard peaches grown from seeds, and the peach-almond hybrid GF 557 grown from rooted cuttings, were also tested under DIP. The root-knot nematode populations included *M. arenaria* (1 pop.), *M. incognita* (3 pop.), *M. javanica* (1 pop.), and *M. hispanica* (1 pop.). DIP did not affect the resistance of P. 1079 and P. 2175 and very low nematode numbers were recovered in both genotypes but high root galling and mortality were observed for most populations in P. 2032. GF 305 root galling and nematode numbers under DIP were high but, presumably because of the age of plants, plant survival was much better than in P. 2032. Under DIP, Nemaguard and GF 557 harboured few nematodes except for the *M. javanica* population that produced moderate galling in Nemaguard and heavy galling, as expected, in GF 557. Nevertheless, root galling, nematode numbers and plant mortality in Nemaguard and GF 557 were always higher than in P. 1079 and P. 2175. Minor resistance genes could be involved in the differences observed between Nemaguard and both resistant Myrobalan genotypes. The effect of 32 °C temperature in comparison with 25 °C was evaluated for the same three Myrobalan genotypes after 4 months under SIP for *M. arenaria* (1 pop.), *M. incognita* (2 pop.), and *M. javanica* (1 pop.). For all nematode populations, resistant genotypes were free of galls at both temperatures whereas root galling in P. 2032 was lower at 32 than at 25 °C. The genotypes P. 1079 and P. 2175 confirmed their broad spectrum of resistance when exposed to a high and durable inoculum pressure or to a high temperature.

**Résumé** – Effets d'une pression continue d'inoculum et d'une température élevée sur l'indice de galles, les effectifs des nématodes et la survie de génotypes de prunier myrobalan (*Prunus cerasifera* Ehr.) hautement résistants à *Meloidogyne* spp. – L'effet d'une pression durable d'inoculum (DIP) de *Meloidogyne* spp. appliquée par transfert dans chaque conteneur de *Prunus* d'un plant de tomate préalablement inoculé en conditions contrôlées, est évalué en comparaison avec une pression courte d'inoculum (SIP) appliquée par inoculation directe de 10 000 J2 par plant. L'indice de galles, les effectifs de nématodes et la survie des plants sont évalués après 4 mois à 25 °C ± 4 °C en conditions de serre sur trois génotypes micropropagés de prunier Myrobalan dont deux hautement résistants (P. 1079 et P. 2175) et un hôte (P. 2032). Trois porte-greffe de un an, les pêchers GF 305 et Nemaguard issus de semis, et l'hybride pêcher-amandier GF 557 issu de boutures racinées, sont également évalués sous DIP. Les populations de nématodes utilisées appartiennent aux espèces *M. arenaria* (1 pop.), *M. incognita* (3 pop.), *M. javanica* (1 pop.) et *M. hispanica* (1 pop.). La résistance de P. 1079 et P. 2175 n'est pas affectée par la DIP et des effectifs très faibles de nématodes sont détectés dans ces deux génotypes mais un indice de galles et une mortalité élevés sont observés pour la plupart des populations chez P. 2032. L'indice de galles et les effectifs de nématodes chez GF 305 sous DIP sont élevés mais vraisemblablement du fait de l'âge des plants, la survie est plus élevée que chez P. 2032. Sous DIP, Nemaguard et GF 557 hébergent peu de nématodes à l'exception de la population de *M. javanica* responsable d'un indice de galles modéré chez Nemaguard et, comme attendu, élevé chez GF 557. Néanmoins, indice de galles, effectifs de nématodes et mortalité des plants de Nemaguard et GF 557 sont toujours plus élevés que chez P. 1079 et P. 2175. Des gènes mineurs de résistance pourraient être impliqués dans les différences observées entre Nemaguard et les deux génotypes résistants de Myrobalan. L'effet d'une température de 32 °C comparé avec celui d'une température de 25 °C est évalué chez les trois mêmes génotypes de myrobalan sous SIP pour *M. arenaria* (1 pop.), *M. incognita* (2 pop.), et *M. javanica* (1 pop.). Quelle que soit la population, les génotypes résistants sont indemnes de galles aux deux températures tandis que chez P. 2032 l'indice de galles est plus faible à 32 °C qu'à 25 °C. Les génotypes P. 1079 et P. 2175 confirment donc leur large spectre de résistance lorsqu'ils sont soumis à une pression élevée et continue d'inoculum ou à une haute température.

**Key-words** : Temperature, inoculum pressure, *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, *M. hispanica*, *Prunus cerasifera*, *P. persica*, *P. amygdalus*, resistance, symptom.

*Prunus cerasifera* Ehr. (Myrobalan) genotypes range from susceptible to highly resistant to *Meloidogyne arenaria* (Esmenjaud *et al.*, 1992 *b*). Resistance to this root-knot nematode species appears to be controlled by at least one major dominant gene (Scotto La Massèse *et al.*, 1990). Separate inoculations of diverse populations of *M. arenaria*, *M. incognita*, *M. javanica*, and *M. hispanica* on several Myrobalan plum selections established that the genotypes P. 1079 and P. 2175 were highly resistant whereas the genotype P. 2032 was susceptible (Esmenjaud *et al.*, 1994). Even without taking into account the pathogenic variability among root-knot species on plum, further studies are needed to ensure that this broad-spectrum resistance is not affected by environmental conditions that are known to affect defense mechanisms, such as high inoculum pressure (Davide & Triantaphyllou, 1967), high temperature (Dropkin, 1969; Wehunt, 1972; Griffin & Elgin, 1977; Cardin, 1979; Canals *et al.*, 1992; Fernandez *et al.*, 1993), plant age and type of propagation (Davide & Triantaphyllou, 1967; Canals *et al.*, 1992), and nutrition and soil type (Wallace, 1969; Sarah *et al.*, 1991).

This paper deals with the effect of two major factors, durable inoculum pressure (DIP) and high temperature, that may affect the expression of resistance in *Prunus* genotypes.

## Material and methods

### INOCULUM PRESSURE STUDY

Two resistant (P. 1079 and P. 2175), and one susceptible (P. 2032) Myrobalan plum genotypes, GF 305 (susceptible) and Nemaguard (resistant) peaches [*P. persica* (L.) Batsch] and one GF 557 peach-almond hybrid (*P. persica* × *P. amygdalus* Batsch) (resistant to *M. arenaria* and *M. incognita*) were evaluated (Scotto La Massèse *et al.*, 1990; Esmenjaud *et al.*, 1994). GF 305 and GF 557 are rootstocks obtained from the French Institut National de la Recherche Agronomique (INRA) and widely used in Europe (Kester & Grasselly, 1987; Layne, 1987). Nemaguard used as a resistant reference rootstock was originally described as a *P. persica* × *P. davidiana* hybrid (Layne, 1987) but it is currently widely considered to be pure *P. persica* without any *P. davidiana* in its parentage (Ledbetter, USDA Fresno, pers. com.).

The Myrobalan genotypes P. 1079, P. 2175 and P. 2032 were obtained from micropropagated plantlets as described by Esmenjaud *et al.* (1993), the peach-almond GF 557 from hardwood cuttings, and the peaches GF 305 and Nemaguard from seedlings. At mid-March, they were transplanted together with GF 557 hardwood cuttings, rooted during the previous year, and 12 month-old GF 305 seedlings into 4 dm<sup>3</sup> individual containers filled with a sterilized 4:1 mixture (v:v) of fine sand (0.1-0.5 mm) and loamy soil. Containers were drip irrigated every 2-3 days as needed,

fertilized once a week with a commercial nutrient solution for ligneous plantlets (Algoflash, Algochimie, Tours, France) at pH 5.8 and maintained in the greenhouse from March to September at 25 ± 4 °C. They were inoculated 2 months after transplant with one galled tomato plant according to the method described by Esmenjaud *et al.* (1992 *b*) to produce a durable inoculum pressure (DIP) modified as follows: the whole live tomato plant and soil (instead of root-system + soil) of a tomato plant grown in a pot and inoculated two months before with 2000 juveniles (J2) (instead of 500), was transplanted into each *Prunus* container. The top part of the tomato plant was cut and removed 2 months after transfer (instead of at the date of transfer). The technique described originally (Esmenjaud *et al.*, 1992 *b*) produced a mean total inoculum of 160 000 J2s + eggs per plant. The juvenile inoculum recovered by monthly sampling in *Prunus* soil varied between 17 000 and 5000 throughout the 4 month-experiment. Consequently, the modified technique provided a higher and more durable inoculum pressure. A short inoculation pressure (SIP) involving inoculation of each plant is described below in the temperature study.

Six of the most aggressive root-knot nematode populations previously tested (Esmenjaud *et al.*, 1994) were used: 1) *M. arenaria* "Monteux", France; *M. incognita*: 2) "Calissanne", France, 3) "Côte d'Ivoire", Ivory Coast, 4) "Landes", France; 5) *M. javanica*, "Oualidia", Morocco; 6) *M. hispanica* "Seville", Spain. All were reared on tomato from one single egg mass. The identity of each species was verified by its isoesterase phenotype (Janati *et al.*, 1982) and their infectivity by abundant galling observed on the tomato plant transplanted into *Prunus* containers.

There were ten replicates of each rootstock-nematode population-level of inoculum pressure combination plus ten uninoculated replicates of each genotype or rootstock as control. Pots inoculated with the same population were arranged in a completely randomized design, in greenhouse benches. The groups of pots corresponding to different populations were separated from each other with transparent antispash screens. Mortality of plants was noted once a week. Plants were considered dead when all their leaves had fallen. These were harvested, their root-system carefully washed and given a 0-5 root-knot index according to the following scale derived from Barker (1985): 0 = 0%; 1 = 1-10%; 2 = 11-30%; 3 = 31-70%; 4 = 70-90%; 5 = more than 90% of the root system galled. After rating, the root system was frozen at -20 °C until the nematode number was estimated. Four months after inoculation, live plants were harvested, the galling level was assessed and their root systems frozen at -20 °C. Before extraction, frozen root systems were transferred to a refrigerator (5 °C) to be slowly defrosted. Ten grams of progressively defrosted roots from each plant were obtained by random sampling. Nematodes were extracted using first an ultra

grinder (20 000 rpm) for 2 s then a 250 µm-pore sieve to collect the freed stages into a beaker. Nonground roots and rootlets were recovered and were ground two more times. Then, the content of the beaker was centrifuged twice (Jenkins, 1964). Females, males, J3-J4, J2 and eggs were counted under a binocular microscope. Data were analyzed using a one way analysis of variance. Nematode densities were  $\text{Log}_{10}(x + 1)$  transformed for analysis (Noe, 1985). Means were compared by Newman-Keuls multiple range tests at  $P \leq 0.05$ .

#### TEMPERATURE STUDY

The two resistant genotypes (P. 1079 and P. 2175), and the susceptible (P. 2032) genotype were produced and treated as previously described. They were transplanted into 250-ml individual containers. One month later, one half of the containers were kept in the greenhouse at a mean temperature of  $25 \pm 4$  °C (soil temperature 22-28 °C). This set also served as SIP controls for three genotypes in the inoculum pressure study. The other half was transferred to a growth chamber kept at a mean temperature of  $32 \pm 1$  °C. Both groups of plants were watered and fertilized as previously reported. However, for the high temperature treatment, irrigation water and nutrient solution were supplied at 32 °C. Soil temperature variation inside the containers was also  $\pm 1$  °C.

Nematode juveniles (J2) of the *M. arenaria* "Monteux", *M. incognita* "Calissanne" and "Landes", and *M. javanica* "Oualidia" populations were extracted in a mist chamber from tomato roots previously inoculated with the various tested isolates. About 10 000 juveniles, 24 to 72 h-old, were pipetted into four 2-cm deep holes in the soil, 1 cm from the stem of each plant. There were

ten replicates of each population-genotype-temperature combination. Four months after the inoculation, plantlets were harvested and given a root-gall index according to the above 0-5 scale. Mean gall indices per genotype were compared by Newman-Keuls multiple range test ( $P \leq 0.05$ ).

## Results

### INOCULUM PRESSURE

No galls were observed and no nematodes were recovered from roots of resistant P. 1079 and P. 2175 under SIP (Tables 1, 2). No galls were observed and few nematodes were recovered in these genotypes under DIP. Most nematodes were juveniles (J2) but third or fourth stage larvae (J3-J4) were occasionally detected. Mortality was observed only on one plant of P. 1079 exposed to *M. incognita* "Landes" and one plant of P. 2175 exposed to *M. hispanica* "Seville" (Table 3). Growth of micropropagated plantlets of both genotypes under DIP was not quantified but was visually reduced in comparison with SIP. As expected, susceptible P. 2032 had more galls and nematodes under DIP than SIP. However, the ranking of gall index ratings was the same for both DIP and SIP. *M. hispanica* "Seville" and *M. javanica* "Oualidia" produced the highest gall index under DIP. A high percentage of P. 2032 died under DIP whereas no plant died under SIP. Mortality in this genotype was significantly correlated ( $r = 0.93$ ) with the mean gall index of the nematode populations. Because of the high level of mortality in P. 2032, nematode numbers are only indicative. These were presumably underestimated and thus cannot be statistically interpreted.

**Table 1.** Influence of inoculum pressure of *Meloidogyne* spp. on root galling of the *Myrobalan plum* genotypes (P. 1079, P. 2175, and P. 2032), and GF 305, GF 557, and Nemaguard rootstocks.

Nematode population	Genotype or rootstock									
	P. 1079		P. 2175		P. 2032		GF 305	GF 557	Nemaguard	
	DIP	SIP	DIP	SIP	DIP	SIP	DIP	DIP	DIP	
<i>M. arenaria</i> "Monteux"	0 a	0	0 a	0	3.2 ab	2.5 a	3.1 ab	0.5 bc	0.8 ab	
<i>M. incognita</i> "Calissanne"	0 a	0	0 a	0	2.8 bc	2.1 a	2.0 b	0.6 bc	0.6 ab	
<i>M. incognita</i> "Côte d'Ivoire"	0 a	nt	0 a	nt	2.3 c	nt	3.8 a	0.3 c	nt	
<i>M. incognita</i> "Landes"	0.1 a	0	0.1 a	0	nt	2.6 a	4.0 a	1.2 b	0.7 ab	
<i>M. javanica</i> "Oualidia"	0 a	0	0 a	0	3.3 ab	2.7 a	1.8 b	3.5 a	1.6 a	
<i>M. hispanica</i> "Seville"	0 a	0	0.1 a	0	3.9 a	nt	2.3 b	0.7 bc	0.4 c	

Ten replicates per genotype or rootstock. Root galling : 0 = no gall; 5 = 100 % root system galled. P. 1079, P. 2175, and P. 2032 are 3 month-old micropropagated plantlets; GF 305 and Nemaguard are 1 year old seedlings; GF 557 are 1 year old hardwood cuttings. DIP = durable inoculum pressure. SIP = short inoculum pressure. Means in columns for each genotype or rootstock followed by the same letter do not differ according to Newman-Keuls multiple range test ( $P \leq 0.05$ ). nt = not tested.

**Table 2.** Influence of inoculum pressure of *Meloidogyne* spp. on total nematode numbers per gram of roots in *Myrobalan* plum genotypes (P. 1079, P. 2175, and P. 2032), and GF 305, GF 557 and Nemaguard rootstocks.

Nematode population	Genotype or rootstock								
	P. 1079		P. 2175		P. 2032		GF 305	GF 557	Nemaguard
	DIP	SIP	DIP	SIP	DIP	SIP	DIP	DIP	DIP
<i>M. arenaria</i> "Monteux"	2.5 a	0	5.0 a	0	2 080	350 a	5 010 ab	60 bc	40 b
<i>M. incognita</i> "Calissanne"	0.5 a	0	7.0 a	0	2 550	400 a	2 470 b	70 bc	25 b
<i>M. incognita</i> "Côte d'Ivoire"	1.0 a	nt	1.5 a	nt	1 050	nt	5 420 a	20 c	nt
<i>M. incognita</i> "Landes"	3.5 a	0	7.0 a	0	nt	300 a	6 830 a	250 b	55 b
<i>M. javanica</i> "Oualidia"	1.5 a	0	5.0 a	0	1 630	650 a	2 040 b	5 800 a	900 a
<i>M. hispanica</i> "Seville"	4.0 a	0	5.5 a	0	900	nt	4 220 ab	100 bc	15 b

Ten replicates per genotype or rootstock. Root galling: 0 = no gall; 5 > 90 % root system galled. P. 1079, P. 2175, and P. 2032 are 3 month-old micropropagated plantlets; GF 305 and Nemaguard are 1 year old seedlings; GF 557 are 1 year-old hardwood cuttings. DIP = durable inoculum pressure. SIP = short inoculum pressure. Means in columns for each genotype or rootstock followed by the same letter do not differ according to Newman-Keuls multiple range test ( $P \leq 0.05$ ). nt = not tested.

**Table 3.** Percentage of plant survival following the application of a durable (DIP) or short (SIP) inoculum pressure of *Meloidogyne* spp. to *Myrobalan* plum genotypes (P. 1079, P. 2175 and P. 2032), and to GF 305, GF 557 and Nemaguard rootstocks.

Nematode population	Genotype or rootstock								
	P. 1079		P. 2175		P. 2032		GF 305	GF 557	Nemaguard
	DIP	SIP	DIP	SIP	DIP	SIP	DIP	DIP	DIP
<i>M. arenaria</i> "Monteux"	100	100	100	100	50	100	90	100	100
<i>M. incognita</i> "Calissanne"	100	100	100	100	70	100	100	100	100
<i>M. incognita</i> "Côte d'Ivoire"	100	nt	100	nt	70	nt	80	100	nt
<i>M. incognita</i> "Landes"	90	100	100	100	nt	100	90	90	100
<i>M. javanica</i> "Oualidia"	100	100	100	100	50	90	100	70	90
<i>M. hispanica</i> "Seville"	100	100	90	100	40	nt	100	100	100

Ten replicates per genotype or rootstock. P. 1079, P. 2175, and P. 2032 are 3 month-old micropropagated plantlets; GF 305 and Nemaguard are 1 year-old seedlings; GF 557 are 1 year-old hardwood cuttings. DIP = durable inoculum pressure. SIP = short inoculum pressure. nt = not tested. Uninoculated controls of the *Myrobalan* genotypes or rootstocks have a 100 % survival.

GF 305 was severely galled and harboured very high nematode numbers under DIP of *M. incognita* "Landes" and "Côte d'Ivoire" but plant survival was much higher than for P. 2032. With Nemaguard, galling and nematode numbers were low but not null and no plant died except with *M. javanica* when nematode numbers were high and all the developmental stages were recovered. For the other populations, nematodes were mainly J2s but their numbers per gram of roots were clearly higher than in the genotypes P. 1079

and P. 2175. Nevertheless, for the same population, no statistical comparison of nematode numbers produced in the *Myrobalan* genotypes and in the rootstocks were made because of the differences in the type of multiplication (*in vitro* propagation, seeds and cuttings) and in the age of plants. As expected, GF 557 was severely galled and highly infested only by the *M. javanica* population that gave 30 % mortality. For the other populations, nematode numbers were equivalent to those recovered in Nemaguard.

## TEMPERATURE STUDY

Resistance of genotypes P. 1079 and P. 2175 was not influenced by the high temperature. Susceptible genotype P. 2032 was less affected by root galling at 32 °C than at 25 °C (Table 4).

**Table 4.** Effect of high temperature on root galling of four populations of root-knot nematodes on Myrobalan plum genotypes.

Nematode population	Average temperature (°C)	Genotype		
		P. 1079	P. 2175	P. 2032
<i>M. arenaria</i> "Monteux"	25	0	0	2.5 a
	32	0	0	1.6 b
<i>M. incognita</i> "Calissanne"	25	0	0	2.1 a
	32	0	0	1.5 b
<i>M. incognita</i> "Landes"	25	0	0	2.6 a
	32	0	0	1.8 b
<i>M. javanica</i> "Oualidia"	25	0	0	2.7 a
	32	0	0	1.7 b

Ten replicates per genotype. Gall index : 0 = no gall; 5 = 100 % root system galled. Means in columns for each genotype followed by the same letter do not differ according to Newman-Keuls multiple range test ( $P \leq 0.05$ ).

## Discussion

Durable inoculum pressure did not result in root galling and had no significant effect on the survival of resistant Myrobalan genotypes. The observation of some J2s in roots of P. 1079 and P. 2175 confirms that juveniles do penetrate the roots of highly resistant Myrobalan genotypes but rarely develop into J3-J4s or adults (Esmenjaud *et al.*, 1992 a, b; Fernandez *et al.*, 1994). The GF 305, GF 557 and Nemaguard control rootstocks were less affected than the susceptible Myrobalan P. 2032. From the data reported here, P. 1079 and P. 2175 appear to have a good level of tolerance but, although not quantified, it was evident that their growth was reduced by DIP. This effect may be due to the direct injury caused by those juveniles that do penetrate the roots. The observation of equivalent numbers of juveniles in roots of resistant P. 1079, P. 2175 and susceptible P. 2032, 48 h after their inoculation into the soil (Esmenjaud *et al.*, 1992 a), would explain the decreased plant growth rate observed in the DIP treatments. Further studies are needed to determine the mechanisms (hypersensitivity, juvenile penetration and migration) involved in the resistance of *P. cerasifera*. The rootstocks GF 305, Nemaguard, and GF 557 expressed a higher tolerance than the susceptible P. 2032 to DIP. However, the *P. cerasifera* genotypes used were only

3 month-old whereas the other rootstocks, rooted and grown during the previous season, were about 1 year-old. Age of *Prunus* plants is known to affect the expression of resistance to *Meloidogyne*. For susceptible almond as well as resistant peach-almond, Canals *et al.* (1992) observed that 1 month-old plantlets were significantly more damaged by *M. incognita* than 1 year-old plants. In our experiment, the greater tolerance of the 1 year-old rootstock may be partly attributed to their age. The type of propagation is also an important factor and seedlings generally show a higher vigor than cuttings. Under DIP, the rootstock GF 305 inoculated with the populations "Côte d'Ivoire" and "Landes" of *M. incognita*, survived better than GF 557 with *M. javanica* "Oualidia", although their gall indices and nematode numbers were equivalent.

High temperature (32 °C) did not reduce the resistance of the Myrobalan plums. Temperature effect was only studied under short inoculum pressure and should be confirmed under durable inoculum pressure. The larger number of galls and higher nematode reproduction observed by Canals *et al.* (1992) on resistant peach-almond hybrid G × N no. 1 maintained at 31 °C and inoculated with *M. javanica* was not observed for the genotypes tested in our study. Peach-almond hybrids appear to be more affected by partial loss of resistance due to high soil temperature. Our findings also agree with results obtained by Fernandez *et al.* (1993) in which the expression of resistance in Marianna GF 8.1 and Myrobalan 29C plum rootstocks was not affected by high soil temperatures (30 and 31 °C, respectively). The latter rootstock, also a highly resistant *P. cerasifera* genotype, expressed the same behaviour as the genotypes P. 1079 and P. 2175 and could share the same major resistance genes (Scotto La Massèse *et al.*, 1990).

We observed that the resistance of Nemaguard to *M. javanica* (Sharpe *et al.*, 1969) was decreased by the high and durable pressure whereas the Myrobalan genotypes P. 1079 and P. 2175 were not. This difference may be attributed to minor resistance genes present in both resistant Myrobalan genotypes and lacking in Nemaguard. The higher juvenile numbers of the other tested root-knot species recovered in Nemaguard roots in comparison with P. 1079 and P. 2175, may illustrate a difference in the ability of juvenile penetration or migration into the root tissues. We conclude that resistance of P. 1079 and P. 2175 Myrobalan plums confirmed its broad spectrum which was not overcome when exposed to a high and durable inoculum pressure or to a high temperature.

## Acknowledgements

This work partly financed by the Commission of the European Communities via the AIR Program of Research and Technological Development "Agriculture and Agro-industry, including fisheries" (Research project no. "AIR1 CT-920312"). The authors wish to thank A. Bonnet and

G. Salesses for supplying Myrobalan genotypes, R. Lorrain and R. Poupet for their help in micropropagation. They are also thankful to M. H. Simard and G. Ollivier, J. Pinochet and C. Fernandez, Lafont Nurseries (Valréas, France) for supplying GF 305 seedlings, Nemaguard seeds, and GF 557 cuttings, respectively.

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