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Effect of Cutting Age on the Resistance of *Prunus cerasifera* (Myrobalan Plum) to *Meloidogyne arenaria*[1]

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Abstract: The response of softwood cuttings of Myrobalan plum infested after 50 and 105 days with 3,000 second-stage juveniles (J2) of *Meloidogyne arenaria* was compared to 15-month-old hardwood cuttings in 13 genotypes ranging from highly resistant to susceptible. Gall index and number of galls were recorded 30 days after infestation. Fifty-day-old cuttings rooted in perlite developed many rootlets, but had only incipient galls after infestation. In sand, rooting of 50-day-old cuttings not treated with indolebutyric acid (IBA) hormone was so variable that their resistance could not be assessed. Similar cuttings rooted with IBA developed more galls, but neither number of galls per plant nor gall index was a reliable criterion for determination of host suitability. Because of the better rooting results with IBA treatment, 105-day-old cuttings were first rooted with IBA in perlite and then transferred into sand for nematode inoculation. Known highly resistant genotypes of Myrobalan plum were gall-free and the responses of other genotypes paralleled that of the reference hardwood cuttings, although the test was less discriminating. Expression of *M. arenaria* host suitability in Myrobalan plum depends on root tissue maturation and cannot be reliably evaluated with 50-day-old cuttings.

Key words: hardwood cutting, host suitability, *Meloidogyne arenaria*, nematode, *Prunus cerasifera*, resistance, root galling, root-knot nematode, softwood cutting.

Fast and reliable methods to evaluate resistance to *Meloidogyne* spp. are particularly useful in studies involving perennial plants (4,7,9,10). The host response in *Prunus cerasifera* (Myrobalan plum) genotypes to *M. arenaria* ranges from susceptible to highly resistant (5,13). A greenhouse method with high levels of inoculum of *M. arenaria* was assessed (6) and established that a root-knot nematode gall index rating was a reliable criterion to evaluate host suitability, allowing statistical differentiation of resistant, susceptible, and intermediate genotypes. Nevertheless, although reliable, the evaluation time is too lengthy because it requires rooting of hardwood cuttings during the first year and infestation during the second year. The objective of the work reported here was to evaluate the reliability of a screening technique involving symptom ratings, a shorter test period, and softwood cuttings of Myrobalan plum.

MATERIALS AND METHODS

Thirteen genotypes of Myrobalan plum were selected for this study, including seven genotypes from a diallel cross with varying degrees of nematode host suitability (1079, 2175, and 2980, highly resistant; 18 and 2794, intermediate; and 2646 and 16.5, susceptible) and six F1 hybrids ((2175 × 2794)15, (1079 × 18)8 and (1079 × 18)13, highly resistant; (2794 × 18)2 and (2794 × 2646)5, intermediate; and (16.5 × 2794)(3, susceptible). Two different tests of softwood cuttings were made. In the first test, cuttings were aged 50 days (i.e., with 50 days between rooting and infestation), and in the second test, cuttings were aged 105 days. Both were compared to reference 15-month-old hardwood cuttings. The 50-day-old softwood cuttings were rooted and tested in 1992, whereas 105-day-old softwood cuttings were rooted and tested in 1993, and 15-month-old hardwood cuttings were rooted in 1992 and tested in 1993.

Fifty-day-old softwood cuttings: Myrobalan plum genotype softwood cuttings (12 cm long and 6-mm d with four nodes) were obtained from the field-grown trees at the end of May 1992. The leaves of the lower portion were removed and the upper two leaves were retained but reduced

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in size to lower transpiration. Two opposing 1-cm cuts were made just below the basal node (8). Cuttings of each genotype were divided into two groups of 48, one control group without hormone treatment and the other treated with indolebutyric acid (IBA). Twenty-four untreated cuttings were individually planted, 3 cm deep, in 150-ml containers filled with 0.1–0.5 mm particle size sand (15) for *Meloidogyne* tests, whereas the other 24 untreated cuttings were planted in similar containers filled with perlite. In the second group, all 48 cuttings were soaked at the base of the stem for 2–3 seconds in a 50% ethanol solution containing 1,500 ppm IBA. Half of the cuttings were then planted individually into sand and the other half into perlite as described. Individual containers grouped in tanks (50 × 30 × 15 cm) of 24 cuttings were then watered with a 10 g/liter thiram solution and placed on greenhouse benches under an automatic mist (8) regulated to keep leaves slightly moist during the first 2 weeks, then spraying was gradually reduced until the date of infestation. Fifty days after planting, rooting was verified by gently pulling the cuttings and only the vigorous, well-rooted cuttings with two shoots were selected, arranged in a randomized design, and inoculated. Inoculum was produced on tomato from the Monteux isolate of *M. arenaria*. The isolate code number AN7 (2) was reared from single egg mass culture on tomato cv. St Pierre and identified by perineal patterns and isoesterase electrophoretogram (11). Second-stage juveniles (J2) were extracted from tomato roots with an adapted mistifier technique (14). Three thousand J2 aged 24–72 hours were deposited into four 1-cm deep holes at the base of the stem of each plant, 1 cm from the cutting stem. *Prunus* containers were irrigated 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 at 25 ± 4 C. Plants were harvested 30 days after inoculation, at which time 10 plantlets of each genotype with similar top growth and root development were selected for gall ratings. Root-galling was assessed by visual estimation of the percentage of root system galled according to the 0–5 rating scale (1) as follows: 0 = 0%; 1 = 1–10%; 2 = 11–30%; 3 = 31–70%; 4 = 71–90%; 5 = more than 90% of the root system galled, with 0.5 step increments included when root galling was estimated to be at the limit between two classes. Root symptoms were also evaluated by number of galls per plant. Galls of two or three plants per genotype were dissected under a binocular microscope to verify that galling was the result of nematode infection and not of physiological reactions such as excessive salinity in the nutrient solution. Nematode counts within galls were not recorded.

One-hundred-five-day-old softwood cuttings: In early June 1993, 24 softwood cuttings of the same Myrobalan plum genotypes, all treated with IBA, were propagated under the same conditions as for the 1992 trial in tanks (50 × 30 × 15 cm) filled with perlite. After 45 days, rooted cuttings were transplanted into 250-ml containers filled with sterilized fine sand and loamy soil (4:1, v:v). The greenhouse could no longer maintain favorable temperatures for *Meloidogyne* development, therefore containers were transferred after 1 month into a growth chamber at 25 ± 1 C (16-hour photoperiod) and arranged in a randomized design. One-hundred-five days after rooting, cuttings were inoculated with 3,000 J2 of the *M. arenaria* Monteux isolate per container as described. Plants were harvested 30 days after inoculation, at which time 10 homogenous cuttings were given a root-gall index as described. Then, as for the 50-day-old cuttings, galls of two to three plants per genotype were dissected under a binocular microscope to verify the presence of nematodes.

Fifteen-month-old hardwood cuttings: Dormant hardwood cuttings of Myrobalan plum (35–45 cm long) were planted in January 1992 in a field previously disinfested with methyl bromide, and harvested in January 1993. Rooted cuttings were individually planted in 5-liter containers filled with sterilized sandy soil in March 1993.

Pots of tomato cv. St Pierre grown to the 5-leaf stage in 250-ml pots filled with sandy soil were simultaneously inoculated with 500 J2 of the *M. arenaria* Monteux isolate. Two months following inoculation, the content of each tomato pot (galled roots and soil) was transferred into a ca. 250-ml hole dug into the soil of each *Prunus* container. Ten replicates of each genotype were arranged in a completely randomized design, ordered side by side, and inoculated. The assessment of this inoculation method (6) established that mean total inoculum (Pi) produced per container at inoculation was 160,000 and that J2 recovered in the soil of each container from 1–4 months after inoculation varied between 17,000 and 5,000, thus providing a high and durable inoculum pressure. Containers were drip irrigated every 2–3 days as needed, fertilized once a week, and maintained in the greenhouse at 25 ± 4 C. Root galling was evaluated after 4 months according to the same 0–5 scale as described. Gall index rating per genotype and number of galls were analyzed by a one-way ANOVA and means were compared by the Newman-Keuls multiple range test ($P \leq 0.05$).

Results

Fifty-day-old cuttings treated with IBA had more advanced root growth than untreated cuttings. Most cuttings treated with IBA formed tertiary rootlets, whereas untreated cuttings had mainly primary rootlets and a few secondary rootlets. Rootlets started along the vertical 1-cm cuts at the base of IBA-treated cuttings, whereas they started mainly around the basal section of the untreated cuttings. These were consequently more fragile and more heterogenous than IBA-treated cuttings. Cuttings rooted in perlite had rootlets more branched, whiter, and more grouped than those in sand.

In perlite, direct inoculation with *M. arenaria* of 50-day-old cuttings failed. Very few incipient galls were observed even in susceptible genotypes (2646 and 16.5). However, galls were observed in both IBA and untreated cuttings established in sand. Due to the high heterogeneity of untreated plantlets, satisfactory sets of 10 replicates could not be obtained and data are not reported for this group. Based on these results, the 105-day-old cuttings tested in 1993 were rooted with IBA (for homogeneity) in perlite substrate (for root development) and repotted into sand before inoculation (for galling response).

Dissection of galls of both 50- and 105-day-old cuttings confirmed the presence of nematodes. As expected, all the developmental stages except eggs were easily observed 30 days after inoculation.

Based on gall indices, genotypes propagated by the reference hardwood-cutting method were ranged into five significantly different classes (Table 1). Fifty-day-old cuttings rooted with IBA were ordered differently than the hardwood cuttings; all the genotypes were classified in the same statistical group for number of galls except that two, 2646 and 18, had more galls and higher gall indices. Genotype 16.5, the most susceptible one in the hardwood cutting test, had a gall index that was not significantly different from highly resistant genotypes such as 1079 and its hybrids in the 50-day-old softwood cutting test.

Number of galls, which was less discriminating than gall index, was not recorded for the 105-day-old cuttings. Cuttings tested at 105 days had the same rank as hardwood cuttings, except that 16.5 and 2646 were not different from each other. Highly resistant genotypes that scored "0" as hardwood cuttings were also completely free of galls in the 105-day-old cutting test. Nevertheless, the range of variation in gall indices was limited to 0–2.1 and several significantly different groups of genotypes identified in the hardwood cutting test were not observed.

Discussion

Meloidogyne arenaria J2 directly added to perlite did not induce galling in young cuttings, indicating that perlite was not a suitable substrate for this type of root-knot

TABLE 1. Response cuttings of Myrobalan plum genotypes of three ages to infection with *Meloidogyne arenaria*.

Genotype	Hardwood cuttings 15-month-old	Softwood cuttings[d] 105-day-old	Softwood cuttings[d] 50-day-old	
	Gall index[a]	Gall index	Gall index	Number of galls per plant
16.5	4.4 a[b]	1.8 ab	1.2 bc	17.2 b
(16.5 × 2794) 3	3.5 b	—[c]	0.9 bc	10.8 b
2646	3.2 b	2.1 a	2.9 a	49.0 a
(2794 × 2646) 5	2.3 c	1.3 bc	0.8 bc	8.4 b
2794	2.1 c	—	0.5 c	6.4 b
(2794 × 18) 2	1.4 d	1.3 bc	1.0 bc	13.0 b
18	1.3 d	0.8 cd	1.5 b	35.6 a
(1079 × 18) 13	0.0 e	0.0 d	0.3 c	1.8 b
(1079 × 18) 8	0.0 e	0.0 d	0.2 c	0.6 b
(2175 × 2794) 15	0.0 e	0.0 d	0.7 bc	5.6 b
1079	0.0 e	0.0 d	0.3 c	1.4 b
2175	0.0 e	0.0 d	—	—
2980	0.0 e	0.0 d	—	—

Data are means of 10 replicates.
[a] Gall index rating: 0 = 0%; 1 = 1–10%; 2 = 11–30%; 3 = 31–70%; 4 = 71–90%; 5 = more than 90% of the root system galled; 0.5 step are included when root galling is estimated to be at the limit between two classes.
[b] Means in columns followed by the same letter do not differ according to Newman-Keuls multiple range test ($P \leq 0.05$).
[c] — = not tested.
[d] Treated with indolebutyric acid (IBA).

nematode evaluation test. Hormone (IBA)-treated cuttings produced more homogenous root systems; however, nematode inoculation of young IBA-treated cuttings grown in sand did not differentiate among the various levels of host suitability of the tested genotypes. Therefore, 50-day-old softwood cuttings are not reliable for evaluation of Myrobalan plum resistance to *M. arenaria*. The 105-day-old cuttings, although less discriminating than those of hardwood cuttings, responded to root-knot nematode infection as expected, based on previous knowledge of the genotypes. Therefore, the use of 105-day-old cuttings appears to be more reliable to confirm the behavior of *M. arenaria*-resistant plant material such as hybrids of the homozygous-resistant genotype 1079 (5,13). Our study indicates that the older the plant material was, the more reliable was its resistance response. Nevertheless, authentication of resistance requires further testing with the reference hardwood-cutting method that exposes germplasm to a high and durable inoculum pressure.

The unreliability of young cuttings for resistance evaluation was observed by Canals et al. (3) for *M. javanica*-resistant peach-almond hybrids in comparisons of 1-month and 1-year-old cuttings. Canals et al. (3) used exogenous IBA (2,000 ppm for 5 seconds) to facilitate rooting, and this rooting hormone may be involved, at least partly, in the modification of host suitability of the 1-month-old cuttings by delaying cell differentiation in the stele tissues, thus favoring J2 penetration and development. Kochba and Samish (12) demonstrated that *M. javanica* could reproduce on the resistant peach rootstock Nemaguard that was wick-fed with 1-naphtaleneacetic acid (NAA), another rooting hormone. Consequently, like resistant peach-almond cuttings, reliable expression of the host suitability of Myrobalan plum requires root tissue maturation.

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