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Method and criteria to evaluate resistance to *Meloidogyne arenaria* in *Prunus cerasifera* Ehr.

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Summary — The inoculation of *Prunus cerasifera* hardwood cuttings in containers by a population of *Meloidogyne arenaria* was done by transfering roots and soil of a tomato plant grown in pot that was inoculated 2 months before with 500 juveniles (J2) of this nematode. Containers with a non host clone of *P. cerasifera* were first used to estimate the release of J2 inoculum into the soil from rotting tomato roots. Mean total inoculum produced in one container was 160 000 J2s + eggs. Juvenile inoculum recovered monthly in *Prunus* soil varied between 5000 and 17 000 all along the 4-month experiment thus providing a high and durable inoculum pressure. Then eleven other *P. cerasifera* clones (five hosts and six non hosts) inoculated with the same method were harvested after 4 months to determine suitability of several criteria of evaluation of resistance. Gall index was highly significantly correlated with the $\log_{10}(x + 1)$ transformed numbers of the different nematode stages in roots of host clones. The best linear correlation was observed with females, followed by eggs and J2s. Presumably because of the extraction technique, gall index, root eggs and root J2s resulted the best criteria to differentiate statistically weakly-galled clones from non host clones. Gall index is therefore a good criterion to evaluate resistance in *P. cerasifera*.

Résumé — ***Méthode et critères d'évaluation de la résistance à* Meloidogyne arenaria *chez* Prunus cerasifera *Ehr.*** — L'infestation de boutures ligneuses de *Prunus cerasifera* en conteneur par une population de *Meloidogyne arenaria* est assurée par le transfert du système racinaire et du sol d'un godet de tomate inoculé 2 mois auparavant avec 500 juveniles (J2) de ce nématode. Un clone non hôte de *P. cerasifera* est d'abord utilisé pour estimer l'évolution des effectifs de J2 libérés dans le sol par les racines de tomate en décomposition. L'inoculum total moyen produit par conteneur est de 160 000 juvéniles et œufs. Les effectifs de J2 retrouvés mensuellement dans le sol des *Prunus* varient de 5000 à 17 400 durant les 4 mois d'expérimentation et assurent donc une pression élevée et durable d'inoculum. Ensuite onze autres clones de *P. cerasifera* (cinq hôtes et six non hôtes) sont inoculés avec la même méthode et analysés 4 mois plus tard pour préciser la valeur des différents critères d'évaluation de la résistance. L'indice de galles est corrélé de façon hautement significative avec les effectifs endoradiculaires transformés en $\log_{10}(x + 1)$ des différents stades du nématode dans les clones hôtes. La meilleure corrélation linéaire est obtenue avec les adultes femelles, suivies des œufs et des J2. Vraisemblablement du fait de la technique d'extraction, l'indice de galles, les effectifs endoradiculaires d'œufs et de J2 s'avèrent les meilleurs critères de différenciation des clones portant peu de galles par rapport aux clones non hôtes. L'indice de galles est donc un bon critère d'évaluation de la résistance chez *P. cerasifera*.

Key-words : *Meloidogyne, Prunus,* resistance.

Methods to study resistance of plants to root-knot nematodes (*Meloidogyne* spp.) are based on inoculation of a defined number of juveniles (J2) at a determined development stage of the plant. But screening of strong sources of resistance needs very high levels of inoculum and repeated inoculations to apply a high pressure at different development stages of the plant, particularly with ligneous plant material. In a study of host suitability of the genus *Prunus* to *M. arenaria.*, we used a technique that was presumed to provide a high and durable pressure of *Meloidogyne* J2s during several months (Scotto La Massèse *et al.*, 1990).

The first objective of this study was to estimate the progressive release of J2 inoculum in soil by this technique.

Furthermore, resistance in *Prunus* spp. stocks to different species of *Meloidogyne* can be evaluated by several criteria e.g. visual appearance of galls or gall index (Sharpe *et al.*, 1981; Kochba & Spiegel-Roy, 1975; Sherman *et al.*, 1981) coupled with an estimation of final numbers of nematodes (Kochba & Samish, 1971; Scotto La Massèse *et al.*, 1984; Pinochet *et al.*, 1989). Using some of these criteria, resistance of *P. cerasifera* Ehr. (Myrobalan plum) to *Meloidogyne arenaria* resulted very variable (Scotto La Massèse *et al.*, 1990). This self-incompatible species is used alone or crossed with

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other plum, peach or almond trees as a rootstock for stone fruit trees (Bernhard *et al.*, 1979; Salesses *et al.*, 1988).

The second objective was to determine the suitability of the gall index or numbers of nematode to study resistance in Myrobalan plum especially to determine " resistant " and " susceptible " classes.

Material and methods

Host suitability of several *P. cerasifera* clones to the " Monteux " population of *M. arenaria* has been established by Scotto La Massèse *et al.* (1990). *Prunus* clones harbouring females and eggs of *M. arenaria* were considered " hosts " and others were considered " non hosts ". Inoculation method was the following. Rooted hardwood cuttings of the clones to be tested were individually planted in a glasshouse in 5 dm^3 containers in sterilised sandy soil. Tomato cv. " St Pierre " grown to the 5-leaf stage in sterilized soil in 250-ml pots were simultaneously inoculated with 500 J2s of the *Meloidogyne* population. After 2 months, the top of each tomato plant was cut off, and the content of its pot (galled roots and soil) was transfered into a hole of the same volume dug with a trowel into the soil of each *Prunus* container. Nematodes of each *Prunus* plant were extracted 4 months later to evaluate resistance of the tested clones. During our experiments, containers were drip irrigated with a nutrient solution, and temperature varied from 20 to 28 °C.

Release of J2 inoculum from tomato roots in *Prunus* soil

Twenty four rooted hardwood cuttings of a non host *P. cerasifera* clone followed the procedure described above. Thirty tomato plants were inoculated with *M. arenaria* " Monteux " population. After 2 months, the tomatoes were transfered into the *Prunus* container and the six more tomato containers were analysed on the date of transfer for an estimation of the mean nematode numbers they harboured. In this objective the content of each tomato container was individually and carefully placed into a bucket and the roots were washed under tap water above it to prevent any loss of nematodes. The six tomato root systems were cut in 2-cm pieces, placed into a mist chamber and eggs and J2s counted every 3 day. After 30 days, they were ground and centrifuged with the grinding-centrifugation technique (Coolen & D'Herde, 1972) before counting. Mean soil numbers of J2 were estimated from the six buckets analysed individually by three sedimentations each followed by sieving on a 40 µm pore sieve and then using a double centrifugation (Jenkins, 1964).

Each month, from 1 month to 4 months after the transfer of tomato roots and soil into *Prunus* containers, nematodes of six *Prunus* containers, taken randomly, were extracted. The content of each container was carefully placed into a bucket and (non host clone) *Prunus* roots taken off. Then the six tomato root systems (or remaining part of them) were individually placed into a mist chamber and eggs and J2s counted every 3 day. When counting had become null, tomato roots were ground and centrifuged in the same conditions as described above. J2 mean soil numbers per container were also estimated from the 6 buckets as previously described.

Evaluation of *Prunus* resistance to *Meloidogyne*

Five host and six non host clones of *P. cerasifera* from the collection of the " Station de Recherches Fruitières " of INRA at Bordeaux (France) were selected for this study. Host clones (S1 to S5) except " 1090 " (S1) were intraspecific hybrids : " (2032 × 26 466) 11 " (S2); " (2032 × 2646) 13 " (S3); " (2175 × 18) 32 " (S4); " (2175 × 18) 27 " (S5). Non host clones (R1 to R6) were all intraspecific hybrids : " (2175 × 1079) 16 " (R1); " (2175 × 1079) 29 " (R2); " (1079 × 18) 37 " (R3); " (1079 × 18) 32 " (R4); " (2175 × 2032) 39 " (R5); " (2175 × 18) 2 " (R6).

Eight rooted hardwood cuttings of each *Prunus* clone were individually inoculated as described at the beginning of this chapter. Four months after inoculation, *Prunus* plants were removed from soil and their roots carefully rinsed under tap water to eliminate any remaining tomato root. Gall index of each cutting was noted on a 0-5 scale (0 = no galls; 5 = fully galled roots) modified from Barker (1985) with steps of 0.5. Nematodes were then extracted from a 10 g representative sample of roots, and 1 kg of soil per container respectively by grinding-centrifugation (Coolen & D'Herde, 1972) and flotation-centrifugation (Jenkins, 1964) techniques.

Females, males, third and fourth stage juveniles (J3-J4), J2s and eggs were estimated from two 1-ml aliquots from 50-ml suspensions. Nematode numbers were $\log_{10}(x + 1)$ transformed, subjected to a single classification analysis of variance (factor " clone ") and means compared by Newman-Keuls multiple range test at $p \leqslant 0.05$. Gall index was submitted without transformation to the same statistical procedure.

Results

Release of J2 inoculum from tomato roots in *Prunus* soil

Table 1 gives the evolution of numbers of J2s and eggs per container. On the date of transplanting, the initial total inoculum given by tomato roots and soil was about 160 000 J2s + eggs. After 1 month, rotting roots yielded very few J2s and soil J2s were more numerous than on transplanting date. After 2 and 3 months, tomato roots were rotten and after 4 months there were no longer available roots for nematode extraction while J2s recovered from soil were between 5000 and 7000 per container.

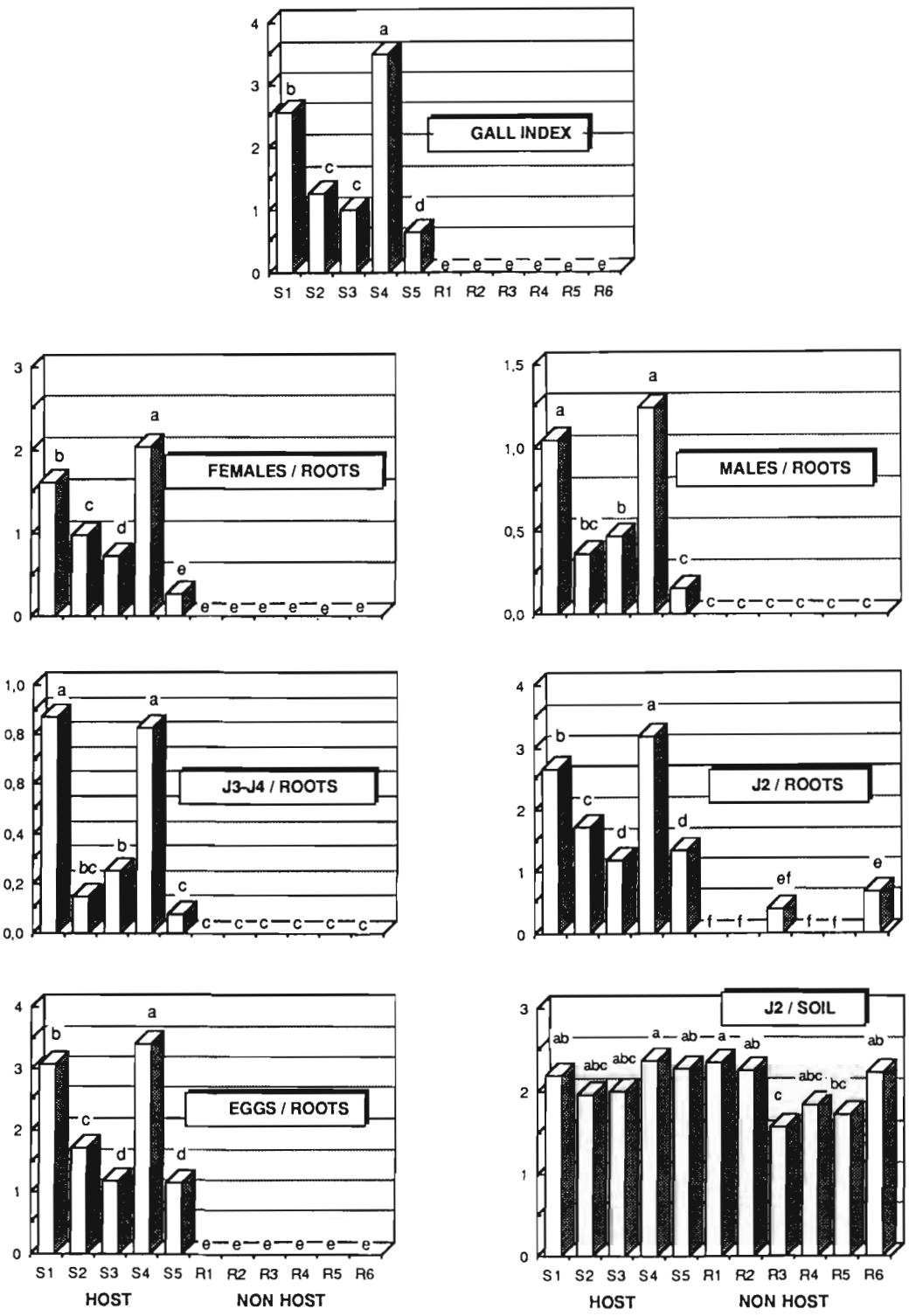

Fig. 1. Gall index, numbers of nematodes at different development stages in roots (10 g), and numbers of second stage juveniles in soil (J2/100 g) in host and non host clones of *P. cerasifera*. Nematode numbers reported on the figure are $\log_{10}(x + 1)$ transformed. Bars with the same letter do not differ at $p \leq 0.05$ using Newman-Keuls test.

Table 1. Evolution of numbers of *Meloidogyne arenaria* recovered from 5 dm³ containers inoculated by transplanting the root system and soil of a tomato plant infested 2 months before with 500 J2s. Values are means and standard deviation (in brackets) of six replications.

Months after transplantation	Roots		Soil	Total inoculum
	J2	Eggs	J2	
Initial* inoculum	127 576	24 070	10 217 (5365)	161 863 (29 502)
1	2588	542	17 392 (4630)	20 522 (4455)
2	5	20	5063 (1087)	5088 (1083)
3	1	0	5706 (1970)	5707 (1970)
4	no longer roots		6984 (1877)	6984 (1877)

* Estimated in the 250-ml tomato pot on the date of transplanting.

EVALUATION OF *PRUNUS* RESISTANCE TO *MELOIDOGYNE*

Nematode densities in the soil

Nematodes recovered from soil were almost exclusively J2s, as the extraction method was not suitable for obtaining eggs or eggmasses (Fig. 1). A maximum of two males per 100 g were counted. Variation in host *Prunus* clones was lower than in non host clones. Although significant differences appeared, no major variation was observed between densities in the soil with host and non host clones.

Gall index and nematode densities in the roots

Mean gall index varied from 0.5 to 3.5 in host clones and was zero in non hosts (Fig. 1). In host clones, eggs and J2s were the most numerous followed by females, males and lastly J3-J4. Female, egg and J2 numbers were ranged into the same order as gall index. J2s were the only stage detected in the roots of non host clones (R3 and R6) but densities were very low.

Female numbers were ranged into the same order as males and J3-J4, except in clones S1 and S3. Sex ratio varied from 0.08 to 0.21, except in clone S3 where it reached 0.43. Linear correlation coefficients were calculated for all host clones (5 clones × 8 replicates = 40 couples of data) between gall index and $\log_{10}(x + 1)$ transformed densities of females ($r = 0.78$), males (0.66), J3-J4 (0.60), J2s (0.68) and eggs (0.71). All the r values are highly significant.

Discussion

Initial total inoculum brought by tomato roots transfer was probably under-estimated because only J2s were obtained from soil. Moreover survival of entire root system in soil is longer than that of root pieces in the mist chamber and consequently advanced J4 and young females might have a higher chance of achieving their development and laying eggs. Most inoculum was certainly eggs in eggmasses whose diapause is removed progressively (de Guiran, 1979). Consequently the release of J2 is progressive and keeps relatively high and stable from 2 to 4 months.

Numbers of J2s in the soil at harvest were almost similar under host and non host clones and thus it is concluded that J2s in soil came mainly from eggmasses that developped on tomato. Consequently the release of J2 from tomato continued to be higher than that of *Prunus* 4 months after inoculation, confirming our previous results.

The best linear correlation between gall index and nematode stages in roots was observed with females followed by eggs and J2s. While host suitability of the clones was very variable, the proportion of different nematode stages in roots was very similar. Moreover the five clones can be ranged into three genetically different groups : S2 and S3, S4 and S5 (brother clones), S1. Despite the high level of initial inoculum, even S4 roots with the highest gall index were not heavily invaded. So multiplication did not occur to an extent on the best hosts; in another experiment not reported here, we observed that in almond seedlings (considered excellent hosts), roots had the highest gall index and leaves had fallen several weeks before those of *P. cerasifera* clones.

These experiments demonstrate that a clear separation between hosts and non hosts was observed in this *Prunus* species. No host-parasite relation of an intermediate type with formation of small galls associated with incomplete development of the nematode, as Malo (1967) observed it on resistant " Okinawa " and " Nemaguard " stocks *(P. persica)* towards *M. javanica*, was found. Although penetration of J2s occurred in some non host clones, no developing larvae were obtained.

The method reported here indicates that a durable inoculum pressure can be used to select non hosts in ligneous plant material. It can also be adapted to study tolerance.

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