Faculty of Agriculture, Technological Education Institute (TEI), P.O. Box 140, 71110 Heraklion, Crete, Greece and
Plant Protection Institute, National Agricultural Research Foundation, P.O. Box 1802, 71110 Heraklion, Crete, Greece

REPRODUCTION OF POPULATIONS OF *MELOIDOGYNE* SPECIES ON *IN VITRO* PRODUCED BANANA PLANTLETS

by
G. GRAMMATIKAKI and E. A. TZORTZAKAKIS

Summary. Reproduction of two populations of *Meloidogyne javanica* and one of *M. incognita*, from Crete, did not differ on Goldfinger (FHIA 01) and Dwarf Cavendish banana plantlets produced *in vitro*. However, the intensity of root galling differed significantly, being higher on Goldfinger than on Dwarf Cavendish at both the two initial (1000 or 5000 eggs/pot) population densities tested.

Banana (*Musa* AAA *cavendish* subgroup: Dwarf Cavendish) is the most important subtropical crop in Crete where it is grown in plastic houses along the coastal areas of the island. A survey on 28 locations in the most representative banana growing areas during 1990-91 indicated the presence of *M. javanica* in nearly 95% of the sampled sites (Vovlas *et al.*, 1994).

Goldfinger is a banana cultivar developed by the Queensland Department of Primary Industries for subtropical cultivation. It has exceptional cold tolerance, is highly resistant to Black Sigatoka (*Mycosphaerella fijensis*), Panama disease races 1 and 4 (*Fusarium oxysporum* f.sp. *cubense*) and the burrowing nematode (*Radopholus similis*) (Information Bulletin, Department of Primary Industries, Queensland). The exceptional cold tolerance and resistance to Black Sigatoka may offer advantages for Cretan conditions and the FHIA lines could replace the traditionally grown clones of cv. Dwarf Cavendish. For this reason *in vitro* plants of the Goldfinger line (FHIA 01) were imported from Tropical Technologies Ply Limited, Tzaneen, Republic of South Africa, for multiplication and agronomic investigation. One line of investigation was an assessment of the reproduction of Cretan populations of root-knot nematode (*Meloidogyne* spp.) on Goldfinger FHIA 01 and the Dwarf Cavendish, the results of which are presented here.

Materials and methods

Both banana cvs were micromultiplied *in vitro* using vegetative shoot apices and the MS culture medium (Murashige and Skoog, 1962) containing myo-inositol (100 mg/l), thiamine HCl (2 mg/l), 6-benzylaminopurine (BAP) (5 mg/l) and sucrose (30 gr/l). The pH was adjusted to 5.8 and the medium was solidified with agar (8 gr/l). Cultures were maintained at 26 °C and 16 h photoperiod with fluorescent light of 3500 Lux. For bud proliferation of cv. Dwarf Cavendish the above mentioned medium was used, while for the Goldfinger line (FHIA 01)

multiple shoots and buds were found to be better stimulated by supplementing the medium with 6 mg/l of BAP. Repetitive dissection of the aggregated adventious buds and new placement in the media was made until a sufficient population of buds was obtained. Buds were then transferred to the rooting medium (MS medium containing 100 mg/l myo-inositol, thiamine HCI 2 mg/l, indole-3-butiric acid 4 mg/l and sucrose 30 gr/l). After incubation for four weeks (26 °C, 16 h photoperiod and 3500 Lux) plantlets exhibited numerous roots. Acclimatization in an insect-proof conditioned glasshouse was carried out for about 20 days and then plants were individually transplanted into 0.6 l pots filled with steam sterilized soil (72% sand, 9% clay, 19% loam, EC=3.75, pH=7.183) and distributed on a glasshouse bench.

After 3 weeks (28 July) each pot was inoculated with egg masses of root-knot nematode populations which were buried in 4-5 cm deep depressions around the stem and covered with soil. The egg masses had been picked from infested roots of tomato plants maintained in pots in a growth room. The average number of eggs/egg mass was estimated by dissolving several egg masses in 1% NaOCI (Hussey and Baker, 1973). Two rates of inoculum were applied for each banana variety X nematode combination, namely c. 1,000 (low) or 5,000 (high) eggs per pot. Both inoculation levels were higher than the tolerance limit for height (0.32 eggs and J2/cm^3) and relative weight (0.146 eggs and J2/cm^3) defined for *M. javanica* on Dwarf Cavendish (Vovlas *et al.*, 1993).

The nematode populations used were *M. javanica* (Treub) Chitw. (populations 1 and 2) and *M. incognita* (Kofoid *et* White) Chitw. collected from vegetable crops of different areas of Crete. One population (2) of *M. javanica* was highly pathogenic to nematode resistant tomatoes (Tzortzakakis and Gowen, 1996).

Pots were randomized in a glasshouse without supplementary light or heat for 14 weeks after inoculation (air temperature ranged from 18-28 °C) with each treatment replicated five times. Then the plants were uprooted, roots washed free from soil, weighed, rated for root-galling intensity from 0 to 10 (Bridge and Page, 1980) and chopped into 1-2 cm pieces. Each root was individually comminuted in a kitchen blender (for three periods of 10-15 seconds separated by two intervals of 10 seconds) and eggs were collected after washing the slurry on a 38 µm sieve. A 150 µm sieve was used to collect coarse root material. The eggs collected on the sieve were washed in a beaker and the concentration was estimated in representative samples of 2 ml under a stereoscope (X 25).

Data on egg production were transformed to square roots before analysis to standardize the variances (Mead and Curnow, 1990). Effects were assessed by ANOVA following the design of a two factor experiment.

Results and discussion

The roots of control plants of Golgfinger (FHIA 01) were longer and heavier (9.1 g) than Dwarf Cavendish (6.5 g). Nematodes caused galls and reproduced in both cultivars. The intensity of root-galling differed significantly between the two cultivars being higher in Goldfinger than in Dwarf Cavendish at both nematode densities. At low nematode density galling was dependent on nematode population (Table I).

Nematode reproduction (number of eggs/g of root) did not differ between the cvs at both densities but was different between populations (Table II). This may be explained by inter or intraspecies differences on reproduction of *Meloidogyne* on a susceptible host (Khan and Haider, 1991).

Our results confirm that the banana cv. Goldfinger (FHIA 01) can be severely damaged and support high reproduction rates of *M. javanica* and *M. incognita*. Nevertheless, of particular interest would be a study on the effect of nematode damage on yield and other agronom-

TABLE I - *Root galling intensity of two banana cvs grown in soil infested by* Meloidogyne *spp.*

Pi =	1000 eggs/pot		5000 eggs/pot	
	Goldfinger	Dwarf Cavendish	Goldfinger	Dwarf Cavendish
M. javanica 1	5.2	5.4	5.0	4.4
M. javanica 2	4.8	4.0	4.8	4.8
M. incognita	5.6	4.0	6.0	4.2
	SE=0.48 Cv. effect P<0.01 Nematode effect P<0.05 Interaction P>0.05		SE=0.56 Cv. effect P<0.05 Nematode effect P>0.05 Interaction P>0.05	

TABLE II - *Egg mass production (eggs/g root) on two banana cvs grown in soil infested by* Meloidogyne *spp.*

Pi =	1000 eggs/pot		5000 eggs/pot	
	Goldfinger	Dwarf Cavendish	Goldfinger	Dwarf Cavendish
M. javanica 1	620 (24.9)	1681 (41.0)	420 (20.5)	1253 (35.4)
M. javanica 2	2180 (46.7	2079 (45.6)	1289 (35.9)	912 (30.2)
M. incognita	2421 (49.2)	5640 (75.1)	2362 (48.6)	4251 (65.2)
	SE=13.47 Cv. effect P>0.05 Nematode effect P<0.05 Interaction P>0.05		SE=9.70 Cv. effect P>0.05 Nematode effect P<0.01 Interaction P>0.05	

ANOVA on square root transformed data ( ).

ical characteristics of both cultivars, as root-knot nematodes are widely distributed and abundant in Crete.

Literature cited

BRIDGE J. and PAGE S. L. J., 1980. Estimation of root-knot infestation levels on roots using a rating chart. *Tropical Pest Management, 26*: 296-298.

HUSSEY R. S. and BARKER K. R., 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Disease Reporter, 57*: 1025-1028.

KHAN W. M. and HAIDER S. R., 1991. Comparative damage potential and reproduction efficiency of *Meloidogyne javanica* and *M. incognita* on tomato and egg plant. *Nematologica, 37*: 293-303.

MEAD R. and CURNOW R. N., 1990. *Statistical methods in agriculture and experimental biology.* Chapman and Hall: 104-124.

MURASHIGE T. and SKOOG F., 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant, 15*: 473-497.

TZORTZAKAKIS E. A. and GOWEN S. R., 1996. Occurrence of a resistance breaking pathotype of *Meloidogyne javanica* on tomatoes in Crete, Greece. *Fundam. appl. Nematol., 19*: 283-288.

VOVLAS N., AVGELIS A., GOUMAS D. and FRISULLO S., 1994. A survey of banana diseases in sucker propagated plantations in Crete. *Nematol. medit., 22*: 101-107.

VOVLAS N., DI VITO M. and GRAMMATIKAKI G., 1993. Growth response of *in vitro* produced banana plantlets to *Meloidogyne javanica* in pots. *Nematropica, 23*: 203-208.

Accepted for publication on 28 May 1998.