RELATIONSHIPS BETWEEN INITIAL POPULATION DENSITIES OF *MELOIDOGYNE ETHIOPICA* AND GROWTH OF VINIFERA GRAPE IN POTS

M. Di Vito[1], J.C. Magunacelaya[2], T. Ahumada[2] and F. Catalano[1]

[1] *Istituto per la Protezione delle Piante, C.N.R., 70126 Bari, Italy*
[2] *Universidad Católica Valparaíso, Valparaíso, Chile*

Summary. The effect of initial population densities of a Chilean population of *Meloidogyne ethiopica* on the growth of vinifera grape, *Vitis vinifera*, cv. Merlot Noir, was investigated in a glasshouse experiment using 350 cm³ clay pots containing pasteurized sandy soil. Each pot was artificially inoculated with 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 or 512 eggs and second stage juveniles/cm³ soil (*Pi*) and planted with one grape seedling. The plants were arranged on a bench in a glasshouse at 26 ± 3 °C for 45 days, with six-fold replication of each *Pi*. Plant response to *Pi* fitted the Seinhorst model, $y = m + (1 - m)z^{P-T}$. Tolerance limits to the nematode were 1.3, 0.6 and 0.45 eggs and second stage juveniles/cm³ soil for height, top weight and root weight of the plants, respectively. Minimum relative yields were 0.2, 0.06 and 0 at $Pi \geq 64$ eggs and second stage juveniles/cm³ soil for height, fresh top and root weight of the grape seedlings, respectively. Final population densities and reproduction rates of *M. ethiopica* were larger at initial population densities of 8-16 eggs/cm³ soil and less at larger *Pi*. The highest nematode reproduction rate, 34.4-fold, occurred at *Pi* of 0.25 egg/cm³ soil. Root gall index increased with the increase of *Pi*.

Key words: Pathogenicity, root-knot nematode, tolerance limit, yield loss, *Vitis vinifera*.

Vinifera grape (*Vitis vinifera* L.) is one of the most economically important crops in many countries where grapes are grown for the production of fresh fruit, raisins, and wine. In 2007, about 7,501,872 ha were used for grape production, yielding 66,271,676 t of fruit and 26,444,731 t of wine (FAO, 2008). Chile has about 182,000 ha of vineyards producing 2,350,000 t of fruit and 827,746 t of wine, which are mainly exported to national and international markets (FAO, 2008). In Chile, as in other grape producing areas, the crop is severely damaged by several diseases and pests, including some plant parasitic nematodes. The most damaging of these nematode pests include *Xiphinema index* Thorne *et* Allen (Magunacelaya *et al*., 2004a), the root lesion nematode (*Pratylenchus vulnus* Allen *et* Jensen) and the citrus nematode (*Tylenchulus semipenetrans* Cobb). Many species of root-knot nematodes (*Meloidogyne* spp.) have been also reported on grape in Chile (Managucelaya and Dagnino, 1999). In the past, some aggressive root-knot nematode populations associated with severe plant damage were considered erroneously as virulent populations of *M. hapla* Cobb. Subsequent morphological and molecular analyses clarified the taxonomic status of these root-knot nematodes, which were identified as *Meloidogyne ethiopica* Whitehead (Carneiro *et al*., 2007). In recent years, this root-knot nematode has emerged as one of the major widespread parasites of vinifera grape in the main grape growing areas of Chile (Carneiro *et al*., 2007). Presently, the vineyards infested by *M. ethiopica* are located in an area delimited in the north by Copiapó (800 km north of Santiago) and in the south by Talca (350 km south of Santiago).

Although field observations indicate that *M. ethiopica* plays an important role in the decline of vinifera grapes, there is a lack of information on the host response of vinifera grape to increasing population densities of this pest. Information on the damaging levels of initial populations of this pathogen is crucial to the implementation of appropriate management practices. A study was conducted in clay pots in a glasshouse to evaluate the effect of initial population densities of a Chilean population of *M. ethiopica* on the growth of grapevine and on the dynamics of the nematode populations on this host.

The population of *M. ethiopica* was collected from grapevine cv. Chardonnay at Casablanca Valley in central Chile, 60 km west of Santiago, and reared on tomato (*Solanum lycopersicum* L.) cv. Rutgers in a glasshouse at 26 ± 3 °C. When large egg masses had formed, the roots were gently washed free of adhering soil and finely chopped (0.5-1 cm long pieces). Ten 5 g root samples were separately shaken for 4 min in jars containing 100 ml of 1% aqueous solution of sodium hypochlorite (NaOCl) to disperse the egg masses and sieved through a 70 µm sieve nested onto a 10 µm sieves (Hussey and Barker, 1973). Eggs remaining on the10 µm sieve were placed in a beaker, appropriately diluted and the contents of three 1 ml aliquots counted and expressed as number of eggs and second stage juveniles in 5 g of roots. The total number of nematodes on the total amount of roots was then estimated. The tomato roots were then thoroughly mixed with 3 kg of steam sterilized sandy soil and used as inoculum (Di Vito *et al*., 1986; Di Vito *et al*., 2004).

Seventy clay pots were each filled with 350 cm³ of steam sterilized sandy soil (sand 88%, silt 5%, clay 7%, organic matter 2.5%). Appropriate amounts of the inoculum were thoroughly mixed into the soil of each pot to give initial population densities (*Pi*) of 0, 0.125, 0.25,

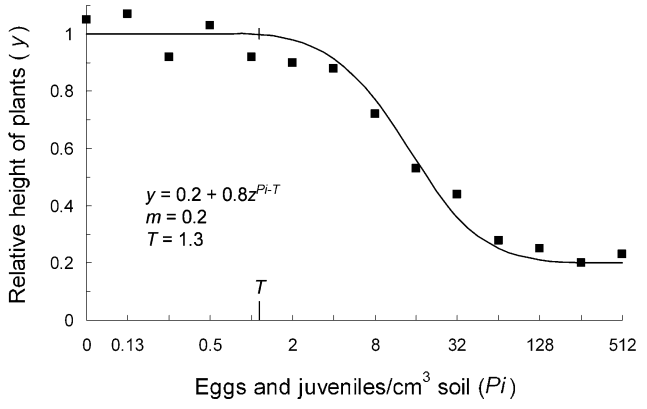

Fig. 1. Relationship between initial population densities (*Pi*) of the Chilean population of *Meloidogyne ethiopica* and relative height (*y*) of grape cv. Merlot Noir grown in pots in a glasshouse at 26 ± 3 °C for 45 days.

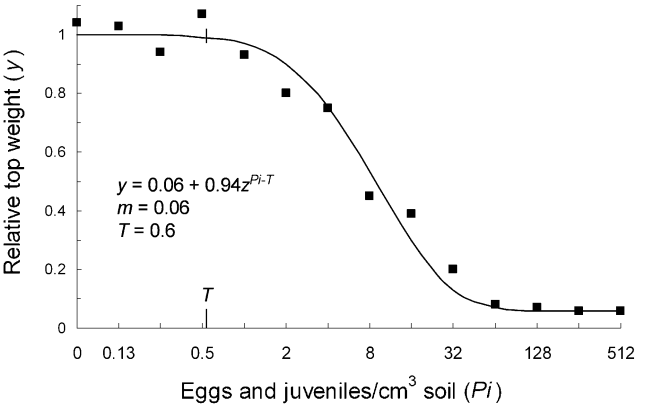

Fig. 2. Relationship between initial population densities (*Pi*) of the Chilean population of *M. ethiopica* and relative fresh top weight (*y*) of grape cv. Merlot Noir grown in clay pots in a glasshouse at 26 ± 3 °C for 45 days.

0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 or 512 eggs and second stage juveniles/cm³ soil.

A single two-month-old grape seedling, cv. Merlot Noir, about 5 cm tall, was transplanted into each clay pot on 24 January 2007. The pots were arranged in a completely randomized design, with six replicates per population density, on a bench in a glasshouse at 26 ± 3 °C. Forty-five days after transplanting, height and fresh top weight of the plants were recorded. The root systems were gently washed, weighed and gall indices assessed according to a 0-5 scale, where 0 = no gall, 1 = 1-2 galls, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 = more than 100 galls (Taylor and Sasser, 1978). Eggs and second stage juveniles of the nematode were extracted from the roots by shaking them in jars containing 200 ml of a 1% aqueous solution of NaOCl for 4 min (Hussey and Barker, 1973). Eggs and second stage juveniles of the nematode present in the soil were extracted by a modification of Coolen's method (Coolen, 1979; Di Vito *et al.*, 1985). The number of eggs and second stage juveniles in the soil plus those found in the roots of the same pot was considered as the final population density (*Pf*) per pot.

Data of plant height, top and root weight at different *Pi*s were fitted to the model $y = m + (1 - m)z^{Pi-T}$ proposed by Seinhorst (1965, 1979). In this model, *y* is the relative yield (the yield at a given $P > T$ divided by the average yield at all values of $P \leq T$) with $y = 1$ at $P \leq T$, *m* is the minimum relative yield (= *y* at very large population density), *P* is the nematode population density at transplanting expressed as eggs and second stage juveniles/cm³ soil, *T* is the tolerance limit of the crop to the nematode (= value of *P* up to which no crop damage occurs), and *z* is a constant with $z^T = 1.05$. Data of root gall indices were analysed by analysis of variance and the means compared using Duncan's Multiple Range Test.

Environmental conditions in the glasshouse (26 ± 3 °C, natural daylight and 14 h daylength) during the experiment were suitable for both grape plant growth and nematode infestation and reproduction.

Meloidogyne ethiopica suppressed the growth of grape cv. Merlot Noir (Figs 1-3). Symptoms of nematode attack were evident at the initial population density (*Pi*) of 8 eggs and second stage juveniles/cm³ and consisted of a marked reduction of plant top growth.

By fitting the data to the Seinhorst (1965, 1979) model, values of tolerance limits, relative yields (*y*) at different population densities and minimum yields (*m*) were derived (Figs 1-3). The tolerance limits for height, fresh top and root weight of grape plants to *M. ethiopica* were 1.3, 0.6 and 0.45 eggs and second stage juveniles/cm³ soil, respectively (Figs 1-3). The minimum relative yields (*m*) were: 0.06 at $Pi \geq 64$ eggs and second stage juveniles/cm³ soil for plant fresh top weight; 0 at $Pi \geq 64$ eggs and second stage juveniles/cm³ soil for root fresh weight; and 0.2 at $Pi \geq 128$ eggs and second stage juveniles/cm³ soil for plant height (Figs 1-3).

The final population density (*Pf*) increased to a maxi-

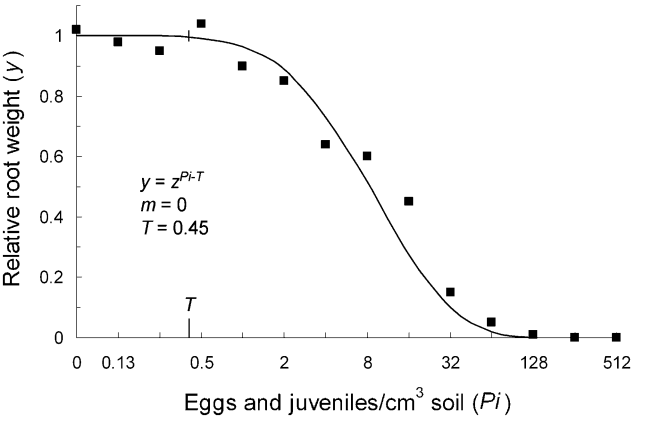

Fig. 3. Relationship between initial population densities (*Pi*) of the Chilean population of *M. ethiopica* and relative fresh root weight (*y*) of grape cv. Merlot Noir grown in pots in a glasshouse at 26 ± 3 °C for 45 days.

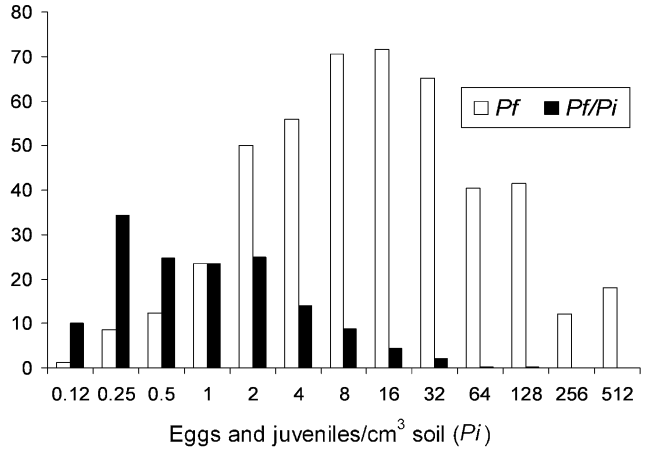

Fig. 4. Effect of initial population densities of *M. ethiopica* at transplanting (*Pi*) on the nematode population at harvest (*Pf*) and on its reproduction rate (*Pf/Pi*) in pots planted with grape cv. Merlot Noir grown in a glasshouse at 26 ± 3 °C for 45 days.

mum of about 72 eggs and second stage juveniles/cm³ in the pots with a *Pi* of 16 eggs and juveniles/cm³ and thereafter declined with the increase of *Pi*. The maximum reproduction rate (*Pf/Pi*) of the nematode (34.4-fold) was observed at *Pi* of 0.25 eggs and juveniles/cm³ soil (Fig. 4). Thereafter, the reproduction rate declined with the increase of *Pi* and was nearly 0 at Pi ≥ 64 eggs and second stage juveniles/cm³ soil. The root gall index was low (0.2) at the smallest *Pi* and highest (5.0) at *Pi* ≥ 64 eggs and second stage juveniles/cm³ soil (Table I). The increasing severity of root galling and suppression of plant growth with increase in *Pi* in the soil confirm field observations indicating a close relationship between grape plant decline and nematode infestation level in the vineyards.

The tolerance limit for fresh top weight of vinifera grape cv. Merlot Noir to *M. ethiopica* (0.6 eggs and juvenile/cm³ soil) is very close to that of cv. Italia to *M. incognita* race 1 (0.78 eggs and juvenile/cm³ soil) reported in Italy by Sasanelli *et al.* (2006). However, Sasanelli *et al.* (2006) reported a minimum yield (*m*) for top fresh weight of grapevine to *M. incognita* of 0.55 (at *Pi* = 64 eggs and juveniles/cm³ soil), while in our experiment *m* was as low as 0.06, with complete plant mortality at *Pi* ≥ 64 eggs and juvenile/cm³. Differences in susceptibility of grapevine cultivars, virulence of the nematode species and climatic conditions may account for the observed differences.

Our results demonstrated that the Chilean population of *M. ethiopica* is highly pathogenic to grape and, therefore, severe crop losses might be expected in nematode infested fields. Growth reduction of plant tops would start at soil population densities as low as 0.6 eggs and second stage juveniles/cm³ soil and would reach 50% in soil infested with 8 eggs and second stage juveniles/cm³ soil. The rate of growth suppression would be less rapid at more than 16 eggs and second stage juveniles/cm³ soil, but plant mortality would occur at 64 eggs and second stage juveniles/cm³ soil. We used small seedlings as the experiment was conducted in small pots for a short time (45 days) and under constant and optimal conditions. In Chile, rooted cuttings, much larger than our short seedlings, are routinely transplanted in the vineyards. Also, under field conditions the temperature varies from rather low in spring to hot in summer to mild in autumn. In addition, temperature fluctuates daily between day and night. These differences in plant size and temperature fluctuation would certainly affect both the extent of damage caused by and the reproduction of the nematode. Field experiments to assess the response of vinifera grape to *M. ethiopica* infections are needed for comparison with these glasshouse results. Nevertheless, our experiment has confirmed the severity of the nematode to grapes and, therefore, the need for a management programme to avoid yield losses of grape, especially in conditions conducive to nematode damage, such as in sandy soil. In addition, vinifera grape is a perennial that should be planted in soil free from *M. ethiopica*. Nematode soil population densities as low as 0.12-0.25 eggs and second stage juveniles/cm³, which are difficult to detect, are expected to reach damaging levels shortly after planting (Fig. 4).

Satisfactory nematode control and yield performance of grape would be achieved with pre-plant soil fumigation with 1,3 dichloropropene (Magunacelaya *et al.*, 2004b). Also, on an established grape crop, good con-

Table I. Effect of initial population densities of *Meloidogyne ethiopica* at transplanting (*Pi*) on the root gall index of vinifera grape cv. Merlot Noir grown in pots in a glasshouse at 26 ± 3 °C for 45 days.

Eggs and juveniles/cm³ soil (*Pi*)	Gall index (0-5)
0.125	0.2 a*
0.25	0.8 a
0.5	1.8 b
1	2.0 b
2	2.8 c
4	3.2 cd
8	3.8 de
16	4.0 de
32	4.5 ef
64	5.0 f
128	5.0 f
256	--
512	--

* Means sharing a letter are not significantly different according to Duncan's Multiple Range Test (for P ≤ 0.05).

trol of the nematode was obtained in Chile with aqueous extract of the soap tree (*Quillaja saponaria* Molina) (Martin and Magunacelaya, 2005) and with nematicides and other practices in California (McKenry *et al.*, 2004).

Finally, *M. ethiopica*, as with most species of *Meloidogyne*, seems to have a rather large host range, which includes other trees (such as kiwi) and annual plants (such as vegetables). Therefore, the impact of the nematodes on other crop plants of importance in Chile should also be investigated.

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