SYSTEMATICS OF *MESOCRICONEMA XENOPLAX* REVISITED: COMBINED ANALYSIS OF MORPHOLOGICAL AND MOLECULAR MARKERS.

Irma Tandingan De Ley,[1] Qi Li,[1] Joaquín Abolafia-Cobaleda,[2] Michael McKenry,[1] Isgouhi Kaloshian[1] & Paul De Ley.[1] [1]Department of Nematology, University of California, Riverside CA 92521, USA;
[2]Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Jaén, 23071, Spain

Abstract

The ring nematode *Mesocriconema xenoplax* is a widespread species whose systematics is complicated by numerous taxonomic and diagnostic problems. Field data suggest that California isolates of *M. xenoplax* respond differently on identical grape rootstock varieties. Microplots were therefore established to test the interaction of 5 isolates (three inland isolates Parlier, Belmont, and Livingston; and coastal isolates Los Alamos and Mendocino) in 2 different rootstocks and with or without their associated soil biota. Vines that grew the greatest biomass provided highest nematode build-up. The less vigorous 420A rootstock supported vines that yielded least or were in decline by the fifth year . Generally, the Parlier isolate had the highest reproductive potential in the presence or absence of suspected suppressive soils and regardless of host genotype.

In order to determine whether differences in reproduction between these populations correspond to detectable morphological and/or molecular differences, we analyzed individual nematodes with Video Capture and Editing followed by Polymerase Chain Reaction of the rDNA Internal Transcribed Spacer region (ITS) and large subunit D2D3 domain. Sequence analysis revealed the existence of two prevalent variants, differing from one another by eight substitutions in ITS and corresponding to a coastal haplotype versus a Central Valley haplotype. The latter included a population from the type locality of *M. xenoplax*. A few of the analyzed individuals exhibited single nucleotide polymorphisms matching the character state of either haplotype for the corresponding nucleotide positions. Both ITS and D2D3 phylogenetic analyses suggested that the 2 coastal populations formed a cluster within a clade that also included the 3 central valley populations. Observed genetic differences corresponded with a phenotypic difference in stylet length, with the coastal populations possessing smaller stylets compared to the Central Valley populations.

Rationale of the study

Previous trials with grape rootstock and peach trees indicated that ring nematodes can cause substantial damage to both these hosts in some parts of California. We compared different nematode populations using a multidisciplinary approach, so as to distinguish more clearly between virulence factors attributable to differences in the soil environment, versus those caused by biological diversity within the nematode species itself. The following research activities were undertaken:

1) Collection of five field populations of ring nematodes

During spring of 2002 approximately 1000 lb of soil was collected from each of five commercial sites infested with ring nematode, *Mesocriconema xenoplax*. Four of these sites had previously been involved in long-term studies involving use of rootstocks or nematicides. Experiences with these trials are in conflict: the morphology of ring nematodes from each of these sites appears similar but plant responses indicate possible biological differences.

Site 1: **Parlier.** The original description of *M. xenoplax* by D. J. Raski came from a vineyard 4 miles east of Fresno. Populations of *M. xenoplax* from that vicinity (Peach Ave. Research Station of USDA-ARS) have been under study in microplots at Kearney Ag Center for 10 years.

Site 2: **Belmont.** Near Belmont, thirty miles due west and across the San Joaquin River from site 1. A grape rootstock trial has been underway at this site for 22 years. Ring nematode was present on Freedom rootstock but its population never reached high levels, although the soil was physically suitable for its development.

Site 3: **Livingston.** A vineyard near Livingston along the Merced River about 90 miles north of Fresno. This site was planted on Harmony rootstock (a sibling of Freedom rootstock) but after 10 years ring nematode had caused serious vineyard decline. This problem of vineyard decline was completely corrected with two years of nematicide treatments.

Site 4: **Mendocino.** A grape rootstock trial in Mendocino County along the Russian River near Ukiah, CA. On Freedom rootstock the population levels of ring nematode reached very high levels after 12 years. In this site the rootstock 420A appears to provide the first example of a grape rootstock with resistance to *M. xenoplax*.

Site 5: **Los Alamos.** A vineyard of own-rooted Chardonnay near Los Alamos north of Santa Barbara, CA where repeated applications of nematicide resulted in notable plant growth benefit. The prevailing nematode was *M. xenoplax.*

2) Microplot trials on Freedom and 420A rootstocks

At Kearney Ag Center we established sterilized sandy soil in 100 microplot settings. These microplots are 24 inches in diameter and reach four feet down to native soil with open top and bottom. Fifty 420A and 50 Freedom rootstocks with Pinot Noir scion were planted to the microplots in a completely randomized design. Microplots that were inoculated received 10,000 ring nematodes. Half the nematode inoculum was washed free of soil, the other half was inoculated with the original soil suspected to contain suppressive organisms.

In January 2003 all vines were pruned back to two buds and top growth weighed. In April 2003 first soil samples were collected from each of the 100 microplots. These microplots were sampled again in October 2003 and April/June 2004. Thus, we now have five geographically separate ring nematode populations with or without associated soil being reared on susceptible and resistant grape rootstocks. We quantified nematode population development over a period of 18 months as they increased to a peak level and then declined to a plateau.

Results

Growth and general appearance of Pinot Noir grapevine on two rootstocks did not differ during four years of exposure to five populations of *Mesocriconema xenoplax*. Vine yield and build-up of the five populations was always greatest on Freedom rootstock compared to 420A rootstock (Figs 1-4). Nematode population was generally low on 420A with or without suppressive soil (Figs 1 & 2).

Mean nematode pressures differed significantly during the four years. The Parlier isolate provided significantly higher nematode pressure regardless of the rootstock and the presence or absence of original soil suspected to have suppressive properties (Figs 1-4). The Fresno population was significantly less successful than the Parlier isolate but increased its numbers more than the coastal isolates or the Livingston isolate.

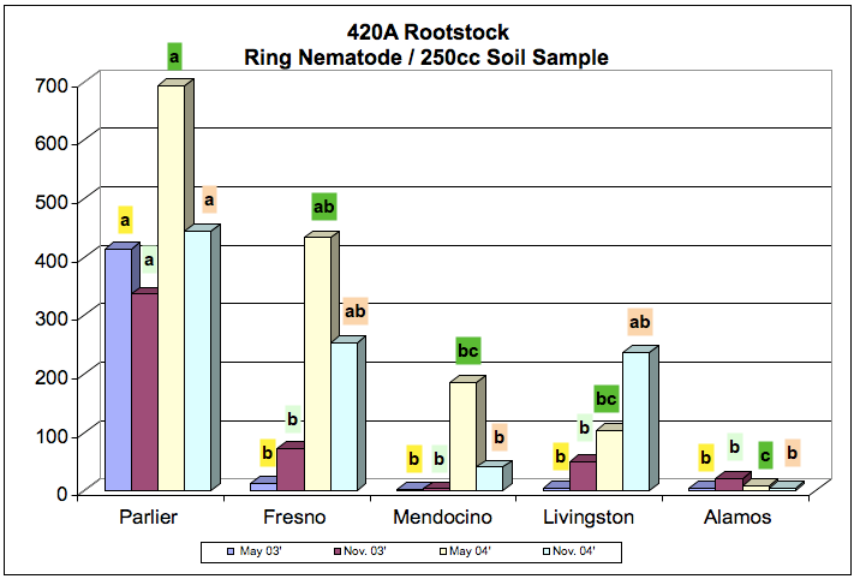

Fig 1. Population dynamics of *M. xenoplax* on 420A rootstock without suppressive soil.

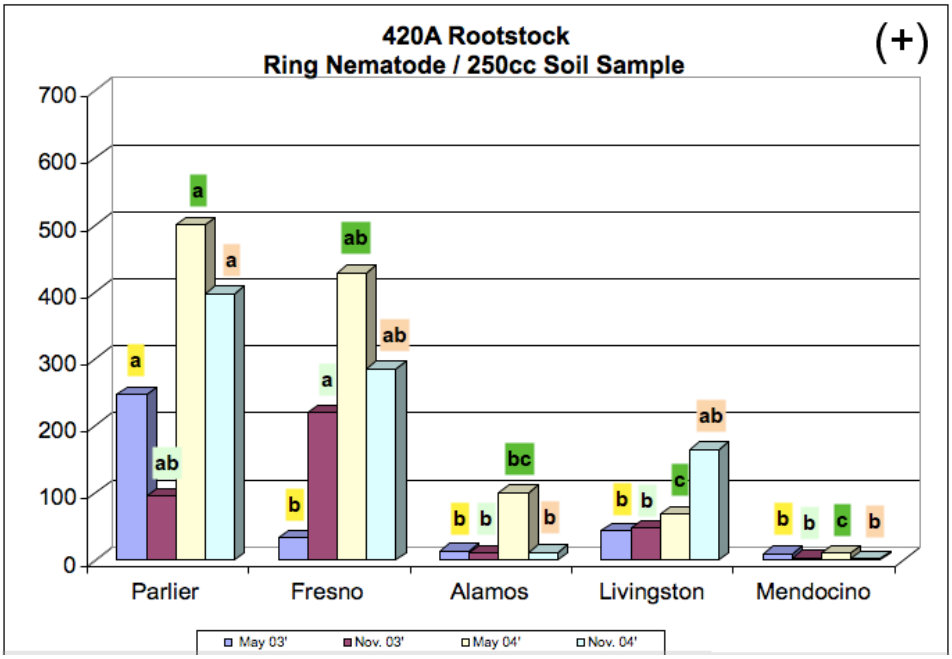

Fig 2. Population dynamics of *M. xenoplax* on 420A rootstock with suppressive soil.

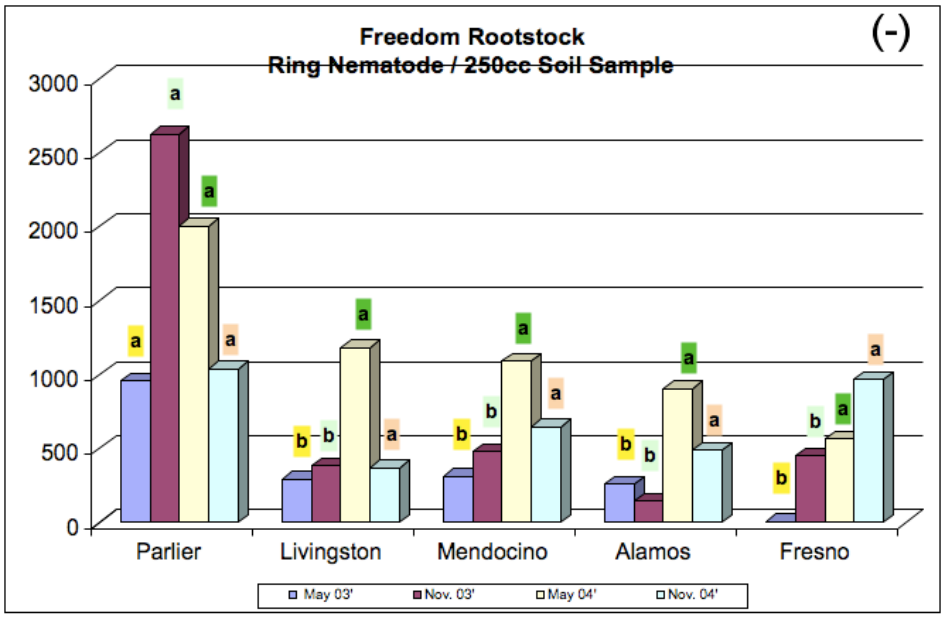

Fig 3. Population dynamics of *M. xenoplax* on Freedom rootstock without suppressive soil.

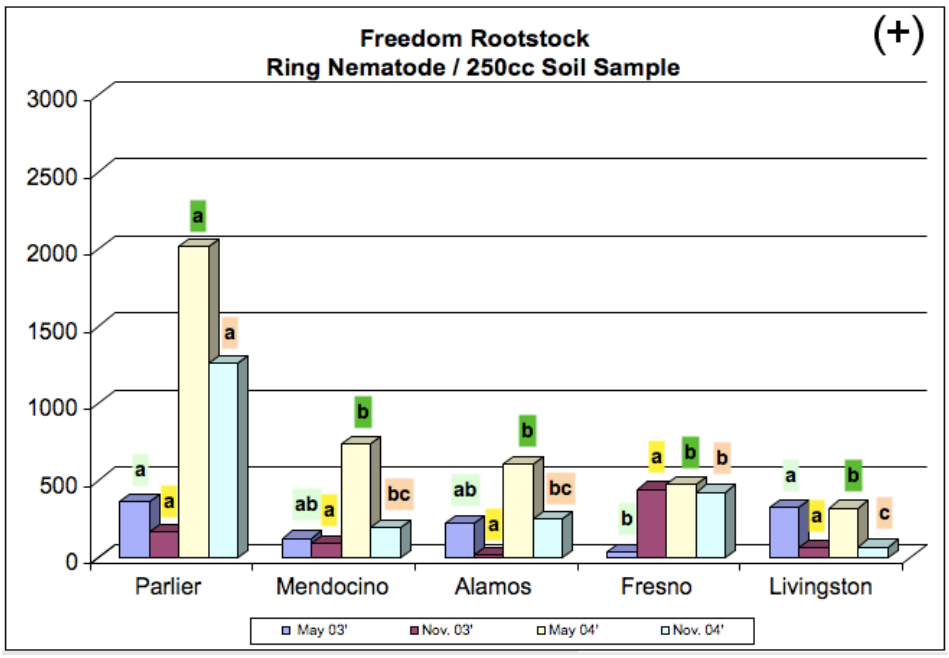

Fig 4. Population dynamics of *M. xenoplax* on Freedom rootstock with suppressive soil.

Conclusion

Vines that grew the greatest biomass provided highest nematode build-up. The less vigorous 420A rootstock supported vines that yielded least or were in decline by the fifth year in the presence of the most aggressive *M. xenoplax* populations. Generally, the **Parlier** isolate had the highest reproductive potential in the presence or absence of suppressive soils and regardless of host genotype.

3) Morphological and molecular analysis

From each site 20 lbs of soil were transferred to UCR for storage and periodic extraction of live nematodes. Five or more live nematodes from each of sites 1 and 3-5 were subjected to Video Capture and Editing (VCE) microscopy for morphological archiving, and then transferred to lysis buffer and frozen at –80 C in preparation for optimized PCR. Additional nematodes were individually transferred directly to lysis buffer and used for preliminary PCR analysis, or stored at –80 C for future use.

A separate number of nematodes were set aside for scanning electron microscopy and light microscopy for morphological and morphometrical studies.

Preliminary PCRs were conducted on individual ring nematodes to assess amplification consistency of different primer combinations for DNA loci, known to exhibit intra- or interspecific variation in other nematodes. To date, primers were tested for the D2/D3 expansion segments of the large subunit (LSU and the Internal Transcribed Spacer (ITS) region) of the ribosomal DNA, the InterGenic Spacer (IGS) region, and the mitochondrial NADH Dehydrogenase subunit 4 gene (ND4).

Results

Scanning Electron Microscopy. No consistent differences were observed on the scanned lip regions, tail, and other external features of the 5 isolates.

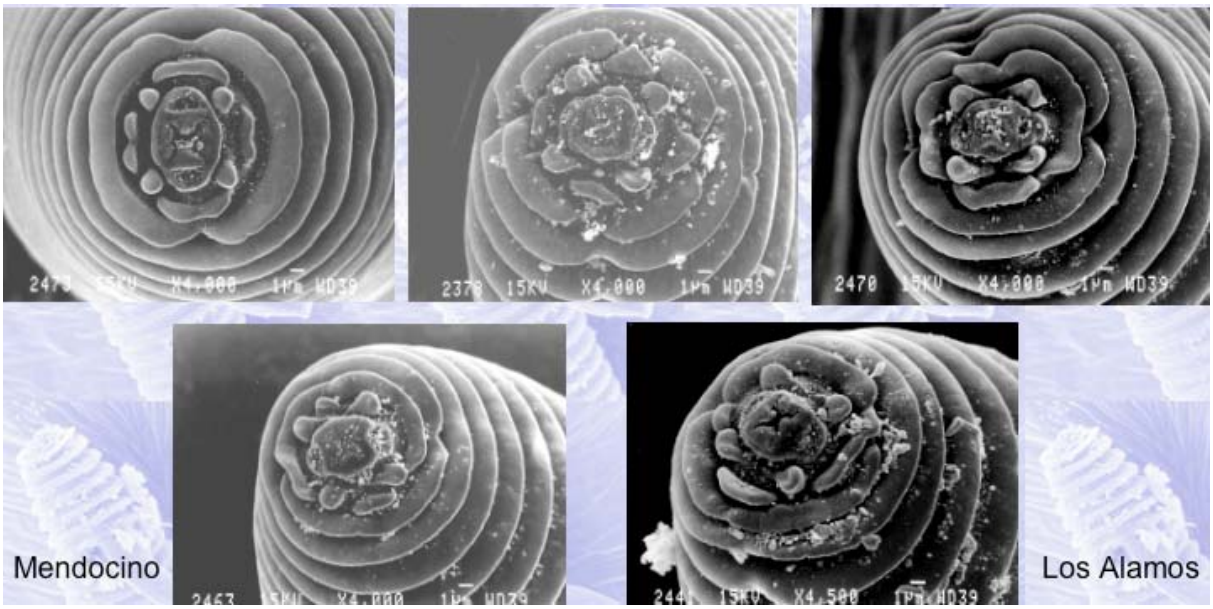

Fig. 5. Comparative scanning electron micrographs of the lip region of *Mesocriconema xenoplax* isolates.

Light microscopy. Measurements were taken from 20 individuals per isolates. **Parlier** isolate is bigger on average compared to the four other isolates. It has an average length of 647 µm (range of 579-735 µm) versus 537-586 µm for the other isolates.

The three inland isolates (Parlier, Belmont, and Livingston) have bigger stylets ranging from 84-99 µm. The coastal isolates (Los Alamos and Mendocino) have stylets of 65-80 µm long. (The original range by Raski = 71-86 µm).

Molecular analyses. Sequences of the ITS regions reveal a very high (>99%) sequence similarity among two coastal isolates Los Alamos and Mendocino. Similarly, there were near identical (>99%) ITS sequences for the three central valley (Parlier, Belmont, and Livingston) isolates. Comparatibly, there was less (97-99%) sequence similarity between the coastal and central valley isolates. Analysis of these 2 ITS variants reveal usually 2 base pair differences in ITS1 and 6 bp in ITS 2 regions.

Polymorphism (sequence variations between and within individuals) was observed in the ITS regions of all isolates with the exception of Los Alamos. For example, one individual from Mendocino has 4 single nucleotide polymorphisms, 3 of these correspond exactly to differences between the two variants. Such observation may suggest genetic exchange between both variants.

Bayesian analysis of the D2D3 sequences reveal that with the exception of a single clone, the 2 coastal isolates Mendocino and Los Alamos form a clade. Inland isolates Belmont and Parlier form a cluster including also the coastal clade. The same trend is apparent with the ITS sequence analyses. (Examples of gene trees in Figures 6 & 7 below).

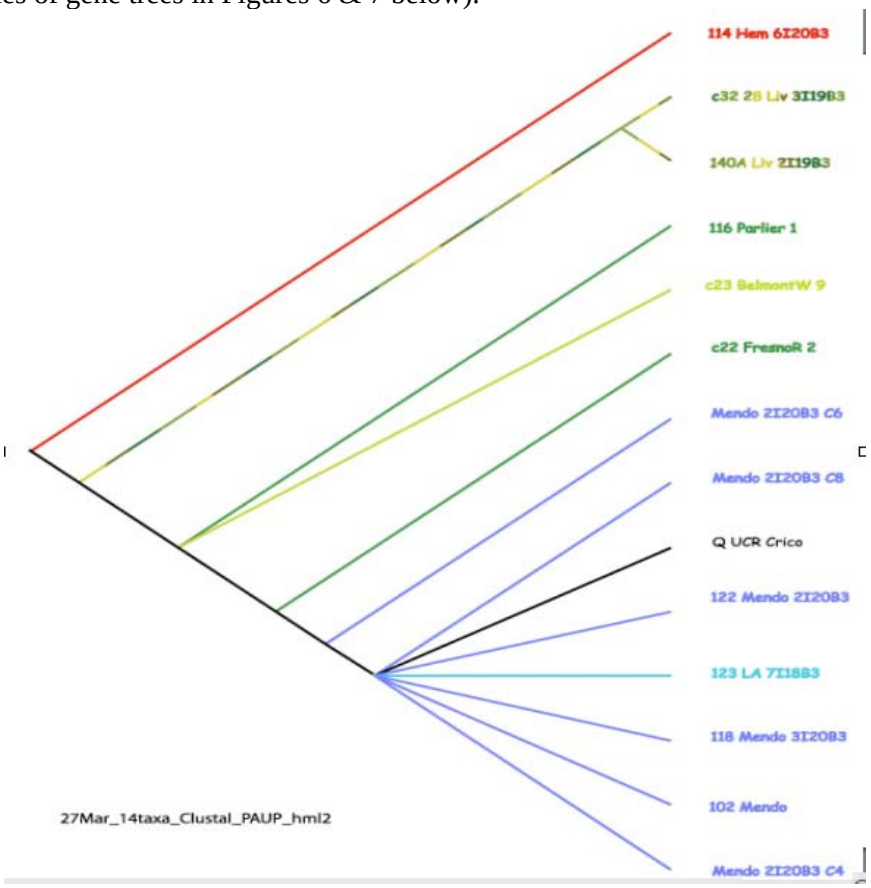

Fig. 6. Example ITS maximum likelihood tree, *Hemicycliophora* as the outgroup.

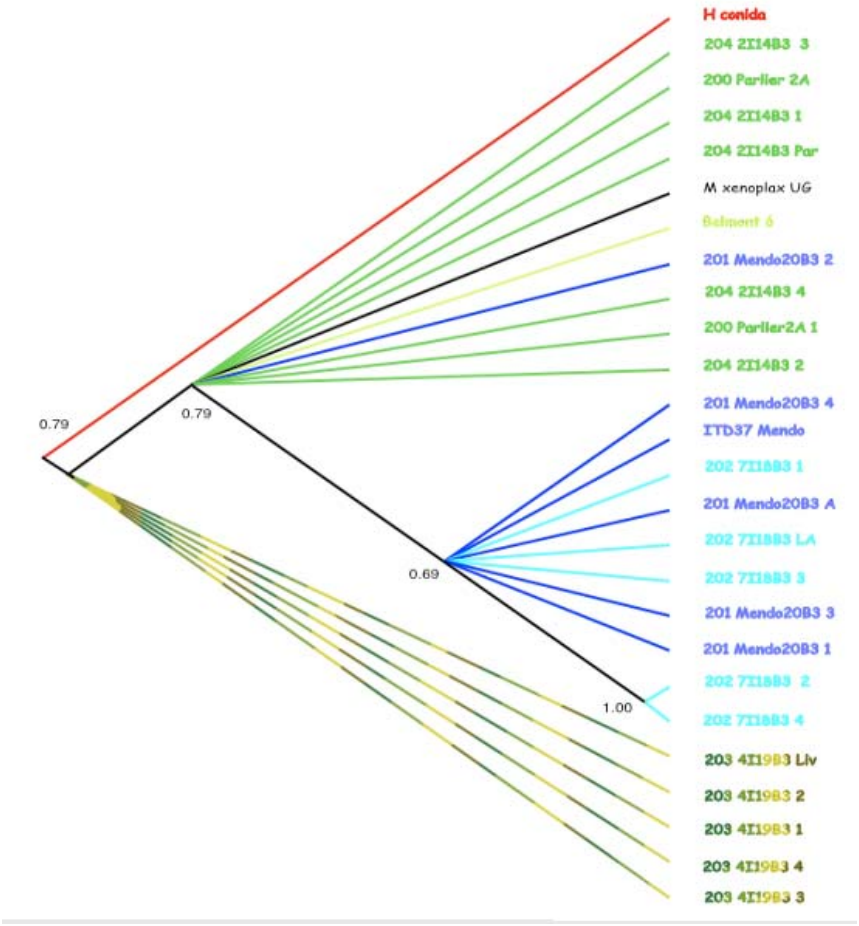

Fig. 7. D2D3 tree, Bayesian analysis. *Hemicycliophora* is the outgroup.

Conclusion

ITS and D2D3 rDNA sequence differences correspond with one morphological difference (stylet length). However, these two molecular markers do not distinguish Parlier from the other isolates.

Overall, our data indicate that *M. xenoplax* populations in southern California are not genetically homogeneous, with some differentiation between coastal versus inland populations that may correlate with differences in reproductive capacity on both tested rootstock varieties.