

Nematodes and Citrus IPM

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Entomopathogenic nematodes (EPNs) to manage citrus root weevils

Plant parasitic nematodes that attack citrus



Weevil IPM **EPNs** Insecticides (monitoring) **Fumigation Rootstocks Cultural practices** Soil drainage pH **Physical barriers**

Regional considerations (EPN conservation)













Diaprepes root weevil is difficult to control because:

- Adults lay eggs that hatch and fall to soil all year long
- ✓ The larvae in soil emerge as adults all year long
- ✓ But the chemicaland bio-pesticides are non-persistent.





EPNs function in association with entomopathogenic bacterial symbionts that provide nutrients and protection from competitors.







EPNs also function as nonpersistent pesticides. Kill insects in first 1-2 weeks following application, but natural enemies of nematodes soon reduce their numbers.







© The Society of Nematologists 1996.



Twenty years of research has demonstrated that *Steinernema riobrave* is the best known EPN for managing citrus root weevils.





BioVector 355 Steinernema riobrave 25 EPNs per cm² soil surface

> 70-90% mortality of sentinel weevil larvae.

Non-persistent one to two weeks

EPNs control Phytophthora complex





4-fold to 11-fold return on treatment cost depending on whether marketed fresh or processed





Second trial, similar results

Note the increased yield

Note the reduction of adult weevils by half



Timing EPNs less obvious

0.3

0.2

JFMAMJJA SONDJFMAMJJA SON

A.

Resulte Revend Education

Timing chemical insecticide applications requires monitoring



\$43.60 12 ft dia \$30.29 10 ft dia 09

<u>Cost</u>

One package of 3 billion infective juvenile nematodes (IJ) will treat 15 acres assuming 25% coverage by irrigation emitters.

@18,365 IJ/ft² soil surface Or 0.26 cents per ft²

For grove of 150 trees/A, 25% coverage requires 9.6 ft diameter spray pattern.

Cost to achieve same dose for some other spray pattern diameters shown here.

<u>Cost</u>



The effectiveness of EPNs is a function of the nematode species, the application rate, the soil conditions and the <u>frequency of application</u>.

Growers should consider increasing the frequency of application as much as can be afforded. E.g., at 12 ft. dia. irrigation pattern x <u>2 applications</u> = \$87/yr. A 6 foot pattern could apply <u>8</u> <u>applications</u> per year for the same cost. Weevil management **EPNs** Insecticides (monitoring) **Fumigation** Rootstocks **Cultural practices** Soil drainage pН **Physical barriers**

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RUS Research Education Center **Cultural Practices**

Landscape fabric can be installed as a barrier to prevent larvae from entering soil and adults from exiting soil

McKenzie et al. 2001 Duncan et al 2008





The integrity of these barriers was maintained for 5-6 years. Herbicide savings equaled the fabric cost.



This barrier is made from a single piece of 15-foot wide fabric. Holes cut with branding iron.



Reflective mulch for psyllid control

Hamlin on Swingle June 2016 Heavily infested by DRW in 2006





Hamlin on Swingle June 2016 Root system typical of scattered declining trees





Conserving EPNs

In addition to augmenting soils with the exotic *Steinernema riobrave* to manage *Diaprepes* root weevil, there are options to enhance and exploit naturally occurring native EPN communities:

- ✓ Reduce soil pH to inhibit bacterial ectoparasites of EPNs
- ✓ Coarsen soil texture in tree planting holes to increase EPN efficacy
- Employ composted animal manure mulch to increase biological control by EPNs
- ✓ Introduce Sx into depauperate flatwoods orchards
- Plant germplasm that respond to herbivory with semiochemical attractants for EPNs



Nematode-induced tree declines in Florida

- Citrus nematode, Tylenchulus semipenetrans (Slow decline)
- Burrowing nematode, *Radopholus similis* (Spreading decline)
- Coffee lesion nematode, *Pratylenchus coffeae* (Citrus slump)
- Sting nematode, *Belonolaimus longicaudatus*
- Dagger nematode, *Xiphinema vulgare*





Integrated Management of Plant Parasitic Nematodes on Citrus

- ✓ Sanitation
- ✓ Host plant resistance/tolerance
- ✓ Cultural practices
 - рΗ
 - Salinity
 - Supplemental irrigation-fertigation Avoid cultivation for weed control
- ✓ Chemical and biological pesticides







Spreading decline caused by the <u>citrus race</u> of *Radopholus similis* the burrowing nematode





Pratylenchus Lesion nematode

Which 2 are cousins?



Phylogeny of Tylenchida





Journal of Applied Ecology 2002 39, 915–923 The effect of transgenic nematode resistance on non-target organisms in the potato rhizosphere

SUE E. COWGILL, RICHARD D. BARDGETT*, DAAN T. KIEZEBRINK and HOWARD J. ATKINSON

001-101111011364.3703 2013 00792



Transformed potato in 2002 to express a <u>cystatin</u> that disrupts digestion of certain proteins and also a <u>peptide</u> that interferes with sensory function of cyst nematodes.

Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY (2012) 13(8), 842-851

Generation of transgenic plantain (Musa spp.) with resistance to plant pathogenic nematodes

HUGH RODERICK', LEENA TRIPATHI², ANNET BABIRYE², DONG WANG^{1,+}, JAINDRA TRIPATHI², PETER E. URWIN^{1,*} AND HOWARD J. ATKINSON¹



In 2012 transformed banana with these genes to confer resistance not only to burrowing nematode, but to lesion, and root-knot nematodes as well.



Lilley et al. 2004 & 2011. Plant Biotechnology Journal 2: 3-12 Plant Biotechnology Journal 9:151-161

AtMDK-20 (root cap promoter)



Tub-1 promoter









RB-7 promoter

Gene constructs - Manjul Dutt and Jude Grosser

Gene	Construct	Function	No pf plant produced
Cowpea thionin II	2300-1867-CII	Inhibition of trypsin	12
OC-IAD86	2300-1867-OCI	Modified rice cystatin	20
Maize Oryzacystatin	2300-1867-ZM	cysteine proteinase inhibitor	5

Cloned into pC2300-1867, a plant transformation vector containing a root specific promoter.

Used to transform Carrizo citrange rootstock.

Evaluate regenerated transgenic plants.







Evaluation of lines is ongoing.

14 lines with no detectable protein expression support burrowing nematode reproduction.

Two lines (ZM and CII) exhibited expression and neither supported burrowing nematodes.

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Increase lines with high gene expression and nematode suppression.

Stack genes.



Transform Kuharski Carrizo to express these genes.





Next generation rootstocks transformed for nematode resistance

Inexpensive

Durable resistance (multigenic targets)

May confer protection against multiple nematode species

No known environmental or safety risk



Effects of nematicides to control citrus nematode in a productive HLB affected grove







Effects of nematicides to control citrus nematode in a productive HLB affected grove







Sudden collapse symtoms during 2015 season









Adult weevil discovered during harvest in February 2016 and structural root damage typical of the declining trees

















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Bayer DuPont BASF















Questions?









