

Project briefing no. 2: Exploring alternatives to HHP nematicides in pineapple *Updated, April 2017*

Trials on biological and other alternatives to ethoprophos for nematode control

Key points

- Many pineapple farm rely on HHP nematicides ethoprophos or oxamyl, which are extremely toxic to humans and known to harm non-target soil organisms
- Ethoprophos is one of the commonest causes of acute poisoning in Costa Rica and found to contaminate surface water and drinking supplies
- Safer, non-chemical methods available include commercial biopesticides, based on fungal biocontrol agents, and 'wood vinegar', which can be prepared on-farm
- A pilot trial comparing these alternatives with the widely used ethoprophos product found no significant difference in nematode infestation between treatments, albeit at very low nematode levels
- Plant weight was significantly lower in plots treated with ethoprophos, suggesting harmful side-effects on soil organisms needed for growing a healthy crop
- Alternatives are much cheaper than ethoprophos and can be applied without special equipment or extensive training

Background

Pineapple production in Costa Rica can be prone to serious infestations of soil-dwelling nematode worms, which can damage the root system of young pineapple plants. Common pathogenic groups are root-knot nematodes (*Meloidogyne* species), lesion nematodes (*Pratylenchus* spp.), *Rotylenchus* species, which partly penetrate the roots, and *Radophulus* species which can cause open wounds to roots, leading to serious rot diseases. *Praty-lenchus* species tend to be the most problematic nematodes in Costa Rican pineapple fields.

Nematodes can be controlled using:

- **good cultural controls**, e.g. careful soil preparation before planting to expose nematodes to sunlight, along with good crop rotations (continuous monoculture makes nematode problems worse)
- **biological control**, mainly via the use of certain fungal species which feed on nematodes
- **chemical control,** via nematicide applications incorporated into the soil pre-planting or as soil drenches around young plants

In conventional chemical control practice in Costa Rican pineapple production, growers usually first apply nematicide just before, or shortly after, planting, followed by a second application, around 2-3 months later. This is the most susceptible stage of the crop as nematodes can cause damage to the young roots while they are still developing. Nematicide active ingredients **ethoprophos** and **oxamyl** are commonly used. In Costa Rican pineapple,

there tends to be little variation in nematicide use patterns. In northern Costa Rica, smallholder pineapple production usually follows Del Monte's protocols for contract growers.

Unfortunately, these two active ingredients and most other synthetic nematicides used in pineapple qualify as Highly Hazardous Pesticides (HHPs), due to their very high acute toxicity to humans (mainly WHO Class 1a or 1b) and negative effects on soil life and the environment. Several sustainability standards, such as Fairtrade, have prohibited or severely restricted use of the highest toxicity nematicides (e.g. oxamyl) in their supply chains in pineapple, banana and other crops.

Hazards and concerns about pineapple nematicides: ethoprophos

The project team identified the nematicide ethoprophos (*aka* ethoprop) as one of the priority HHPs of concern in pineapple production, and for which there is some grower interest in finding IPM alternatives. Ethoprophos is a broad-spectrum organophosphate compound, used for combating soil-dwelling organisms (nematodes, wireworms, white grubs, etc.) in a variety of tropical and temperate fruit and vegetable crops. Well known branded products are Mocap 10G, Sanimul and Prophos. The hazard classifications for which ethoprophos qualifies as an HHP in the PAN International HHP List are described in Annex A, along with its legal status in the EU. Annex B summarises information from Costa Rica and other Central American countries on ethoprophos use, risks and impacts on human health, non-target organisms and environmental contamination.

Exploring safer but effective alternatives to ethoprophos

A small, pilot trial of three non-chemical alternatives was set up in November 2015 by the IRET project team on pineapple plots belonging to large scale grower Fertinyc. Fertinyc cultivates 150 hectares (ha) of land in Pital de San Carlos district, Alajuela, in central Costa Rica. This grower is very interested in ways to reduce pesticide and fertiliser applications and Fertinyc is one of the first conventional farms to make some of its own inputs - biofermentation products, based on local microorganisms + selected mineral nutrients. These on-farm produced fertilisers have enabled farm manager Wilberth Gomez to cut his fertiliser purchase costs by 15-20%. He has experimented with biological control agents and already succeeded in reducing nematicide applications. He agreed to set up a demonstration trial to expand his current experimentation with biopesticide products based on the fungus *Paecilomyces* for nematode control.

Treatments to test and compare

Three alternative methods were tested, comparing the results with the current nematicide use and a totally untreated control:

- 1. Commercial practice standard *Mocap 10G* (ethoprophos) (dose rate: 35kg/ha)
- PA-ECO biopesticide based on the fungal biocontrol agent Paecilomyces lilacinus (4 kg/ha)
- 3. 'Wood vinegar' extract distilled from wood smoke (40 litres/ha)
- 4. *Klamic* biopesticide based on the fungal biocontrol agent *Pochonia chlamydosporia* (0.83 kg/ha)
- 5. Untreated control (zero nematicides or alternatives)

Both biopesticide products are commercially available in Costa Rica. Pyroligneous acid or 'wood vinegar' contains over 300 constituents (including acetic acid, methanol, phenol, esters, ketones and formic acid), some of which have bactericidal and fungicidal properties, while others stimulate plant growth and promote certain beneficial microbes¹. Its use has been pioneered in recent years in Japan and Korea. Wood vinegar is known to be very effective against nematodes, by killing them directly as well as encouraging microbes that feed on them. In Costa Rica its application has given good results in vegetable crops. It can be easily prepared on farm by collecting the distillate from burning soft wood species.

The trial did not include nematicides which do not qualify as HHPs- until recently there were none available. One option which growers could use is the new Verango® product (active ingredient fluopyram), now in use by some banana plantations. Fluopyram is not considered an HHP (according to hazard data available to date) and in Costa Rica products containing fluopyram carry the national 'green' (i.e. least toxic), classification for acute toxicity by the country's regulatory agency for pesticide product labels.

Trial protocol details and agronomic management: Plots were located in a block of commercial farmland under cassava in the previous cropping cycle. Land was prepared mechanically with standard ploughing and turning into raised beds, planted with asexual pineapple suckers of cultivar M-2 ('Golden pineapple', the most commonly grown variety) at a density of 60,000 plants per hectare (ha).



The trial was laid out in a standard randomised block design, with 5 replicates for each treatment of 20m² each. Treatments were applied as a soil drench at 15 days after planting, using an electronic knapsack sprayer of 18 litre capacity, at a drench rate of 3,000 litres/ha. Nematode counts in samples of soil and within pineapple plantlet roots were made at 75 and 135 days after planting.

Project coordinator Fernando Ramirez supervising application of trial treatments, FERTINYC farm, Alajuela. Credit: IRET

Trial results and treatment costs

Nematodes from six different genera were extracted from soil and root samples, including *Pratylenchus* and *Helicotylenchus*, the two most important genera affecting pineapple production in Costa Rica. Numbers of both these genera increased over the study period (Nov. 2015 to Feb. 2016), however there were no significant differences in nematode levels between any of the treatments (Table 1). A likely reason was that the nematode population levels in all the treatment plots turned out to be unusually low- well below the root damage threshold of 1,000 *Pratylenchus* nematodes per 10g roots. These fields were previously under grazing pasture and cassava and this was the first season of pineapple crop grown, which may explain the very low nematode populations found. In fact, none of the treatment plots reached nematode levels which would have justified any treatment, either with synthetic nematicides or alternatives.

Table 1. Nematode counts in trial treatments (individuals per 100 g of soil or 10 g of root)

Treatment	Pratylenchus sp.		Helicotylenchus sp.	
	Soil	Root	Soil	Root
1. HHP nematicide (ethoprophos)	6	27	8.3	17.3
2. Fungal biopesticide (<i>P. lilacinus</i>)	2	195	24,7	15,3
3. Wood vinegar	1.7	101	15.3	40.7
4. Fungal biopesticide (P. chlamydosporia)	1	48	36.3	16.7
5. Untreated control	0.7	217.5	15.3	15.7

This trial was terminated before fruit harvest (18-24 months after planting) so final yield could not be assessed. Nevertheless, measuring plant size at 75 days after planting, the team noticed that the smallest plants were those on the plots treated with the HHP nematicide and this difference proved to be significant (Table 2). One explanation could be that ethoprophos was causing harmful effects not only to the target nematodes but to beneficial microorganisms in the soil, with adverse consequences for biomass production of the pineapple plants. HHP nematicides are very potent, with broad spectrum activity and known to cause damage to a wide range of non-target organisms living in the soil, including those that contribute to soil health, nutrient cycling and biological control of soil-dwelling pests and diseases.

Table 2. Differences in plant weight between treatments	(at 7	75 days after planting))
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Treatment	Pineapple plant weight (grams)
1. HHP nematicide	1,015*
(ethoprophos)	
2. Fungal biopesticide (P. lilacinus)	1,463
3. Wood vinegar	1,585
4. Fungal biopesticide (P. chlamydosporia)	1,445
5. Untreated control	1,355

(*= statistically significant from treatments 2-5 at P<0.05 level)

Contrary to widely held perceptions that alternatives to synthetic pesticides are always more expensive, all three non-chemical methods used in this trial were considerably cheaper than the HHP nematicide (Table 3). The cheapest treatment was for *Klamic* biopesticide based on *Pochonia chlamydosporia*. Costs were calculated for a single soil drench treatment although most pineapple growers usually make two applications.

Table 3. Comparison of treatment costs (in Costa Rican colones)

Treatment	Unit cost	Cost per hectare
1. HHP nematicide Mocap ®	7,570 per 1.5kg	176,633
(ethoprophos)		
2. Fungal biopesticide (P. lilacinus)	5,000 per kg	20,000
3. Wood vinegar	5,000 per gallon	52,910
4. Fungal biopesticide (P. chlamydosporia)	5,500 per kg	4,565
5. Untreated control	0	0

US\$ = 526 colones in Nov. 2015

These alternatives for nematode control do not require specialised equipment, expert advice nor intensive training. Production costs for preparing wood vinegar are not excessive and

on-farm preparation is easy, making this a viable option for large farms. Care needs to be taken when burning, boiling and distilling soft woody material, in terms of fire prevention and using a simple mask to avoid inhaling wood smoke. Application of these biopesticide products is not really more complicated than using conventional nematicides although it is best to avoid peak periods of high temperature and solar radiation because these can harm the living fungal spores. Applying biological products early in the morning or late afternoon is recommended good practice to make most effective use of microbial biopesticides. Shelf-life is shorter than for chemical products and biopesticides are best stored under refrigeration.

Conclusions and next steps

It is hard to draw firm conclusions from this short duration trial and further trials are needed under medium or high nematode infestation levels across a range of pineapple growing conditions and including eventual fruit weight and quality comparison. These limited results suggest that alternatives could be as effective as HHP nematicides and cheaper and are certainly worth experimenting with. IRET and Fertinyc plan to repeat and extend assessment of the biopesticide and wood vinegar alternatives during 2017.

Combining one application of conventional nematicide and one non-chemical treatment may also be an option for IPM growers, which would enable them to reduce HHP use and costs, without the perceived risks or challenges associated with completely changing their usual practice. The trial also reinforces the message that, under good IPM practice, growers should certainly be sampling fields to monitor nematode levels before planting pineapple, to avoid unnecessary applications when numbers are low.

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Footnotes

1. The use of wood vinegar in reducing the dependence on agrochemicals, 2011. Via: http://www.agrowingculture.org/the-use-of-wood-vinegar-in-reducing-the-dependence-onagro-chemicals/

Annex A Hazard and legal status summary on ethoprophos

Ethoprophos is a neurotoxicant and cholinesterase inhibitor, disrupting the normal nerve signalling mechanisms, not only of the target soil-dwelling pests but also of a wide range of non-target organisms, including humans. The key concern for human health is operator exposure as this chemical is very toxic by inhalation and dermal absorption. Ethoprophos qualifies as an HHP (both under the FAO/WHO proposed hazard criteria and under PAN International's more comprehensive and precautionary HHP criteria), for the hazard criteria summarised below.

Active ingredient	CAS # *	Pesticide type	HHP: Acute toxicity to human health classifications	HHP: Chronic human health classifications	HHP: Environmental concerns
Ethoprophos	13194- 48-4	Nematicide; Insecticide	WHO Class 1a: 'Extremely hazardous'	Probable carcinogen: US EPA 'probable/likely'	
			GHS/EU ' Fatal by inhalation' operator hazard (H330 code)		

Hazard classifications which qualify ethoprophos as a Highly Hazardous Pesticide

*CAS # = Chemical Abstracts Service unique identifying code for individual chemicals

This nematicide is highly soluble and very persistent in water but is classified¹ as 'nonpersistent' in soils, with a degradation half-life in field conditions of around 2-3 weeks. Ethoprophos exhibits high acute toxicity to mammals, birds and aquatic crustaceans; and moderate toxicity to fish, other aquatic invertebrates, honeybees and earthworms. Data on its ecotoxicity to key natural enemies shows potential for 100% mortality to predatory beetles, for the ground beetle *Poecilus cupreus* (at dose rate of 7kg/ha) and the rove beetle *Aleochara bilineata* (at 10kg/ha).

Legal status in Europe: Ethoprophos is currently approved in the EU until July 2018. It was reviewed in 2013 and the risk assessment highlighted serious risks for operators and non-target organisms, not all of which can be fully mitigated using risk reduction measures. As a result it was given a limited approval of only 5 years (rather than the usual 15 years) and for extremely limited uses. The EU requires considerable restrictions to mitigate ethoprophos risk to operators and the environment, notably use under **closed transfer application systems only** (which significantly reduce exposure of the people handling and applying the chemical). Its use is now restricted to pre-planting band row or in-furrow application rate of 6kg active ingredient per hectare.

Member States must consider the following when assessing/approving products containing ethoprophos:

> Only uses in soil application can be authorised and limited to professional users

¹ As per hazard data and interpretation in the 'FOOTPRINT' Pesticide Properties Database managed by Univ. Hertfordshire. Via: http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm

- To protect operators, national use and labelling requirements must demand adequate personal and respiratory protective equipment and other risk mitigation measures, e.g. the use of closed transfer system for the distribution of the product
- To protect birds, mammals, aquatic organisms, surface and groundwater under vulnerable conditions, national approvals must include risk mitigation measures, e.g. buffer zones and ensuring complete incorporation of granules in the soil

Ethoprophos and the international chemical conventions: Ethoprophos is not listed under either the Stockholm or Rotterdam conventions nor is it in the candidate process for addition to either convention.

Annex B Relevant information from Costa Rica and other Central American countries

Ethoprophos ranked 18th in the volumes of pesticides imported into the Central American region during 2000-2004 and 10th in Costa Rican imports during1977-2006 (3,081 tons). Survey data from Costa Rican pineapple growers in 2015-2016 as part of this project documented an average annual application of 9.28 kg ethoprophos active ingredient per hectare.

Human health effects: Ethoprophos is one of the most common causes of acute or lethal pesticide poisoning incidents. In Costa Rica it was detected in dust inside houses and schools close to banana plantations in Limón province (in 2002 studies). In Panama it has been detected in swab tests and in Honduras ethoprophos residues were found in vegetables (in 1994)².

Survey of pesticide use practices by indigenous peoples in high poverty areas in Costa Rica's Atlantic Coast showed that over 60% of households interviewed use pesticides on plantain smallholdings, and of these, 84% applied nematicides. Only 31% reported using some type of protective clothing during application³.

IRET research to generate hazard indicators for human health effects in Costa Rica has assessed volumes used in specific crops and known international hazard classification data for acute and chronic effects. In the results, ethoprophos was identified as one of seven

² Information translated from the database in IRET's Pesticide Manual for Central America, via http://www.plaguicidasdecentroamerica.una.ac.cr/

³ Polidoro et al. (2008) Pesticide application practices, pest knowledge, and cost-benefits of plantain production in the Bribri-Cabécar Indigenous Territories, Costa Rica. *Environmental Research* 108(1):98-106

active ingredients recommended for use monitoring in relation to extreme or high acute toxicity⁴.

Ecotoxicology: *Costa Rican studies:* Ethoprophos was found in drinking water in Sixaola basin in 2006 and in surface waters of River Suerte river basin and the Tortuguero conservation areas during 1993-1998 at concentrations which represent low acute risk but very high chronic risk for aquatic organisms. It was found in 25% of water samples in the conservation areas. Residues were also found in 2001 and 2007 in surface waters in canals, streams and rivers in pineapple cultivation areas in Pocora Siquirrez and the Caribbean zone. In non-target organisms, residues have been documented in fur samples from sloths living around banana and pineapple farms in the Caribbean zone and in aquatic organisms following mass kill incidents.

Recent research on cholinesterase activity testing of the native tropical fish *Astyanax aeneus* as a biomarker for pesticide exposure in Costa Rican banana plantations showed that significant cholinesterase inhibition takes place in brain and muscle tissue of fish exposed to ethoprophos in lab tests⁵. IRET has also conducted acute toxicity testing of ethoprophos and chlorpyrifos on *Daphnia* spp. water fleas and on the guapote fish *Parachromis dovii*⁶. Chlorpyrifos is more toxic to both groups than ethoprophos. Cholinesterase inhibition in guapote fish was observed using contaminated water collected from the field, at levels below the LC50 for both chemicals. The native *D. ambigua* was found to be more sensitive to both chemicals than the standard test organism *D. magna* and could serve as a useful indicator species for Costa Rican risk assessment.

⁴ Bravo et al. (2013) Agriculture pesticides use as a tool for monitoring health hazards. *Uniciencia* 27 351-376 (in Spanish).

⁵ Mena Torres et al. (2014). Use of cholinesterase activity as a biomarker of pesticide exposure used on Costa Rican banana plantations in the native tropical fish Astyanax aeneus (Günther, 1860) *Journal of Environmental Biology* 35(1)35-42

⁶ Diepens et al. (2014). Effect of pesticides used in banana and pineapple plantations on aquatic ecosystems in Costa Rica. *Journal of Environmental Biology* 35 73-84.