Prospects for application of entomopathogenic nematodes to soil

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Introduction

The compatibility of entomopathogenic nematodes with conventional spray equipment has certainly contributed to their success in biological control. At the same time, however, little effort was spent to develop more efficient techniques for applying nematodes to soil. When sprayed, nematodes may dye in the spray-tank or when passing the nozzles. They suffer from UV-.radiation and desiccation on the way to the ground especially when trapped in the canopy. Finally, they have to penetrate through barriers at the soil surface like the thatch layer on turf which involves severe losses (Smits, 1996) or might desiccate on the canopy before they will even hit the ground. In row-crops, a substantial amount of nematodes are wasted between the rows if applied by broadcast spraying. For larger area crops like turf, sugar beet or corn, more efficient ways to deliver the expensive biological active ingredient are desired. This review summarises the current directions of alternative application techniques and gives some suggestions for future work.

Irrigation systems

In field experiments, EPN have been successfully applied with centre-pivot irrigators and furrow irrigation in corn, furrow irrigation in cotton and trickle irrigation systems. When compared to conventional spraying, delivering nematodes by irrigation was generally more successful (Wright *et al.* in press). If done properly, excellent application of EPN through irrigation systems can be achieved and EPN rates can be substantially reduced, for example from 5 to 2 billion IJ/ha in strawberries (Kramer and Grunder, 1998). In drip irrigation systems it is recommended to pre-run the irrigation tubes with water for cooling and to add thickener like Carboxy-Methyl-Cellulose (CMC) to slow down nematode sedimentation in the irrigation tubes (Peters & Backes, 2003. Problems arise with leakages in irrigation tubes which cause significant nematode losses. In broad acre crops, water-gun chariots ?? are most common for irrigation but they move too slowly across the field. Nematode would have to be added over a period of several hours and could dye in the supply tank during that time. This problem is yet to be solved.

Root dipping

Dipping plants into a nematode suspension is a logical way to apply nematodes in a targeted way to the sphere where the pest attacks. When problems with the efficiency of *H. bacteriophora* against *Otiorhynchus sulcatus* were recorded in German tree nurseries, growers dipped cuttings into a nematode-solution prior to transplanting into the field. This method gave improved control and reduced the number of EPN applied per ha by 60%. Thickeners (e.g. 0.5% CMC) can be used to increase the amount of nematode solution retained by plant roots following dipping. Frozen strawberry runners have been treated with 2 ml *Heterorhabditis bacteriophora* suspension with 0.5% CMC added. In the first two weeks after application nematode recovery in treated plants was superior to plants treated with a drench of nematodes at the same application rate.

Retarded release techniques

Application costs could be lowered significantly if nematodes were applied during sowing. Nematode action, however, is often required several weeks or even months after sowing. In these instances a formulation is required that shelters the nematodes until their action is needed. The most logical slow release formulation is an insect cadaver, where the nematodes have reproduced in. Buried infected insects have been successfully used to control the banded cucumber beetle, *Diabrotica balteata* (Creighton & Fassuliotis, 1985) and the sweet potato weevil, *Cylas formicarius* (Jansson, *et al.*, 1993). Recently, the formulation of infected cadavers was improved to avoid desiccation of compression of densely packed cadavers Shapiro, *et al.*, 2001. The price for host insects on the current market makes this application method relatively costly (Tab. 1). On the other hand, it substitutes any other formulation procedure. This technique is therefore only interesting for companies producing nematode in insects. For large scale producers other slow release techniques are required.

Tab. 1 Prices for insects which can be used for a retarded application of entomopathogenic nematodes to soil. Costs do not include shipment, labour or equipment. The cost for the non-insect production medium in liquid culture production is given. Prices/ha are calculated assuming a rate of 2 billion nematodes/ha. All calculations are based on the reproduction rate of *Heterorhabditis bacteriophora*.

Insect	Price [€/g]	emerging nematodes [million/g]	nematode costs [€ / million]	[€/ha]	reference
Galleria mellonella 0.25g/piece	0.030	0.600	**0.050**	**100.00**	Shapiro-Ilan *et al. 2001*
Tenebrio molitor	0.012	0.900	**0.013**	**26.67**	Shapiro-Ilan et al. 2002
T. molitor 0.1g/piece	0.012	0.100	**0.120**	**240.00**	e-nema
T. molitor 0.01g/piece	0.010	0.300	**0.033**	**66.67**	e-nema
Liquid culture			**0.0012**	**2.46**	e-nema

In Finland, a slow release formulation was tested against flea beetle (*Phyllotreta* sp.) and pollen beetle (*Meligethes aeneus*) in oilseed rape (Menzler-Hokkanen & Hokkanen, 2003). This formulation is based on polyacrylate. A concentrated nematode suspension is mixed with polyacrylate beads and sealed into permeable bags (tea bags). This formulation with 15000 nematodes/m² was applied one week after sowing of canola in spring and reduced flea beetle to a similar level than if nematodes were sprayed at 450 million/m² one week after sowing. None of the treatments affected the pollen beetle as opposed to treating in July with 1 million nematodes/m² which killed 94,4% of *M. aeneus*.
Surely, this formulation needs further development. Applying the nematodes at sowing is not only attractive for summer-canola but also for sugar beet to control the weevils *Temnorhinus mendicus* and *Bothynoderes punctiventris* and for maize to control the western corn-root worm, *Diabrotica virgifera virgifera.* No nematodes would be wasted between the rows and

no extra work is needed for application. The development of a slow-release granule for entomopathogenic nematodes is therefore warranted. Possibly, the granular formulation used for *S. carpocapsae* could be developed further into a slow-release granule.

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