
Summary
The compatibility of EPNs with biological control agents and agrochemicals that are likely to be used in an integrated pest management programme for citrus in South Africa was investigated. This study is the first to report on the possible negative effects of employing EPNs against Cryptolaemus montrouzieri, which is a commercially produced biocontrol predatory insect that is used against mealybugs. Tolerance of Heterorhabditis zealandica and Steinernema yirgalemense to aqueous solutions of Cyperfos 500 EC*, Cryptogan™, Helicovir™, Nu-Film-P™ and Zeba™, for purposes of infectivity and survival, was evaluated. Heterorhabditis zealandica has proved to be highly compatible to all products tested, with no significant increase in nematode mortality. Infectivity of both species showed no decrease in the ability of the nematodes to infect insect hosts. Results from this study indicated that nematodes should possibly be applied prior to the release of C. montrouzieri in citrus orchards.

Introduction
In South Africa, citrus is attacked by a wide range of economically damaging insect pest species, such as false codling moth, Thaumatotibia leucomotreta (Meyrick), citrus thrips, Scirtothrips aurantii, American bollworm, Helicoverpa armigera, and a variety of mealybug species, of which Planococcus citri is the most common, as well as the most destructive. To apply EPNs as a biological control agent efficiently and effectively as part of an integrated pest management (IPM) programme for citrus in South Africa, their compatibility with biological control agents, and with other agrochemicals, needs to be known.

EPNs in the order Rhabditida, from the families Steinernematidae and Heterorhabditidae, are valuable biological control agents for a wide range of economically important insect pests. Although traditionally applied to control the soil stages of insects, nematodes have been proven to be able to control some foliar pests. The broad host range of EPNs is advantageous, as the possibility exists of controlling more than one pest species by means of a single IPM programme. However, when nematodes are applied to the aerial parts of trees, their broad host range could also be problematic, if the beneficial insects that are present during application are susceptible to them.

High mortality among the larvae of the two-spotted lady beetle, Adalia bipunctata, and among those of the lacewing species, Chrysoperla carnea, when exposed to Steinernema feltiae, S. carpocapsae, and Heterorhabditis bacteriophora, has previously been reported (Rojht et al., 2009). In contrast, other researchers found four ladybug beetle species to be significantly less susceptible to H. bacteriophora and S. carpocapsae than was the black cutworm, Agrotis ipsilon, which is a known susceptible host (Shapiro-Ilan & Cottrell, 2005). The above-mentioned studies show that the susceptibility of beneficial insects to nematodes varies, depending on the nematode, the insect stage, and the combination tested. Such beneficial insects as the predatory lady beetle, Cryptolaemus montrouzieri, and the parasitoid, Leptomastix dactylopii, are made commercially available to citrus farmers in South Africa, after having been mass reared. The beneficial organisms involved have been shown to play a vital role in the effective implementation of IPM programmes for citrus. The susceptibility of such beneficial insects to a specific nematode species should first be determined, before nematode application is considered as part of an IPM programme.

Nematodes are exposed to a variety of agrochemicals, and to formulations of biological control agents, when they are applied as part of an IPM programme that could be toxic, and which could impair nematode performance. Although some pesticides are known to retard nematode persistence and infectivity, a previous study showed nematodes as tolerating short periods (2–4 h) of exposure to the majority of the 75 commercial pesticides tested. When applying a specific nematode species, one should also consider the fact that nematodes tend to vary in reaction to pesticides, depending on the nematode species, and on the pesticide formulation, concerned. Nematode tolerance to...
agrochemicals allows for the tank-mixing of nematodes together with other agrochemical and biopesticide formulations, thus enabling the saving of both time and labour costs, and/or facilitating improved control of a target pest species (Koppenhöfer & Grew, 2005).

In previous studies, Van Niekerk and Malan (2012, 2013, 2014) screened local South African EPN isolates so as to be able to select the two best candidates, *H. zealandica* (strain SF41) and *Steinernema yirgalemense* (strain 157-C), for use against the citrus mealybug. The objective of the study described in this article was to evaluate the compatibility of the two selected nematode species concerned with a biological control insect, with biocontrol formulations, and with agrochemicals to which they would most likely be exposed in an IPM programme for citrus in South Africa.

**Results and discussion**

In South Africa, citrus production is plagued by a complex of major and minor insect pest species. To save time and labour costs, to achieve enhanced control of a single pest, or to target more than one pest, it would be desirable to tank-mix, and then to apply, agrochemicals with a biocontrol agent, or simultaneously with more than one biocontrol agent. The latter form of application, combining pesticides and EPNs, is possible, due to some species having been proven tolerant to short periods of exposure to certain pesticides. Tolerance of agrochemicals is also advantageous, as nematodes can be applied within a short time interval after the application of chemicals. Tolerance of *H. zealandica* and *S. yirgalemense* to aqueous solutions of Cyperfos 500 EC®, CryptogranTM, HelicovirTM, Nu-Film-P® and Zeba®, for purposes of infectivity and survival, was evaluated (Table 1).

**Susceptibility of *C. montrouzieri* to nematodes**

*Cryptolaemus montrouzieri* is a coccinellid beetle, which is also known as the mealybug lady beetle, or as the mealybug destroyer. It is considered to be a valuable biological control agent aimed at *P. citri*. Both the adult (Fig. 1) and the larvae of the beetle (Fig. 2) are voracious feeders, preying predominantly on mealybugs. In South Africa, *C. montrouzieri* is produced by a commercial company, Du Roi IPM, Letsitele, South Africa.

Results of the susceptibility of *C. montrouzieri* larvae to *H. zealandica* and *S. yirgalemense* showed beetle larvae to be highly susceptible to both nematode species, with an infection rate of 80% for *H. zealandica*, and of 92% for *S. yirgalemense* (Fig. 3). The adult beetle was also found to be susceptible to nematode infection, with an average mortality of 30% in the case of *H. zealandica*, and of 64% for *S. yirgalemense* (Fig. 3).

Unlike adult beetles on control plates, beetles exposed to nematodes were observed to excrete an obnoxious smelling yellowish liquid, with its secretion being a defence mechanism aimed at preventing nematode infection. The high mobility of adult beetles would further tend to impair nematode infection. The results mentioned here indicate that the susceptibility of natural enemies to nematodes depends on the nematode and insect species interaction, as the adults involved were found to be twice as susceptible to *S. yirgalemense* as they were to *H. zealandica*. Other researchers have, in the past, found comparable results using different beetle and nematode species (Rojht et al., 2009).

The high susceptibility of beetle larvae, and even of adult beetles, to both nematode species, which is especially high for *S. yirgalemense*, should be taken into consideration when both of the biocontrol agents concerned are to be applied in a citrus orchard. Adults of *C. montrouzieri* can live up to two months, with a female producing up

<table>
<thead>
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<th>Product</th>
<th>Active ingredient</th>
<th>Use</th>
<th>Concentration/L</th>
</tr>
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<tbody>
<tr>
<td>Cyperfos 500 EC®</td>
<td>Chlorpyrifos and Cypermethrin</td>
<td>Insecticide</td>
<td>1.00 ml</td>
</tr>
<tr>
<td>CryptogranTM</td>
<td>Cryptophlebia leucotreta granulovirus (CriLeGV-SA)</td>
<td>FCM control</td>
<td>2.50 ml</td>
</tr>
<tr>
<td>HelicovirTM</td>
<td>Nucleopolyhedrovirus</td>
<td>Bollworm control</td>
<td>0.12 ml</td>
</tr>
<tr>
<td>Nu-Film-P®</td>
<td>Poly-1-P-menthene</td>
<td>Spreader, sticker</td>
<td>0.60 ml</td>
</tr>
<tr>
<td>Zeba®</td>
<td>starch-g-poly (2-propenamide-co-2-propenoic acid) potassium salt</td>
<td>Anti-desiccant</td>
<td>3.00 g</td>
</tr>
</tbody>
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Table 1. List of products tested. Recommended dosage of different products and target use.
to 500 eggs, and with the potential of being persistent. As nematodes have only a short window period of a few hours of prevailing high humidity for mealybug infection after application, they should be applied prior to the release of *C. montrouzieri*. Van Niekerk and Malan (2012) showed low concentrations of *H. zealandica* and *S. yirgalemense* to obtain up to 89% control of adult female *P. citri*, indicating the *C. montrouzieri* larvae to be just as susceptible to nematodes as were the target pest species. The results also indicated that the nematode species should not be applied when the *C. montrouzieri* larvae are already present in high numbers on the trees concerned.

**Influence of pesticides and adjuvants on nematode survival and persistence**

Nematode tolerance to three agrochemicals and to two biocontrol formulations, to which they are likely to be exposed in an IPM programme for citrus in South Africa, showed *H. zealandica* mortality, after exposure to Zeba®, Nu-Film-P®, Cryptogran™ (Fig. 4), Helicovir™ (Fig. 5), and Cyperfos 500 EC®, over a 24 h period to be unaffected by any of the products tested. Significant increases in nematode mortality were, however, observed for *S. yirgalemense* after 24 h exposure to Helicovir™, Nu-Film-P® and Cyperfos 500 EC®. The results indicate that *S. yirgalemense* should not be tank-mixed with the above-mentioned products for prolonged periods before application.

A significant increase in *S. yirgalemense* mortality was also observed after 6 h exposure to Nu-Film-P® (Fig. 6), while a significant increase in nematode mortality from > 5% to 11% was recorded for nematodes after 12 h exposure to Cyperfos 500 EC® (Fig. 7). The increase in mortality noted was also significantly higher (P = 0.001) than the 3% mortality recorded for the control after 12 h. Nematode mortality significantly (P = 0.001) increased to 27% after an additional 12 h exposure to Cyperfos EC®.

To determine exactly how long *S. yirgalemense* is tolerant to Nu-Film-P® and Cyperfos 500 EC®, the same experiment should be conducted again, but this time with nematode mortality being recorded every hour, for 12 h. Although *S. yirgalemense* proved to be sensitive to some of the formulations tested, the results that were obtained for nematode infection showed that none of the products tested influenced the ability of either nematode species tested to infect hosts after 24 h.
Die Ballie Wahl-meriete toekenning vir die beste Hortologie student in Sitrusproduksie

Helen Marais, ’n vierdejaar student van die Departement Hortologie aan die Universiteit Stellenbosch, is vanjaar die eerste ontvanger van die Ballie Wahl-meriete toekenning. Hierdie toekenning gaan jaarliks oorhandig word aan die beste derdejaar student in die sitruskursus wat deur die Departement Hortologie aangebied word. In dié sitruskursus word aandag gegee aan aspekte soos kultivarseleksie, invloed van klimaat op produksie, asook die vestiging en bestuur van ’n sitrusboord. Verder word die onderliggende fysiologie wat vrugkwaliteit kan beïnvloed, asook boommanipulasie en die gebruik van plantgroeireguleerders om vrugte van optimum interne en eksterne kwaliteit te produeer ook in diepe bestudeer.

Die “Citrus Technical Association” (CTA), ’n vrywillige assosiasie van tegniese kundiges wat sedert 1984 bestaan en direk betrokke is by sitrusnavorsing, produksie en uitvoere, borg die nuutingeetstelde toekenning aan Helen. Met die toekenning van hierdie meriete prys poog die sitrusbedryf om erkenning aan uitmuntendheid te verleen asook om verdere studies in sitrus aan te moedig.

Ballie Wahl is nie net in Suid-Afrika nie, maar ook wêreldwyd een van die bekendste name in die sitrusbedryf. Sy tegniese kennis en vermoë om al die komplekse aspekte met betrekking tot produksie van sitrusvrugte te integreer, word nasionale en internasionaal hoog aangeslaan. Ballie is ’n alumnus van die Universiteit Stellenbosch. Nadat hy sy BSc (Agric) graad verwerf het in 1966, het hy by die sitrusbedryf geraak as deel van die Sitrusbeurs se tegniese span. Sedertdien het hy die SA sitrusbedryf in verskeie posisies gedien en in 2006 ’offisieel’ afgetree. Die goue draad regdeur Ballie se loopbaan is die onbaatsugtige manier waarop hy sy kennis deel, asook die moeite wat hy nog steeds bereid is om te doen om nuwe toetreders tot die sitrus tegniese gemeenskap van die Suider-Afrikaanse sitrusbedryf met raad en daad by te staan.

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Conclusion

The current study illustrates some of the factors that should be taken into consideration before nematodes are applied in commercial orchards as part of an IPM programme. Pest complexes that attack citrus tend to vary from one orchard to the other, and from one year to the next. Thus, the required agrochemicals and biological control agents also vary.

When EPNs become available as commercial biological control agents in South Africa, a database of data relating to nematode species’ tolerance of agrochemicals, as well as to the susceptibility of pests and the commercially available natural enemies used in IPM programmes for citrus, should
In southern Africa some 4 450 hectares are at the moment planted with Star Ruby grapefruit. This comprises about 8% of the total citrus plantings in the region.

Given the meagre beginnings with this variety in 1974, the growth in the contribution of Star Ruby to the SA industry is nothing but phenomenal as illustrated by the fact that the industry is capable of an annual export volume of 6 to 7 million Star Ruby cartons - at a DIP value of R50 per carton this provides our growers with an estimated annual R350 million turnover.

Star Ruby was introduced to South Africa in 1974 when Dr. Doug Stanton of the SA Cooperative Citrus Exchange organised the procurement of 12 seeds from the United States. Dr. Anton Hough received these irradiated seeds from Dr. Pete Timmer who collected the seeds near the town of Weslaco in Texas. The seeds were sown by Ferdi Esselen at his nursery in Malelane who nurtured them and produced 10 trees from each seedling. He used Troyer Citrange and Rough lemon as rootstocks. The trees were planted in 1976 for evaluation. With the first evaluation in 1979 under the scrutiny of Steve Burdette it was established that only 3 of the 12 seedling sources were true to type. The rest varied from having white flesh to very small fruit or fruit with excessive seeds.

The first true to type Star Ruby nursery trees were produced in 1981 and planted on various farms in the Onderberg area (Komatipoort - Malelane); amongst others on the farms of TSB, Piet Maritz and Jacob de Villiers.

After production of the initial propagation material, the original twelve Star Ruby trees were planted in an experimental block at Malelane nursery. The trees survived in this experimental block until 1996 when they had to be removed due to excessive Tristeza virus infection. Budwood from these Star Ruby trees were distributed to many nurseries and private estates before the establishment of the citrus foundation block at Uitenhage.

Taking into account plant densities, replacement programmes etc, it can be calculated that at least 3 million trees were propagated in SA from those first 12 seedlings.

Ferdi Esselen was honoured for his tremendous contribution to the welfare of the SA Citrus Industry when the first prestigious Technical Merit award given by Citrus Research International was awarded to him in August 2006 at the biennial RSA CRI Citrus Research Symposium.

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References


