Evaluating the efficacy of a polymer-surfactant formulation to improve control of the citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), using entomopathogenic nematodes under simulated natural conditions

S van Niekerk & A P Malan*
Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland, 7602 South Africa


Traditionally, entomopathogenic nematodes (EPNs) of the Heterorhabditidae and Steinernematidae targeted the soil stages of insect pests. To improve control of the citrus mealybug, *Planococcus citri*, which occur in the canopy of citrus trees, EPN application suspensions require the addition of adjuvants. In a growth chamber assay, simulating glasshouse conditions of 75 ± 8% relative humidity and 22°C, all treatments with *Heterorhabditis zealandica* and *Steinernema yirgalemense* achieved significantly higher mortality of adult *P. citri* females than did the control. The addition of 0.3% Zeba® and 0.6% Nu-Film-P® to EPN suspensions of both species did not increase mortality significantly. The ability of the above-mentioned formulation to prolong the ability of *S. yirgalemense* to infect *P. citri* and to prolong survival of infective juveniles was also evaluated under the same conditions and showed the formulation to improve both infectivity and survival for up to 2–3 h post-application. In the semi-field trial, significantly higher control of *P. citri* was achieved with the application of *S. yirgalemense* together with 0.3% Zeba® compared to the control, resulting in up to 53% control. The study conclusively showed the polymer product Zeba® to improve the ability of *S. yirgalemense* to infect *P. citri* by retarding desiccation and by buffering EPNs from suboptimal environmental conditions.

**Key words:** adjuvants, citrus mealybug, *Heterorhabditis zealandica*, Nu-Film-P®, *Steinernema yirgalemense*, Zeba®.

The family Pseudococcidae, commonly referred to as mealybugs, comprise about 2200 species in almost 274 genera (Ben-Dov et al. 2010), of which the citrus mealybug, *Planococcus citri* (Hemiptera: Pseudococcidae), is considered the most destructive (Cadee & Van Alphen 1997; Blumberg & Van Driesche 2001). *P. citri* is highly polyphagous and is known to infest such commercially produced hosts as citrus, coffee, grapevine and a variety of ornamental plants (Cadee & Van Alphen 1997; Mustu et al. 2008).

In South Africa, seven mealybug species, of which *P. citri* is the most important, are regarded as economically damaging citrus pests (Hattingh et al. 1998). Mealybugs feed on all parts of citrus trees, except the roots (Canhilal et al. 2001), causing both direct and indirect damage (Hattingh & Tate 1996), such as wilting, premature fruit and flower drop, growth deformation and sooty mould growth (Blumberg et al. 1995). During winter, mealybugs occur throughout the tree canopy, generally residing in cracks and crevices or leaf axils (Hattingh 1993; Smith et al. 1997). First-generation nymphs emerge during spring or early summer, move to the foliage to colonise fruitlets and young growth (Martinez-Ferrer et al. 2006) and ultimately settle in protected sites, such as under fruit calyces or in between fruit clusters (Hattingh & Moore 2003). Multiple overlapping generations of *P. citri* occur during a single growing season (Wakgari & Giliomee 2003), with the highest population numbers occurring between mid- and late summer, parallel with fruit growth intensity (Franco et al. 2004).

Although mealybugs are generally controlled with chemicals (Franco et al. 2004), this method of control is not ideal. Mealybugs are known to develop resistance (McKenzie 1967; Blumberg & Van Driesche 2001; Mahfoudhi & Dhouib 2009) to such products and the continuous applications of broad-spectrum pesticides has proven to be partially responsible for pest outbreaks (Michelakis & Hamid 1995), as they disrupt natural enemies that usually keep mealybug populations under control (Hattingh 1993; Hattingh & Tate 1996; Hattingh et al. 1998; Hattingh & Moore 2003). The success of chemical control is further impaired by mealybugs being covered with protective waxes, displaying cryptic behaviour and residing in protected sites where they cannot be reached by chemicals (McKenzie 1967; Michelakis & Hamid 1995; Franco et al. 2004). Growing public awareness of detrimental environmental impact and of health risks associated with pesticides has further pressured citrus growers into trying to find alternative control methods (Hussaini 2002).

The application of natural enemies is considered the most feasible alternative to chemical insect control (Hussaini 2002). In citrus orchards, mealybug populations are usually controlled by means of natural enemies, if the behaviour of the latter is not disrupted by the application of pesticides (Hattingh 1993; Hattingh & Tate 1996; Hattingh et al. 1998; Hattingh & Moore 2003). After winter, however, natural enemy population numbers tend to increase slowly and early spring population densities are usually insufficient to prevent early feeding damage (Hattingh 1993; Franco et al. 2004).

Entomopathogenic nematodes (EPNs), classified in the order Rhabditida and families Steinernematidae and Heterorhabditidae, are fatal pathogens of insect and are used as inundatively applied biological control agents against a wide variety of economically important insect pests (Grewal et al. 2005). EPNs are, however, primarily applied to control the soil stages of insects (Arthurs et al. 2004). Controlling foliar pests with nematodes in orchards is still a relatively new field of study and is extremely challenging since nematodes require a water film to maintain mobility and to ensure survival (Wright et al. 2005).

Above-ground conditions are not optimal for nematode survival (Mráček 2002; Tomalak et al. 2005), since EPNs are exposed to limiting abiotic factors (Gaugler & Boush 1979) such as ultraviolet radiation (Gaugler et al. 1992), extreme temperatures (Lacey et al. 2005), low ambient humidity and wind (Unruh & Lacey 2001).

EPNs are usually applied to foliage as an aqueous suspension by means of ordinary chemical-spraying equipment (Grewal 2002; Hussaini 2002). Water retention agents can be added to application formulations to retard desiccation (Glazer et al. 1992), thus increasing the duration of EPN survival on foliage (Webster & Bronskill 1968; Shapiro et al. 1985; Glazer & Navon 1990). According to Tomalak et al. (2005), the successful control of western flower thrips, *Frankliniella occidentalis*
(Pergande) (Thysanoptera: Thripidae), in glasshouses is a result of the sensible use of adjuvants, which improve EPN distribution on foliage. A EPN application formulation containing 0.3% Rimulgan® (surfactant) and 0.3% of the polymer xanthan (antidesiccant) achieved more than 90% control at 80% relative humidity (RH) and >70% control at 60% RH of the diamondback moth (DBM), Plutella xylostella (Linnaeus) (Lepidoptera: Plutellidae), on cabbage leaves (Schroer & Ehlers 2005). The same formulation was also evaluated by Schroer & Ehlers (2005) for DBM control on cabbage-leaf disc assays, with results showing EPN to survive 22 h longer at 80% RH and >17 h longer at 60% RH than the control. Further field studies for DBM control on cabbage were conducted by Schroer & Ehlers (2005). The formulation significantly reduced the number of insects per plant, resulting in >50% control after 7 days. No significant effect was, however, recorded when compared to that achieved with a formulation containing EPNs only and with a surfactant, with the difference in effect being attributed to the high ambient humidity that prevailed in the experimental unit and the moist microclimate in the cabbage heads, favouring nematode survival.

In Brazil, 18 strains of EPNs were evaluated for efficacy against P. citri (Barbosa Negrisola et al. 2013) using laboratory bioassays. Of these, only 10 strains were found to be pathogenic against females of P. citri. Best efficacy was achieved with native Heterorhabditis bacteriophora Poinar 1976 and Steinernema feltiae (Fililjiev 1934) Wouts, Mráček, Gerdin & Bedding 1982.

During a previous study by Van Niekerk & Malan (2012), all available local South African EPN isolates were screened for susceptibility of P. citri and the two most promising species selected for further studies. The objective of the present study was to evaluate the potential of a surfactant-polymer formulation added to EPN application suspensions to improve the ability of nematodes to control P. citri on citrus under simulated glasshouse conditions and semi-field trials.

Materials and methods

Source of nematodes and insects

Infected juveniles (IJs) of Heterorhabditis zealandica Poinar 1990 (SF 41) and Steinernema yirgalemense Tesfamariam, Gozel, Gaugler and Adams 2005 (157-C) were produced in last-instar mealworm larvae, Tenebrio molitor (Linnaeus) (Coleoptera: Tenebrionidae), at room temperature, according to the procedures described by Kaya & Stock (1997). After harvest, IJs were stored in horizontally placed, 500 ml vented culture flasks, containing 150 ml distilled water at 14°C. Flasks were shaken weekly to improve aeration and IJ survival. IJs were used within the first three weeks after emerging and harvested from white traps (White 1927). IJs were kept at 22°C for 24 h prior to use in all experiments, except for the semi-field experiment for which IJs were kept at room temperature for 24 h prior to application. Before conducting experiments, IJ concentrations were quantified for all trials by using the method developed by Navon & Ascher (2000).

Mealybugs were laboratory-raised in ventilated cages (650 mm × 350 mm × 590 mm) on butternuts and sprouting potatoes. The identity of P. citri used in this study was verified using morphological (Wakgari & Giliomee 2005) and molecular techniques (Pieterse et al. 2010).

Susceptibility assay on leaves and fruit

Leaves were obtained from a citrus orchard at Welgevallen experimental farm, Stellenbosch, Western Cape Province, South Africa, while citrus fruits were obtained from a local supermarket. To eliminate other organisms, leaves and fruit were washed in a solution of water and 0.01% sodium hypochlorite, rinsed thoroughly in tap water and left to dry before use.

Leaves were cut to fit Petri dishes (13 cm diam.) lined with moist filter paper. Eight female mealybugs were transferred to each of eight leaves for each treatment and represent eight replicates and totalled 64 insects used for each experiment. After adding the mealybugs, the Petri dishes were covered with a lid to keep the mealybugs from escaping and the citrus leaves from drying out.

Citrus fruit were cut in half, with each half being placed in a small, round plastic container (250 ml), with the open end facing to the bottom. Eight fruits with eight mealybugs each were prepared for each treatment, which represent eight replicates and totalled 64 insects for each experiment. The container, with the fruit and insects, was covered with a lid to prevent the mealybugs from escaping. The mealybugs were then left for 24 h to settle on leaves and fruit before treatment.

The two adjuvants used were Zeba® [starch-g-poly (2-propanamide-co-2-propanoic acid) potassium salt, Tongaat Hulett Starch] and Nu-Film-P® (poly-1-pmenthene, spreader/sticker, Hygrotech). Treatments were: 1) water only; 2) H. zealandica; 3) H. zealandica + Zeba® + Nu-Film-P®; 4) S. yirgalemense; and 5) S. yirgalemense + Zeba® + Nu-Film-P®. EPNs were applied to leaves and fruit with the aid of calibrated handheld spray applicators at a concentration of 2000 IJs ml⁻¹. Zeba®, and Nu-film-P® were used in treatments at a concentration of 0.3% and 0.6%, respectively. Treatment formulations were prepared 1 h before each trial. After treatment, plastic containers with fruit were covered with fine-mesh netting to allow airflow, while preventing mealybugs from escaping. Treatments were arranged according to a randomised design in a growth chamber at 22°C and 75 ± 8% RH. Large plastic containers were filled with water and placed at the bottom of a 150 l temperature-controlled incubator, to increase humidity.

Leaves were left for three minutes after treatment to eliminate excess runoff and placed in small pockets made out of the same fine-mesh netting that covered the plastic containers with fruit. Pockets with leaves were then hung in a complete randomised design in the same growth chamber as was used for the fruit. After 48 h, mealybugs were removed from the leaves and the fruit and their mortality were assessed. The mealybugs were then washed to remove surface EPNs and placed in Petri dishes (13 cm diam.) lined with moistened filter paper, and incubated for a further 48 h at 25°C. After further incubation, each insect cadaver was dissected with the aid of a dissection microscope to confirm mortality due EPN infection. Temperature and humidity levels were monitored with Hobo® H8 Pro Series data loggers, which were placed inside the growth chambers. The experiment was repeated on a separate test date and data of both experiments were pooled for statistical analysis.

Effect of adjuvants on IJ infectivity after different time intervals

The ability of a polymer-surfactant formulation to increase the infectivity of S. yirgalemense under simulated glasshouse conditions, as described in the growth chamber assay, was evaluated. The same procedure for preparing leaves before treatment and for determining mealybug mortality in the growth chamber assay was followed. EPNs were applied to leaves with the aid of calibrated handheld spray applicators at a concentration of 2000 IJs ml⁻¹. A suspension of S. yirgalemense only was compared to a suspension containing S. yirgalemense,
0.3% Zeba® and 0.6% Nu-film-P®. Five leaves were prepared for each treatment at each time interval. To estimate nematode infectivity potential after treatment application, leaves were left in a growth chamber for 0, 60, 120, 180 and 240 min, after which the leaves, at each time interval, were removed and cut into smaller pieces to fit into Petri dishes (3 cm diam.). Five adult mealybugs were added to five Petri dishes each for a treatment and represented five replicates and totalled 25 insects. The Petri dishes were covered with cling wrap to ensure an airtight seal and left in the growth chamber for 48 h, after which insect mortality was assessed, as described in the growth chamber assay. The experiment was repeated on a separate test date and data of both experiments were pooled for statistical analysis.

Effect of adjuvants on IJ survival after different time intervals

The ability of a polymer-surfactant formulation to retard EPN desiccation under simulated glasshouse conditions described in the growth chamber assay was evaluated. A suspension containing S. yirgalemense only was compared with a suspension containing S. yirgalemense, 0.3% Zeba® and 0.6% Nu-film-P®, EPNs were applied to leaves with a calibrated handheld spray applicators at a concentration of 2000 IJs ml⁻¹. Three leaves were prepared for each treatment at each time interval. To determine percentage EPNs mortality, two 2 cm² leaf discs were cut out of each leaf (3 replicates; 6 leaf discs), rinsed in 5 ml tap water and the number of live and dead EPNs recorded. Mortality of EPNs on citrus leaves was recorded 0, 30, 60, 120 and 240 min after applying EPNs to leaves. EPN mortality was determined as a percentage of the total number of nematodes recorded on each individual leaf disc, with those that did not respond to gentle prodding being recorded as dead. The experiment was repeated on a separate test date and data of both experiments were pooled for statistical analysis.

Semi-field trial

The efficiency of adjuvants to increase the ability of S. yirgalemense to control P. citri females under semi-field conditions described in a citrus orchard on Welgevallen experimental farm, Stellenbosch. The semi-field experiment was conducted in the early evening during spring (6 October 2011). The experimental layout represented a complete randomised design, with seven rows that each contained six treatment trees, except for one row that contained four treatment trees and represented five replicates and totalled 80 insects per treatment. Between the individual treatment trees stood two buffer trees, with two buffer rows separating the treatment rows from each other.

Pockets made out fine-mesh netting containing citrus leaves, each containing 10 P. citri females, were used for insect containment in the semi-field experiment. Treatments were: 1) water only; 2) EPNs; 3) EPNs + Nu-Film-P®; 4) EPNs + Zeba®; and 5) EPNs + Zeba® + Nu-Film-P®. EPNs were applied to leaves using calibrated handheld spray applicators at a concentration of 4000 IJs ml⁻¹, 0.3% Zeba® and 0.6% Nu-film-P®. Treatment formulations were prepared 1 h before application.

Pockets with leaves containing mealybugs were fastened onto the scaffold branches 1 m above-ground of each of the 40 treatment trees on the day of the trial before applying the different treatments. After 14 h, the leaves were removed from the trees and transported the laboratory. The mealybugs were then removed from the leaves and rinsed to remove surface EPNs, and mealybugs from each leaf were placed in individual Petri dishes (9 cm diam.) lined with moistened filter paper. The Petri dishes were then incubated for a further 48 h at 25°C, after which the mealybug mortality was assessed. Hobo® H8 Pro Series data loggers were placed in the middle of every second treatment row to document temperature and humidity in the orchard throughout the trial period.

Data analysis

All statistical analyses were performed with Statistica 9.0 software (StatSoft Inc. 2009). The data were analysed using ANOVA, with post-hoc comparison of means using Bonferroni’s method, or a bootstrap multi-comparison test if residuals were found not to be evenly distributed (Efron & Tibshirani 1993). Significant differences were determined at a 95% probability level.

Results

Susceptibility assay on leaves and fruit

A two-way ANOVA analysis showed no interaction between main effects, part of tree (leaves and fruit) and five treatments (F(4,150) = 0.60, P = 0.66). The mortality of mealybugs on fruit and leaves was consistent with that encountered in the treatments and no significant differences were observed between the mortality of P. citri on leaves and fruit for any of the treatment suspensions tested. The one-way ANOVA for mortality observed separately on the fruit and leaves were pooled and showed the average percentage mortality (30%) on fruit not to be significantly higher (F(1,150) = 0.84, P = 0.36) than the average percentage mortality (28%) observed on leaves. A one-way ANOVA of the pooled insect mortality obtained from the treatment of the fruits and leaves indicated significantly higher mortality than the control (F(5,150) = 16.59, P = 0.001) with an average percentage mealybug mortality of 11% (Fig. 1). The combined addition of 0.6% Nu-Film-P® and 0.3% Zeba® to H. zealandica (T3) suspensions did not significantly increase mealybug mortality. The highest average percentage mortality of 45% was achieved when mealybugs were treated with a suspension of S. yirgalemense, 0.6% Nu-Film-P® and 0.3% Zeba® (T5); the mortality was significantly higher than that of mealybugs treated with suspensions of H. zealandica alone.

Effect of adjuvants on IJ infectivity after different time intervals

Analysis of S. yirgalemense infectivity under simulated glasshouse conditions on citrus leaves by a two-way ANOVA showed interaction between the main effects (treatment and formula) and time (0, 30, 60, 120, 180 and 240 min) (F(5,108) = 6.14, P = 0.001), indicating that the treatments did not behave consistently over time. No significant differences in IJ infectivity were observed during the first 60 min after applying EPNs to leaves, with mealybug mortality recorded above 70% and no significant difference being recorded. Infectivity of IJs treated with 0.3% Zeba® and 0.6% Nu-Film-P® was significantly higher (P = 0.007) after 120 min than with IJ applied in water only and resulted in high mealybug mortality of 86%. Infectivity of IJ treated with 0.3% Zeba® and 0.6% Nu-Film-P® only started to decrease after 180 min, with 60% mealybug mortality being recorded; however, the percentage mortality was still not significantly lower than the mortality of 84% (P = 0.21) reached directly after applying IJ. The infectivity potential of IJ applied in water only, with 24% control after 180 min, was still significantly lower (P = 0.001) than that of IJ applied with 0.3% Zeba® and 0.6%
Nu-Film-P®. The lowest infectivity potential of IJ was observed in both treatments after 240 min, with 10% mealybug control being recorded.

Effect of adjuvants on IJ survival after different time intervals

The analysis for S. yirgalemense survival under simulated glasshouse conditions on citrus leaves using a two-way ANOVA showed interaction between main effects, treatment (formula and water) and time (30, 60, 120 and 240 min) ($F_{3,88} = 4.77, P = 0.004$) indicating that the treatments did not behave consistently over time. Low IJ mortality (<8%) for both treatments was observed 30 min after applying IJ to the leaves (Fig. 3). The mortality of IJ applied with water only increased significantly to 38% ($P = 0.001$) 60 min after application, while the mortality of nematodes applied with Zeba® and Nu-Film-P® increased only slightly, to 17%. After 120 min, the mortality (51%) of IJ applied with Zeba® and Nu-Film-P® was lower than the mortality (74%) of IJ applied with water only, but it did not differ significantly.

Semi-field trial

Moderate temperatures, ranging between 9°C and 26°C, with an average of 11°C, were recorded throughout the 14 h semi-field trial period. The relative humidity in the orchard was average (~52% RH) at the time of application and started to increase after 1 h, as the temperature dropped after sunset (Fig. 4). The average temperature and humidity for the first 5 h post-application was 12°C and 71% RH, respectively. From approximately 03:00 dew formed on the trees, as the ambient humidity rose to 100% and the dew point equalled the temperature (±10°C). The ambient humidity remained at 100% until 08:00. At 10:00, when pockets containing leaves were retrieved, the trees were still wet from the morning dew.

Mortality (<33%) for P. citrifemales achieved in the field trials, with a suspension of S. yirgalemense only (T2) and one containing of S. yirgalemense and Nu-Film-P® (T3), analysed by one-way ANOVA (Fig. 5), were not significantly higher than was that achieved with the control (11% mortality). The addition of 0.6% Nu-Film-P® to S. yirgalemense suspensions increased

![Fig. 1](image1.png)

**Fig. 1.** Mean percentage mortality of Planococcus citri females on leaves after exposure to different formulations of Heterorhabditis zealandica and Steinernema yirgalemense during a growth chamber assay at 75 ± 8% RH, 22°C and 2000 IJs ml⁻¹. Treatments were: T1, water only; T2, H. zealandica; T3, H. zealandica + Zeba® + Nu-Film-P®; T4, S. yirgalemense; and T5, S. yirgalemense + Zeba® + Nu-Film-P®. Bars with the same letter do not differ significantly ($P \geq 0.05$).

![Fig. 2](image2.png)

**Fig. 2.** Mean percentage infected Planococcus citri adults after exposure to Steinernema yirgalemense for different time intervals during growth chamber assays at 75 ± 8% RH, 22°C and 2000 IJs ml⁻¹. Data points with the same letter do not differ significantly ($P \geq 0.05$).
mortality only by 2%. The average percentage mortality increased substantially to 50% (T4) with the addition of 0.3% Zeba®, the mortality being significantly higher than in the control ($P = 0.003$). The highest average percentage mortality of 53% was seen in mealybugs treated with a suspension of EPNs, 0.6% Nu-Film-P® and 0.3% Zeba® (T5).

**Discussion**

EPN desiccation, accelerated by such abiotic factors as high temperatures, wind and low humidity, limits the effectivity of EPN to control above-ground insect pests (Wright et al. 2005). To retard EPN desiccation, water retention agents can be added to nematode application suspensions (Glazer et al. 1992). The sensible use of adjuvants, by combining water retention agents with surfactants, has resulted in the successful control of western flower thrips in glasshouses (Tomalak et al. 2005). In the current study, the ability of a polymer product, Zeba®, and a surfactant, Nu-Film-P®, to improve the ability of *H. zealandica* and *S. yirgalemense* to control *P. citri* was evaluated. These two adjuvants were shown to have no effect on mortality and infectivity of IJ after exposure shorter than 6 h (Van Niekerk 2012). De Waal et al. (2011, 2013) achieved a significant improvement in the control of codling moth by adding adjuvants to the nematodes application suspension.

During a growth chamber assay, simulating glasshouse conditions of 75 ± 8% RH and 22°C, both *H. zealandica* and *S. yirgalemense* were able to increase *P. citri* mortality significantly compared to the control. When applying suspensions of the two nematode species against *P. citri* in water only, compared with adjuvants (0.3 Zeba® and 0.6 NuFilm®) added to the water, a slight increase in mortality was obtained, but it did not differ significantly. However, with the addition of adjuvants, the *S. yirgalemense* treatment was able to achieve significantly higher control than did the *H. zealandica* treatments. The better performance of *S. yirgalemense* is in accordance with results obtained by Van Niekerk & Malan (2012), which indicated that *S. yirgalemense* was able to locate and infect *P. citri* faster than *H. zealandica*, as well as being more tolerant towards lower free water conditions.
improving the ability of S. carpocapsae to control DBM in a field
and 0.3% of the polymer xanthan (antidesiccant) aimed at
sions containing S. yirgalemense were unable to achieve sig-
results were obtained by Schroer & Ehlers (2005), who evalu-
experiment showed that without the addition of Zeba
combined influence on EPN performance. Results of the field
in the current field study, in which mealybugs were much more
exposed to a harsh macro-environment.

As S. yirgalemense performed significantly better with the
addition of adjuvants, only this species was investigated
further. Under the same conditions as those mentioned above,
adjuvants improved both the infectivity and the survival rate of
S. yirgalemense 2–3 h post-application. When the ability of
S. yirgalemense to infect mealybugs post-application was evaluated, the infection potential of EPNs in water significantly
decreased 2 h post-application. The first decrease in infectivity
potential of nematodes applied with the adjuvants was observed
3 h post-application, although the decrease was not
significant. The infectivity potential of EPNs applied with adju-
vants decreased drastically 4 h post-application, achieving
only 10% control. When the mortality of IJs was investigated, the average mortality of IJs applied with water only increased
significantly 1 h post-application, while a significant increase in the mortality of IJs applied with adjuvants was observed 2 h
post-application. The mean mortality of 51% observed for IJs
applied with adjuvants was considerably lower than the mortal-
ity of 74% observed for IJs applied with water only 2 h
post-application, but this was not significantly different. No live IJs were recorded 4 h post-application. The death of IJs
due to low humidity after 4 h explained the loss of IJ infectivity
observed.

The ability of the formulation to improve control of P. citri was
further investigated during a semi-field trial. The adjuvants were added separately in order to compare their individual and
combined influence on EPN performance. Results of the field
experiment showed that without the addition of Zeba®, suspens-
sions containing S. yirgalemense were unable to achieve sig-
nificantly higher effect on P. citri than in the control. The addition of 0.6% Nu-Film-P® to suspensions had minimal effect on EPN
performance, increasing P. citri control by only 2%. The highest
mealybug mortality of 53% was achieved with S. yirgalemense
applied together with both Zeba® and Nu-Film-P®. Similar
results were obtained by Schroer & Ehlers (2005), who evalu-
ated a formulation containing 0.3% Rimulgan® (surfactant) and
0.3% of the polymer xanthan (antidesiccant) aimed at
improving the ability of S. carpocapsae to control DBM in a field
study conducted on cabbage heads. The number of insects
observed on cabbage heads declined significantly, resulting in ≥50% control after 7 days. However, their formulation did
not prove to have a significant effect, compared to that of the
EPNs only, as the moist microclimate in cabbage heads,
combined with high ambient humidity, favoured EPN survival.

Moderate temperatures ranging between 9°C and 26°C prevailed during the field trial, with a mean of 11°C. EPNs have
proven to be most active at temperatures ranging between 15°C and 32°C (Lacey et al. 2005), indicating that the low
temperatures of between 10°C and 15°C, which occurred
during the night, suppressed the performance of S. yirgale-
mente. In Van Niekerk & Malan (2012), the exposure time
experiment showed the first 2–4 h post-application to be the
most important. According to the findings of Lacey & Unruh
(1998), the ability of EPNs to infect hosts was found to be
greatly impaired when exposed to ambient humidity lower than
95%, thus suboptimal humidity levels ranging between 52% and 87% with an average of 72% prevailing during the first 4 h
post-application would have suppressed EPN infectivity even
further. In spite of the suboptimal environmental conditions dur-
ing the semi-field trial undertaken in the current research, 53% control of P. citri was still achieved by a suspen-
sion of S. yirgalemense, Zeba® and Nu-Film-P®.

The ability of EPNs to control P. citri should be investigated
by applying nematodes to citrus trees that have been naturally
infected with mealybugs. The insect containment method used
in the field trial was very limited. Although mealybugs occur on
foliage, they are cryptic in nature and usually occur in more
protected sites, such as between leaves, in bud-mite-induced
growth deformations or in between fruit clusters (Wakgari
& Giliomee 2003), which are sites that also provide a much more
favourable microclimate for nematode infection than does the
exposed leaf surface. Arthurs et al. (2004) collected data from experiments conducted over the last two decades in order
to develop a linear model for testing the efficacy of S. carpocapsae to control insect pests. His model showed EPN
efficacy to be dependent on pest target habitat, with
efficiency decreasing in the following order: firstly, bore holes;
secondly, cryptic foliage; and thirdly, exposed foliage. Thus,
control of P. citri achieved under natural conditions should
teoretically be considerably higher than the control achieved
in the current field study, in which mealybugs were much more
exposed to a harsh macro-environment.

The cost-effect efficiency of increasing nematode applica-
tion concentrations should also be investigated, as the concen-
tration of 4000 IJs ml⁻¹ used in the field trial is relatively high.
Also, mealybugs tend to cluster together and often to infect only
a few adjacent trees, which can be treated as hotspots, with the application of a high concentration of nematodes. Van Niekerk & Malan (2012) discussed the feasibility of irrigating citrus trees pre- and post-application to increase humidity and to improve nematode performance, with it being concluded that, in many cases, such irrigation would, most probably, not be practical, as water is a limited natural resource in South Africa. Furthermore, the waxy coatings that cover citrus leaves and fruit impair the ability of nematode application suspensions to stick to their surface. Irrigating trees before application could increase application runoff, while nematodes would most probably be washed off from the exposed leaf surface if trees were to be irrigated after application. It was suggested that such loss of nematodes could be compensated for by applying them during the late afternoon, as was done in the current field trial. However, if, theoretically, EPNs had been applied at 05:00 on 7 October 2011, the nematodes would have been subjected to approximately 7 h of moisture, allowing them to detect and infect their hosts. Dew formed at 03:00, when the relative humidity reached 100%. The ambient humidity remained 100% until 08:00. At 10:00, when treated leaf pockets were retrieved, the trees were still wet from the morning dew. If the nematodes had been applied at 05:00, they would have had at least 5 h of moisture before the desiccation-retarding abilities of Zebra® would have been required. Consequently, at least an additional two hours would have been added to the lifetime of the EPNs, providing them with a total of seven hours in which to locate and infect P. citri. During this time, the temperature would also have increased, adding to the EPN infection potential.

EPNs have been found to be best used to control above-ground pests in an IPM system (Wright et al. 2005). As mealybugs tend to occur in all life stages on citrus trees throughout the year (Wagkari & Gilmoree 2003), EPNs can be applied at any time when environmental conditions are favourable. Adult mealybugs are difficult to control with chemicals, as, in addition to tending to hide in protected sites, where insecticides cannot reach them, they are also covered by protective waxes (McKenzie 1967; Michelakis & Hamid 1995; Franco et al. 2004). Crawlers that emerge from protected hiding sites in search of food during early spring, despite being less susceptible to EPNs than are adults (Stokwe 2009), tend to be more susceptible to chemicals (Hattingh & Moore 2003). Although the use of chemicals is not desirable, EPNs can be applied in combination with an insecticide during early spring to target all life stages of mealybugs. Control of mealybug populations early in the growing season will tend to reduce the pesticide load that might otherwise be experienced later in the season. Furthermore, natural enemy populations recover slowly after winter, resulting in population levels that are inefficient in preventing feeding damage to young fruitlets (Hattingh 1993; Franco et al. 2004). EPNs can thus be applied during this period to fill the gap left by natural enemies. If high mealybug numbers are recorded later in the growing season during late and midsummer, EPNs alone can be applied for control, without problems of residues remaining on the fruit. Average temperatures will also be higher during the night at such times of the year, which will increase EPN efficiency.

In laboratory bioassays, simulating natural conditions, S. yirgalemense and H. zealandica showed promising control potential for P. citri. The addition of water retention agents showed a significant improvement in the survival and infectivity only of S. yirgalemense. During the first three critical hours of the late afternoon application in the field trial, suboptimal RH of lower than 80% prevails. Unfortunately for the duration of the trial the night temperature was low (<15°C). However, in spite of negative field conditions, significant control of P. citri was achieved with S. yirgalemense and water retention agents. More research is needed to devise suitable containment methods to indicate the immediate effect of an EPN application under field conditions. We conclude that more field trials, in more conducive environmental conditions to EPN infection, should be performed to gauge the real potential of S. yirgalemense and other EPN species as an aerial biological control agent of the citrus mealybug.

Acknowledgements

The authors would like to thank Citrus Research International (CRI), the Citrus Academy and the National Research Foundation of South Africa (NRF-THRIP TP2011060100026) for funding of the project and D Nel for assistance with statistical analysis.

References

XVIII. International Plant Protection Congress

Mission possible: food for all through appropriate plant protection

24–27 August 2015 • Berlin (Germany)

www.ippc2015.de