Effects of *Verticillium lecanii* (Zimm.) Viegas on *Toxoptera citricida* Kirkaldy (Homoptera: Aphididae) and its Parasitoid *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae)

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Abstract

Balfour A., Khan A. (2012): **Effects of** *Verticillium lecanii* (Zimm.) Viegas **on** *Toxoptera citricida* Kirkaldy (Homoptera: Aphididae) **and its parasitoid** *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae). Plant Protect. Sci., **48**: 123–130.

The preponderance of susceptible sour orange (*Citrus aurantium* L.) rootstock has facilitated the spread of *Citrus tristeza virus* (CTV) in Trinidad and Tobago. CTV is transmitted by the brown citrus aphid *Toxoptera citricida* (Kirkaldy), which establishes large colonies on new flushes of citrus plants. As the colonies become highly populated, winged (alate) aphids are produced which can migrate to uninfected neighbouring citrus trees and hence transmit CTV. In the present study different concentrations of the entomopathogenic fungus *Verticillium lecanii* (Zimm) Viegas in water-based formulations were applied to *T. citricida* and the pathogenic effects were analysed. Bioassays were also conducted on the major parasitoid *Lysiphlebus testaceipes* Cresson to test the pathogenic effects of *V. lecanii* on mortality and the percent emergence from mummies. The LC_{50} values for *T. citricida* and *L. testaceipes* were 2.26×10^{10} spores/ml and 1.09×10^{9} spores/ml, respectively. Statistical analyses indicated that there was no significant difference between the two LC_{50} values. At the highest concentration (1.49×10^{9} spores/ml) percent mortality peaked at 78.9% after 12 days and 1.95×10^{9} spores/ml, mortality of *L. testaceipes* reached 95.1% after 6 days. Emergence of *L. testaceipes* at the highest concentration of 1.95×10^{9} spores/ml was 57.8%.

Keywords: brown citrus aphid; alate aphids; *entomopathogenic fungus; natural enemy;* mummies

Citrus crops constitute a substantial sector of tree crop production worldwide. In the United States of America the annual value of the citrus industry was estimated over US$ 8 billion in 1996 (Lee & Rocha-Peña 1992) while the Caribbean region produced 58 million tonnes of fruit in 1992 (Aubert *et al.* 1992). This includes the large and top producers such as Mexico (3 081 000 t), Venezuela (485 000 t), Cuba (898 000 t), and Jamaica (104 000 t) and smaller producers like Trinidad (16 000 t).

In the last decade, the thrust for increased citrus production in Trinidad has been fuelled by the expansion of three major estates, which covered about 5000 ha of oranges (*Citrus sinensis* L.) and grapefruit (*Citrus paradisi* Macfad.) (Aubert *et al.* 1992). Most of these citrus trees were established on the sour orange *Citrus aurantium* L. rootstock.

The brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphididae), is one of the economically important pests of citrus crops. The severity of this pest stems from its ability to debilitate tree production by feeding on young plant material and to transfer *Citrus tristeza virus* (CTV). The devastating effects caused by CTV were reported in the 1940's in Argentina, where 16 million citrus trees on the sour orange rootstock were killed (Fasulo & Halbert 2009).

T. citricida is native to Asia and until 1990 the insect was confined to citrus growing areas of Asia, Australia, New Zealand, Pacific Islands, Sub-Saharan Africa, and South America (Yokomi 2009).

Since then it has been detected in Puerto Rico (1992), Cuba (1993), and Florida (1993) (Yokomi *et al.* 1994). *T. citricida* is now widely distributed in the Caribbean, including Guadeloupe, Martinique, St. Lucia, and Dominican Republic (Leclant *et al.* 1992) and in St. Kitts and Jamaica (Etienne *et al.* 1994). In Trinidad and Tobago, *T. citricida* was reported as early as 1985 (Yokomi & Tang 1994).

The ability of *T. citricida* to colonise the major economic citrus species has been of great concern in the Americas and the Caribbean. Aphids are dispersed to these regions *via* commerce and tourism (Yokomi & Tang 1995). *Citrus tristeza virus* transmitted by *T. citricida*, which is 8–20 times more efficient than other citrus aphid species (Aubert *et al.* 1992), has become one of the most serious challenges faced by the citrus industry. CTV causes two important disorders. The most serious damage is decline of scions which affects specific scion-stock combinations, such as orange (*Citrus sinensis* L.), mandarin (*Citrus reticulata* Blanco), tangelo (*Citrus* × *tangelo* Ingram & Moore) or tangor (*Citrus reticulata* × *sinensis*), grafted onto the sour orange rootstock. The disease hinders the movement of starch down into the roots thus causing root decay, subsequent decline and death (Aubert *et al.* 1992). The second type of disorder causes stem pitting symptoms on twigs, young branches, stems, and roots. Despite being less severe than the decline of scions, stem pitting can result in long-term debilitation that reduces yields of sweet orange and grapefruit by 5–45% (Yokomi & Tang 1995).

With *T. citricida* established locally and regionally as a major pest of citrus, and the potential for CTV transmission, many control practices have been employed. Systemic insecticides have been used on budwood material and even in established field plants. Several foliar insecticides have adverse effects on non-target and beneficial insects and it has been reported that foliar applications were followed by significant re-colonisation of *T. citricida* after 15 days (Shevale *et al.* 1987). The use of granular formulations or soil drenches on fruiting trees is restricted to certain seasons so as not to pollute groundwater systems (Pickett *et al.* 1992). These factors support arguments to favour the use of biological control and bio-pesticides to manage *T. citricida* populations. Aphidophagous wasps such as *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphidiidae) naturally reduce *T. citricida* populations in citrus fields (Stary *et al.* 1988). Entomopathogenic fungi such as *Verticillium lecanii* (Zimmerman) Viegas are also effective natural enemies of *T. citricida.* Although studies have been done on *V. lecanii* as a biocontrol agent, it is not clear as to its effectiveness either in different formulations or its effect on major parasitoids of *T. citricida.* However it has been shown that biological control and integrated control of citrus aphids is economically profitable (van Lenteren 1987). This study was undertaken to determine the effect of water formulation of *V. lecanii* on the mortality of *T. citricida* and the pre- and post-emergent mortality of the parasitoid *L. testaceipes.* This information could prove useful to establish an effective Integrated Pest Management program which could reduce *T. citricida* populations below economic thresholds, while minimising the use of synthetic chemicals especially during production periods. Additionally, the formulation of a fungal bio-pesticide could limit the spread of alate aphids, which could reduce the spread of *Citrus tristeza virus*.

MATERIAL AND METHODS

***Rearing of** Toxoptera citricida.* Two large cages (1.25 × 0.6 × 1.25 m) covered with nylon organza mesh (250 mesh/cm^2) were constructed to house 5 potted rough lemon, *Citrus jambhiri* Lush, plants in each cage. No pesticides were applied to these plants and manual weed removal from pots was practiced throughout the experiment. Each plant was fertilised weekly with a foliar application of Nutrex® (Bream Corporation, Chicago, USA) (N:P:K – 20:20:20 plus micronutrients) at a rate of 3.3 g/l, which promoted flushing of plants and proliferation of aphids.

Citrus flushes (1–12 cm) with *T. citricida* were collected from grapefruit (*Citrus paradisi*) and tangerine (*Citrus* × *tangerina*) trees at the Citrus Research Station of the Ministry of Agriculture, Lands and Marine Resources located at Farm Road, Curepe, Trinidad. The collected shoots were gently placed on the flushes of the caged citrus plants and *T. citricida* nymphs and adults moved onto caged plants. Aphids were allowed to develop until a minimum of two colonies (a single shoot with aphids was considered a colony) were produced on each plant.

Agar preparation and inoculation. Approximately 15.6 g of dehydrated Potato Dextrose agar (Oxoid®, Basingstoke, UK) powder was suspended in 400 ml of distilled water in a 1-l Pyrex flask and boiled for 1 min to completely dissolve the

powder. The flask was loosely plugged with cotton wool and the mixture was autoclaved at 121°C for 15 minutes. The liquid medium was cooled by running tap water over the flask while simultaneously swirling. Sixteen sterile Petri plates (9 cm diameter) were placed in an Envair HLF/4/8® (NuAire Corporation, Minneapolis, USA) laminar flow cabinet and the PDA medium was poured into the plates. *Verticillium lecanii* (ARSEF 6145) was streaked onto each of the 16 plates which were sealed with Parafilm® around their periphery and left inverted for 14 days at room temperature for fungal growth and colony formation.

Verticillium lecanii ***conidial suspensions***. A solution comprising 70 ml of sterile water and 0.05 ml Tween 80® (Acumedia Manufacturers, Inc., Michigan, USA) was made and 5 ml was poured onto the PDA plates with mature colonies of *V. lecanii*. *V. lecanii* fungal growth was carefully scraped off the PDA plates using a sterile, stainless steel spatula and the fungal spore/mycelia suspension was poured over another plate with sporulating colonies of *V. lecanii.* This procedure was repeated with 8–10 plates until the highest concentration of spores was produced in the original volume of sterile water-Tween 80®. The suspension was placed on a magnetic stirrer (COSLAB Model CLE-108; Cosmo Laboratory Equipment, Haryana, India) for 5 min in order to produce a homogenous suspension of spores. The spore concentration was determined using a Neubauer haemocytometer. The spore/mycelia concentration was determined to be 1.49×10^{10} spores/ml and four 10-fold serial dilutions were made from this. A control with Tween 80® and sterile water was also prepared.

Inoculation of *T. citricida*. There were five treatments (four concentrations and a control) and each was applied randomly to five shoots with a *T. citricida* colony on different host plants. The number of aphids on each shoot was counted and 2.5 ml of the appropriate spore suspension was immediately applied using a 20 ml plastic spray bottle, which produced a light mist on the aphid colonies. The applications were done starting with the lowest concentration, then proceeding to the higher concentrations. Aphids on each of the 25 treated colonies were isolated by securing a nylon mesh (250 mesh/cm^2) bag that covered the entire shoot. The number of aphid cadavers was recorded daily for 12 days and the percent corrected mortality determined. Aphid cadavers were placed on moist, sterile filter paper in Petri plates (5 cm diameter). Probit analysis was conducted on the data using EPA Vers. 1.3 software (US Environmental Protection Agency).

Inoculation of *L. testaceipes* ***mummies***. Shoots with aphids mummified by *L. testaceipes* were collected from the Citrus Research Unit of the Ministry of Agriculture, Lands and Marine Resources, St. Augustine, Trinidad and placed inside cages with *T. citricida*. Adult *L. testaceipes* emerging from the aphid mummies parasitised the healthy aphids and produced mummies 7 days post inoculation. Spore suspensions of *V. lecanii* were prepared and applied as above to the mummies. The number of adult *L. testaceipes* emerging from these treated mummies was recorded daily and percent emergence determined. Probit analysis was conducted on the data using EPA Vers. 1.3 software.

Inoculation of adult *L. testaceipes*. *L. testaceipes* adults were collected daily from each mummy treatment described above using an insect aspirator and placed on shoots which were surface sterilised with 0.5% Clorox® (Clorox Company, Oakland, USA), immediately rinsed thoroughly in sterile water and then sprayed with a suspension of *V. lecanii* at a concentration of 1.49×10^9spores/ml. The experiment was repeated daily with newly eclosed *L. testaceipes*. All dead adult *L. testaceipes* were placed on moist filter paper to confirm that mortality was due to *V. lecanii.* Probit analysis was conducted on the data using EPA Vers. 1.3 software. A difference between the two probit slopes was considered significant ($P = 0.05$) if it was > 1.96 times the standard error of either slope. This was calculated and used to determine the variability of results between mortality for *T. citricida* and *L. testaceipes.*

The Selectivity Ratio (Metcalf 1972) was used to determine whether the treatment was more toxic to the parasitoid or the pest:

$$\text{Selectivity ratio} = \frac{LC_{50} \text{ of parasitoid}}{LC_{50} \text{ of pest}}$$

A Selectivity Ratio > 1 favours the parasitoid, while a ratio < 1 favours the pest.

RESULTS

Mortality of *Toxoptera citricida* by *Verticillium lecanii*

The highest mortality (91.0%) of *T. citricida* was recorded 12 days post application of 1.49×10^9 spo-

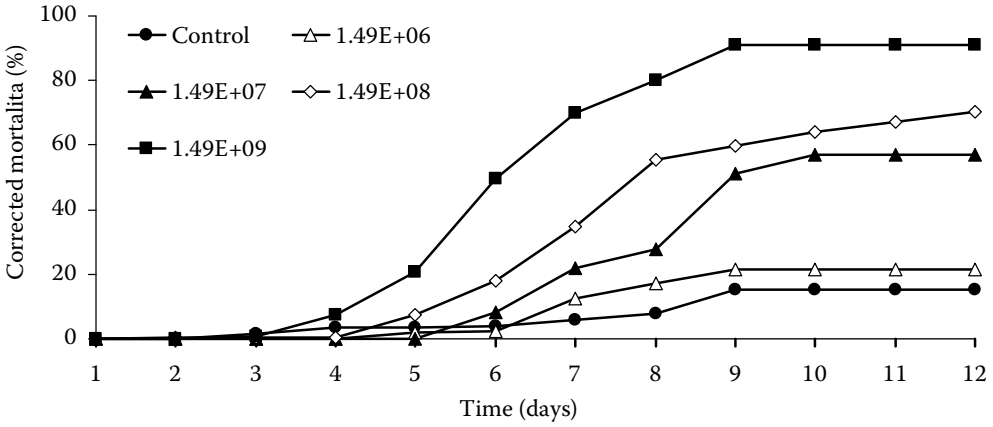

Figure 1. Effect of different concentrations of *Verticillium lecanii* spores on the percentage mortality of *T. citricida* over 12 days after inoculation

res/ml of *V. lecanii* (Figure 1). The LT_{50} at this concentration was 6 days. Neither the control nor the lowest concentration of 1.49×10^6 spores/ml produced LT_{50} values since their highest percent corrected mortality values were less than 50% (Figure 1). Probit analysis revealed that there was no difference between the LC_{50} values of *T. citricida* and *L. testaceipes* when exposed to *V. lecanii* (Table 2).

Emergence of *Lysiphlebus testaceipes* from *Verticillium lecanii* treated mummies

Adult *L. testaceipes* emerged from *V. lecanii* treated mummies until Day 6 of the seven days of testing. There was a gradual increase in the emergence of *L. testaceipes* over time among all treatments, with the control having the highest overall emergence (66.7%) (Figure 2). However, the percentage emergence for 1.95×10^9 spores/ml was higher than that of 1.95×10^8 spores/ml between Day 2 and Day 7 (Figure 2). This trend was also observed for *L. testaceipes* mummies treated with *V. lecanii* at a concentration of 1.95×10^7 spores/ml, which had greater eclosion between Day 1 and Day 3 as compared to a concentration of 1.95×10^6 spores/ml (Figure 2).

The time for 50% emergence (ET_{50}) of *L. testaceipes* mummies treated with *V. lecanii* spores increased with increasing spore concentration. The lowest ET_{50} occurred in the Control (3.47 days) but this was not significantly different ($P = 0.05$) from *L. testaceipes* mummies exposed to 1.95×10^6 spores/ml and 1.95×10^7 spores/ml. *L. testaceipes* took significantly ($P = 0.05$) longer (5.7 and 6.4 days) than the control to achieve 50% emergence when treated with concentrations of 1.95×10^8 spores/ml and 1.95×10^9 spores/ml, respectively (Table 1).

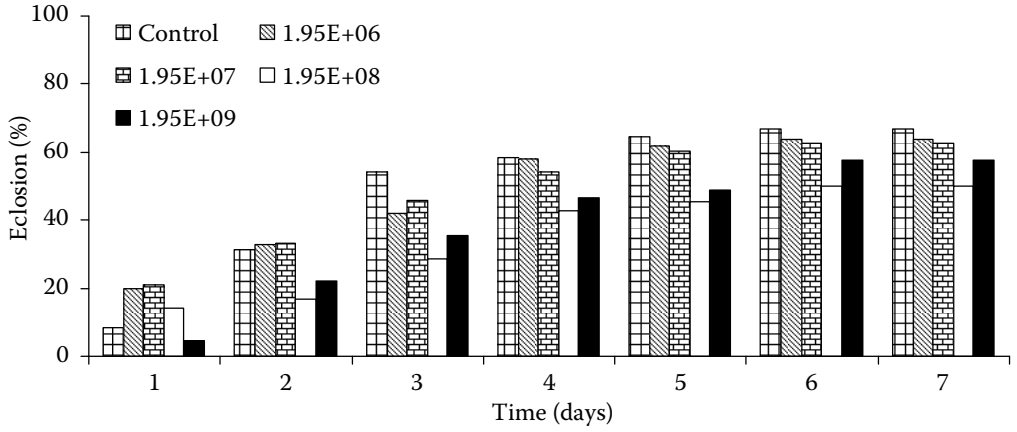

Figure 2. Percentage emergence of *L. testaceipes* mummies treated with *V. lecanii* over time

Table 1. Effect of *Verticillium lecanii* spore concentration on time for 50% emergence (ET_{50}) of *Lysiphlebus testaceipes*

Concentration (spores /ml)	ET_{50} ± SE (days)	χ^2	Probit equation	95% C.I. of ET_{50}
0 (Control)	3.47 ± 1.05[a]	8.47	$Y = 2.02x + 3.91$	3.09, 3.88
1.95×10^6	3.59 ± 1.08[a]	2.78	$Y = 1.55x + 4.14$	3.12, 4.15
1.95×10^7	3.65 ± 1.08[a]	1.12	$Y = 1.44x + 4.19$	3.14, 4.25
1.95×10^8	5.71 ± 1.06[b]	2.04	$Y = 2.21x + 3.51$	5.29, 7.72
1.95×10^9	6.39 ± 1.09[b]	4.34	$Y = 1.47x + 3.82$	5.42, 7.53

ET_{50} values followed by the same letter are not significantly different from each other (Tukey-Kramer Multiple Comparisons test, $P = 0.05$)

Mortality of adult *Lysiphlebus testaceipes* treated with *Verticillium lecanii*

There was a direct relationship between the mortality of *L. testaceipes* adults and increasing spore concentration of *V. lecanii* (Figure 3). The three highest concentrations caused significantly higher mortality compared to the control and concentration of 1.95×10^6 spores/ml (Figure 3). The highest percentage corrected mortality (95.1%) occurred at a concentration of 1.95×10^9 spores/ml (Figure 3). At the highest concentration (1.95×10^9 spores/ml) the LT_{50} was achieved in the shortest time of 2.42 days and was significantly different ($P = 0.05$) from all treatments including the control. Concentrations of 1.95×10^7 spores/ml and 1.95×10^8spores/ml had LT_{50} values which were not significantly different ($P = 0.05$). The control took a significantly ($P = 0.05$) longer time to cause 50% mortality compared to all treatments except 1.95×10^6 spores/ml (Table 3). The Selectivity Ratio of 0.048 was found to be in favour of *T. citricida* (Table 2).

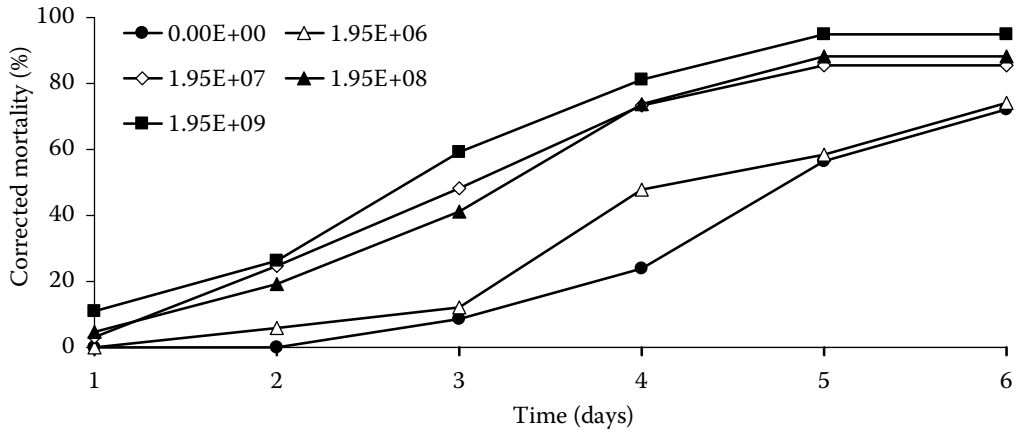

Figure 3. Effect of different concentrations of *V. lecanii* on the percent corrected mortality of *L. testaceipes* adults over time

Table 2. Comparison of LC_{50} values for *Toxoptera citricida* and *Lysiphlebus testaceipes* when treated with *Verticillium lecanii*

Species	LC_{50} (spores/ml)	S.E. of LC_{50}	χ^2	Probit equation	95% C.I. of LC_{50}
T. citricida	2.26×10^{10a}	1.29	3.56	$Y = 0.517x - 0.355$	1.36×10^{10}, 3.73×10^{10}
L. testaceipes	1.09×10^{9a}	5.59	2.43	$Y = 1.191x + 3.275$	3.74×10^8, 3.17×10^{10}

LC_{50} values followed by the same letter are not significantly different from each other (Tukey-Kramer Multiple Comparisons test, $P = 0.05$)

Table 3. LT_{50} (days) for *Lysiphlebus testaceipes* treated with *Verticillium lecanii*

Concentration (spores /ml)	LT_{50} (days)	S.E of LT_{50}	χ^2	Probit equation	95% C.I. of LT_{50}
0 (Control)	4.88[a]	1.02	1.55	$Y = 6.89x + 0.26$	4.66, 5.11
1.95×10^6	4.43[a]	1.03	5.63	$Y = 4.96x + 1.79$	4.18, 4.69
1.95×10^7	2.92[b]	1.04	1.45	$Y = 4.07x + 3.10$	2.73, 3.13
1.95×10^8	2.98[b]	1.04	10.35	$Y = 4.06x + 3.07$	2.79, 3.19
1.95×10^9	2.42[c]	1.04	12.65	$Y = 3.86x + 3.52$	2.24, 2.60

LT_{50} values followed by the same letter are not significantly different from each other (Tukey-Kramer Multiple Comparisons test, $P = 0.05$)

DISCUSSION

The results presented in this study indicate that the application of *V. lecanii* spores at concentrations of 1.49×10^9 spores/ml and 1.49×10^8 spores/ml caused 78.9% and 68.8% mortality to *T. citricida*, respectively. These results were similar to the early findings by Rondón *et al* (1981), who also showed over 80% mortality on *T. citricida* nymphs and adults (alate and apterous) by *V. lecanii.* In comparison with Mycotrol®, a commercial formulation of *Beauveria bassiana,* mortality of 79.8% and 94.4% was observed on *T. citricida* at rates of 2.5×10^{13} and 5×10^{13} conidia/ml (Poprawski *et al.* 1999). At the highest concentration of 1.95×10^9 spores/ml of *V. lecanii,* the observed mortality of *L. testaceipes* was 95.1% (Figure 3). The highest percent mortality between the species may not be comparable as the life span of *L. testaceipes* is approximately seven days and by Day 6 the natural mortality was approaching 75% due to age (Figure 3).

The Selectivity Ratio of 0.048 indicated that *V. lecanii* was more pathogenic to *L. testaceipes* than *T. citricida*. However, the present study also revealed that there was no significant difference ($P = 0.05$) between the LC_{50} values of *T. citricida* and *L. testaceipes* (Table 2). This indicated that the concentrations of *V. lecanii* were causing similar mortality in both species despite the Selectivity Ratio which was favourable for the pest. One limitation of the study which might affect the mortality of *L. testaceipes* was the use of sleeve cages that enclosed the parasitoid thus ensuring constant contact with the treated foliage and therefore it might increase the mortality of *L. testaceipes.* However, under field conditions the parasitoid is not trapped with *V. lecanii* treated foliage, but has greater mobility and consequently the probability of contact with entomopathogenic fungi may be reduced and hence allow for higher levels of survival and parasitism.

At the highest concentration of *V. lecanii* (1.49×10^9 spores/ml) used on *T. citricida*, the time for 50% mortality (LT_{50}) was 6 days (Figure 1), which is less than one third of the aphid's life cycle of 21 days (Michaud 1998). This is promising as rapid mortality could reduce the amount of adults which reproduce by parthenogenesis, as well as reduce the population of alates which can migrate and increase the rate of CTV spread. Although the LT_{50} at 1.95×10^9 spores/ml for *L. testaceipes* adults was approximately 3 days, it is almost half of the parasitoid's life cycle of 7.5 days (Figure 3). From this perspective there is a relative advantage for *L. testaceipes* in relation to the LC_{50} and the life cycles of both species. There can still be a certain level of parasitism that could take place prior to Day 3 since some of the 50% of the parasitoids that died from the application of *V. lecanii* could still have parasitised *T. citricida* before dying. Additionally, the 50% of survivors would still be active up to Day 3 and subsequently as this population declines between Day 3 and Day 6 there would be continuing parasitism by the survivors at lower levels as their reproductive capability may be impaired by *V. lecanii.*

Previous reports on natural emergence were not consistent with the findings in this study. Trials by Yokomi and Tang (1996) indicated that less than 5% of viable *L. testaceipes* adults emerged from *T. citricida* mummies. Michaud (1998) reported few emergence holes, whereas the control in this study yielded 66.7% adult parasitoids. Although there was a relatively high level of *L. testaceipes* emergence, at the highest spore concentration (1.95×10^9 spores/ml) the ET_{50} was twice as high as in the control and generally increased as the

spore concentration increased (Table 1 and Figure 3). Hansen and Steenberg (2007) obtained similar results using the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin against the stored product pest *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) and its parasitoids *Lariophagus distinguendus* Förster (Hymenoptera: Pteromalidae) and *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae). They concluded that although the parasitoids were negatively impacted by *B. bassiana*, the parasitoids still gave the control of the pest between 83% and 98%.

There were also decreases of percent emergence between the control and the highest concentration (1.95×10^9 spores/ml) of 18.6% on Day 3 and 8.9% on Day 6 (Figure 2). These decreases over time indicate a decrease in the pathogenic effect of *V. lecanii* on *L. testaceipes*. Hence the longer the time after application when *L. testaceipes* came into contact with the entomopathogenic fungus, the lower effect *V. lecanii* had on *L. testaceipes* compared to the control. This study provides evidence that *V. lecanii* can cause sufficiently high mortality on *T. citricida* in a relatively short time, which could be useful in an Integrated Pest Management (IPM) program to manage this pest. At a concentration of 1.49×10^9 spores/ml there was 78.9% mortality of *T. citricida* which is comparable to successful trials, where traditional insecticides, botanicals, natural, and commercial applications of *V. lecanii* and other entomopathogenic fungi were used (Tao & Wu 1969; Rondon *et al.* 1981; Michaud 1998; Rashki *et al.* 2009).

The practical aim of this study was to reduce the spread of *Citrus tristeza virus* (CTV) through the management of *T. citricida* populations and thus to reduce the production of alates which are able to migrate and spread this disease. The LT_{50} of 6 days as a result of the application of 1.49×10^9 spores/ml of *V. lecanii* suspension can reduce *T. citricida* populations to low levels that could minimize alate production. The key to success is the application of *V. lecanii* before development of high populations which normally coincides with the commencement of a rainy period and the subsequent emergence of new citrus flushes. This may be achieved by the application of *V. lecanii* at a rate of 2.26×10^{10} spores/ml at the onset of first rains as an inoculative treatment. Upsurge in *T. citricida* populations may be managed by inundative application(s) of *V. lecanii*. Yokomi (2009) also noted that entomopathogenic fungi can reduce a population of *T. citricida* with great speed and that high humidity is an essential requirement for efficacy. The LC_{50} (2.26×10^{10} spores/ml) of *T. citricida* was not significantly different ($P = 0.05$) from that of *L. testaceipes* ($LC_{50} = 1.09 \times 10^9$ spores/ml) (Table 2) and consequently with similar mortalities there is a good possibility that surviving *L. testaceipes* would manage populations of *T. citricida*. The LT_{50} for *T. citricida* at 1.49×10^9 spores/ml was 6 days, while that at 1.95×10^9 spores/ml the LT_{50} for *L. testaceipes* was approximately 3 days (Figures 1 and 3). Hence with a life span of the adult *L. testaceipes* lasting up to six days (Aziz & Khan 2008), the surviving 50% of adults would continue to parasitise aphid colonies, the duration of which coincides with the LT_{50} of *T. citricida*. This hypothesis supports the likelihood that the presence of *L. testaceipes* in citrus fields where *V. lecanii* is applied could assist in the suppression of *T. citricida* populations. One aspect of this study that supports this suggestion is the relatively high emergence (57.8%) of *L. testaceipes* mummies of *T. citricida* 6 days post treatment with *V. lecanii* at a rate of 1.95×10^9 spores/ml (Table 1 and Figure 2). The impact of *V. lecanii* on the fecundity of adult *L. testaceipes* is one factor which would influence the parasitoid's effectiveness. If the fecundity of *L. testaceipes* is drastically reduced by *V. lecanii* applications, then populations of *L. testaceipes* would decrease thereby negatively impacting the ecological balance and causing a possible rapid increase of *T. citricida* populations. However, if the fecundity is not adversely affected, the parasitism by *L. testaceipes* could justify the integration of *L. testaceipes* in a pest management program to control *T. citricida* using *V. lecanii*.

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