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RESEARCH ARTICLE

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The addition of molasses to the Cryptophlebia leucotreta granulovirus formulation improves its efficacy against *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae), a pest of citrus in South Africa

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Thaumatotibia leucotreta (Meyrick) (Lepidoptera: Tortricidae), commonly referred to as false codling moth, is an important pest of citrus in southern Africa. Given its quarantine status in markets to which a large portion of the fresh produce is exported, its management in citrus orchards is essential. This management typically follows a systems approach in which baculovirus-based biopesticides, such as Cryptogran™ (*Betabaculovirus cryleucotreta*), are considered an important component of pre-harvest control strategies. It has previously been demonstrated that adding molasses with Cryptogran™ improves field performance, further reducing the number of *T. leucotreta* infested fruit. However, the reason for this improvement was never determined. This study aimed to understand the role that molasses may have on the behaviour of *T. leucotreta* first instars under laboratory conditions, leading to this improved field efficacy, and to confirm conclusively if the addition of molasses to the virus formulation improved efficacy across a further six field trials. In the laboratory, larvae exposed to molasses-treated oranges at either a concentration of 0.25% or 0.50% moved around the surface of the oranges less and also penetrated the oranges less compared to larvae exposed to non-molasses-treated oranges. This suggested that molasses, as a phagostimulant, encouraged larval feeding quicker and longer. This will promote the uptake of the biopesticide infective units and is likely the reason for the recorded improved field efficacy of Cryptogran™ when combined with molasses across all six field trials compared to its application alone. These two sets of experiments confirm conclusively that the addition of molasses to the virus formulation improves its efficacy and should be standard practice in the industry.

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INTRODUCTION

Thaumatotibia leucotreta (Meyrick) (Lepidoptera: Tortricidae), commonly referred to as false codling moth, is an important pest of citrus in southern Africa. Although all cultivars, with the exception of lemons and limes, are susceptible to infestation, *T. leucotreta* has developed a preference for Navel oranges (Grout and Moore 2015; Moore et al. 2015a). While it can cause significant damage to citrus fruit, resulting in yield loss, its pest status is largely one of quarantine importance. This is attributed to its highly polyphagous feeding behaviour, endemism to sub-Saharan Africa, and its current absence in countries to which citrus fruit are exported, resulting in the placement of quarantine regulations on *T. leucotreta* by export markets (Moore et al. 2016; Hattingh et al. 2020; Adom et al. 2021; Moore and Manrakhan 2022). Given that the majority of citrus production in South Africa is destined for export (Citrus Growers' Association 2023), the management of this pest typically follows a systems approach (Moore et al. 2016; Hattingh et al. 2020). This approach includes the use of registered pre-harvest control options as part of an integrated pest management (IPM) plan, of which the use of more environmentally sustainable options is preferred (Grout and Moore 2015; Malan et al. 2018; Moore 2021).

The baculovirus, *Betabaculovirus cryleucotreta* (van Oers et al. 2023), commonly known as Cryptophlebia leucotreta granulovirus (CrleGV), and hereafter referred to as CrleGV, has been successfully used to manage *T. leucotreta* populations in citrus orchards for over 15 years (Moore et al. 2015b). Currently, four *T. leucotreta* targeted biopesticides, with CrleGV as the active ingredient, are registered for commercial use in South Africa, namely Cryptogran™, Cryptomax™ (both River Bioscience, South Africa), Cryptex® and Gratham (both Andermatt Biocontrol, Switzerland) (Moore and Jukes 2023). The success of baculoviruses against agricultural pests, particularly Lepidoptera, has been reviewed in Africa and elsewhere (Moscardi 1999; Yang et al. 2012; Haase et al. 2015; Knox et al. 2015; Lacey et al. 2015; Moore and Jukes 2023). In South African citrus orchards, the application of CrleGV-based products across a representative sample of 13 field trials, has been documented to reduce *T. leucotreta* infestation between 30–92%, and in most cases to be as effective as chemical alternatives (Moore et al. 2015b).

The use of additives, such as molasses, with commercial biopesticides, either in the formulation or combined in spray tanks prior to application, is not uncommon and serves several purposes, including as a phagostimulant to ensure maximum ingestion of the biopesticide, for protection of the infective propagules from efficacy-affecting environmental factors (e.g. UV radiation), and for adhesion to, and adequate coverage of the target area (Burges and Jones 1998; Grzywacz and Moore 2017). Phagostimulants may be particularly useful for biopesticides that use entomopathogens that require ingestion by the insect in order to be effective, such as CrleGV, as they are known to encourage increased insect feeding (Burges and Jones 1998). The increase in feeding results in the uptake of more viral occlusion bodies (OBs), the infective units of baculovirus-based biopesticides, and so, Cryptogran™, a commonly used CrleGV-based biopesticide in South Africa, has a registration for use with molasses (Moore et al. 2015b; Moore 2022). The application timing of Cryptogran™ is important, as the only targetable life stage of *T. leucotreta* using this product is the first instar. *Thaumatotibia leucotreta* oviposits on the rind of citrus fruit, and on hatching, the first instars spend a brief period on the external surface of the fruit before penetration into the flesh, in which the further development through five instars occurs before pupation in the soil (Newton 1998; van den Berg 2001; Grout and Moore 2015). Any damage caused by larval feeding prior to entry into the fruit may be negligible, thus limiting the probability of fruit decay and a decrease in fruit quality (Moore et al. 2011). The role that molasses played in enhancing CrleGV effectiveness in the field trials conducted by Moore et al. (2015b) is speculative, without empirical evidence from laboratory trials and further field trials.

The aim of this research was to determine how the addition of molasses influenced the behaviour of first instar *T. leucotreta* with respect to time spent on the fruit, as measured by distance travelled, and the number of larvae that penetrated within the observation period, under laboratory conditions. In addition, further field trials were also completed to conclusively assess the efficacy of molasses, in both liquid and powder form, as an additive with the CrleGV-based biopesticide, Cryptogran™ under field conditions.

METHODS AND MATERIALS

Effect of molasses on neonate behaviour

Field-collected Navel oranges that had reached colour break were used for the bioassays. Each orange was submerged in one of three different treatments for approximately 30 s: (1) distilled water, which served as the control; (2) 0.25% molasses, the current rate recommended with Cryptogran™ application and, (3) 0.50% molasses, the previously recommended rate with Cryptogran™ application. Post submersion, the orange was allowed to dry before being placed on a pre-made stand erected in the centre of three Samsung HMX-H400 video cameras, each 6.5 cm from the stand, and positioned strategically to ensure the entire orange was viewed; directly above the orange, to the left of the orange in view of one diagonal half, and to the right of the orange in view of the other diagonal half. For consistency, the navel end was always placed facing the left orientated camera. Thereafter, three first instar *T. leucotreta*, < 24 h old, were carefully placed onto each orange using a sterile 000 soft-bristled paintbrush. Insects were obtained from the mixed continuous laboratory culture (Opoku-Debrah et al. 2013) maintained at the Centre for Biological Control (Department of Zoology and Entomology, Rhodes University, Eastern Cape, South Africa) on an artificial maize-based diet according to Moore et al. (2014). Larval movement on each orange was recorded for 1 h, after which the cumulative distance travelled across the fruit surface per larva using the software package Image J and the number of larvae that penetrated the fruit were logged. For each treatment, 12 oranges were used, and a total of 36 first instar *T. leucotreta* were observed.

Efficacy of Cryptogran™ with and without molasses against *T. leucotreta* in field trials

The efficacy of Cryptogran™ with and without molasses was evaluated across six citrus farms between 2005 and 2011 in the Sunday's River Valley, Eastern Cape province, South Africa (Table 1). Trials 1, 2 and 3 assessed the efficacy of Cryptogran™ with and without liquid molasses. Trial 4 investigated the efficacy of Cryptogran™ with and without two different brands of liquid molasses [Voermolas (Voermol, South Africa) and Molatek (Molatek, South Africa)], whilst trials 5 and 6 investigated the efficacy of Cryptogran™ with and without either liquid or spray-dried condensed molasses soluble powder [Kalori 3000 (Yara Animal Health, South Africa)]. In all trials, a corresponding untreated control was included. With the exception of trial 3, trials were laid out in a single-tree randomised block format, replicated 10 times per treatment. Treatment application to each tree was achieved using a Janisch hand-gun spray applicator powered by a Honda 250 cc engine and towed through orchards using a four-wheel drive utility vehicle. For trial 3, each treatment block (replicated twice) consisted of 60–65 trees, and application was made using a tractor-drawn PTO-driven oscillating tower mistblower (Table 2).

In all trials, efficacy was assessed using fruit drop surveys. Fruit drop surveys are considered the most accurate way to measure *T. leucotreta* infestation as all infested fruit will drop from the trees. These surveys were not initiated earlier than three weeks post-application, as infested fruit only begins to drop from this time onwards (Moore 2022; Moore et al. 2015b). In the single-tree format trials, dropped fruit from each tree were collected weekly and analysed separately. For the block format trial, 10 data trees in the centre of each treatment block were used to monitor infestation. A fruit was considered infested either by the presence of a larva or its frass upon careful dissection (Moore et al. 2004).

Data analysis

For the laboratory experiment, the difference in larval penetration between treatments was analysed using a generalised linear model (GLM) specified using a binomial error distribution and a logit link function. Larval penetration into the fruit was scored as a Bernoulli variable with entered (1) or failed to enter (0). The difference in the distance (mm) the larvae travelled across the fruit surface over a 1 h observation period between treatments was analysed using a GLM specified using a Gamma error distribution and an identity link function. For both analyses, molasses application was specified as the fixed categorical effect (control, 0.25% molasses, 0.50% molasses). Fixed effect parameter significance was computed by means of a likelihood ratio test ($p < 0.05$) using the 'car' R package (Fox and Weisberg 2019). If significant differences were found, pairwise comparisons, with Bonferroni correction, were used to contrast the data set using the 'emmeans' R package ($p < 0.05$) (Lenth 2023).

Table 1: Site details of the trials where Cryptogran™ was applied with and without molasses against *Thaumatotibia leucotreta* across four citrus farms in the Sunday's River Valley, South Africa.

Trial	Farm	Co-ordinates	Orchard age (years)	Orange cultivar and variety	Tree spacing (rows x trees)
1	Lone Tree	33°31′56″ S, 25°41′31″ E	8	Palmer Navels	6 m × 3 m
2	Bernol	33°28′26″ S, 25°36′43″ E	7	Palmer Navels	6 m × 3 m
3	Orange Grove	33°29′29″ S, 25°35′15″ E	10	Palmer Navels	5.5 m × 2 m
4	Lone Tree	33°31′56″ S, 25°41′31″ E	9	Palmer Navels	6 m × 3 m
5	Far Away	33°29′07″ S, 25°40′34″ E	3	Newhall Navels	6 m × 3 m
6	Bernol	33°28′26″ S, 25°36′43″ E	7	Lane Late Navels	6 m × 3 m

Table 2: Details of Cryptogran™ applications with and without molasses in field trials conducted against *T. leucotreta* in citrus orchards.

Trial	Treatments (application rate/100 l)	Volume applied per tree (l)	Application date (time)	Weeks evaluated
1	Untreated control Cryptogran™ (10 ml) Cryptogran™ (10 ml) + molasses (250 ml) + [1]Break-Thru S240 (5 ml)	21.9	10 December 2007	7
2	Untreated control Cryptogran™ (10 ml) Cryptogran™ (10 ml) + molasses (250 ml) + Break-Thru S240 (5 ml)	24.0	19 December 2011	10
3	Untreated control Cryptogran™ (10 ml) Cryptogran™ (10 ml) + molasses (500 ml)	14.0	09 February 2005	3
4	Untreated control Cryptogran™ (10 ml) Cryptogran™ (10 ml) + Voermol molasses (250 ml) + [2]Agral 90 (18 ml) Cryptogran™ (10 ml) + Molatek molasses (250 ml) + Agral 90 (18 ml)	21.9	10 December 2008	6
5	Untreated control Cryptogran™ (10 ml) Cryptogran™ (10 ml) + molasses (250 ml) + Break-Thru S240 (5 ml) Cryptogran™ (10 ml) + molasses (225 g) + Break-Thru S240 (5 ml)	10.7	07 December 2010	9
6	Untreated control Cryptogran™ (10 ml) Cryptogran™ (10 ml) + molasses (250 ml) + Break-Thru S240 (5 ml) Cryptogran™ (10 ml) + molasses (225 g) + Break-Thru S240 (5 ml)	15.0	04 May 2011	8

[1]surfactant: polyether trisiloxane (Evonik Africa, South Africa); [2]surfactant: alkylated phenol-ethylene oxide (Plaaskem, South Africa).

For the field experiments, the mean number of infested fruit per tree per week between treatments was compared using a GLM, specified using a Gaussian distribution and an identity link function. Treatment application was specified as the fixed categorical effect in all trials and the fixed effect parameter significance and pairwise comparisons, with Bonferroni correction, were computed as described above ($p < 0.05$). It should be noted that data related to the untreated control and Cryptogran™ only treatments for trials 1, 4 and 5 has been previously reported in Moore et al. (2015b). In addition, the performance of Molatek molasses and liquid molasses in trials 4 and 5, respectively have also been reported in Moore et al. (2015b). Their inclusion was necessary for comparative purposes of other treatments applied at the same time. All data were analysed using the statistical software R version 4.2.2 (R Core Team, 2022).

RESULTS

Effect of molasses on neonate behaviour

The number of larvae ($n = 36$) recorded penetrating the fruit was found to be significantly different between the control and two molasses treatments ($\chi^2 = 8.7248$, df = 2, $p = 0.01275$). Significantly more larvae penetrated the control fruit (21) than the fruit treated with 0.50% molasses (9) (z-value = 2.801, $p = 0.0153$). Although statistically not significant, fewer larvae (13) also penetrated fruit treated with 0.25% molasses than the control fruit (z-value = 1.872, $p = 0.1835$). The number of larvae penetrating fruit treated with 0.50% molasses and 0.25% molasses was similar (z-value = 1.019, $p = 0.9246$).

The mean total distance travelled by a larva on the control fruit and molasses-treated fruit was significantly different ($\chi^2 = 26.52$, df = 2, $p < 0.0001$). On average, larvae in the control treatment travelled a total distance of 13.77 mm within the 1 h observation period, whilst larvae exposed to fruit treated with 0.25% and 0.50% molasses travelled 3.64 mm and 6.41 mm less, respectively. Statistically, this was significant (0.25% molasses: t-value = 2.448, $p = 0.0482$; 0.50% molasses: t-value = 4.798, $p < 0.0001$). Larvae also spent significantly less time walking around the surface of the fruit treated with the higher molasses concentration (0.50%) than on fruit treated with 0.25% molasses (t-value = 2.513, $p = 0.0406$) (Figure 1).

Efficacy of Cryptogran™ with and without molasses against *T. leucotreta* in field trials

Evident across all field trials is the improved performance of Cryptogran™ when used with molasses (Figure 2). Alone, Cryptogran™ reduced the number of *T. leucotreta* infested fruit by between 27.9% and 47.9%, whilst Cryptogran™ with molasses decreased this even further, with reductions in infested fruit ranging between 48.6% and 82.2%, relative to the untreated control. Significant differences were reported between treatments in all trials (trial 1: $\chi^2 = 29.767$, df = 2, $p < 0.0001$; trial 2: $\chi^2 = 8.424$, df = 2, $p = 0.014$; trial 3: $\chi^2 = 14.800$, df = 2, $p < 0.0001$; trial 4: $\chi^2 = 23.880$, df = 3, $p < 0.0001$; trial 5: $\chi^2 = 9.155$, df = 3, $p = 0.027$; trial 6: $\chi^2 = 31.480$, df = 3, $p < 0.0001$).

In trial 1, the addition of 0.25% molasses with Cryptogran™ recorded significantly less *T. leucotreta* infested fruit per tree compared to the untreated control (t-value = –1.967, $p = 0.0001$) and Cryptogran™ only treatment (t-value = 3.424, $p = 0.0091$). The addition of molasses resulted in a further 52% reduction in *T. leucotreta* infestation per fruit per tree relative to the Cryptogran™ only treatment. In trial 2, only Cryptogran™ with

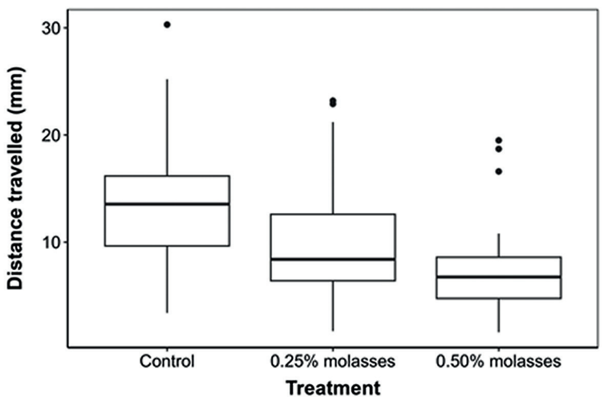

Figure 1: The median total distance travelled for a single larva on the exterior surface of a Navel orange submerged in two different concentrations of molasses or water (control) over a 1 h observation period. Boxes represent the interquartile range, whiskers the minimum and maximum range and black circles the outliers. Different letters represent significantly different results between treatments ($p < 0.05$).

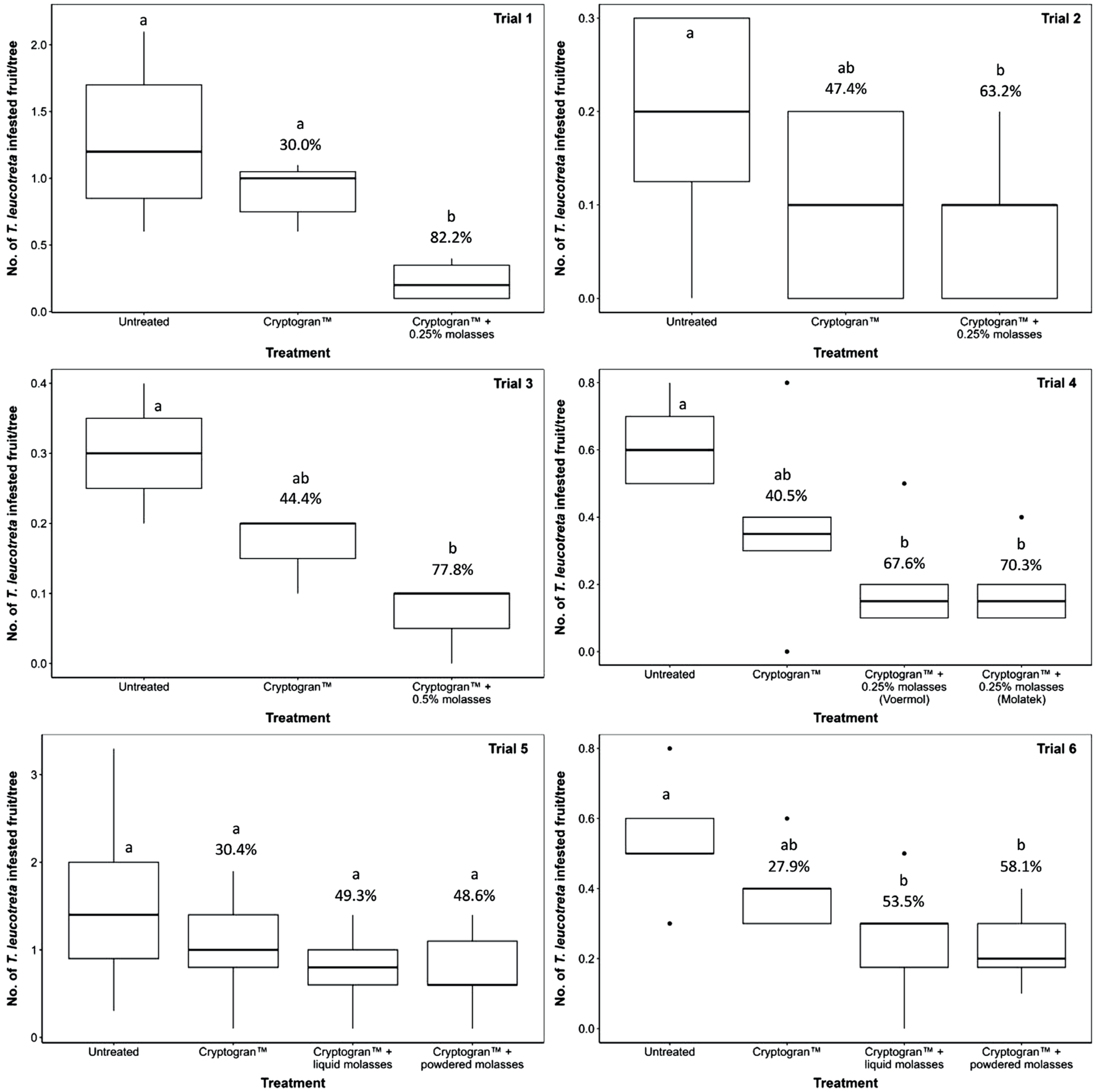

Figure 2: The median number of infested *T. leucotreta* fruit per tree for all six field trials comparing the efficacy of Cryptogran™ application with and without molasses. Boxes represent the interquartile range, whiskers the minimum and maximum range and black circles the outliers. Different letters represent significantly different results between treatments (p < 0.05). Percentages above the boxes represent the reduction in infestation relative to the untreated control.

0.25% molasses recorded significantly less infested fruit per tree compared to the untreated control (t-value = –2.789, p = 0.0287). However, the addition of molasses reduced the number of infested fruit per tree further by approximately 16% compared to the Cryptogran™ only treatment. Statistically this was not significant (t-value = 0.679, p = 1.0000). In trial 3, the addition of 0.50% molasses with Cryptogran™ recorded significantly less infested fruit per tree compared to the untreated control (t-value = –3.834, p = 0.0259). Although statistically not significant (t-value = 1.643, p = 0.4544), the addition of molasses further reduced the number of *T. leucotreta* infested fruit per tree by approximately 35% compared to Cryptogran™ alone. In trial 4, both brands of molasses improved the performance of Cryptogran™ similarly (t-value = –0.165, p = 1.000), and resulted in significantly less infested fruit per tree recorded compared to the untreated control (Voermolas: t-value = –4.132, p = 0.0031; Molatek: t-value = –4.298, p = 0.0021). Compared to the Cryptogran™ only treatment, approximately 25% less infested fruit per tree was recorded when molasses was added. In trial 5, the powdered molasses and liquid molasses performed similarly (t-value = –0.039, p = 1.000). Although statistically not significant, approximately 49% less *T. leucotreta* infested fruit per tree was recorded in the molasses treatments relative to the untreated control (liquid: t-value = –2.637, p = 0.0769; powdered: t-value = –2.598, p = 0.0844). Compared to the Cryptogran™ only treatment, the addition of molasses improved the reduction in infestation by approximately 18%. This was statistically not significant (liquid: t-value = 1.008, p = 1.000; powdered: t-value = 0.969, p = 1.000). In trial 6, again the liquid molasses and powdered molasses performed similarly (t-value = 0.398, p = 1.000) and recorded significantly less infested fruit per tree compared to the untreated control (liquid: t-value = –4.574, p = 0.0005; powdered: t-value = –4.972, p = 0.0002). Compared to the Cryptogran™ only treatment, approximately 26% and 30% less infested fruit per tree was recorded when either the liquid or powdered molasses was added, respectively. This was statistically not significant (liquid: t-value = 2.188, p = 0.2233; powdered: t-value = 2.585, p = 0.0914).

DISCUSSION

In the laboratory study, an increase in molasses concentration resulted in a decrease in the distance the larva travelled across the fruit surface. This relatively stationary behaviour in the presence of molasses, indicates that first instars began feeding shortly after hatching, which did not happen in the absence of molasses. Without molasses, larvae appear more prone to wandering in search of a suitable entry point before beginning to feed. Therefore, when exposed to molasses, larvae are more likely to ingest the number of occlusion bodies (OBs) required to induce mortality and will do so quicker than in the absence of molasses. Thus, not only is virus-induced mortality likely to be higher in the presence of molasses, but there may be more time for the virus to induce death before noticeable damage on the fruit occurs, preventing a loss in yield and fruit quality. A faster uptake may also ensure that more viable OBs are ingested, as their exposure time to potentially adverse environmental conditions would be reduced. This may permit the application of CrleGV at lower rates. Indeed, Moore et al. (2015b) showed that reduced rates of CrleGV were possible without any immediate loss in efficacy, but argued that lowering the recommended dose should be discouraged as it may diminish the residual efficacy, as breakdown (predominately due to ultraviolet irradiation) to below the critical minimum level of viable OB density on the tree for optimal efficacy would be reached sooner. Although molasses has also been suggested to afford some level of UV protection to baculoviruses in the field, Burges and Jones (1998), after an extensive review of the available literature, state that this is unlikely to have a significant impact in improving baculovirus efficacy in the field. In addition, Kirkman (2007) reported that molasses did not offer any UV protection when Cryptogran™ with molasses was exposed to an artificial UV source. Thus, the improved performance of Cryptogran™ with molasses in all the field trials reported in this study is almost certainly related to the role of molasses as a phagostimulant, and therefore the application of molasses with CrleGV is unlikely to improve residual efficacy.

Also evident from the laboratory study was that alone, molasses at both 0.25% and 0.50% led to fewer larvae penetrating the orange than in the absence of molasses, within the 1 h observation period. Thus, molasses encouraged surface feeding for longer, which again would promote the increased uptake of OBs. This in turn would translate into increased efficacy, as is apparent in the field trials presented here and in Moore et al. (2015b) and likely also explains why reduced deep damage was observed by Ballard et al. (2000) when *Betabaculovirus cypomonellae* (van Oers et al. 2023), commonly referred to as the Cydia pomonella granulovirus (CpGV), was applied with molasses in apple orchards. Although all but one of the field trials in this study did not record a statistically significant reduction in efficacy when comparing results between molasses and CrleGV treated trees and CrleGV alone treated trees, the improved efficacy when molasses was included was mathematically and biologically notable across all six field trials. On average, fruit infestation was reduced by 28 percentage points with the addition of molasses relative to CrleGV only. This translated to an average real reduction in *T. leucotreta* infestation of approximately 83% when molasses was added. Furthermore, in five of the six field trials, the addition of molasses led to a significant reduction in infestation, relative to the untreated control, which was never achieved with CrleGV alone.

Cryptogran™, which is one of four products that uses the baculovirus CrleGV as the active ingredient, is effective in reducing *T. leucotreta* infestation in citrus orchards (Moore et al. 2004, 2011, 2015b). The findings of this study support the speculations made by Moore et al. (2015b) that the addition of molasses improves the performance of Cryptogran™ in the field. This was irrespective of the brand or formulation (i.e., liquid or powder). The enhanced performance of insect viruses with the addition of phagostimulants under field conditions has been reported by Sood et al. (2013) and Ballard et al. (2000), amongst others (Burges and Jones 1998; Cisneros et al. 2002; Lasa et al. 2009; Knight et al. 2015; Kour et al. 2022; van Der Merwe et al. 2023). Sood et al. (2013) linked an increase in feeding activity with an increase in larval mortality in leaf-disc bioassays, with 2% boric acid and 2% crude sugar being the most effective. These findings translated to improved performance of Pieris brassicae granulovirus (PbGV) in the field when combined with these additives, compared to PbGV applied alone against the cabbage butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae). Likewise, Ballard et al. (2000) found that the addition of 15% cane molasses combined with CpGV at 10^{12} OBs/ha gave similar control of codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) in apple orchards, compared to treatments where CpGV was applied at higher rates, namely 10^{13} and 10^{14} OBs/ha.

Using a droplet-dose bioassay technique, developed by Pereira-da-Conceicoa et al. (2012), Opoku-Debrah (2012) and Opoku-Debrah et al. (2016), determined average LD_{50} and LD_{90} values for Cryptogran™ against neonates from five different laboratory-reared populations of *T. leucotreta* to be 1.03 and 309.18 OBs/larva. Consequently, to kill 50% of larvae in the field with a Cryptogran™ spray, application must be with a concentration and coverage that will enable larvae to ingest an average of 1.03 OBs each. To achieve 90% efficacy, larvae must ingest more than 300 OBs each. Cryptogran™ (and Cryptomax™) is registered to be applied at a concentration of 5 million OBs per ml. Cunningham and Harden (1998) showed that the maximum volume of spray that can be held on a citrus leaf (and presumably fruit) surface is 2.68 µl/cm^2, which would contain 1.34×10^4 OBs. If a neonate, with a 1 mm long body and a 0.28 mm wide head capsule (Hofmeyr et al. 2016), eats a 0.5 mm^2 hole to enter a fruit, the maximum number of OBs it can ingest is 67. This is equivalent to an $LD_{83.7}$ and will thus kill an average of 84% of larvae in a population, if spray coverage is consistent and uniform across the surface of all fruit. This was determined by using mean mortalities per concentration for all five *T. leucotreta* populations (Opoku-Debrah et al. 2016), to conduct a probit analysis in R, using the drm function (drc package), from which the R code ED(res1, c(LC values)) is used, to iteratively determine the LC/LD value that would give 67. However, if the addition of molasses to the virus spray leads to greater feeding on the fruit surface, a greater ingestion of OBs and thus a higher spray efficacy can be expected. This is the explanation behind the measured superior efficacy of Cryptogran™ sprays in the field that include molasses. The addition of molasses would be even more important with the other CrleGV products, which are registered at a lower OB application rate, equating to an average of only 8.8 OBs per 0.5 mm^2, which would be an $LD_{69.3}$, calculated as described above.

In conclusion, the continuously observed enhanced efficacy of the CrleGV-based biopesticide, Cryptogran™, with molasses is likely owing to the effect that molasses has on the behaviour of first instar *T. leucotreta*, encouraging them to feed soon after hatching, and for longer before penetrating the fruit. This promotes the uptake of sufficient viral OBs to induce a high level of mortality and treatment efficacy, thus limiting further economic damage. Therefore, the recommendation to apply molasses with Cryptogran™ and other CrleGV-based biopesticides, in enhancing performance in the field, is conclusively supported and should be standard practice in the industry.

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AUTHOR CONTRIBUTIONS

Candice Coombes: writing – original draft, writing – review and editing, formal analysis; Storm Hilliar: investigation; Sean Moore: conceptualisation, writing – review and editing, investigation, supervision; Martin Hill: conceptualisation, writing – review and editing, supervision.

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