Oviposition and feeding by *Bactericera cockerelli* (Homoptera: Psyllidae) in response to a solar protectant applied to potato plants

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**Abstract**

The potato psyllid, *Bactericera cockerelli* (Sulc), is a major concern for potato growers as it vectors the pathogen responsible for “zebra chip” disease. Current management practices of potato psyllids in commercial potato (*Solanum tuberosum* L.) fields are based almost exclusively on insecticide applications with as many as 6–8 applications in individual potato fields during the growing season. As a complement, and to reduce the reliance on insecticides, it might be advantageous to apply biorational insecticides, which alter the attractiveness of potato plants to feeding and ovipositing potato psyllids. In this study, we evaluated two biorational insecticides: a limestone particle film (Purshade\(^e\)) and a plant growth regulator (prohexadione-calcium, Apogee\(^e\)), as both have documented repellency to insects when applied to crop plants. Based on experimental applications to potato plants and subsequent no-choice and choice studies with potato psyllids, prohexadione-calcium had no significant effect on leaf probing activity or oviposition. We found that limestone particle film treatment caused a small but significant reduction in oviposition between 7 and 14 days after application, while probing activity was unaffected. Examination of spectral reflectance indicated changes resulting from application of the limestone particle film. We propose that differences in reflectance may indicate changes in photosynthesis and plant physiology that subsequently alters the suitability of potato plants as hosts for potato psyllids. This interesting possibility, and the utility of limestone particle films as part of potato psyllid management strategies in potatoes is discussed.

**Keywords:**
Potato psyllids
Solar protectant
Apogee\(^e\)
Limestone particle film
Prohexadione-calcium
Purshade\(^e\)

1. Introduction

The potato psyllid (*Bactericera cockerelli*) (Sulc) (Hemiptera: Psyllidae) is a sucking insect that vectors *Candidatus Liberibacter psyllaurosus* (aka *Candidatus Liberibacter solanacearum*) (LSO), the purported cause of “zebra chip” (ZC) disease in potatoes, *Solanum tuberosum* L. (Abad et al., 2008; Crosslin and Munyaneza, 2009; Hansen et al., 2008; Liefting et al., 2008; Lin et al., 2009; Munyaneza et al., 2008, 2007a). Effects of ZC disease include: decreased yields, poor tuber quality, and a distinct striped pattern when potatoes are fried (Munyaneza et al., 2008, 2007a). As a result, ZC infected potatoes are discarded, costing the potato industry millions of dollars (Munyaneza et al., 2007a, 2007b). Thus, several major research projects (i.e. https://zebrachipscr.tamu.edu/) are being conducted to develop improved potato psyllid management strategies. The importance of ZC disease in potatoes and need for improved management strategies have further increased after this disease was recently detected in the major potato producing states, including Idaho (Crosslin et al., 2012), Washington and Oregon (Crosslin et al., 2012).

Current potato psyllid management mostly relies on applications of insecticides (Butler et al., 2011; Gharalari et al., 2009), with as many as 6–8 applications in individual potato fields during the growing season in Texas. Therefore, methods to reduce the attractiveness of potato plants to potato psyllids, may serve to complement and reduce reliance on insecticides. Such negative (repellent) changes in attractiveness can result from a tactile response, alteration of an insect pests’ visual evaluation of host plants, or through interference with the host plant physiology (change in olfactory and/or visual cues). LSO can be transmitted from potato psyllids to potato plants within six hours of exposure and feeding (Buchman...
et al., 2011). Consequently, an additional benefit of repellency would be reduced feeding and thereby possible reduction in LSO transfer to crop plants.

Various biorational insecticides have been examined for their repellency to psyllids, including: insecticidal oils (Mann et al., 2010; Yang et al., 2010), plant derived volatiles (Nehlin et al., 1994), and biopesticides such as the growth inhibitors Neemix 4.5® (4.5% azadirachtin) (Weathersbee and McKenzie, 2005) and diminiozide (Westigard et al., 1980). Hexadione-calcium (Apogee, BASF Corp., Research Triangle Park, NC) is a growth regulator that inhibits metabolism of the plant hormone gibberellin. Originally intended as a product to reduce shoot growth (Paulson et al., 2005), it has also been examined as part of integrated pest management programs. Field studies of prohexadione-calcium have shown reduced infestations of aphids (Aphis spiraecola Patch) and ‘Bartlett’ pear (Pyrus communis L.) orchards, putatively due to a reduction in new shoot production (Hall et al., 2007) and pear psyllids (C. pyri). However, these limestone particle films are also used to alter photosynthesis (Glenn and Puterka, 2010).

In this study, we applied a limestone particle film (Puterka et al., 2005; Yee, 2012). In tests of kaolin clay against citrus psyllids, Diaphorina citri (Hemiptera: Psyllidae) (Hall et al., 2007) and pear psyllids (C. pyri) (Erler and Cetin, 2007), both reduced and delayed oviposition was observed. In addition, mid-season application of kaolin clay reduced pistachio psyllid nymph densities and damage (Saour, 2005). Finally, Peng et al. (2011) found that kaolin clay influenced both oviposition and adult settling behavior of potato psyllids on tomato (Solanum lycopersicum L.) under both field and laboratory conditions.

Although not typically applied as pesticides, limestone particle films (Eclipse™ and Purshade™) are also believed to have repellency to insect pests, likely due to a tactile response (Hall et al., 2007; Yee, 2012). However, these limestone particle films are also applied to reduce sun stress, and are expected to alter photosynthesis (Glenn and Puterka, 2010).

In this study, we applied a limestone particle film (Purshade®) and the plant growth regulator prohexadione-calcium (Apogee®) to potted potato plants and used choice and no-choice bioassays of excised leaves to assess affects on oviposition and probing activity by potato psyllids. The results are discussed in the context of developing improved management strategies for this important pest.

2. Materials and methods

2.1. Insects and plants

Potato psyllids used in this study originated from a laboratory culture maintained at the Texas AgriLife Research and Extension Center in Lubbock, Texas. Cultures were maintained on tomato plants at 25 °C and 30–60% RH with an artificial light photoperiod of 14:10 (L:D) (Ecoulux®, F40PL/AQ-ECO, USA). Potato plants (Red LaSoda) were grown under greenhouse conditions in two-gallon plastic pots (Nursery Supplies Inc., blow-molded containers) in Metro-Mix 900 Professional Growing Mix (Sun Gro Horticulture), watered ad libitum and fertilized weekly with Peter’s Professional 20–20–20 growing media. To protect from insect infestation, plants were maintained in mesh tents (60 cm tall, with a 60 × 60 cm base) (BugDorm®, MegaView Science Co., Taiwan) until used in bioassays. After treatments with limestone particle film or prohexadione-calcium, the potato plants were maintained, along with untreated controls, in the insect facility at room temperature (approximately 25 °C) and humidity with a 14:10 (L:D) cycle provided by artificial lighting, and watered ad libitum.

2.2. Limestone particle film and prohexadione-calcium applications

Both the limestone particle film and prohexadione-calcium were applied to individual potted plants using a CO2 backpack sprayer (Bellspray, Inc., R&D Sprayers). Limestone particle film was applied with ConeJet® Visiflo® hollow cone spray nozzles (TXVS-3) at 50.5 L/ha (per. manufacturer recommendation). Prohexadione-calcium was applied using Teejet DG 8000VS spray nozzles at 175.8 L/ha. The adjuvant, Induce®, was used with prohexadione-calcium treatments at a rate of 2.5 ml/L.

Tests of limestone particle film consisted of three treatments: 1) untreated control (water), 2) 10% concentration of limestone particle film to water and 3) 20% concentration of limestone particle film in water. Bioassays of limestone particle film were conducted in two sets. In the first set, application was made 28 days after planting (DAP) (denoted “round 1”), second set application occurred 33 DAP (denoted “round 2”). Prohexadione-calcium was examined following application at two different growth stages. The “early” growth stage application was 21 DAP and the “late” growth stage was 35 DAP. Prohexadione-calcium treatments consisted of: 1) untreated control, 2) 1.1 g/L prohexadione-calcium, and 3) 10.1 g/L prohexadione-calcium.

2.3. Bioassays

For both biorational insecticides examined, no-choice bioassays were conducted in arenas consisting of a 1-L Mason jar covered with a piece of white chiffon fabric approximately 12 cm square. Each arena contained a 40 ml plastic vial secured to the metal disk lid of a Mason jar, filled with tap water, and covered with parafilm. Excised potato leaves with five leaflets were collected from the top third of potato plants; four leaflets were removed leaving the terminal leaflet and petiole was inserted into the vial. In testing the limestone particle film, we also conducted three-choice bioassays with a similar arena that replaced the plastic vial with three 8 ml glass vials. Leaflets were prepared as in no-choice bioassays with one leaflet of each treatment per vial.

In all bioassays, seven (limestone particle film) or five (prohexadione-calcium) unsexed adult (post-teneral) psyllids were used. It was assumed that post-teneral females were both mature and mated; however, neither the exact age nor the sex ratio of psyllids used was determined. All bioassays lasted 72 h. Following exposure, both live and dead psyllids were recovered and numbers of eggs on the leaflets were counted. In addition, leaflets were subjected to McElroy’s staining process (Backus et al., 1988) to count stylet sheaths along the mid rib, which was used as an indicator of probing activity.

Regarding limestone particle film bioassays, both no-choice and 3-choice bioassays were conducted at four time points (immediately prior to application, 7, 14 and 21 days after spray, denoted “DAS”). Prohexadione-calcium no-choice bioassays were also conducted at four time points (immediately prior to application, 14, 28, 42 DAS) after either an early or late stage application.
2.4. Reflectance analysis of leaves from limestone particle film bioassays

Since the limestone particle film is specifically designed for use as a “solar protectant”, it is expected that its application would influence plant physiology and rates of photosynthesis. Moreover, psyllids have been shown to respond differentially to visual cues at different wavelengths (Al-Jabr and Cranshaw, 2007; Wenniger et al., 2009). Therefore, we examined reflectance of bioassayed leaves to determine if there might be associations between potato psyllid behavior and reflectance profiles acquired from potato leaves used in bioassays. We used a hyperspectral push broom spectral camera (PIKA II, Resonon Inc., Bozeman, MT) to acquire reflectance data in 160 spectral bands from 405 to 907 nm from each bioassayed potato leaflet. The objective lens had a 35 mm focal length (maximum aperture of F1.4) and was optimized for the visible and NIR spectra. The main specifications of the spectral camera are as follows: interface, Firewire (IEEE 1394b), output, digital (12 bit), 160 bands (spectral) by 640 pixels (spatial), angular field of view, 7°, and spectral resolution, <3 nm. All hyperspectral images were collected with artificial lighting from 15 W, 12 V light bulbs mounted in two angled rows, one on either side of the lens, with three bulbs in each row. A voltage stabilizer (Tripp-Lite, PR-7b, www.triplite.com) powered the lighting. A piece of white Teflon was used for white calibration, and “relative reflectance” refers to proportional reflectance compared to that obtained from Teflon. Consequently, relative reflectance values ranged from 0 to 1.

2.5. Statistical analysis

All statistical analyses were conducted using PC-SAS 9.2 (Cary, NC). Mortality in no-choice bioassays was examined via linear mixed models (PROC GLIMMIX; LOG-Link function) with a Poisson distribution. The model contained the fixed factors DAS and treatment, and the interaction term for DAS and treatment; a random effect term was included for differences between rounds. Counts of eggs and probing events in no-choice bioassays were examined using similar linear mixed models with a Poisson distribution (eggs) or negative-binomial distribution (probing), chosen based on Akaike Information Criterion (Bolker et al., 2009). The interaction of treatment and DAS was not significant for probing and was excluded from the final model. In all analyses, denominator degrees of freedom were calculated using Satterthwaite’s method (Satterthwaite, 1946). When appropriate, significant effects were further examined via least-square means adjusted using the Tukey-Kramer method. Oviposition and probing data from three-choice bioassays were examined using chi-square tests on eggs or negative-binomial distribution (probing), chosen based on Akaike Information Criterion. The interaction of treatment and DAS was not significant for probing and was excluded from the final model. General linear modeling (PROC MIXED) was used to analyze the difference in reflectance data from untreated leaflets compared to those treated with limestone particle film.

3. Results

3.1. Prohexadione-calcium no-choice bioassays

Neither late nor early applications of prohexadione-calcium influenced oviposition (Early: $F_{2, 116} = 1.11, P = 0.34$; Late: $F_{2, 78} = 1.32, P = 0.27$) or probing (Early: $F_{2, 116} = 0.27, P = 0.76$; Late: $F_{2, 78} = 2.78, P = 0.1$). In absence of significant effects of prohexadione-calcium treatment in no-choice bioassays, it was considered of limited value to conduct three-choice bioassays.

3.2. Limestone particle film no-choice bioassays

There was no effect of treatment ($F_{2, 129.2} = 1.54, P = 0.2$) or DAS ($F_{3, 125.7} = 1.48, P = 0.2$) on psyllid mortality. Overall, oviposition differed significantly with treatment ($F_{2, 162} = 100.50, P < 0.0001$) and DAS ($F_{3, 162} = 130.13, P < 0.001$) (Fig. 1). Post-hoc analyses revealed no differences among treatments in the pre-application time point. Following application, both concentrations of limestone particle film caused a significant reduction in oviposition relative to the untreated control. However, there was no difference between the 10% and 20% concentrations. The post application effect of DAS was due exclusively to the 14 DAS time point, which was significantly different from both the baseline and the 7 DAS time points, but also was subject to greater overall oviposition than other time-points (Fig. 1). There were no significant effects of treatment ($F_{2, 159} = 0.89, ns$) or DAS on probing activity ($F_{3, 159} = 0.00, ns$).

3.3. Limestone film three-choice bioassays

In three-choice bioassays, there were significant differences in oviposition among treatments ($F_{2, 155} = 106.32, P < 0.001$) and with respect to DAS ($F_{3, 155} = 40.31, P < 0.001$) (Fig. 2). However, there was also a significant interaction of treatment and DAS ($F_{6, 155} = 42.58, P < 0.001$) and no consistent or distinguishable trends with respect to treatment or to DAS. Furthermore, there was no evidence of an oviposition preference within bioassays at any time point. Eggs were distributed evenly among leaves and did not differ from an expectation of random distribution (Baseline: $\chi^2 = 1.43, p = 0.4$; 7 DAS: $\chi^2 = 1.43, p = 0.4$; 14 DAS: $\chi^2 = 0.61, p = 0.7$; 21 DAS: $\chi^2 = 1.53, p = 0.49$). Overall, this suggests that significant treatment effects resulted from the combination of DAS and treatment (the interaction) rather than a distinct effect of the limestone particle film.

Fig. 3 shows the average reflectance response to limestone treatment. That is, we divided the average reflectance profile from untreated leaflets with that acquired from limestone treated leaflets, so a reflectance value: 1) close to 1 would suggest a negligible reflectance response to limestone treatment, 2) $>1$ would suggest that limestone treatment caused a decrease in reflectance, and 3) $<1$ would suggest that limestone treatment caused an increase in reflectance. Fig. 3 shows that, as expected, there was a negligible reflectance response at baseline (prior to limestone applications).
activity and oviposition of potato psyllids. Overall, prohexadione-calcium had no significant effect of on potato psyllid behavior or mortality. However, the examined limestone particle film (Purshade®) significantly reduced potato psyllid oviposition, and this may be attributed to a significant change in potato plant physiology, which was indicated based on reflectance profile analysis. Although, changes in reflectance profiles also indicate a change to the limestone material itself overtime.

Neither the limestone particle film nor prohexadione-calcium treated leaflets were associated with greater mortality than untreated leaflets. This contradicts studies of D. citri (Hall et al., 2007) and Psyllacaco psyllapyri L. (Puterka et al., 2005) where kaolin treatment resulted in greater mortality. This kaolin clay induced psyllid mortality was partially attributed to its desiccating properties (Glenn and Puterka, 2010; Glenn et al., 1999; Puterka et al., 2000; Yee, 2012), but our data do not indicate that limestone particle films share this property. This is somewhat reasonable as kaolin and limestone particle films differ in both composition and shape (Tegethoff et al., 2001; Yee, 2012), and Yee (2012) has suggested that such differences may be responsible for differential results observed when bioresponses of Western cherry fruit flies [Rhagoletis indifferens Curran (Diptera, Tephritidae)] were examined.

We found no effect of prohexadione-calcium on potato psyllid oviposition or probing. This contrasts findings in pear psyllids (P. psyllapyri) (Paulson et al., 2005; Shaltiel-Harpaz et al., 2010) and citrus psyllid (Tsagkarakis et al., 2012) in which oviposition behavior was reduced. However, these studies involved tests on fruit trees where new growth is required for aphid and psyllid reproduction, but only occurs as shoots. In contrast, potato psyllids are capable of feeding on nearly all potato tissue including leaves and stems. Further, tests in citrus demonstrated an effect on the timing of oviposition in addition to the number of eggs. Our study did not directly consider the timing of oviposition; eggs were only counted at the end of the assay period. However, we did examine multiple time points following both late and early stage late applications, and found no significant effects.

In no-choice bioassays, the limestone particle film product Purshade® reduced oviposition by 50–70%, but the effect was only observed at seven and 14 DAS. Another particle film product, kaolin clay, has been demonstrated to reduce oviposition in pear psyllids (Puterka et al., 2005), citrus psyllids (Hall et al., 2007), root weevils (Diaprepes abbreviates L.) (Lapointe, 2000; Lapointe et al., 2006), and potato psyllids (Peng et al., 2010). Similarly, previous studies involving limestone particle film (Purshade®) formulations (i.e. Yee, 2012) have demonstrated a slight reduction in oviposition of Western cherry fruit flies.

The limestone particle film had no significant effects on probing activity by potato psyllids. Limited or negligible effect on probing activity is similar to responses of potato psyllids to kaolin clay (Liu and Trumble, 2004). However, studies with kaolin clay have demonstrated reduced feeding or feeding damage by root weevils (Lapointe, 2000), onion thrips (Thrips tabaci Lindeman) (Larentzaki et al., 2008), and codling moths (Cydia pomonella L.) (Glenn and Puterka, 2010). It is worth noting, though, that our bioassays were conducted on excised leaves, and while the number of probing events does not differ between excised and in-tact leaflets (unpublished data), excision may have changed pressure in the phloem and possibly psyllid feeding/probing behavior in a manner not reflected in number of stylet sheaths.

It is interesting that the effect of the limestone particle film on oviposition was not immediate. Typically, particle films function as tactile repellents and/or desiccants (Glenn and Puterka, 2010; Glenn et al., 1999; Puterka et al., 2000; Yee, 2012). These mechanisms should have a near immediate effect, and should be effective in repelling or deterring oviposition.
as long as an adequate number of particles remained on the plant. Conversely, fresh tissue should be uncoated and unaffected. In this study, leaflets for bioassays were specifically chosen from segments of the plant that were treated, and so should have been covered with the material. It is thus unlikely that the limestone particle film's effect on oviposition results from irritation and/or tactile repellency. Instead, it appears that the limestone film (Purshade®) alters plant physiology in a manner that alters reflectance and psyllid host preference. This is suggested by the finding that reflectance at 550 nm is altered by the limestone particle film. The 550 nm band is in the spectral range associated with photosynthesis (Carter, 1993) and 550 has been shown to indicate some stress in D. citri (Wenninger et al., 2009). Two species of psyllids (Ctenarytaina eucalypti Maskell and C. spatulata Taylor) have been shown to respond differently to sticky cards of different colors, preferring yellow cards to those of other colors (Brennan and Weinbaum, 2001). Similar results have been observed for potato psyllids, which preferred neon-green, neon-orange and standard yellow eucalypti. In summary, prohexadione-calcium had no effect on oviposition or probing activity. The limestone solar protectant (Purshade®) reduced potato psyllid oviposition, altered reflectance, but had no effects on probing or on mortality. Potatoes in Texas are subject to multiple insecticide applications, and are often grown under limited water. Therefore, in light of the threat potato psyllids presents to potato crops and the limited market options, Purshade® deserves further study for its potential use in potato, particularly since there may be additional benefits to their application beyond insect repellency, such as increased water efficiency.

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References


Al-Jabi, A.M., Cranshaw, W.S., 2007. Trapping tomato psyllid, Purshade alters plant physiology in a manner that alters repsyllid host preference. This is suggested by the thesis (Carter, 1993) and 550 has been shown to indicate some stress in D. citri (Wenninger et al., 2009). Two species of psyllids (Ctenarytaina eucalypti Maskell and C. spatulata Taylor) have been shown to respond differently to sticky cards of different colors, preferring yellow cards to those of other colors (Brennan and Weinbaum, 2001). Similar results have been observed for potato psyllids, which preferred neon-green, neon-orange and standard yellow eucalypti. In summary, prohexadione-calcium had no effect on oviposition or probing activity. The limestone solar protectant (Purshade®) reduced potato psyllid oviposition, altered reflectance, but had no effects on probing or on mortality. Potatoes in Texas are subject to multiple insecticide applications, and are often grown under limited water. Therefore, in light of the threat potato psyllids presents to potato crops and the limited market options, Purshade® deserves further study for its potential use in potato, particularly since there may be additional benefits to their application beyond insect repellency, such as increased water efficiency.

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References


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