

**Mortality and behavioral responses of adult potato psyllids,
Bactericera cockerelli (Hemiptera: Psyllidae), to insecticides**

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Abstract

It is unarguably important that insecticides suppress target pest populations, but the primary goal for successful management of vectors of plant diseases should be to minimize the feeding by all life stages on the crop. Thus, it is paramount that comprehensive testing of insecticides includes assessments of how they affect probing and feeding behavior and oviposition. In this laboratory study, we counted number of stylet sheaths (indication of probing and feeding), total oviposition, and mortality of adult potato psyllids, *Bactericera cockerelli* (Sulc) (Hemiptera: Psyllidae) when exposed to potato leaves treated with abamectin, flonicamid, novaluron, distilled water, and a surfactant. Treatments with abamectin, flonicamid and novaluron caused significant decreases in both oviposition and numbers of stylet sheaths. Choice test was included to simulate incomplete leaf coverage, and the results highlighted the importance of including the effect of canopy coverage (application method) in performance analysis of insecticides.

Key words: Integrated pest management, insecticide performance, stylet sheaths, oviposition

چکیده

بی‌شک حشره‌کش‌ها منجر به کاهش جمعیت آفت هدف می‌شوند، ولی هدف اصلی در مدیریت موفق ناقلین بیماری‌های گیاهی باید به حداقل رساندن میزان تغذیه آفت هدف در تمام مراحل زیستی باشد. بنابراین، آزمایش جامع حشره‌کش‌ها بر روی آفت هدف باید شامل

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بررسی تاثیر این حشره‌کش‌ها بر روی میزان تخم‌ریزی و رفتار تغذیه‌ای آن‌ها نیز باشد. در این تحقیق آزمایشگاهی، تعداد غلاف‌های استایلتی (که نشانگر میزان تغذیه و پروب بود)، میزان تخم‌ریزی و مرگ و میر حشرات کامل پس‌یل سیب‌زمینی، بر روی برگ‌های سیب‌زمینی تیمار شده با سموم آبامکتین، فلونیکامید، نووالرون، آب مقطر و سورفکتانت بررسی شد. در آزمون انتخاب (بین برگ‌های تیمار شده و برگ‌های شاهد میزان مرگ و میر کاهش داشت. در آزمون‌های انجام شده با دوز توصیه شده مزرعه‌ای سموم آبامکتین، فلونیکامید و نووالرون باعث کاهش میزان تخم‌ریزی و تعداد غلاف‌های استایلتی شدند نتایج تحقیق اهمیت بررسی تاثیر پوشش اندام هوایی (روش کاربرد سموم) در آزمون‌های کارایی تاثیرات سموم را نشان می‌دهد*.

واژه‌های کلیدی: مدیریت تلفیقی آفات، کارایی حشره‌کش‌ها، غلاف استایلتی، تخم‌ریزی.

Introduction

Potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Psyllidae), is a key pest of Solanaceous crops (Daniel 1934, List 1939, Swenk and Tate 1940, Wallis 1951, Liu and Trumble 2004). It has been known for decades that feeding of potato psyllids causes 'psyllid yellows' (Pletsch 1947, Carter 1950, Richards 1928, List 1939, Arslan et al. 1985). Recently, it has been documented that feeding by this pest also can result in transmission of "Zebra Chip" (ZC) disease, which has been associated with the pathogen, *Candidatus Liberibacter* (Munyaneza et al. 2007a,b, Hansen et al. 2008, Liefing et al. 2008, Munyaneza et al. 2008, Abad et al. 2009, Crosslin and Munyaneza 2009, Lin et al. 2009). ZC-infected tubers develop a striped pattern of necrosis that becomes particularly prominent after frying (Munyaneza et al. 2007a, b, 2008). Insecticides constitute the main component of current control strategies for potato psyllids in potatoes, and several recent studies have been conducted to assess their performance in terms of mortality, residual effect under field conditions, effect on behavioral patterns, and their repellency (Liu and Trumble 2004, Liu and Trumble 2005, Gharalari et al. 2009, Walker and Berry 2009). In other words, it is paramount to not only focus on immediate knock-down of insecticides but also to evaluate performance characteristics with

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profound implications for development of successful resistance management strategies and optimized spraying techniques. For instance, if an insecticide relying on contact or feeding to be effective is repellent to target insects, then concerns about incomplete leaf coverage and low canopy penetration are markedly increased, as target insects will likely avoid treated portions of the canopy and therefore will not be exposed to a lethal dosage of active ingredient. In this context, it is important to mention that pesticide applications in dense canopies, like potatoes and soybeans, have been shown only to achieve 0.2-10% coverage in the bottom portion of the canopy (Wolf and Daggupati 2009). Regarding development of management strategies of insect-vectored plant diseases, it is important to remember that while killing the vector is important, the primary objective should be to suppress its probing and feeding on the target crop.

In this study, we presented a comprehensive approach to insecticide performance analysis in the laboratory with adult potato psyllid and potato leaves treated with different insecticides, in which we investigated mortality, number of stylet sheaths (indication of probing and feeding events), total oviposition, and incomplete canopy coverage. This study is part of an on-going project on development and implementation of feasible and effective management strategies against potato psyllid in the potato fields.

Materials and Methods

Insect colony: Potato psyllid was originally obtained from Texas AgriLife Research Station (Weslaco, TX, USA) and USDA-ARS, Yakima Agricultural Research Laboratory (Wapato, WA, USA); but had been reared at Texas AgriLife Research and Extension Center (Lubbock, TX, USA) for over 12 months. The potato psyllid colony was reared inside mesh tents (60 cm tall, with a 60×60 cm base) (BugDorm[®], MegaView Science Co., Taiwan) on tomato plants in an insect rearing room at 25°C and 30-60% RH with a photoperiod of 16:8 (L:D) provided by artificial lighting (Ecolux[®], F40PL/AQ-ECO, USA).

Insecticide screening: A spraying device, denoted the “bottle sprayer” (Nansen et al. 2010), was used to apply controlled dosages to individual potato leaves. In brief, the bottle sprayer consists of an airbrush (Master Airbrush model G22, TCP Global[®], San Diego, CA) mounted inside a 2-liter soda bottle, spraying at a fixed distance (20 cm). A compressed air cylinder with corresponding regulator (JO Series, Compressed Gas Regulator, Chudnow, MFG. CO. Inc., Oceanside, NY) and connecting tubes provide a controlled and consistent pressure. Based on a priori calibration, we used a gas pressure of 10 psi and sprayed for 1

second (measured with a metronome). The following insecticides were applied in a water formulation equivalent to 187 L / ha (20 gallons per acre) and surfactant (Activator 90 at a rate of 420 g/ha)(Loveland Products Inc. Greeley, CO), and amounts of active ingredient are listed in brackets: abamectin (710 g/ha) (Agri-Mek[®] 0.15 EC miticide/insecticide, Syngenta Crop Protection, Greensboro, NC), flonicamid (199 g/ha) (Beleaf[®] 50 SG, FMC Corporation, Agriculture Products Group, 1735 Market Street, Philadelphia, PA), and novaluron (Rimon[®] 0.83 EC, Chemtura USA Corporation, Middlebury, CA).

All potato leaflets except the terminal leaflet were excised, and insecticides were applied to both sides of the potato leaflets. As controls, we included untreated potato leaves (negative control) and leaves treated with water, and surfactant in a water formulation as positive controls. All treatments were repeated 10 times. In a no-choice test, a single leaflet was placed with the petiole in a 5-ml glass vial, filled with distilled water, which was glued to the bottom of a Mason jar (16.75 cm tall and 9.50 cm diameter). Five unsexed adult potato psyllids were released into each Mason jar, which was covered with a fine mesh. All tests were conducted for 7 days, and Mason jars were maintained under the same environmental conditions as the potato psyllid culture (see above). Choice test was similar to the no-choice test, however, in each Mason jar there were two glass vials, one containing treated leaflet while the other contained the untreated leaflet.

Performance evaluations: Mortality of adult potato psyllids was observed 1, 2, 5, and 6 days after treatment (DAT), after which leaflets were collected and treated according to the McBryde staining method (McBryde 1936) to count the number of stylet sheaths and eggs. The number of stylet sheaths corresponds to the combined probing and feeding events by potato psyllids, and it was used as an indicator for the likelihood of pathogen transfer to potato leaves. In brief, McBryde staining consists of placing potato leaves for 24 h in McBryde's stain (0.2% acid fuchsin in 95% ethanol and glacial acetic acid (1:1)), followed by heating at 65 °C while they were in a clearing agent [distilled water, 99% glycerine, and 85% lactic acid (1:1:1)] (Backus et al. 1988). The eggs and stylet sheaths (Fig. 1) were observed under a stereomicroscope at a magnification of $\times 45$.

As part of the performance evaluation of insecticides applied at the labeled field rate, we conducted a choice test, in which potato psyllids were offered a choice between one treated leaf and one untreated potato leaf. These choice tests were included to simulate partial canopy coverage during field applications. The experimental protocol for these choice tests was identical to that of the no-choice test.

Statistical analysis: We used logistic regression models in PC-SAS 9.1 (SAS Institute Inc., Cary, NC) (PROC GENMOD: binomial distribution, logit link function) to compare adult potato psyllids mortality rates among treatments. In these regression models, numbers of dead adult potato psyllids were events and total numbers of adult potato psyllids were trials. If a model was converged and showed differences among treatments, it was followed by a two-by-two comparison of treatments (CONTRAST statement). A mixed model analysis of variance (PROC MIXED) was used to compare the egg numbers and stylet sheath numbers among treatments. The Satterthwaite method was applied for determining the denominator degrees of freedom (df). Comparisons among treatments were made using the Tukey-Kramer test where analysis of variance showed significant differences among means. The oviposition and feeding rates of adult potato psyllids were subjected to parametric t-test (PROC TTEST) in choice tests. Spearman correlation coefficient analysis (PROC CORR) was used to examine correlations between mortality rate, total oviposition, and number of stylet sheaths. In all tests, $P < 0.05$ indicated significant differences in mean values of treatments. To get more information about the above statistical methods, please refer to Gharalari (2008) and Gharalari et al. (2009).

Results and Discussion

Residual mortality effect at labeled field rates: Mortality of adult potato psyllids was significantly different among treatments at each of the observation days (1, 2, 5 and 6 DAT) (Logistic Regression: $98.23 < \chi^2_5 < 168.61$, $0.0001 < P < 0.0358$) (Fig. 2). Across days of observation, there was a consistent pattern with abamectin treatment providing highest mortality followed by flonicamid and novaluron. We observed an increase in mortality rate over time for all three insecticide treatments (Logistic Regression: $10.34 < \chi^2_3 < 25.12$, $0.0184 < P < 0.0286$). On all observation days, the lowest mortality rates were observed on untreated, water treated and surfactant treated leaves, which were not significantly different from each other ($P > 0.05$).

Mortality in choice test: The mortality of adult potato psyllids was significantly different among treatments at each of the observation days (1, 2, 5 and 6 DAT) (Logistic Regression: $53.21 < \chi^2_4 < 93.68$, $0.0001 < P < 0.0215$) (Fig. 3). On all observation days, the sequence of mortality rates among treatments was similar with highest mortality on tests involving abamectin, and lowest mortality on positive controls (water and surfactant).

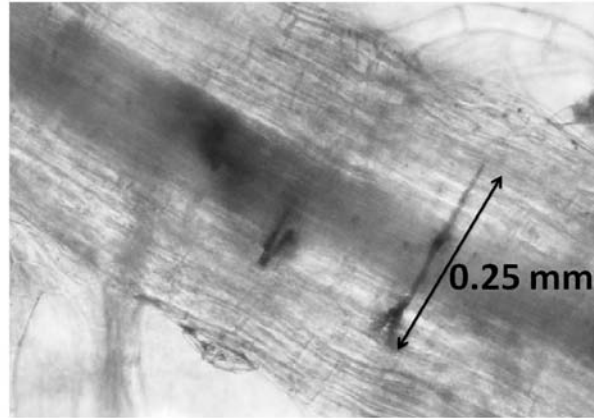


Fig. 1. An image of a stylet sheath of adult potato psyllids, *Bactericera cockerelli*, in a vein of a potato leaflet

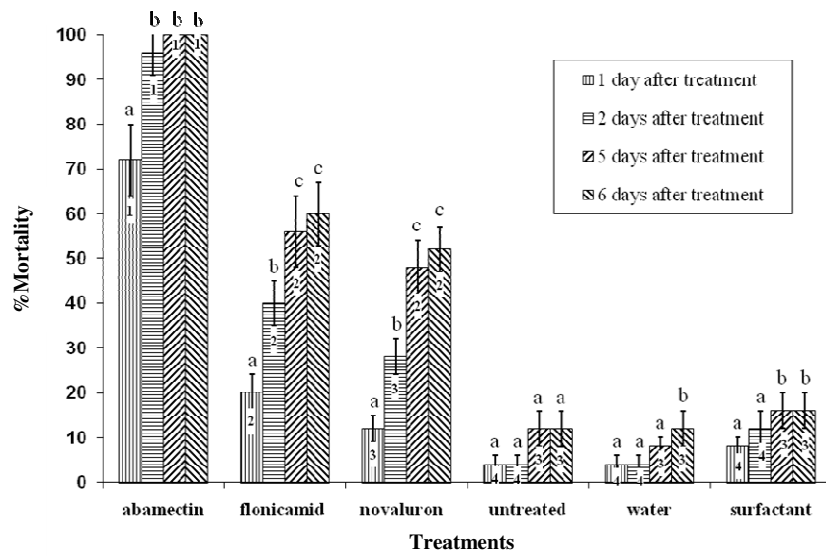


Fig. 2. Mean (\pm SE) percentage of mortality rates of adult potato psyllids, *B. cockerelli*, over 1 week, when exposed to different treatments on potato leaves in the laboratory. Mean mortality rates followed by the same letter inside a 'treatment' category or followed by the same number inside a 'day after treatment' category are not significantly different ($P < 0.05$)

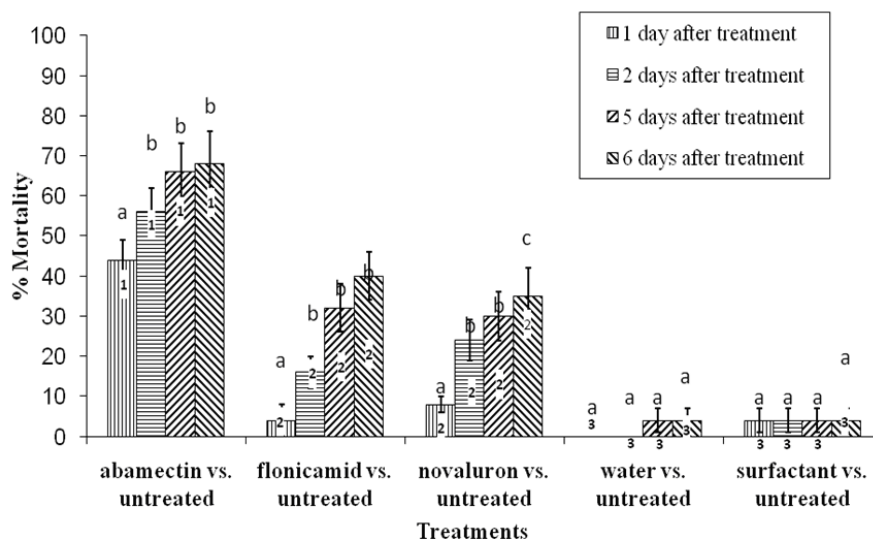


Fig. 3. Mean (\pm SE) percentage of mortality rates of adult potato psyllids, *B. cockerelli*, over 1 week, when exposed to different treatments on potato leaves in the laboratory in a choice test consisting one treated vs. one untreated leaf inside an experimental unit. Mean mortality rates followed by the same letter inside a 'treatment' category or followed by the same number inside a 'day after treatment' category are not significantly different ($P < 0.05$)

Insecticidal effects on number of stylet sheaths and total oviposition in no-choice test: The oviposition rate and feeding events were different among treatments (MIXED ANOVA: $13.21 < F < 24.09$, $0.0001 < P < 0.0231$). The highest number of stylet sheath were observed on untreated, water treated and surfactant treated leaves, which were not significantly different from each other, and the lowest number of stylet sheaths was observed on abamectin-treated leaves (Fig. 4). The highest number of eggs was observed on untreated, water treated and surfactant treated leaves, which were not significantly different from each other (Fig. 5). There were negative correlations between mortality rate and number of stylet sheaths ($r_s = -0.74$, $P = 0.0248$, and $r_s = -0.83$, $P = 0.0109$, respectively), and also between mortality rate and number of eggs ($r_s = -0.87$, $P = 0.0159$, and $r_s = -0.84$, $P = 0.0184$, respectively). There were positive correlations between number of eggs and number of stylet sheaths ($r_s = 0.64$, $P = 0.0387$, and $r_s = 0.70$, $P = 0.0301$, respectively).

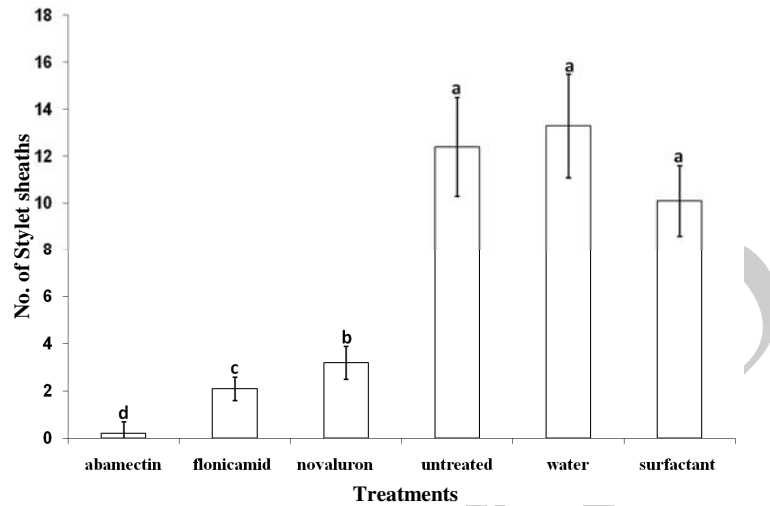


Fig. 4. Mean (\pm SE) number of stylet sheaths of adult potato psyllids per main vein of Atlantic potato leaflets, when exposed to different treatments on potato leaves in the laboratory. Mean number of stylet sheaths followed by the same letter are not significantly different ($P < 0.05$)

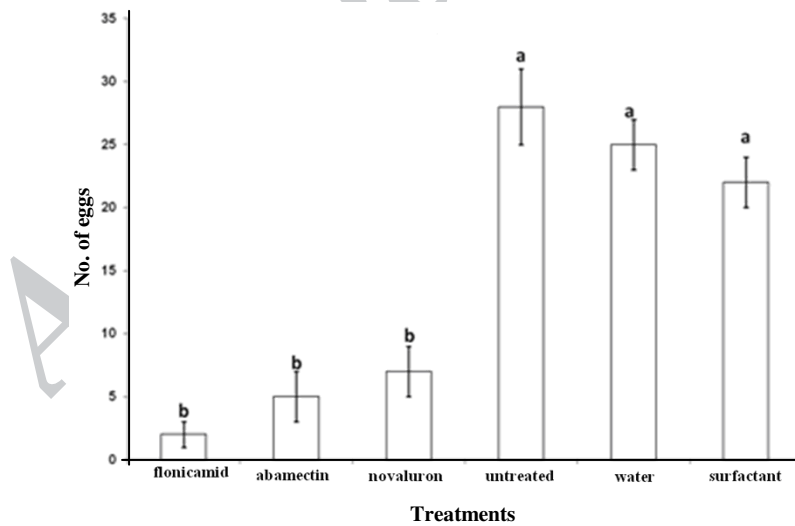


Fig. 5. Mean (\pm SE) number of eggs of adult potato psyllids laid on Atlantic potato leaflets, when exposed to different treatments on potato leaves in the laboratory. Mean number of eggs followed by the same letter are not significantly different ($P < 0.05$)

Insecticidal effects on feeding and oviposition behavior in choice test: Total oviposition rate did not differ significantly between treated and untreated leaves for any of the treatments ($df = 18$, $-0.87 < t < -0.62$, $0.127 < P < 0.5543$). The only significant effect of insecticide treatment on number of stylet sheaths was observed between untreated and flonicamid-treated ($df = 18$, $t = 2.29$, $P = 0.0148$) (Fig. 6).

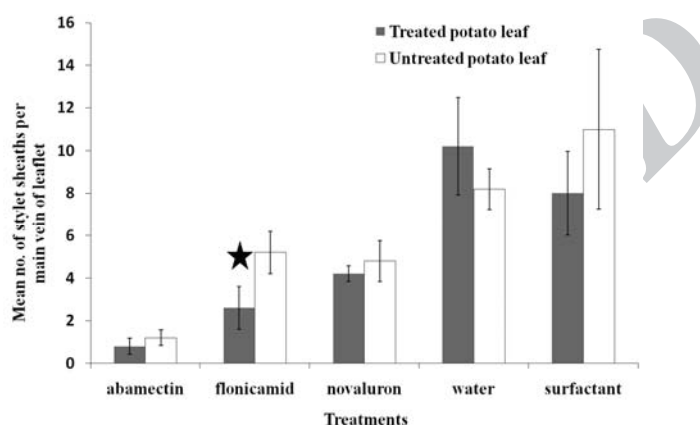


Fig. 6. Mean (\pm SE) number of stylet sheaths and eggs of adult potato psyllids in a choice test between untreated and treated potato leaves with different chemicals in the laboratory. The star sign indicates a significant difference between mean values at $P < 0.05$

Although it is unarguably important that insecticides suppress target pest populations, the primary goal for successful management of vectors of plant diseases should be to minimize the feeding by all life stages on the target crop. Thus, it is paramount that comprehensive testing of insecticides includes assessments of how they affect probing and feeding behavior and also oviposition. In this study, we demonstrated that: abamectin, flonicamid, and novaluron provided high level of mortality at labeled field rates, noticeable decreases in mortality were observed for all three insecticides when adult potato psyllids were offered a choice between treated and untreated potato leaves, and treatments with abamectin, flonicamid, and novaluron caused significant decreases in both oviposition and numbers of stylet sheaths.

Choice test, with treated and untreated potato leaves, were included in this study to highlight an often neglected aspect of the performance evaluation of insecticide treatments – how effective is a given insecticide treatment under incomplete canopy coverage? That is,

many soft-bodied pests, like potato psyllids, are predominantly found on the underside of crop leaves, and the canopy of virtually all potato varieties is quite dense, so what dosage are target pests being exposed to? According to the manufacturers, abamectin (Syngenta Crop Protection, Greensboro, NC) has limited systemic activity, but it does exhibit translaminar movement, flonicamid (FMC, Philadelphia, PA) is both translaminar and systemic, and novaluron (Chemtura, Middlebury, CA) is non-systemic but has translaminar movement. In other words, all three insecticides would be expected to adversely impact adult potato psyllids if at least portions of potato leaves were treated through translaminar movement. Studies Such as Wolf and Daggupati 2009 suggest that leaf coverage after commercial applications may be as low as 10% or less. We only tested effects of 50% and there was a marked decrease in mortality. Thus, we emphasize that although it is obviously important to identify insecticide with high efficacy, it seems equally important to develop improved application methods under field conditions to increase the likelihood of high leaf coverage within the entire canopy (including underside of leaves). In addition to markedly reducing the immediate efficacy of insecticide applications, it also reasonable to assume that low leaf coverage increases the risk of resistance development within pest populations as they may only acquire sub-lethal dosages and move to untreated leaf surfaces to recover (Kamminga et al. 2009). In our choice tests, we did not observe any significant effect on total oviposition, but increase egg sterility may have occurred and has been reported in other studies (Ascher 1993, Weathersbee and Tang 2002).

Several studies have used electrical penetration graph (EPG) (Collar et al 1997, Harrewijn and Kayer 1997) or simple counting of feeding scars (Kamminga et al 2009) to study how insecticide treatments affected the feeding behavior of phloem and xylem feeding insects. Initially, we found that it was not possible to accurately detect probing and feeding events by adult potato psyllids without *a priori* staining of leaves. However, this method was found to be quite easy and greatly improved our insight into the effect of insecticide treatments on adult potato psyllids. However, it is important to mention that we grouped probing and feeding events, although they probably represent quite different behavioral events (Joost et al 2006), and this probably explains our fairly poor correlation between number of stylet sheaths and total oviposition. That is according to the preference-performance hypothesis (Thompson and Pellmyr 1991), one would expect a positive correlation between amount of feeding by adults and total oviposition. However, one could envision a negative correlation between total number of probing events and total oviposition, as a high number of

probing events could indicate low unsuitability of the host. Simple counting of stylet sheaths (without discriminating between feeding and probing events) was used in this study, as it is not known whether actual feeding is required for transfer of *Liberibacter* from psyllid saliva to potato leaf.

Overall, this study may be considered a model for comprehensive evaluation of insecticide performance testing, as we provided insight to several of the key aspects, including: controlled dosage applications to individual crop leaves, mortality within 7 DAT, assessment of the role of incomplete leaf coverage, and insecticide effect on both stylet sheaths and oviposition.

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