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Functional nano-dispensers (FNDs) for delivery of insecticides against phytopathogen vectors†

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Imidacloprid is a widely used insecticide against a variety of insect vectors. Imidacloprid may have a negative environmental impact, as well as toxic effects to animals and humans. In this work, we describe Functional Nano-Dispensers (FNDs), based on polymeric encapsulation of imidacloprid, into poly(lactic-*co*-glycolic acid) particles 5–10 μm in size, using the solvent-evaporation method. Our goal was to develop a formulation that may decrease the negative environmental impact of this widely used insecticide. *In vivo* experiments were conducted comparing the efficacy of FNDs against Asian citrus psyllids (*Diaphorina citri*) with a commercial formulation of imidacloprid. FNDs releasing imidacloprid caused equivalent mortality of insects as compared with the current commercial formulation, yet at a dosage 200 times lower.

Current insect vector management focuses on the dispersion of insecticides against pests that pose a threat to humans, animals or food crops. An example vector of a phytopathogen is the Asian citrus psyllid (*Diaphorina citri*), which has created a critical impact in the citrus industry worldwide. It transmits a phloem-limited bacterial pathogen (*Candidatus liberibacter* sp.) between trees during feeding. The pathogen causes citrus greening disease or Huanglongbing (HLB), which kills trees.[1–4]

It is reported that close to 100% of all trees in Florida (approximately 65 million trees) and approximately 20% of all citrus trees in Brazil (40 million trees) are infected with HLB transmitted by this vector. The survival of the current U.S. citrus industry is threatened.[3] Typical dispersion of insecticides requires spraying a solution that contains an active ingredient, providing efficacy over a targeted period of time. One of the challenges of pest control over large areas is to perform a cost-effective dispersion that would provide protection against the targeted vector without producing a negative environmental impact.

Imidacloprid is one of the most widely used insecticides and is also implemented for psyllid management by citrus industries worldwide.[3] Imidacloprid is also widely used for other agricultural applications. It is widely used against aphids, beetles, locusts; in arboriculture (*e.g.* maple, oak, birch) against emerald ash borer; home protection against termites, carpenter ants; and for domestic animals against fleas.

This insecticide is a neonicotinoid, acting as a neurotoxin on the central nervous system of insects, binding irreversibly to specific insect nicotinic acetylcholine receptors.[5–7] Recent research suggests that imidacloprid may have negative environmental impacts, including the worldwide decline of honey bee colonies, as well as, having possible toxic effects to animals and humans.[8–13]

During the past 15 years, an explosion in research in micro and nanotechnologies (MNT) has led to the development of a variety of techniques that allows to control the matter at the micron (1 micrometer = 10^{-6} meter) and nano (1 nanometer = 10^{-9} meter) scale. MNT have opened a new era in delivery of pesticides through the development of micro and nanosize controlled release systems, such as micro and nanoparticles, including nanospheres, nanocapsules, nanogels and micelles, using a wide variety of materials.[14] In particular, polymers have been employed for the synthesis of these nanoparticle systems due to their scalability, versatility, biocompatibility, and low cost.

Polymers that have been used for pesticide encapsulation include polyethylene-glycol, poly-methyl-methacrylate, polycaprolactone, polylactic acid, alginate and lignin.[14] For example, the use of amphiphilic polymers as micro-containers, allows entrapment of hydrophobic pesticides inside a polymeric matrix surrounded by a hydrophilic shell. This improves pesticide solubility in water, which is an important requirement for pesticide dispersion. Nanoencapsulation provides high surface to volume ratio, increasing the amount of active ingredient per unit area and decreasing the total amount of pesticide dis-

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persed. This should result in less negative environmental impact.

In addition, micro and nanoparticles provide protection against harsh environments (radiation, high temperature), improving chemical stability of the pesticide, thus providing sustained pesticide bioactivity over time. In this work, we investigated a formulation based on micro-encapsulation of imidacloprid using PLA, to decrease negative environmental impact, yet achieve similar performance to commercially formulated imidacloprid. Our work demonstrates that the amount of active ingredient can be drastically reduced, while maintaining equivalent efficacy to the current commercial standard.

Micro and nanoencapsulation of insecticides has been proposed previously.[14–28] In pioneering work. Allan *et al.*,[22] developed controlled release devices for insecticides to manage insects pests in forestry. Polymers containing amide groups, known to be powerful solvents for pesticides, were loaded with hexamethylphosphoric triamide (HMPT) as a model for organophosphate insecticides. In addition, 2,4-dichlorophenoxyacetic acid was attached to a polymer *via* the carboxylic group of the acid reacting with a replaceable hydrogen group. Both approaches provided either an extended period of protection at equivalent dosages of application (expressed as mg), or the same period of protection at significantly lower dosages of application, with both methods reducing potential environmental hazards.

Nano emulsions (micelles) based on fatty acids of different length have been investigated for encapsulation of pesticides.[23] Encapsulation efficiency and bioactivity of the insecticide were found to be proportional to fatty acid length. This observation suggested that further investigation is needed on the interaction between the active ingredient and the matrix in which the insecticide is embedded.[23]

Takei *et al.*[24] developed polylactic acid (PLA) microspheres in aqueous media for controlled release of the insecticide, acetamiprid. With increasing amounts of PLA, entrapment efficiency and release rate of acetamiprid decreased.[24] Hydrophobicity of PLA was thought to prevent water from penetrating the polymer, which is a critical step required to allow pesticide diffusion from the polymer microsphere.[24] The addition of polycaprolactone to the PLA polymer matrix resulted in increased microsphere hydrophilicity, thereby improving insecticide diffusion.[24]

A sodium chitosan alginate microparticle impregnated with a photocatalytic compound consisting of SDS/TiO_2/Ag was developed through layer by layer self-assembly and tested against *Martianus dermestoides*.[25] The microparticle formulation was more toxic to the target pest than the commercial formulation.[26] In addition, less insecticide was accumulated in treated soybean leaves and soil with the nanoformulation compared to the commercial formulation.[25]

Micro and nanoencapsulation of imidacloprid has also been explored previously. Various materials have been used for encapsulation of imidacloprid, including chitosan-poly(lactic), lignin, alginate-bentonite, lignin-polyethylene glycol-ethyl cellulose and polyethyleneglycol. For example, a controlled release system for imidacloprid has been developed using a copolymer containing poly citric acid, polyethyleneglycol, and aqueous solvents.[26] Imidacloprid released from the nanoformulation, was more toxic to the target pest than free imidacloprid.[26] Finally, a nanocapsule formulation of imidacloprid has also been developed using an amphiphilic co-polymer based on poly-ethyleneglycol and aliphatic diacids that self-assembled in aqueous media into nanomicelles.[27] The release rate of the co-polymer formulation was lower than the commercially used formulation of this widely used insecticide.[27]

To our knowledge, *in vivo* testing using a formulation containing imidacloprid inside PLA based microparticles has not been attempted previously. Herein, we describe the first *in vivo* test using polymeric particles based on poly lactic-*co*-glycolic acid (PLGA) that contain imidacloprid, against a phytopathogen vector. This particle technology for insecticide dispersion was named Functional Nano-Dispensers (FNDs). Encapsulation consists of using PLGA to create particles of micrometer dimensions, which optimize release kinetics as the ratio of surface area over volume is dramatically increased allowing for smaller dosages of active ingredient to achieve high release-kinetic efficacies.

A schematic diagram of a FND particle containing imidacloprid is shown in Fig. 1. In this work, the PLGA particle diameter range was 5–10 μm. Fig. 2A and B shows an image of the final product. *In vivo* experimental results indicated that FNDs were an effective release device for optimal delivery of imidacloprid to target insects and caused mortality at concen-

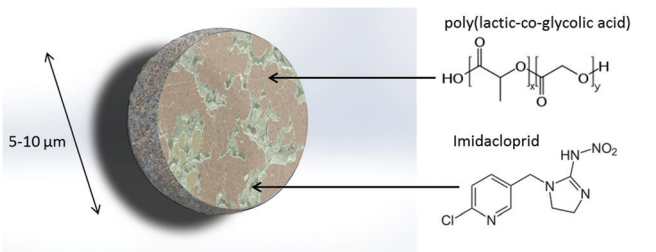

Fig. 1 Sketch of cross-sectional view of particle containing uniform distribution of PLGA (not in scale) for controlled release of imidacloprid.

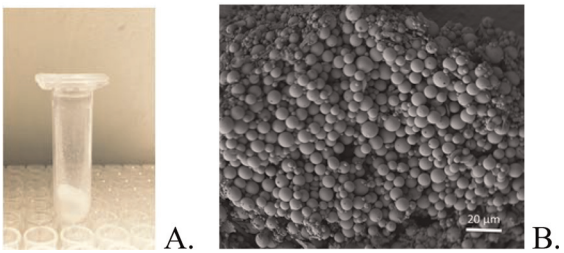

Fig. 2 A. Lyophilized formulation. B. Scanning electron microscope (SEM) image of PLGA particles of approximately 5–10 μm in size.

trations at least 200 times lower than the current commercial formulation of this insecticide.

FNDs were produced using an emulsion-solvent evaporation method, as described in the ESI.† Three *in vivo* assays were performed to test efficacy of the FNDs as a function of concentration in solutions. Each assay compared a positive control of commercially formulated imidacloprid (Provado 1.6F, Bayer Crop, LP) at a concentration of 3.06 mg mL^{-1} with varying concentrations of imidacloprid formulated in FNDs: 1 μg mL^{-1}, 13.4 μg mL^{-1}, and 10.9 μg mL^{-1}. Mortality of psyllids was assayed with a standard leaf disk method.[28] Approximately 5 mL of agar was put into 35 mm diameter Petri dishes and allowed to solidify. Leaf discs were made from sweet orange leaves to fit within the dishes. The leaf discs provide a sub-

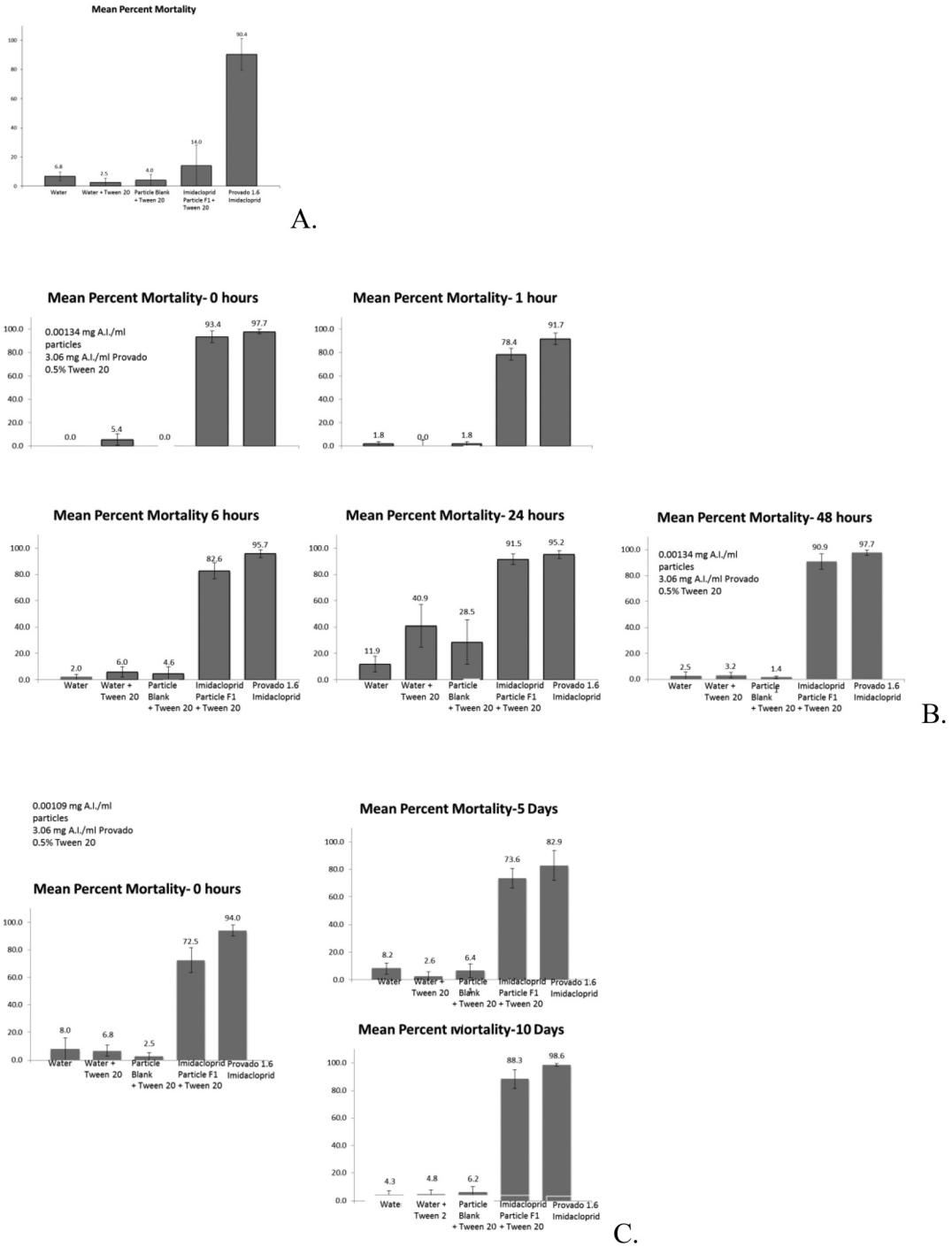

Fig. 3 *In vivo* experimental results showing efficacy of FNDs releasing imidacloprid compared with commercially formulated imidacloprid (Provado, 3.06 mg mL^{-1}) after (A) 24 hours at 1 μg mL^{-1}; (B) (0, 1, 6, 24, 48 hours) at 13.4 μg mL^{-1}; and (C) (0, 5, 10 days) at 10.9 μg mL^{-1}.

strate for adult psyllid feeding. The following treatments were prepared in 10 mL of distilled water: water alone, water + 0.5% Tween 20, 13.4 mg particle alone + 0.5% Tween 20, FNDs with imidacloprid (with aforementioned concentrations for each assay) + 0.5% Tween 20 or 160 μL Provado 1.6 F (commercial formulation). The leaf discs were dipped for 30 s in each solution and left to air dry for 1 hour in a fume hood. The discs were then placed into the Petri dishes. For the first assay, treatments were tested for 24 hours after preparation. For the second assay, treatments were tested 0, 1, 6, 24 and 48 hours after preparation. For the third assay, treatments were tested 0, 5, 10 days after preparation. Approximately 10 psyllids were anesthetized with CO_2 and transferred into the Petri dishes. The dishes were sealed with parafilm and left out at room temperature (23.0 °C) for the duration of each assay. During each assessment of mortality, the number of live and dead psyllids was recorded. A psyllid was considered dead if there was no movement when touched with a fine probe. There were 5 replicates for each treatment.

Results for the three assays are shown in Fig. 3A and C. Experimental results with FNDs containing imidacloprid against psyllids compared very favourably with the commercial formulation of this insecticide. Concentrations of imidacloprid within FNDs in the 10–20 μg mL^{-1} range were similar in efficacy against psyllids over the time course of the investigation. However, FNDs containing imidacloprid at a 1 μg mL^{-1} concentration showed limited efficacy. Mortality of psyllids was comparable between the higher concentrations of imidacloprid in FNDs to that observed with commercially formulated imidacloprid (Provado 1.6F) after 48 hours. Similarly, high mortality (*ca*. 80%) was observed with FNDs after 10 days in the third assay.

The advantage of FNDs was an approximate 200 fold reduction in the amount of imidacloprid required to achieve approximately the same mortality of psyllids as compared with the commercial formulation. Such a reduction in insecticide concentration, while maintaining similar efficacy, shows promise to significantly reduce negative environmental impact of this important pest management tool. It also suggests that release kinetics of other insecticides could be similarly optimized. FNDs can also be lyophilized allowing imidacloprid to be stored under stable conditions, while reducing overall volume. FNDs can be quickly reconstituted for use as an insecticide by simply adding water and surfactant. Additionally, FNDs can be used with standard spraying technologies, without requiring changes in the current dispersion/application methods used for standard insecticides.

Further *in vivo* assays will be performed to evaluate performance of FNDs in the field to take environmental factors such as UV exposure, temperature and humidity variations into account. The method for particle encapsulation is known and can be readily scaled to provide cost-effective solutions. Additionally, registration of such a formulation should be straightforward as PLGA particles are widely used in a number of medical applications approved by the Food and Drug Administration,[29] while imidacloprid has long-standing approval by the Environmental Protection Agency (EPA).[30] Therefore, registration of this proposed product should be focused on the combination of particle and active ingredient.

Imidacloprid is one of the most important currently used tools for insect pest control worldwide, including vectors of pathogens.[6,7] Given the recent concern of the environmental impact of imidacloprid, particularly on pollinators, a technology that reduces the amount of active ingredient required for efficacy, but maintains efficacy, could lead to a breakthrough novel insecticide release device.[31]

FNDs containing imidacloprid were tested using *in vivo* experiments against an insect phytopathogen vector. Our results indicate that FNDs are an effective release device for insecticides as compared with a standard commercial formulation, providing an acceptable level of protection for at least 10 days. A key advantage of FNDs as a release device for imidacloprid is that the exposed surface area is maximized allowing a 200 fold dosage reduction of active ingredient as compared with the current commercial formulation of this ubiquitously used insecticide. Additionally, FNDs can be supplied in lyophilized form, which provides a stable formulation that can be stored and quickly reconstituted, without affecting the standard spraying method. FNDs have great potential as a cost-effective solution against a number of vector threats.

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References

1 H. Schneider, *Phytopathology*, 1988, **78**, 687.
2 L. Vasconcellos and W. S. Castle, *J. Am. Soc. Hortic. Sci.*, 1994, **119**, 185.
3 E. Grafton-Cardwell, L. L. Stelinski and P. A. Stansly, *Annu. Rev. Entomol.*, 2013, **58**, 413.
4 D. Hall and T. R. Gottwald, *Outlooks Pest Manage.*, 2011, **22** (4), 189.
5 *Pesticide Information Profiles: Imidacloprid*, Extension Toxicology Network, 2012.
6 J. A. Gervais, B. Luukinen, K. Buhl and D. Stone, *2, 4-D Technical Fact Sheet*, National Pesticide Information Center, 2012.
7 I. Yamamoto, Nicotin to nicotinoids, in *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*, ed. I. Yamamoto and J. Casida, Springer-Verlag, Tokyo, 1999, p. 3.
8 USDA Forest Service, Final Report, 2005.
9 National Pesticide Information Center, *Imidacloprid: General Fact Sheet*, 2010.

10 D. A. Herms, D. G. McCullough, D. R. Smitley, C. Sadof, R. C. Williamson and P. L. Nixon, *North Central IPM Center Bulletin*, 2012.
11 D. Carrington, The Guardian on line, http://www.theguardian.com/environment/2012/mar/29/crop-pesticides-honeybee-decline [accessed, March 12, 2015].
12 P. R. Whitehorn, S. O'Connor, F. L. Wackers and D. Goulson, *Science*, 2012, **336**, 351.
13 C. Lu, K. M. Warchol and R. A. Callahan, *Bull. Insectology*, 2012, **65**, 99.
14 B. Perlatti, P. L. de Souza Bergo, M. F. das Graças Fernandes da Silva, J. B. Fernandes and M. R. Forim, in *Insecticides - Development of Safer and More Effective Technologies*, ed. S. Trdan, InTech, Croatia, 2013, ch. 20.
15 S. Kumar, G. Bhanjana, A. Sharma, M. C. Sidhu and N. Dilbaghi, *Carbohydr. Polym.*, 2014, **101**, 1061, DOI: 10.5772/53355, Rijeka, 2013, ch. 20.
16 F. L. Yang, X. G. Li, F. Zhu and C. L. Lei, *J. Agric. Food Chem.*, 2009, **57**, 10156.
17 S. B. Laoa, Z. X. Zhanga, H. H. Xua and G. B. Jiang, *Carbohydr. Polym.*, 2010, **82**, 1136.
18 B. H. Feng and L. F. Peng, *Carbohydr. Polym.*, 2012, **88**, 576.
19 F. J. Garrido-Herrera, E. González-Pradas and M. Fernández-Pérez, *J. Agric. Food Chem.*, 2006, **54**, 10053.
20 Z. E. Bahri and J. L. Taverdet, *Powder Technol.*, 2007, **172**, 30.
21 J. Li, J. Yao, Y. Li and Y. Shao, *J. Environ. Sci. Health, Part B*, 2012, **47**, 795.
22 G. G. Allan, C. S. Chopra, A. N. Neogi and R. M. Wilkins, *Nature*, 1971, **234**, 349.
23 H. Casanova, P. Araque and C. Ortiz, *J. Agric. Food Chem.*, 2005, **53**, 994.
24 T. Takei, M. Yoshida, Y. Hatate, K. Shiomori and S. Kiyoyama, *Polym. Bull.*, 2008, **61**, 391.
25 H. Guan, D. Chi and J. Yu, *Pestic. Biochem. Physiol.*, 2008, **92**, 83.
26 N. Memarizadeh, M. Ghadamyari, M. Adeli and K. Talebi, *Ecotoxicol. Environ. Saf.*, 2014, **107**, 77.
27 T. Adak, J. Kumar, N. A. Shakil and S. Walia, *J. Environ. Sci. Health, Part B*, 2012, **47**(3), 217.
28 D. R. Boina, E. O. Onagbola, M. Salyani and L. L. Stelinski, *Pest Manage. Sci.*, 2009, **65**, 870.
29 Food and Drug Administration website: http://www.accessdata.fda.gov/cdrh_docs/pdf12/k10556.pdf [accessed, March 1, 2015].
30 Environmental Protection Agency website:websitepro http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2012-0204-0005 [accessed, March 1, 2015].
31 G. Rondeau, F. Sanchez-Bayo, H. A. Tennekes, A. Decourtye, R. Ramirez Romero and N. Desneux, *Sci. Rep.*, 2014, **4**, 1.