Pre-Proposal to EPA - IRAC

Project title

Resistance risk assessment in populations of the Asian citrus psyllid (*Diaphorina citri*) to recommended insecticides: resistance monitoring for suppression in Texas, California, Florida and Arizona and establishment of the Asian Citrus Psyllid (ACP) resistance website portal.

Principal Investigators

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and

Cecilia Tamborindeguy, Assistant Professor, Plant Vector Biology

Department of Entomology
Texas AgriLife Research
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Critical Personnel

Raul Villanueva, Weslaco, TX- Texas AgriLife Extension
Other collaborators in Florida, California, Arizona: to be determined

July 29, 2010

Contact Information

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Statement of interest and purpose for EPA participation

This pre-proposal was shared with IRAC, Insecticide Resistance Action Committee and received interest from the whole IRAC group as long as has a National scope on May 4, 2010. Please see bold text at the bottom of this pre-proposal. I am showing an email exchange with the IRAC representative from Monsanto, Graham P. Head.

We are willing to pursue this collaborative venue but the funds that IRAC will eventually provide may serve only to produce vials for monitoring and the salary of a technician for preparation and shipping to collaborators from the mentioned states for two-three years.

We are looking at EPA to provide additional funds to complement this project. We would minimally need from EPA the two-month salary for the computer designer to establish the ***Asian Citrus Psyllid Portal*** *for dissemination of information in the already existing Insecticide Resistance TAMU website, and for a graduate student to maintain this Asian Citrus Psyllid Insecticide Resistance database through probit data analysis, production of graphs and tables, etc. Supplies will be also needed such as updates or purchase of new georeferenced software, server maintenance, travel to conference and office supplies (printer cartridges, paper, dvds for data storage, etc.).*

Budget is also requested from EPA to support the collaborators in other states, about $4,000 per collaborator to obtain samples and perform the vial assays and fax information to Pietrantonio's lab for analysis.

Text highlighted in yellow in next page was modified from the original text sent to IRAC.

Project Narrative

This proposal fits within the IPM management strategy of **PA<u>MS</u>:** Protection, Avoidance, **<u>Monitoring and Suppression</u>**

Rationale: Pesticide use remains a keystone on the battle against ACP populations because they vector the pathogen causative of the **Citrus greening (CG)** disease. Low volume and ultra low volume sprays are particularly recommended as these approaches are economically more feasible and environmentally-friendlier than normal sprays: These sprays save time, water and gas when compared with alternative insecticide treatments. Two major products used as broad-spectrum foliar insecticides are Malathion (organophosphate) and Danitol® (pyrethroid: fenpropathrin). Provado® (neonicotinoid: imidacloprid) is largely used as systemic pesticide because of its long persistence.

Aggressive and coordinated treatment of groves is being recommended to ensure that ACP populations are maintained as low as possible and no abandoned grove will remain untreated and act as an insect reservoir. However, as this strategy is used, insecticide resistance in the field will invariably appear. The appearance of resistance must be monitored to ensure that: first, control with insecticides is being effective in the field, second, the detection of potential heterozygotes in bioassays may suggest that application rates utilized by growers may be suboptimal (not according to label) and third, to try to delay the onset of resistance by recommending products with different modes of action in an effective rotation. Therefore resistance monitoring may reveal suboptimal management practices identifying opportunities for grower education. During the last decade, ACP was not specifically targeted in Texas but with the discovery of **CG** in Louisiana and Mexico, aggressive chemical control is now being recommended (Setamou, 2009).

In order to start monitoring the appearance of resistance in ACP populations in Texas, California, Florida and Arizona, first, the population's current status of susceptibility to insecticides must be evaluated (baseline). For this, it is necessary to have a standard reference susceptible population for following the evolution of resistance in future years. Unfortunately, due to quarantine restrictions we cannot import into Texas susceptible laboratory colonies or populations from states where the disease is present. A susceptible colony from field-collected insects must be established and maintained for future insecticide resistance monitoring and research. Field populations of ACP will be collected to assess the current status of susceptibility (or resistance).

Materials and methods: Laboratory bioassays using the three insecticides (malathion fenpropathrin and imidacloprid) will be carried out. Dr. Pietrantonio's laboratory already has malathion ULV in hand. Fenpropathrin (Valent, USA) will be purchased as active ingredient. Late instars of ACP will be collected in Texas groves from different host-plants (sweet orange, grapefruit, lime and tangerine) and at different dates, and shipped to the Department of Entomology at Texas A&M University, College Station, TX where they will be kept until they become about one week old adults. For the two foliar insecticides, malathion and fenpropathrin the adult vial test will be performed. Control vials will be coated with dehydrated acetone only. Stock solutions will be prepared with malathion and fenpropathrin in acetone, the latter previously dehydrated for at least 48 h on 4-Å molecular sieves (EM Science, Gibbstown, NJ). Insecticide coated vials will be prepared with insecticide solutions in acetone covering at least three log-concentrations. One to five psyllids will be placed in each vial, and the vials will be stored at 27°C. Equal numbers of psyllids will be tested for each concentration. Mortality will be evaluated after 24 h, and psyllids will be categorized as alive or dead (includes knock-down). We estimate that each bioassay will use 200 insects and these will be repeated at least 3 times for a total of 600 insects per insecticide and per location. Currently a laboratory colony is available to us from Texas A&M University Kingsville, Citrus Center in Weslaco, TX; these insects will be also tested. Lethal concentrations (LC_{50} and LC_{90}) will be estimated for all populations and resistance ratios will be calculated using the most susceptible population as a reference. The susceptibility status of the laboratory colony is currently unknown, but these insects have been in culture for an extended period (3 years) so it is likely that this colony will be the most susceptible.

To determine the susceptibility baseline to imidacloprid (Provado; Bayer CropScience LP, Research Triangle Park, NC), 1–2-month-old citrus seedlings, *Citrus aurantiifolia* (Christm.) cv. Swingle, grown in a greenhouse will be used. Plant roots will be completely immersed for 48 h in 30 mL glass vials containing aqueous solutions of imidacloprid (0.15, 0.3, 1.0, 3.0, 10 and 100 μg AI mL^{-1}) prepared by serial dilution of commercial imidacloprid 240 g L^{-1} SC in tap water. Control plants will be immersed similarly in tap water for the same period. After 48 h of immersion, plants will be transferred to new vials with tap water, and 10 Asian citrus psyllid adults will be released onto each plant, including controls. Plants with insects will be caged and placed at 25°C. Mortality counts will be taken at 24, 48 and 72 h after release of adults. Each treatment, including the controls, will be replicated 3 times.

All bioassay data will be analyzed using PoloPlus, Probit and Logit Analysis programs (LeOra Software, Petaluma, CA) (Robertson *et al.*, 2007) and dosage mortality regressions will be plotted using SigmaPlot software. The LC_{50} and LC_{90} values will be used to calculate resistance ratios. The results of chi-square tests (χ^2) will be used to estimate how well the data of each concentration-mortality curve fit the assumption of the probit model. Data will be corrected for control mortality in the PoloPlus program (Abbott, 1925). Resistance ratios (RRs) will be determined at a given response level (50% or 90%) between the susceptible and resistant populations. In order to determine if there are statistically significant differences between the compared lethal concentrations, the 95% confidence intervals for the resistance ratios will be calculated. In this pairwise comparison, lethal concentrations will be considered significantly different if the value '1' does not fall within the confidence interval for the ratio (Robertson & Preisler, 1992; Robertson *et al.*, 2007). The overlap of the confidence intervals for lethal concentrations is not used to determine significant differences between them because this method lacks statistical power (Robertson *et al.*, 2007). This same methodology will be used to compare populations between different dates, and thus, if the 95% CI for the ratio includes the number one, they will be considered equal. Test of equality and parallelism will be also conducted. Upon analysis and comparison of bioassays from different locations we will determine the most susceptible field population and insects from these trees/fields will be collected and brought into the laboratory to establish a permanent susceptible reference colony.

Survivors of dosages that kill the majority of the insects in the susceptible population will be kept stored in the -80C freezer for future elucidation of mechanisms of resistance.

<u>Expected results and pitfalls:</u>

This survey will determine the location of insecticide resistant populations of ACP in Texas. The detection of resistance to a particular compound may trigger the recommendation for a change of active ingredient, frequency of applications or change in their rotation. Pesticide resistant populations present a higher risk for disease introduction and spread. The selection pressure on this pest will probably increase once the pathogen responsible for CG is found in the state. It is crucial to monitor the appearance of resistance in order to maintain adapted control strategies. The susceptibility baseline will be determined using insects collected at different time-point and in different host-plants. If different biotypes are identified as a result of experiments in Objective 1, then more detailed experiments will be carried out for each of the biotypes to verify their susceptibility or resistance to the recommended pesticides. For suppression is important to determine if a particular biotype (genotype) is associated with insecticide resistance in the eventuality that the "best vector biotype" would be also the most resistant, increasing risk of disease transmission.

Pitfalls and alternatives: Laboratory assays can detect resistant individuals even when at relatively low frequencies in the population. Resistant populations identified in laboratory assays should be further followed up in the field to determine if field failures are occurring. Therefore resistance may be evolving although it may not be apparent to growers in the field. This proactive approach provides some time to modify management practices with grower participation.

<u>Principal investigators</u>:

Dr. Pietrantonio is an expert in insect toxicology and she also manages the state-wide program for bollworm that uses similar techniques. She also has experience with leaf bioassays with whiteflies and imidacloprid (drench, dip, etc.). No problems are expected for this objective.

Dr. Tamborindeguy is an expert in vector biology, she has extensive experience in psyllids and bacterial transmission and she has a network of collaborators working in this pest.

EMAIL EXCHANGE WITH IRAC ABOUT PROPOSAL

HEAD, GRAHAM P [AG/1000]" <graham.p.head@monsanto.com

From Graham Head on 5/4/2010

Pat,

I can get you names if you do have the contacts. If you know people that you feel are suitable, that's great.

Total dollars? We do not tend to go over 25-30 per year, and 2-3 years, but we will consider whatever is reasonable and we can think about other partners if the total is much larger.

Thanks,

Graham
----- Original Message -----
From: Patricia Pietrantonio <p-pietrantonio@tamu.edu>
To: HEAD, GRAHAM P [AG/1000]
Sent: Tue May 04 09:59:13 2010
Subject: RE: A question from: Entomology at Texas A&M University

Wonderful. Do you prefer any people in particular before I start making phone calls?

Also how much money are you willing to put down because I cannot call people without an idea of what they can count on. At least approximately.

Thanks, Pat.

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>>> "HEAD, GRAHAM P [AG/1000]" <graham.p.head@monsanto.com> 5/4/2010 6:23 AM >>>
Thanks Patricia - good to hear about Brad, though sorry that he accepted a position at Dow!

As promised, we also had the discussion within IRAC on your new "pre-proposal". The group is supportive of funding IRM-related work in this area along the lines of the objective that you excerpted. We would like to see work that spans across the affected area - i.e., CA, FL, AZ, TX - and therefore we would particularly like to a proposal with suitable collaborations built it. Does that make sense?