FINAL REPORT **YEAR**: **2013**

Project Title: Protein-based foam for applying lacewings eggs to fruit trees by ATV

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Other funding sources
WTFRC/ Apple Crop Protection**/Predator Releases 2010-2012**
Year 3 of 3 (2012): $80,680. The lacewing portion of this grant stimulated the request for Technology funds and the two projects overlapped in 2012.

Pending: WTFRC Crop Protection/ New approaches to aphid biocontrol in apples
Total Request $ 89.931

Budget History

Organization Name: USDA-ARS

Item	**2012-Unruh**	**2012-Dunlap**	**TOTAL**
Salaries			
Benefits			
Wages GS-3 (90/90 days)	$7431	$7431	
Benefits	$569	$569	
Equipment	$ 400		
Supplies	$600	$1200	
Travel		$800	
Miscellaneous			
Total	$9000	$10000	$19, 000

Footnotes:

OBJECTIVES

1. **Test formulations of various foaming agents using a foam generator and adapt foam generation to a modified 12-volt pump sprayer suitable for use on an ATV**
   We have tested keratin and whey protein hydrolysates, saponin-containing Yucca extract and Qullaja sopninaria extract as foaming agents were tested using off-the-shelf foam sprayers and a sprayer under development. The latter device drops dry lacewing (LW) eggs into the foam stream after it leaves the pressurized portion of the sprayer and appears close to a final product. The remaining problem is in the geometry of the egg and foam mixing downstream of the foam spray stream which allows the eggs to be blown out ahead of the foam and falling before the target is reached.

2. **Test adhesion of foam to waxy, water repellent, surfaces and leaves of seedling apples and on bark**
   Initial efforts have been restricted to tests on artificial surfaces including Tyvec sheets plastic cafeteria trays (Wapato) and Plexiglass (Peoria. We have found that the foam produces by keratin, Yucca and Quillaja stick well to tree trunks and in droplets the size of quarters or smaller also to leaves

3. **Test survivability of lacewing eggs in laboratory conditions when eggs are immersed in and sprayed with these foams**
   With each new formulation of foam producing liquid, measurement of survival after 30 minute submersion in the product is compared to submersion in water. With new spray technique where eggs are dropped into a trough and swept up in a stream of foam, survival has been tested with egg sprayed onto Tyvec surface or sprinkled into foam

4. **Test adherence of LW eggs in foam on apple, pear and cherry trees in the greenhouse and the field and estimate hatch rates of eggs in those settings.**
   Using foam gun with Keratin and Quillaja foam we get excellent retention of foam to both bark and leaf substrates. However, we have yet to achieve adequate mixing of the LW eggs in the foam to allow testing hatch rates in this setting. Hatch rates exceed 60% in foam in the laboratory.

5. **Estimate colonization rates (proportion of eggs recollected as larvae) on test trees.** .
   Preliminary studies using sprayer were not made in 2012 because of lack of adequate numbers of aphids in our experimental farmnd. In 2013 we conducted a proof of concept experiment without foam and discovered recovery rate of aphids in aphid-infested buds was less than 3% over 3 separate studies.

SIGNIFICANT FINDINGS

- ✓ Keratin and whey protein hydrolysates, *Qualia* and *Yucca* saponins can produce rich foam suitable for initial contact adherence to water repellent surfaces and tree trunks
- ✓ Passage of eggs through rotary diaphragm pumps damages >25% eggs requiring eggs be introduced into the stream of the foaming agent distal to the pump
- ✓ Eggs e introduced in a suspension medium separate from the foaming medium using Venturi aspirator has proven problematic for accurate metering of eggs.
- ✓ Eggs can be dropped into the spray stream of foam after foam leaves spray nozzle.
- ✓ Mixing of eggs with dry bulking material is necessary to meter eggs for above gravity feed.

- ✓ Long term adherence depends on volume deposited, concentration of foaming agent and presence of other additives
- ✓ Psyllium husk (Metamucil), a potential bulking agent, expands on wetting, absorbs water as foam collapses and causes eggs to stick securely to Tyvec substrate
- ✓ A two trigger spray gun has been developed which has a sliding plate that collects a fixed amount of eggs with bulking material, drops eggs into a trough, and toggles the spray.
- ✓ The addition of eggs to the foam stream after it leaves the spray nozzle eliminates mechanical damage from pressure and shearing in the pump, and from long term submersion of eggs in the foaming agent or other liquids. Optimization of this spray device is required.

RESULTS & DISCUSSION

Objective 1 - Initial testing evaluated the suitability of a variety of natural products and proteins to serve as a foaming agent in this application. A variety of food grade or OMRI approved proteins or natural surfactants were evaluated to serve as foaming agents. Preliminary screening evaluated keratin hyrdolysate, egg albumin, gelatin, whey protein isolate and concentrate (Glanbia inc.), β-lactoglobulin and α-lactalablumin (Davisco foods inc.) and *Yucca schidigera* extract and recently *Quillaja saponaria* extract. The suitability was evaluated by measuring their physical properties including dynamic surface tension, expansion ratio, half-life, and density using standard procedures. This analysis was conducted us a pestifoamer PF-2 (Richway Industries) to generate foam in continuous mode.

Figure 1. Richway Pestifoamer.

The protein hydrolysates (keratin hydrolysate and whey protein isolate) provided better surface tension and better foam due to their smaller molecular size and faster diffusion rates than the crude proteins. Keratin hydrolysate was superior compared to whey hydrolysate. Keratin hydrolysate, while derived from agricultural products (bovine hooves and horns), is not currently OMRI approved. In addition, while it is produced on a commercial scale for fire-fighting foams, it is not readily available without antimicrobial biocides included as preservatives. S

These limitations and the need to use OMRI products in some field testing sites caused to take a closer look at existing OMRI certified surfactants that could be adapted with other adjuvants to serve as suitable foaming agents. This search identified *Yucca schidigera* and *Quillaja saponaria* extracts, both of which are OMRI approved agricultural surfactants (with a reputation of undesirable tank foaming in standard spray applications). Initial spray trials in IL and WA show them to having acceptable dynamic surface tension to meet our requirements. Preliminary screening of hatch rate of eggs after being submerged in the yucca

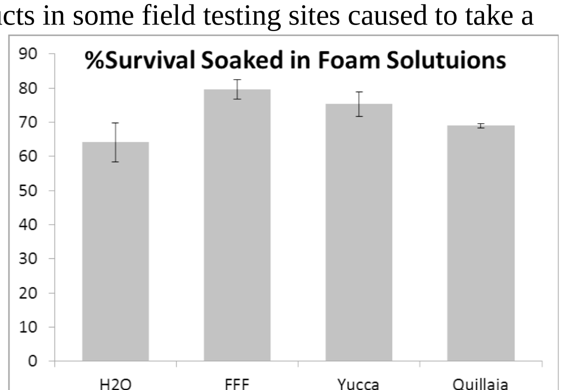

Figure 2. Survival rates of LW eggs soaked for 30 min. in water firefighter foam, yucca and quillaja extractand

extract showed no appreciable differences from water controls Figure 2. The hatch rate in Quillaja and yucca extracts is slightly lower but both are comparable to that of the Keratin firefighter foam. Unfortunately, when eggs pass through a foam inducing spray nozzle (T-jet air jet)at 60 psi setting

Because OMRI certified products based on proteins/peptides which make excellent foam were not readily available and OMRI certified, we expanded our search to all classes of OMRI certified surfactants which include Quillaja and Yucca extracts. During 2013, the no-cost extension period of the grant, we were able to identify additional surfactants that met OMRI certification. These additional "small molecule" surfactants are described in table 1. The ability of these surfactants to produce foam was evaluated using a common foamer used in pest control applications.

Table 1. Foaming properties of tested surfactants.

Product Name	Expansion ratio[1]
Yucca Ag-Aide	6.0 ± 1.3 A
Armak 2042	4.7 ± 0.5 B
Armak 2079	2.7 ± 0.2 C
Armak 2081	≤ 2 D
Armak 2087	2.8 ± 0.2 C
Silwet Eco Spreader	≤ 2 D

The results show Yucca Ag-Aide and Armak 2042 are moderate foam producers with expansion ratios in the 4-6 range. The others were poor foaming agents. Armak 2079 and Armak 2087 gave expansion ratios in the 2-3 range,while Armak 2081 and Silwet Eco did not produce foams. Since Yucca Ag-Aide and Armak 2042 were moderate successful foam producers, we evaluated the longevity of the produced foam. For Yucca Ag-Aide 85% of the initial foam remained as foam 1 hour later, while Armak 2042 had 20% of the initial foam after 1 hour. For sprays on to bark relatively rapid collapse of the foam is fine but on leaves it may be less tenable. The survival of LW eggs in Armak 2042 has not been evaluated.

The second overall goal of this project was to determine the best method to introduce lacewing eggs to foam. The solution to this objective has been confounded by the competing engineering requirements for foam generation versus introducing the lacewing eggs. One approach tested in 2012/2013 was to suspend eggs in a gum solution and introduce that solution into the foaming agent using a venturi injector just before passage through an air induction spay nozzle. This approach worked to generate great foam but two problems interfered with our goals: 1) LW eggs sink in water, addition of gums to slow sinking was inadequate, and no suitable submersible agitator could be found to resolve this problem. 2) Passage of eggs through the terminal spray nozzle reduced survival rate if eggs by 20-50%.

After much trial and error, we concluded that the ideal system would produce a pulse of foam and introduce the LW eggs into the foam in a batch mode as it leaves the gun. Two prototype sprayer designs were constructed and tested. They both consist of two functions: 1) A stream of pressurized foaming agent in water is turned to foam by passage through an air induction spray nozzle; and 2) a sliding plate attached above the barrel of the sprayer which drops dry LW eggs into a tube through which the pulse of foam will travel after leaving the spray nozzle. This design is shown in Figure 3. This design has proven to produce adequate foam with fire fighter foaming agent (keratin hydrolysate) but not yet with the soap-like sapponins and the Armak 2042 has not been tested.

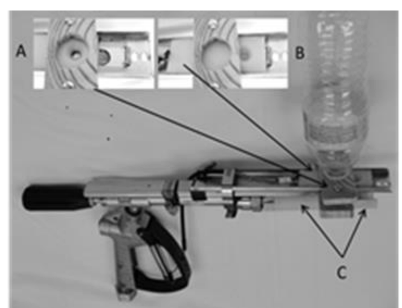

Figure 3. Sliding plate delivery of LW eggs into a trough and passage of foam from sprayer through trough to acquire and project eggs

A superior design entails adding a compressed air assist to the liquid stream and a sparging system to enhance foam formation. Together these changes allow the use of the sapponin-based materials which have a lower foaming capacity. We are currently rebuilding this sprayer and sliding plate to make it more reliable and lightweight. It will retain a similar sliding plate for dropping eggs.

One problem with the air assisted foam formation is that it can blow the eggs out the trough ahead of the foam reducing mixing with the foam and thus reducing adhesion to the tree. This is being addressed by adding a longer tube/trough through which foam passes after picking up dropped dry eggs. This work will continue under ARS base funding and successful results will be reported to the Technology Committee in a supplementary report or presented at the crop protection meetings if it proves successful.

Objective 2 was to test of adhesion of foam to waxy, water repellent surfaces in the laboratory in Peoria using a foam generator/sprayer. Fulfilling this objective was limited by the ability to settle on a preferred method of foam generation and egg introduction, which greatly impact the physical properties (such as velocity and droplet size) of the emitted foam solution. However, efforts were made to identify suitable materials that mimic the properties of apple tree surfaces. A literature survey and analysis of local tree stock determine apple leaves are generally considered easy to wet with water contact angles in the 60-80o range. The bark of local apple trees, at the estimated site of application, was variable with an average water contact angle of $74 \pm 9\,^{o}$. It was decided to use Plexiglas with a water contact angle of $76\,^{o}$ as the leaf mimic, due to its low cost and wide availability. The branches of the canopy will imitated with small diameter polyvinyl chloride pipe, which has a water contact angle of $85\,^{o}$. Once a suitable foam generation system has been identified, these mimics will be used to evaluate the influence of additional adjuvants on adhesions. These adjuvants will include viscosity modifiers, polymers to promote egg suspension and adhesion.

Recent tests using psyllium as a dry bulking agent for metering out eggs in the sliding plate design shows exceptional promise in assisting sticking of the foam because the water leaving the foam as it collapses is taken up by the psyllium. In preliminary tests it appears the only problem from psyllium is using too much, then eggs become trapped in a mat of cross-linked psyllium fibers. Finer grinding of the psyllium husks may also alleviate this problem.

Objective 4 and 5. A preliminary study was conducted at the Moxee farm which consisted of sprinkling dry eggs onto fresh foam solution, wet white glue and water alone painted onto leaves. Best retention of eggs occurred on leaves with glue, followed by foam and no retention at 3 days was observed with water. Improvements in adhesiveness of foam is provided using bulking agents (Metamucil) as observed by greenhouse studies subsequent to field tests. Objectives 4 and 5 remain incomplete until we have an optimal foam and egg sprayer and until we determine vigor of lacewings being sold by insectary. See closing studies described below.

Proof of concept of lacewing colonization and survival on trees when applied as eggs

This idea was suggested by Jim McFerson and was tested on three dates in spring of 2013 at the USDA Moxee Farm. Lacewing eggs glued to light cardboard were stapled on trees and colonization was determined by sampling fruit or vegetative shoots nominally harboring aphid colonies a week to ten days later. The experiments suffered from very heavy predation by ants on the LW eggs but in our third study, we applied tanglefoot to the trunks of half of the trees. Three treatments were made, no eggs applied, 1x rate of eggs (50-100 eggs) stapled to a scaffold near to shoot with aphids and 2x rate of eggs. Terminal clusters removed and counted in the laboratory. The results are summarized in Figure 2. It is evident that

2013 field studies of LW movement to aphid colonies from egg cards

	12-Apr	18-Apr	5-May*
# shoots	36	59	160
# infested	30	17	78
# with LW	1	2	7
lw/shoot	3%	3%	4%
lw/Aphid colony	3%	12%	9%
	* Protected from ants		

Table 2. Percentage of shoots terminals at the Moxee farm found to have lacewings nymphs 7 to 10 days after the placement of lacewing eggs

very few nymphs colonized the shoots on trease receiving the eggs (no LWs were retrieved in the control treatments). Four percent or less of the shoots were found to have LW nymphs. LW did seem to favor shoots with aphids and up to 12% of aphid-infested shoots harbored a LW nymph.

These observations raised concerns about LW nymph mobility and their capacity to search nearby shoots. A second experiment was conducted to observe departure rates of nymphs from the LW cards as provided by the insectary (50-75 LW eggs. We found that of only 2 nymphs left 19 cards with already hatched nymphs while the remainder stayed on the card trying to eat unhatched eggs or just-hatched LW nymphs. This cannibalism behavior argues for spraying eggs on trees singly, and not placing large groups together.

A possible positive spinoff of this work.

Studies searching g for foaming agents have identified an OMRI approved wetting agent that may have useful insecticidal properties which we will test in the laboratory. In addition to modifying the physical properties of a formulation, surfactants also have the ability to become a biologically-active component of the formulation. Surfactants themselves have been shown to be insecticidal to a variety of pest species. Silwet L-77, a closely related trisiloxane surfactant of Silwet Eco, has been reported in to kill a variety of insect species. It has also been reported to kill Asian citrus psyllid, *Diaphorina citri* nymphs, while exhibiting no activity against its parasitoid *Tamarixia radiate*. No data can be found on the insecticidal activity of any of the surfactants in the current study. However, the surfactants presented in Table 1 will be evaluated with pear psylla.

EXECUTIVE SUMMARY

Project Title: Protein-based foam for applying lacewings eggs to fruit trees by ATV

Participants: Tom Unruh and Christopher Dunlap; USDA-ARS

Budget: $19,000 for 2012 We did not request 2nd year funding and received no-cost extension of yr 1 funding; extended to September 2013 by Dunlap and to August 31, 2014 by Unruh.

OVERVIEW

Our three objectives were: 1) chemical - find OMRI-approved foaming agent(s) that preserves the health of the lacewing (LW) eggs and provides adhesion to foliage or tree bark; and 2) mechanical - develop a sprayer that both produces the foam and delivers the eggs to the trees with a spray gun from an ATV, and 3) demonstrate efficacy of aphid control. Three good foaming agents were found and some progress has been made with development of foam plus LW sprayer. We have experienced two shortfalls: first, the foam application of the eggs, while promising, is not reliable, and second, proof-of-concept studies have shown poor colonization of, and dispersal by, LW nymphs on trees, even when applied on paper cards. With funding extending through summer of 2014, we will continue studies to better understand causes of poor dispersal by LWs from cards and try to optimize sprayer.

Accomplishments:

- 2012: Keratin hydrolysates, *Yucca* and *Quillaja* saponin extracts produce suitable foam that adheres to foliage and tree bark but only the latter two saponin extracts are OMRI approved.
- Survival of lacewing eggs in foaming agents exceeds 80% in many trials in the laboratory.
- 2013: The OMRI approved Armak 2042 was added to our list of suitable foaming agents.
- 2012-2013: A hand gun that sprays foam through a cylinder into which dry lacewing eggs are dropped can accurately deliver the eggs in foam to a target 6-8 feet away.
    - Addition of compressed air thickens foam but reduces mixing of eggs with foam
- Addition of bulking agent (ground rice hulls or sphagnum) together with a sticking agent (dry chopped psyllium hulls) helps meter eggs and increases adhesion of eggs, respectively.
- In three field experiments, application of cards containing 40-100 LW eggs/card resulted in little to no recovery of LWs in aphid-infested flower buds nearby or distant to cards
    - Similarly egg cards to glued to walls showed little movement of nymphs
- **Work Needed:**

The mechanical sprayer needs to be optimized to have reliable retrieval and carrying of the eggs from the spray gun and in metering the egg numbers accurately.

Demonstration of the utility and efficacy of application of LW eggs in foam must be demonstrated in the field. This will be done on ARS base funding and reported to the committee.

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