Proposal Title: Crop Protection Product Efficacy Testing for Codling Moth - Laboratory
Report Type: Final Project Report

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Project Duration: 2-Year

Total Project Request for Year 1 Funding: $42,500
Total Project Request for Year 2 Funding: $37,500

Other related/associated funding sources: Requested:
Funding Duration: 2023
Amount: $20,460
Agency Name: WSCPR
Notes: Wages for time-slip

WTFRC Collaborative Costs: NONE

Budget 1
Primary PI: RT Curtiss
Organization Name: Washington State University
Contract Administrator: Office of Research Support and Administration
Telephone: 509-335-9661
Contract administrator email address: ORSO@wsu.edu
Station Manager/Supervisor: Chad Kruger
Station manager/supervisor email address: cekruger@wsu.edu

Item	2023	2024	
Salaries	$8,500.00	$8,840.00	
Benefits	$2,754.00	$2,864.00	
Wages	$15,600.00	$16,224.00	
Benefits	$1,592.00	$1,655.00	
RCA Room Rental			
Shipping			
Supplies	$11,062.00	$4,806.00	
Travel			
Plot Fees			
Miscellaneous			
Total	$39,508.00	$34,389.00	$0.00

Footnotes: [1]RT Curtiss, Project lead, Salary+Benefits (@0.1 FTE); [2]Time-slip employee Wages+Benefits (@$20/hr, 15hr/week); [3]Supplies (year 1 equipment: Autoclave ($5000), Shelves, Rearing cages, Diet heater and mixer, Air purifiers; year 1 and 2 supplies: artificial diet, exposure arenas, misc. consumables, and lab supplies). Tobin Northfield does not require salary for this project, but will provide research space and guidance at TFREC, assistance with analysis and WSU processes.

Budget 2
Co PI 2: Louie Nottingham
Organization Name: WSU
Contract Administrator: Office of Research Support and Administration
Telephone: 509-335-9661
Contract administrator email address: ORSO@wsu.edu
Station Manager/Supervisor: Carol Miles
Station manager/supervisor email address: milesc@wsu.edu

Salaries1	$2,260.00	$2,350.00	
Benefits	$732.00	$761.00	
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	$2,992.00	$3,111.00	$0.00

Footnotes: [1](Louie Nottingham was RT Curtiss' postdoc advisor and provided project guidance on WSU processes for obtaining funds from insecticide companies) Salary+Benefits (@0.02 FTE)

ORIGINAL PROJECT OBJECTIVES:

1) Establish a codling moth colony available for use in new crop protection product testing
2) Lab-test current and new conventional and organic materials and strategies' effectiveness as CM control tactics
3) Supply moths to researchers and companies to sustain outside funding (prevent future requests to WTFRC)
4) Update extension resources to include new product information

SIGNIFICANT FINDINGS:

Objective 1

– 2023 Key Findings

- Of the 2190 cardboard bands placed in apple trees on commercial farms in 2022, 599 final instar larvae and pupae were extracted in the spring of 2023 and emerged as adults in the laboratory shortly thereafter.
- No mating occurred in the mating arenas we constructed based on previous literature, and the adults slowly died apparently without mating or laying eggs.
- We changed tactics and began extracting larvae from infested apples collected on commercial farms in Chelan, Douglas, Okanagan, and Grant Counties. Between June and September 2023, we extracted a total of 1546 larvae from infested apples.
- The adults that emerged from June and July collections, like those that overwintered, failed to mate and no eggs were produced.
- In Mid-July, PI Curtiss designed a new mating arena to test, and by the end of July, reared adults were mating and laying eggs in the new arenas.
- By September, 302 F1 Generation larvae were produced in the colony. They all entered diapause in the cold chamber, and will have diapause broken beginning in late January
- In addition, several thousand cardboard bands were placed on commercial apple farms and collected for pupa/larva extraction. Most have high numbers of individuals.

– 2024 Key Findings

- 14496 larvae were added to the colony from cardboard bands, apples, and eggs laid in mating arenas
- The mating arenas used in 2023 continued to induce moths to lay eggs
- The most important potential finding of this study was unexpected. While extracting larvae from cardboard bands we discovered three new entomopathogenic fungi that may be developed as biological insecticides. Their study is the subject of a pending WTFRC proposal for 2025.

Objective 2

– 2023 Key Findings

- Due to difficulty inducing mating in the laboratory early in the season, limited numbers of F1 Generation moths were produced. They were all reserved for mating and creation of the F2 generation.
- No new products were able to be tested in 2023.
- However, we began testing new products in early 2024 as mating and egg laying occured in the new mating arenas.
- In addition, we began testing some insecticides on larvae being extracted from cardboard bands in late January 2024.

– 2024 Key Findings

- Although egg laying through a fourth generation was induced, with successive generations we have observed reductions in egg production, thus it is critical to constantly introduce new individuals from the field.
- Several products were tested in 2024 in partnership with insecticide companies
- We tested survival of eggs to potentially ovicidal compounds, adult responses to potentially repellent compounds, and efficacy of final instar toxicants
- In addition, we used larvae from the colony to test the three newly discovered codling moth entomopathogenic fungi.

Objective 3 – 2023/2024 Key Findings

- Now that we have solved the mating and egg laying issues, we are prepared to continue producing enough moths to provide them for other research projects such as the pending entomopathogenic fungi project.

Objective 4 – 2023/2024 Key Findings

- This objective will be addressed following final negotiation with insecticide companies on publication of findings.

METHODS

Objective 1: Establish a codling moth colony

Codling moths, sourced and aggregated from Washington State apple farms, were reared in the laboratory on artificial codling moth diet (Frontier Agricultural Sciences product #F9370B) using well-established protocols to ensure that the colony would establish and grow to sustainable levels within one year. The research colony was housed at WSU TFREC in Wenatchee, WA. Apple trees in commercial and research orchards were banded for final instar codling moth caterpillars to colonize in summer 2022 and 2023. Bands were removed from cold storage in January-February 2023 and 2024. We recovered several hundred codling moth final instar larvae and pupae from these bands to establish and sustain the colony. Upon returning colonized bands to the laboratory, individuals were prepared for a break in diapause and caged for emergence and mating. Eggs (F0 generation) from field-collected mated females were then placed on commercial pre-mixed diet. Larvae were fed upon the diet until they were of sufficient size to pupate. Emerging F0 generation adults laid the eggs of the F1 generation, the first generation potentially available for research use. All generations were reared at constant day length (16L:8D), temperature (24-28 °C (75-82 °F)), and RH (50-70%) to synchronize development.

Through the project we continued banding in other WA locations to maintain the colony's genetic diversity. There was a quarantine process much like initial colony establishment before they were incorporated into the main colony to ensure we did not introduce diseases into the colony. Beyond the end of the project, we will also periodically re-collect moths to maintain representative genetics.

Although past WSU codling moth colonies were maintained, this proposed codling moth colony was not be managed exactly the same way. Previous WSU colonies used a codling moth pinto bean diet from made from scratch, and instead, we used a commercial premixed diet because it requires less space, equipment, and personnel. We followed the rearing protocols for codling moth recommended by the diet company (Frontier Agricultural Sciences).

Potential ongoing problem: Occasionally colonies crash and many of the individuals will die. There are often simple explanations for a colony crash, including poor genetics, and proliferation of disease through the colony. Though there is a possibility that we will continue to experience a colony

crash, we will continue to take steps, such as sterilizing equipment, limiting entry into the growth rooms, and treating egg surfaces with dilute bleach to minimize the risk. We are prepared to field collect new individuals if necessary to replenish the colony.

Objective 2: Lab-test current and new conventional and organic materials and strategies

In 2024 the Washington representative codling moth colony grew to a large enough size to accommodate removal of individuals. Thus, we began testing new and current insecticides' efficacies. We employ direct contact assays and choice assays for adults. Against eggs, ovicidal compounds were tested using topical assays. Survival following exposure to insecticides was monitored for up to 14 days.

Topical ovicides were tested by directly spraying eggs laid on egg deposition substrates. Egg hatch was monitored after exposure to insecticides for up to 14 days.

Funds obtained from industry will contribute to the long-term viability of the colony as well as the salaries of personnel tasked with maintaining the colony.

Beyond the end of this project, the codling moth colony will be continued. The primary source of funds for this colony will be from chemical company contributions for testing new products. Secondary sources of funding will come from research projects (both our research and sales of moths to other researchers' projects). In addition, the colony will be scaled up or down as funds/needs dictate. If there are times where demand lags, it can easily be scaled down without elimination. There is usually a several month negotiation with insecticide companies before moths are needed (i.e., discussions with chemical companies began in August 2022 for spring 2023 trials). We do not intend for the colony to be dissolved while we are at WSU. In addition, our process for quarantine of new genetic strains will allow us to collect and grow the colony quickly from new field-collected individuals when needed.

Objective 3: Supply moths to researchers and companies to sustain outside funding

When there are excess moths produced for our research needs, we will provide them to other labs for research use. Because there is a cost associated with production of moths, we will establish a per moth cost structure for researchers. Eventual costs from a robust colony may be as low as $0.02/moth, though initially it will cost as much as $0.10 to produce each moth. Sterile moths produced by the Okanagan Kootenay Sterile Insect Release facility will cost researchers $0.02/moth in 2023, and the only stage available is adult, so our target cost is comparable, and our facility will be able to provide all life stages to researchers. This codling moth colony at WSU-TFREC will become an invaluable resource for discovery of new management tactics and techniques and understanding codling moth biology and behavior in Washington orchards.

Objective 4: Update Extension resources to include new product information

As new products and solutions are tested, approved for use, and found to be effective as management tools, we will continue to incorporate suggestions for their use in extension talks and online resources. We will develop strategies for incorporating new chemistries into the management recommendations found in the crop protection guide and on the Decision Aid System. In addition, we will publish our findings in peer reviewed journal articles, on the Tree Fruit Website, and in other fruit industry publications.

RESULTS AND DISCUSSION

Objective 1: Establish a codling moth colony available for use in new crop protection product testing

2023 Results

Trees that were cardboard banded in fall 2022 in Grant and Chelan Counties were used to obtain codling moth larvae in puparia. 2,115 cardboard bands from four Grant County locations yielded 215 codling moth larvae, while approximately 75 cardboard bands from Chelan County yielded 384 moth larvae (Fig. 1). Diapause was broken for these overwintering larvae beginning in May, and by June 20 all larvae had been extracted from bands, allowed to pupate, and placed in mating arenas. Unfortunately, no successful mating occurred, and no viable eggs were laid from either the Grant or Chelan County collections.

Figure 1. Checking cardboard bands for CM larvae

Figure 2. CM larva to be collected from infested apple

Figure 3. Some mating arena set-ups tested

Because it was possible that the cardboard band process negatively impacted moth fitness, beginning in July 2023, we changed tactics and began extracting codling moth larvae from infested apples collected on farms in Chelan, Douglas, and Grant Counties, while placing cardboard bands on farms in Chelan, Douglas, Grant, Yakima, and Okanagan Counties (Fig. 2). Larvae extracted from

apples and cardboard bands, 766, were fed on artificial diet, pupated, and emerged as adults. The first several rounds of adult moths were once again placed into the mating arenas, and neither mating nor egg laying occurred. The mating arena design that our colony failed to mate in was based on descriptions found in White and Hutt 1970, 1971; Hathaway et al. 1971, 1972, 1973; Howell and Clift 1972; Bathon et al. 1991; Toba and Howell 1991; Dyck 2010. Again, we changed tactics and tested several new and modified mating arena setups (Fig. 3). By the end of July, one of the tested mating arenas successfully allowed mating, egg deposition, and produced viable larvae (Fig. 4,5). From these mating individuals we obtained 302 F1 generation larvae by the middle to end of September. The majority of these individuals entered diapause and are housed in cold storage and will not be used until late January 2024. Cardboard bands from several on-farm locations will be processed through winter 2023/24.

Figure 4. Transferring F1 Generation CM larvae from mating arena to artificial diet

Figure 5. First successful F1 generation CM larva

2024 Results

In the second year of this project, we added 14496 codling moths to the colony. These moths came from several sources, including cardboard bands, apples, and progeny of colony-reared moths. We had very high emergence from live codling moths extracted from bands, ca. 93% of the extracted larvae emerged as adults. From those field-collected moths, we successfully reared four generations from late 2023-late 2024 (Generations F0-F3). Moving forward with the colony beyond the end of the WTFRC funding, we now know that extracting adults from bands is a viable method of quickly building up the colony. In the mating arenas PI Curtiss designed in 2023, eggs were consistently laid by F0-F3 moths, however, the egg to adult production decreased with each successive generation (Table 1).

	F0	F1	F2	F3
% Moths Emerged	81.95	15.36	2.61	0.07

Table 1. The percentage of each lab generation of adult moths that emerged over 2024.

Improvements to our rearing process still need to be made. In the future we plan to test other commercial diets and compare them with the pinto bean diet. In addition, we have recently observed a high proportion of F2 and F3 larvae ceasing to develop when the diet conditions begin to degrade. We recently started transferring them to fresh diet to determine if percent successful pupation and

emergence can be increased by providing new food. That test will be completed several months following the end of the WTFRC project.

Objective 2: Lab-test current and new conventional and organic materials and strategies' effectiveness as CM control tactics

2023 Results

Due to the lack of mating in the first mating arena tested, much of the first half of the year yielded no larvae upon which to test insecticides. However, now that we have successful rearing occurring in the colony, we will be able to begin testing new materials in 2024. In addition, we will utilize individuals from cardboard bands in some insecticide tests.

2024 Results

In 2024 we had enough colony-reared moths to begin product testing. We conducted three studies with colony-insects.

1) An egg mortality assay that compared two new products efficacy at causing mortality in topical applications with applications of 440 superior oil. The products were organic, botanical oils that are used against other pests as topical ovicides. For this study we tested all the products in 1% solutions. The 440 oil prevented ca. 95% of the eggs from hatching, test product A prevented ca. 55% of the eggs from hatching, and test product B prevented ca. 40% of the eggs from hatching. At this time, we do not have permission to release additional information about these products, however at the tested concentrations the new products were not more effective than 440 oil. In 2025, we are negotiating with the company to conduct additional tests with the products at higher concentrations.
2) An adult oviposition choice assay that tested the repellent effects of a botanical compound that contained garlic oil. For this cage assay, we compared moths' oviposition on apples treated with the test product or water. The assay allowed moths to choose between only two apples for oviposition. The findings were somewhat inconclusive, but the data suggests that there may be some repellency of the garlic oil. More replication is needed. In 2025, we may conduct more testing if the company wishes to pursue this study again.
3) A final instar larvae mortality/sublethal effect assay using five different test compounds compared to a positive control (Malathion), and negative control (water). One of the test compounds contained a neem product. Our findings suggest that this stage is particularly difficult to affect. It took over 20 days for the last malathion-exposed moth to die, and no other test compounds caused significant mortality or prevented pupation. More studies in 2025 will be conducted with the newly found codling moth entomopathogenic fungi, and compared with other products.

Objective 3: Supply moths to researchers and companies to sustain outside funding (prevent future requests to WTFRC).

2023 Results

We only produced 302 F1 Generation final instar larvae in 2023, however, when they break diapause, they will be used to produce enough eggs and larvae to provide to other projects.

2024 Results

Although we produced over 14,000 moths in 2025, we only provided a few hundred to other researchers. As this colony continues to develop, we expect to provide more moths to other researchers in the future.

Objective 4: Update extension resources to include new product information

No new updates were produced in 2023 due to the previously described issues. Updates from 2024 testing will be found in 2025 documents such as the crop protection guide, and 2-3 arthropod management tests publications.

References:

Bathon, H, et al. "Rearing Methods." Tortricid Pests: Their Biology, Natural Enemies and Control. , vol. 5, World Crop Pests, The Netherlands, Amsterdam, 1991, pp. 283–293.

Dyck, V. A. "Collecting, Holding, Marking and Sexing Adults." Rearing Codling Moth for the Sterile Insect Technique, Food and Agriculture Organization of the United Nations, Rome, 2010, pp. 75–79.

Hathaway, D. O., A. E. Clift, et al. "Development and fecundity of codling moths reared on artificial diets or immature apples." Journal of Economic Entomology, vol. 64, no. 5, 1971, pp. 1088–1090, https://doi.org/10.1093/jee/64.5.1088.

Hathaway, D. O., L. G. Schoenlfber, et al. "Codling moths: Plastic pellets or waxed paper as oviposition substrates." Journal of Economic Entomology, vol. 65, no. 6, 1972, pp. 1756–1757, https://doi.org/10.1093/jee/65.6.1756.

Hathaway, D. O., L. V. Lydin, et al. "Monitoring mass rearing of the Codling Moth." Journal of Economic Entomology, vol. 66, no. 2, 1973, pp. 390–393, https://doi.org/10.1093/jee/66.2.390.

Howell, J. F., and A. E. Clift. "Rearing codling moths on an artificial diet in trays." Journal of Economic Entomology, vol. 65, no. 3, 1972, pp. 888–890, https://doi.org/10.1093/jee/65.3.888.

Toba, H H, and J F Howell. "An improved system for mass-rearing codling moths." J. Entomol. Soc. British Columb, vol. 88, 1991, pp. 22–27.

White, L. D., and R. B. Hutt. "An inexpensive transparent holding cage for insect oviposition and longevity studies." Journal of Economic Entomology, vol. 64, no. 2, 1971, pp. 551–552, https://doi.org/10.1093/jee/64.2.551.

White, L. D., and R. B. Hutt. "Codling Moth catches in sex and light traps after exposure to 0, 25, or 40 krad of gamma irradiation." Journal of Economic Entomology, vol. 64, no. 5, 1971, pp. 1249–1250, https://doi.org/10.1093/jee/64.5.1249.

White, L. D., and R. B. Hutt. "Effects of gamma irradiation on longevity and oviposition of the codling moth." Journal of Economic Entomology, vol. 63, no. 3, 1970, pp. 866–869, https://doi.org/10.1093/jee/63.3.866.

EXECUTIVE SUMMARY

Project Title: Crop Protection Product Efficacy Testing for Codling Moth – Laboratory

Keywords: *Cydia pomonella*, management, research colony, new materials

Abstract: Codling moth management in Washington State requires effective mating disruption and application of conventional or organic insecticides. New insecticide products and tactics need to be tested under laboratory conditions, but without access to a colony of codling moths for assays, new product testing is severely hampered. This project established a research codling moth colony at WSU-TFREC in Wenatchee to be used to test new crop protection products and tactics. The first year of the project was constrained by repeated failures to induce oviposition in the laboratory, but by the second year, that issue was resolved and eggs were consistently laid in mating containers. By the project end, we performed assays with new topical ovicides, antifeedants, and potential repellants using the colony. There are still improvements to be made in the rearing process, however, moving forward we have established protocols that will allow for future testing of new products and tactics.