

FINAL PROJECT REPORT

Project Title: Identification of chemical lure for spotted wing drosophila

PI: Peter Landolt
Organization: USDA, ARS
5230 Konnowac Pass Rd.
Wapato, WWA 98951
Telephone: (509) 454 6570
Email: peter.landolt@ars.usda.gov

Co-PI (2): Helmuth Rogg
Organization: Oregon Dept. Agric.
635 Capitol Street NE
Salem, OR 97301
Telephone: (503) 986-4662
Email: hrogg@oda.state.or.us

Other funding sources

Agency Name: NIFA, NE RIPM Program

Amount awarded: \$24,000

Notes: Grant awarded to CT Ag. Exp. Station with subcontract to USDA, ARS Wapato

Total Project Funding: \$101,000

Budget History:

| Item | Year 1: | Year 2: | Year 3: |
|---------------|---------|---------|---------|
| Salaries | | | |
| Benefits | | | |
| Wages | 20,300 | 20,300 | 20,300 |
| Benefits | 7,700 | 7,700 | 7,700 |
| Equipment | | | |
| Supplies | 5,500 | 4,500 | 2,500 |
| Travel | 1,500 | 1,500 | 1,500 |
| Plot Fees | | | |
| Miscellaneous | | | |
| Total | 35,000 | 34,000 | 32,000 |

RECAP OF ORIGINAL OBJECTIVES:

The overall objective of the project was to develop a reliable detection system for cherry growers, which would allow them to react to a spotted wing drosophila infestation early and at low population densities. Technical objectives were to:

1. Isolate and identify attractants from wine and vinegar.
2. Determine an optimized combination of attractive chemicals.
3. Develop a controlled release dispenser for use as a lure in a trap.

SIGNIFICANT FINDINGS

1. A blend of four chemicals from wine and vinegar were found to be strongly attractive to female and male adult SWD. These four chemicals are acetic acid, ethanol, acetoin, and methionol.
2. The same blend of four chemicals is much more selective to SWD, and attracts much fewer non-target insects.
3. In a comparison between the chemical lure and food bait materials used by pest managers, the chemical lure was more effective in attracting the first flies in the spring in Hood River.
4. A sachet system of dispensing the chemicals was worked out to provide a controlled release dispenser to place in a trap.
5. Information on the chemical attractant and means of dispensing the attractant was shared with five companies, and two of those companies (Scentry and Trece) are marketing the attractant. We maintain a dialogue with two other interested companies (Sterling and AgBio).

RESULTS AND DISCUSSION

Fly responses to antennal-active volatiles of wine and vinegar

Thirteen wine and vinegar chemicals, in addition to ethanol and acetic acid, consistently elicited fly antennal responses determined with an electro antennal detector system (EAD) (Table 1). A combination of all 13 of these chemicals tested in the field was not as attractive as the starting material of wine and vinegar, and indeed was quite weak. This problem was anticipated, because the GC-EAD assay determines chemicals detected (smelled) by the antennae, and does not indicate chemical attractiveness. There is potential for chemicals that are “smelled” by the fly to be attractive, repellent, or neutral.

A laboratory assay was developed and used to test 13 EAD-active chemicals for attractiveness and repellency to SWD when added to acetic acid a mixture of and ethanol. Results of this series of assays indicated that seven chemicals reduced the fly response to acetic acid and ethanol, while the six other chemicals either improved the attraction response or were neutral (Table 1).

Table 1. Chemicals from wine and vinegar headspace analysis that elicited electro antennal responses from spotted wing drosophila, and fly response in laboratory bioassay when added to acetic acid and ethanol.

| Chemical | In Wine headspace | In Vinegar headspace | Bioassay Response |
|-----------------------|-------------------|----------------------|-------------------|
| Ethyl acetate | X | X | repellant |
| Acetoin | X | X | attractive |
| Ethyl butyrate | X | | repellent |
| Ethyl lactate | X | X | repellent |
| Hexanol | X | | repellent |
| Isoamyl acetate | X | X | repellent |
| 2-methylbutyl acetate | X | X | repellent |
| Grape butyrate | X | X | neutral |
| Methionol | X | | neutral |
| Isoamyl lactate | X | | neutral |
| Ethyl sorbate | X | | repellent |
| 2-phenyethanol | X | X | neutral |
| Diethyl succinate | X | | neutral |

Fly responses to subsets of the antennal-active volatiles of wine and vinegar.

A second generation chemical blend, consisting of 6 chemicals plus acetic acid and ethanol, was field- tested as a bait for a trap. This blend was improved over the previous blend but was still significantly less attractive than wine plus vinegar.

A series of field tests showed that only three of thirteen EAD-active wine and vinegar chemicals significantly improved fly captures in traps when added to acetic acid plus ethanol. A third generation chemical blend then was comprised of acetic acid, ethanol, and those three chemicals that were EAD-active and also co-attractive in field test. This third generation blend was equal in attractiveness to SWD compared to wine plus vinegar in field tests (Figure 1).

These results provided a basic set of chemicals to use to optimize a chemical lure for use in trapping and detecting SWD. Our previous work showed that the combination of wine and vinegar is much more attractive to the fly than either wine or vinegar. During the duration of this project, that combination of materials has been the strongest food type bait for SWD. Our combination of the four chemicals was initially as attractive as that best bait. This result provides a clear opportunity to develop and use a lure that is powerful in luring both sexes, can be formulated to provide even attractiveness for long periods of time, and can be used in a dry trap or a wet trap. All of the active chemicals are commercially available and are relatively inexpensive.

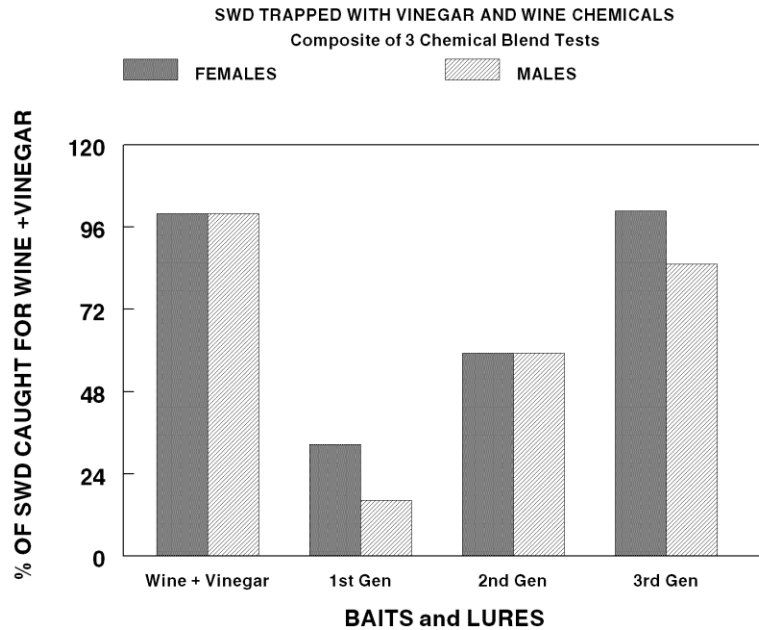


Figure 1. The attractiveness of the three blends of chemicals that were field-tested is shown in this graph, in relation to the attractiveness of wine plus vinegar. The first generation blend was the combination of EAD-active chemicals. The second generation blend was the EAD active chemicals minus the chemicals that were repellent in a laboratory assay. The third generation blend was limited to those chemicals that were co-attractive when tested individually in the field.

2013. Dispenser development. We expected that evaluations of chemical release rates and ratios would show further improvements in the attractiveness of the blend of chemicals. In a series of trapping experiments, increases in the release rates of acetoin and greater doses of acetic acid and ethanol resulted in increases in numbers of SWD captured. Thus, a new dispensing system (sachet lure) with acetoin and methionol released from sachets and acetic acid and ethanol released from the trap drowning solution yielded further improvement in the SWD trap catches. This dispensing system was then compared with the previous lure system that involved dispensing acetoin and methionol from vials, and acetic acid and ethanol from the trap drowning solution. We found that the use of the new sachet lure was significantly more attractive to SWD than the previous system or the wine plus vinegar mixture that was the basis for the research project.

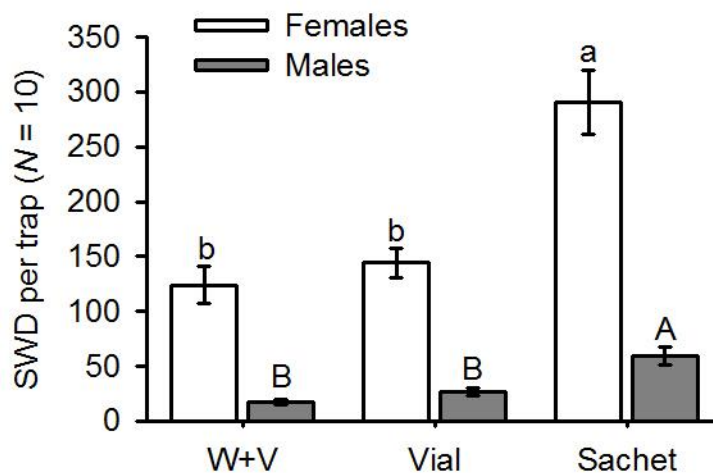


Figure 2. This graph shows SWD flies captured in traps baited with the combination of wine and vinegar (W + V), with acetoin and methionol in a vial and ethanol and acetic acid in the drowning solution (vial), and with acetoin and methionol in two individual sachets and ethanol and acetic acid in the drowning solution (sachet).

Assessment of non-target insect responses. Numbers of the two cutworm moths (spotted cutworm and olive dart) and two armyworms (bertha and true armyworms) were much fewer in traps baited with the SWD chemical lure, compared to the wine/vinegar mixture. Similar results were seen for the two yellow jacket wasp species that were abundant in test 3, and the false stable fly which was abundant in test 2. Numbers of the little house fly were numerically but not statistically less with the chemical lure. These decreases in non-target insects in traps baited with the chemical lure compared to the wine/vinegar mixture (Table 2) are of great potential importance to the costs of detection and monitoring programs. Large numbers of non-target insects in traps greatly increases the time it takes to sort, identify, and tally the spotted wing drosophila in the trap. Also, large numbers of these insects can interfere with the effectiveness of the effectiveness of the drowning solution to retain SWD that enter the trap.

Additionally, numbers of non-target species of *Drosophila* flies were generally reduced in traps with the chemical lure, compared to the wine/vinegar mixture (Table 3). We include here only the data for the species commonly encountered. Again, we suggest that these reductions in non-target catches in SWD traps should reduce the costs of trap checking and trap maintenance. Note that it takes considerable skill to sort and identify SWD from a strong mixture of related *Drosophila* flies. Reducing the numbers of non-target *Drosophila* in traps should make trap checking and SWD detection and monitoring considerable easier.

Table 2. Mean numbers of insects per trap per week, for traps baited with a mixture of wine and vinegar, and traps baited with the SWD chemical lure.

| | Wine + Vinegar | 4-component lure |
|-----------------------|----------------|------------------|
| Test 1. | | |
| Spotted cutworm moth | 10.7 ± 1.0a | 0.3 ± 0.2b |
| Bertha armyworm moth | 6.7 ± 1.1a | 0.0 ± 0.0b |
| Test 2. | | |
| False stable fly | 431.6 ± 97.5a | 82.3 ± 36.3b |
| Little house fly | 8.3 ± 3.6a | 5.0 ± 2.4a |
| Test 3 | | |
| German yellow jacket | 19.0 ± 2.1a | 5.0 ± 1.2b |
| Western yellow jacket | 22.9 ± 5.5a | 5.2 ± 1.3b |
| Test 4 | | |
| Olive dart moth | 21.7 ± 1.9a | 1.9 ± 0.6b |
| True armyworm moth | 1.2 ± 0.4a | 0.0 ± 0.0b |

Means within a row followed by a different letter are significantly different by a paired T-test, at $P < 0.05$.

Table 3. Mean numbers of different species of *Drosophila* flies per trap per week, for traps baited with a mixture of wine and vinegar, and traps baited with the SWD chemical lure. Tests were conducted in the lower Yakima Valley and near Salem, Oregon.

| | Wine + Vinegar | 4-component lure |
|------------------------------|-----------------|------------------|
| Test 1. WA | | |
| <i>D. suzukii</i> (SD) | 92.9 ± 10.0a | 104.0 ± 7.3a |
| <i>D. immigrans</i> | 15.0 ± 3.0b | 5.0 ± 2.9a |
| <i>D. melanogaster</i> group | 1015.0 ± 142.9b | 64.5 ± 18.5a |
| <i>D. obscura</i> | 625.1 ± 91.6b | 146.4 ± 26.2a |
| Test 2 OR | | |
| <i>D. suzukii</i> (SD) | 1489.8 ± 422.4a | 1751.2 ± 457.9a |
| <i>D. immigrans</i> | 3.2 ± 1.9a | 3.1 ± 1.5a |
| <i>D. melanogaster</i> group | 39.1 ± 9.1b | 15.0 ± 3.4a |
| <i>D. obscura</i> | 88.5 ± 33.2a | 72.9 ± 23.0a |

Early season detection of SWD with a chemical lure. A key advantage of a strong lure is the ability to detect either populations of the fly, or their first movement, so as to determine appropriate pest management needs. Working with Peter Shearer at OSU Hood River, a comparison of traps and baits was set up to look at early season catches of SWD in cherry orchards. In that test, traps baited with the chemical lure (Cha lure) caught more SWD than traps baited with apple cider vinegar (ACV) or traps baited with yeast/sugar bait, in early season cherry orchards in Hood River.

Early detection: First week

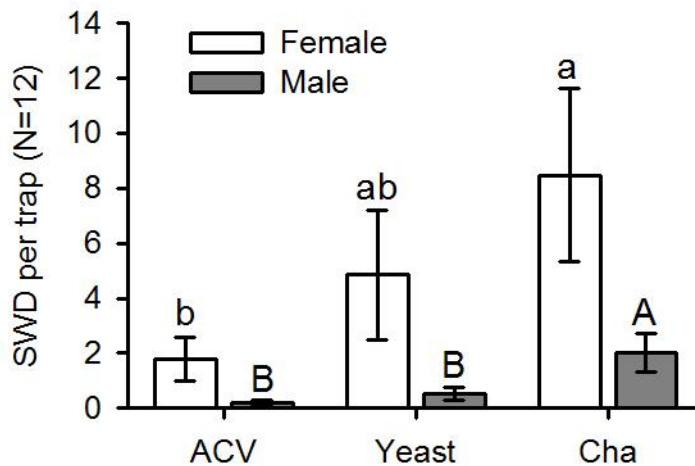


Figure 3. Mean numbers of SWD flies captured per trap per week, for traps baited with apple cider vinegar (ACV), a yeast/sugar formulation (yeast) and the SWD chemical lure (Cha).

Technology transfer.

An important outcome of a successful research project is the availability and use of the information or technology produced. To this end, information on the results of the research has been

shared with multiple companies that manufacture and market lures and traps for the detection and monitoring of insect pests. These companies are AgBio, ChemTiki, the Hungarian Academy of Science, Scentry, Sterling and Trece. At this time, Scentry and Trece are producing and marketing lures based on the results of our studies.

PUBLICATIONS

Cha, D. H., T. Adams, H. Rogg, and P. J. Landolt. 2012. Identification and field evaluation of fermentation volatiles from wine and vinegar that mediate attraction of spotted wing drosophila, *Drosophila suzukii*. J. Chem. Ecol. 38: 1419-1431.

Landolt, P. J., T. Adams, and H. Rogg. 2012. Trapping spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) with combinations of vinegar and wine, and acetic acid and ethanol. J. Appl. Entomol. 136: 148-154.

Landolt, P. J., T. Adams, T. S. Davis, and H. Rogg. 2012. Spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Tephritidae), trapped with combinations of wines and vinegars. Florida Entomol. 95:326-332.

Cha, D. H., T. Adams, C. T. Werle, J. J. Adamczyk, Jr., H. Rogg, and P. J. Landolt. 2013. A four-component blend of fermented bait volatiles is attractive to spotted wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae). Pest Manag. Sci. 70: 324-331.

Cha, D. H., S. P. Hesler, R. S. Cowles, H. Vogt, G. M. Loeb, and P. J. Landolt. 2013. Comparison of a synthetic chemical lure and standard fermented baits for trapping *Drosophila suzukii* (Diptera: Drosophilidae). Environ. Entomol. 42: 1052-1060.

Epsky, N. D., M. A. Gill, D. H. Cha, and P. J. Landolt. 2014. Trapping the African fig fly (Diptera: Drosophilidae) with combinations of vinegar and wine. Florida Entomol. 97: 85-89.

Cha, D. H., T. B. Adams, H. Rogg, and P. J. Landolt. Cooking it right: emission rate dependent synergism between food related fermentation volatiles influence chemical attraction of *Drosophilidae suzukii*. Plos One. (in press).

Cha, D. H., S. P. Hesler, S. park, T. B. Adams, R. S. Zack, H. Rogg, G. M. Loeb, and P. J. Landolt. Simpler is better: a comparison of non target insects trapped with a 4 component chemical lures versus a chemically more complex food-type bait for *Drosophila suzukii*. Entomol. Exp. Et App. (in press).

Cha, D. H., M. A. Gill, N. D. Epsky, C. T. Werle, J. J. Adamczyk Jr., and P. J. Landolt. From a nontarget to a target: identification of fermentation volatiles attractive to African fig fly, *Zaprionus indianus*. J. Entomol. Sci. (submitted).

EXECUTIVE SUMMARY

For project “Identification of chemical lure for spotted wing drosophila”.

Significant Outcomes.

1. This work determined that the combination of four chemicals (ethanol, acetic acid, acetoin, and methionol) can constitute a strong attractant for the spotted wing drosophila fly.
2. Methods were worked out for optimum release of acetoin and methionol from plastic sachets, and optimum amounts of acetic acid and ethanol in water, for making a strong trap bait.
3. It was determined that the chemical lure is as strong or stronger than the starting material of wine and vinegar, which was shown to be a superior food type bait.
4. It was determined that the chemical lure is much less attractive than wine and vinegar to other types of insects (called non-target insects) that interfere with SWD detection and monitoring efforts.

Summary of Findings

These results provide a chemical lure for use in detecting and monitoring SWD. At this time, this combination of chemicals is as attractive as our best food type bait. This result provides a clear opportunity to develop and use a lure that is powerful in luring both sexes, can be formulated to provide attractiveness for long periods of time, and can be used in a dry trap or a wet trap. All of the active chemicals are commercially available and are relatively inexpensive. Two companies are producing and marketing lures based on this work, and we are working with them to assist their efforts and to make the chemical lure available for further study by other researchers and for detection and monitoring efforts.

We expected that isolation of the volatile chemicals from wine and vinegar that attract SWD would also lead to a lure that is less attractive to non-target insects, which would reduce labor and trap maintenance effort when trapping SWD. Our experimental results support this expectation, although the chemical lure is still attractive to other types of insects and it is not a species specific lure.

This work is conducted with the purpose of providing a powerful chemical lure for reliable early season detection of SWD for cherry orchards, information that is needed for making sound pest management decisions. We anticipate that the approach and strategy taken here also will provide a trap/lure combination that is easier to use and more consistent in its attractiveness compared to current monitoring methods in use. The direct practical impact will be to reduce crop damage and losses due to undetected SWD populations, and also to reduce costs of pest control incurred when a fly population is not present

Future Directions.

Work is ongoing at a number of other institutions to develop applications of the lure and to incorporate it into IPM systems. We anticipate further technology developments, such as the incorporation of the chemistry into sprayable baits, and attract-and-kill technologies.