

Northeastern IPM Center – IPM Competitive Grant Program 2012 – Project Description**PD: Richard S. Cowles****Lead Institution: The Connecticut Agricultural Experiment Station****Co-PD(s) and Institutions:****DeWei Li, C.A.E.S.****Peter Landolt, USDA, ARS, Wapato, WA****Cesar Rodriguez-Saona, Rutgers Univ. Steven R. Alm, Univ. Rhode Island****Gregory Loeb, Cornell University****Collaborator: Phyllis A. W. Martin, USDA, ARS, Insect Biocontrol Laboratory, Beltsville Agricultural Research Center, Beltsville, MD****Project Title: Spotted wing drosophila management****Project Type: Agricultural IPM****Proposal Summary**

This is a Research project. The spotted wing drosophila (SWD), *Drosophila suzukii*, is a highly invasive pest that infests small fruits (raspberry, blueberry, strawberry, and grapes) and tree fruits (most notably, cherries). First found in New England on August 19, 2011, this pest over the next month caused \$500,000 - \$1,000,000 in losses to fruit growers in New England due to unsaleable maggot-infested fruit. Late fruiting blueberries, most fall-bearing raspberries, many wine grapes, and day-neutral strawberries were lost. Current SWD management recommendations from the Northwest suggest weekly applications once fruit start to ripen with methomyl, malathion, advanced generation pyrethroids, or spinosyns. Except for spinosyns, sprays of these products will set back decades of IPM progress by eliminating the beneficial predators and parasites in these crops. Such intensive use of pesticides on these crops is expensive, selects intensively for insecticide resistance, and leaves residues on fruit. This project will test in four small fruit crops in three states attractant and phagostimulatory baits combined with insecticides, some of which are organically acceptable, to manage SWD. Methods to compare will be conventional spray, bait spray applied to the crop, attractant plus bait applied to vegetation surrounding the crop, and insecticidal bait stations. Efficacy will be assessed by measuring insect activity and fruit infestation. We will investigate dipteran-specific *Bacillus thuringiensis* and *Entomophthora muscae* for deployment either with bait sprays or stations. Pest management methods developed in this proposal will provide science-based pest management that improves human and environmental health by minimizing the need for sprays of broad spectrum insecticides.

Background and Justification

The spotted wing drosophila (SWD), *Drosophila suzukii*, is similar in appearance to the common fruit fly (more properly called the vinegar fly) used in genetics laboratories, *Drosophila melanogaster*, to which it is closely related (Kopp and True 2002). Details of its life history as observed in west coast fruit crops have recently been described (Shearer et al. 2011). Adults mate within 1 – 2 d of emerging, and can live 21 – 300 d. Females can lay 300 – 600 eggs during their lifetime, usually depositing 1 – 3 eggs per fruit. Eggs are inserted just under the skin of fruit, but paired respiratory filaments are left projecting from fruit, which allow the number of eggs to be assessed microscopically. Eggs develop in 1 – 3 d, larvae in 5 – 7 d, and pupae in 3 – 15 d. Therefore, under optimal conditions of about 20 °C, one generation can complete development in about 10 d. Estimates of 100-fold population increase every 2 wk are believable, based upon the explosive colonization and subsequent increase in fruit crops observed in Connecticut during September, 2011.

SWD is an extremely invasive direct pest, and is one of only two species of agriculturally important pests in the Family Drosophilidae (Hauser 2011). The reasons it can cause extensive economic loss are (1) it has a broad host range, (2) females have an ovipositor equipped with a pair of saw-like appendages, which permit eggs to be deposited in unripe fruit, (3) each female can lay 300 – 600 eggs, and (4) the generation time is 10 – 20 days during the growing season (Walsh et al. 2010). My initial observations from Connecticut farms, based on rearing out flies from infested fruits, suggest that crops severely affected are: raspberry, blueberry, strawberry, and grapes. Fruit growers in the Pacific Northwest initially called this species the small cherry vinegar fly, and so we can expect there to be a high risk to certain species of stone fruits, although cherries in New England may be somewhat protected due to sprays to manage cherry fruit flies. Certain less-common crops are a special concern: table grapes, unlike wine grapes, will have no tolerance for the presence of infested fruit. Day-neutral strawberries ripen through the late summer and into the fall, when they will be subject to higher SWD population pressures. For example, fruits collected September 19, 2011, indicate that as many as 500 eggs may be deposited per strawberry.

Native to mainland Asia, SWD was detected in Japan in the early 1900s and Hawaii in 1980 (Hauser 2011). SWD was first detected in the continental United States in California in 2008 and then the rest of the other Pacific Coast States, BC, and Florida during 2009 (Walsh et al. 2010; Hauser 2011). During 2010 it was detected in Utah, Louisiana, North Carolina, South Carolina, Wisconsin, Michigan, Alberta, Manitoba, Ontario, and Quebec, but the more northern detections were interceptions of individual flies and economic injury was not reported (Hauser 2011). It arrived in the region covered by the Northeast Regional IPM programs in 2011, with the first detection in New Jersey on July 7 in blueberries (Cesar Rodriguez-Saona, Rutgers University, e-mail alert to BMSB group, July 21, 2011). I (RSC) made the first discovery (August 19, 2011), of this pest in New England, from traps baited with whole wheat bread dough designed to study strawberry sap beetles. In the next three weeks, this pest caused \$500,000 - \$1,000,000 in losses to fruit growers in Connecticut and Rhode Island due to unsaleable maggot infested fruit and increased expenses from frequent sprays needed to salvage the crops. Late fruiting blueberries and nearly the entire crop of fall-bearing raspberries were lost in 2011. Within two days of having detected SWD in southwestern Connecticut, a grower received

complaints of maggot-infested raspberries in south-central Connecticut. Since then, there have been several observations of SWD damage to raspberries, blueberries, and day-neutral strawberries and wine grapes throughout CT and RI. Tropical storm Irene traveled through the state soon after the first detection and this pest is now found through the rest of New England. Grape growers have seen 20 – 50% loss of grapes due to sour rot enhanced by SWD infestation, and growers of day-neutral strawberries are looking at complete crop loss from mid-September to the end of harvest (usually in October). Losses to wine grapes are a special concern, as other regions in the U.S. and Canada have not reported serious losses in wine vineyards.

Current recommendations developed by university personnel suggest weekly applications with full coverage of foliage and susceptible fruit, once fruit start to ripen, with methomyl, malathion, pyrethroids, or spinosyns to manage this pest (Isaacs et al. 2010). Recent trials have suggested that use of neonicotinoids may kill larvae within fruit (Lynell Tanigoshi, WSU, personal communication Sept. 20, 2011). My previous experience with tree fruits suggests that carbaryl may also be valuable for killing newly hatched larvae within fruits. However, such a strategy runs counter to growers' desire to minimize direct application of insecticides to ripe fruit, and it is unknown whether this strategy will lead to residues exceeding their tolerance. These sprays, with the exception of the spinosyns, can be anticipated to set back decades of IPM progress by eliminating the beneficial predators and parasites in these crop systems. Fruit growers in New England tend to be very progressive; some have been participating with fruit scouting and IPM programs since the 1980s. They are therefore reluctantly adopting weekly or more frequent full sprays of insecticides to protect their fruit, and are anxious to have tools available that (1) will reduce or eliminate the need to deposit insecticide residues directly on fruit, (2) will maintain the ecological balance of predators and parasitoids that keep secondary pests at low numbers, (3) provide additional options (besides spinosyns) for organically acceptable management of this pest, and (4) will reduce the likelihood of SWD developing resistance to insecticides. The needs of growers in the northeast are critically urgent, and are probably greater than those of fruit growers on the west coast. One reason is that CLIMEX modeling of SWD suggests that this species is better adapted to conditions on the east coast than in most of the west (Walton et al. 2010). A second reason is that fruit crops on the east coast generally are grown in smaller fields with greater crop and non-crop vegetation diversity that could lend itself toward continuous SWD population growth.

One of the grave concerns regarding SWD is the likelihood of insecticide resistance development. Some farms in the northeast have June strawberries, blueberries, and fall-bearing raspberries. This combination permits continuous development of flies as they move from one crop to the next. Pick-your-own operations try to minimize residues of insecticides on fruits, and some attempt to grow fruit with only organically acceptable products. Currently, spinosyns consist of the only organically acceptable product available and effective for targeting SWD (Pyganic, a combination of pyrethrins and azadirachtin, has been ineffective). Growers may rely too greatly upon spinosyns, and could experience insecticide resistance in a short time. Also, growers of day-neutral strawberries have found that they have to spray spinosyns so frequently to control SWD that they reach the maximum allowed product permitted to be sprayed per year, and still have much of a fruit season to harvest.

Cultural management techniques identified for managing SWD have included sanitation, through removal of all overripe or waste fruit from the field, and exclusion with fine insect screens (Walton et al. 2010). Sanitation, unless aided by machinery, will not be practical in the Northeast where labor is very expensive. I (RSC) have already tested screens sold by Dubois Agrinovation, Inc., and have found that the ProtekNet 80 g, but not the ProtekNet 60 g can exclude SWD adults. Such netting is currently available in limited width, but I have started discussions with the manufacturer of this netting material to determine whether it will be practical to manufacture greater widths that could be used for covering high tunnels or entire fields (similar to current use of bird netting or shade cloth).

Biological control under investigation for SWD includes several species of parasitoid wasps (Walsh et al. 2010). However, there may be limited opportunity for parasitoids to interact with internal fruit feeding larvae, and use of insect pathogenic fungi to induce epizootics in the adults may have greater likelihood of matching the explosive population dynamics of this pest.

Infections of *Drosophila* spp. by *Entomophthora muscae* (Entomophthorales) have been long reported (Goldstein 1927). Infected *Drosophila* spp. were collected at a local university by RSC in 2011 and 2012. *E. muscae* overwinters as asexual or sexually produced thick-walled resting spores (azygospores [chlamydospores] and zygozospores, respectively) present in the soil. These spores form infective conidia, which germinate on contact with host cuticle, for example from a teneral fly eclosing from overwintering puparia in the soil. After a few days of hyphal growth through the abdomen of the fly, the fly crawls to the highest point on nearby vegetation and dies. When high humidity conditions arise, the fungus grows through the intersegmental membrane of the abdomen, and the multinucleate conidia are projected into the air, where they may land on the next host. Some resting spores are also produced within hosts throughout the season, which fall to the soil when the host is dislodged from the vegetation.

E. muscae has such complex biology that authors disagree whether it constitutes a single or several cryptic species. Pu and Liang (1996) considered *E. muscae* as a species complex. Within the species complex morphological and nuclear characteristics of the primary conidia are taxonomically important (Jensen et al. 2006). Keller (1984) divided it into four groups (A, B, C, D) according to number (4 – 20) and size of nuclei and size of conidia. Keller (1988) described *E. schizophorae* Keller and Wilding as a new species which is similar to Group A. *E. scatophaga* Giard is close to Group C (Pu and Liang 1996). Thus, Steiner and Kramer (1988) revived *E. scatophaga*. Further phylogenetic study of ITS II and LSU rRNA sequences separated the *E. muscae* species complex into three distinct lineages: *E. schizophorae*, *E. muscae* and *E. syrphi* (Jensen et al. 2006). The isolate we collected in CT that infects *Drosophila* spp. needs to be studied to determine its phylogenetic position within this species complex.

Whatever its true identity, an "*E. muscae*" capable of infecting SWD adults could be of great value. Fungi in the Entomophthoraceae like *Entomophthora* tend to have a high degree of specificity (Roy et al. 2006), and so if a strain of *E. muscae* could be selected with virulence to SWD, it is unlikely that its use would have significant negative environmental consequences. Factors in the *E. muscae* life cycle that make it appealing for biological control of SWD are: (1) the germination of spores and penetration of host cuticle are essentially independent of relative humidity (Carruthers et al. 1985, Watson and Petersen 1993), (2) the fungus most efficiently

converts host to conidia under cool and moderately warm temperatures (Watson and Petersen 1993), the same temperatures most favorable to SWD, and (3) the principle factor determining the likelihood of an epizootic occurring is the combination of highly concentrated inoculum and susceptible hosts (Carruthers et al. 1985), factors that we should be able to manipulate by attracting flies to stations where *E. muscae* on hosts is sporulating. Deployment of *E. muscae* as a biological control agent in this manner should not require EPA registration, because it would be introduced into the field through infected insect cadavers, rather than as an application of the spores themselves.

A Northeast Region Urgent IPM Project "Custom baits to manage spotted wing drosophila" was awarded to RSC, and is expected to be completed by the end of May, 2012. This project examined desiccant dust products (which, because they have a physical mode of action, are anticipated to not be susceptible to insecticide resistance), and also has evaluated how highly attractive baits or phagostimulant-based baits could be effectively combined with various insecticides, some of which are organically acceptable, to manage this pest. An effective bait treatment would require (a) an attractant to elicit directed movement from a distance to the odor source, (b) presence of a phagostimulant to elicit feeding, and (c) an efficient toxicant. A bait system could be made more effective by also applying deterrents to the crop to be protected, using a "push-pull" strategy (Cowles 2004).

Chemical attractants and deterrents. Drosophilids are generally attracted to vinegar, giving the family their common name "vinegar flies." Although SWD can be captured in vinegar traps, initial observations in Connecticut and Washington (Lynell Tanigoshi, pers. comm.) suggest that this species is less responsive to vinegar, which is a signal from rotting fruit, than their close relatives when sound ripe fruit are available. These observations have been reinforced further: trap catches using vinegar as bait dramatically increased once fruits were no longer available (Christopher Maier, CAES, pers. comm.), indicating that vinegar traps are not highly competitive with nearby ripe fruit. The importance of fruit aromas was also demonstrated with greater field catches of SWD in traps containing vinegar or wine compared with traps containing equivalent concentrations of acetic acid or alcohol (Landolt et al. 2011). Use of fermented food materials, such as vinegar or wine, has been successful in the field, but probably can be significantly improved upon with the identification and formulation of the volatile chemical attractants in these baits. The techniques of gas chromatography-electroantennodetection (GC-EAD) and laboratory attraction assays were used to isolate chemicals from fruits and food baits that are attractive to *D. melanogaster* (Baker et al. 2003; Becher et al. 2010; Zhu et al. 2003), and similar work is underway by Dr. Landolt to isolate and identify the volatile chemicals in wines and vinegars that are attractive to *D. suzukii*. In addition, two attractant/phagostimulant fruit flavor/odor compounds have been identified through lab choice bioassays (RSC lab). They are melonal, which is a characteristic aldehyde aroma of melons, and methyl butanoate, an important fruit ester found in apples and strawberries. Preliminary data from screening ten fruit flavor syrups used in the beverage industry indicate that three (peach, pineapple, and tangerine) are attractive to SWD flies, and could be used in baits. We anticipate that the identification and formulation of chemical attractants from food baits, as was done for *D. melanogaster* (Baker et al. 2003, Becher et al. 2010, Zhu et al. 2003) will provide chemical blends that can be used to design lures and baits that will be competitive with ripe as well as damaged and fermented fruit in the field.

Laboratory preference tests (RSC lab) have revealed that SWD adults strongly avoid feeding on substances containing either vinegar or yeasts. This paradox (attraction to but feeding rejection of vinegar and yeast) might result from flies using cues of spoiled fruit to locate habitat where they may then encounter sound fruit suitable for them to colonize. Additional deterrent compounds identified have included many fruit flavors, including methyl anthranilate, ethyl butanoate, and nonanal. These results have to be investigated further: it may be that when presented in a simple mixture that these chemicals, and the doses or concentrations tested, are “out of context” and that their properties may be different when added at very small concentrations to attractant mixtures.

Besides acting as a desiccant dust, Surround WP (kaolin) was found in our lab to be highly deterrent to SWD. Flies exposed to the kaolin rested in areas where they did not encounter dust (until they died, usually about 3 hours following exposure). There could also be some degree of repellency, as a kaolin coating of fruits will interfere with the flies' ability to see dark colored fruit against the light colored foliage background. Although grape growers already have adopted use of Surround WP as a wet spray, we determined (RSC lab) in laboratory tests that this material works better as a desiccant when applied dry – a result that was expected because desiccant activity is related to dislodgeable residue (Ebeling 1971). However, application of kaolin as a dry dust may be hazardous to growers unless they protect their lungs from dust exposure (EPA 1996). Either wet or dry application of kaolin may have advantages over use of other compounds, such as methyl anthranilate (registered for use on fruit as a bird repellent) to repel SWD. Because it is a mineral, it will not volatilize, and so it will remain on plant surfaces until washed off by rain or consumers. Furthermore, it will not impart an objectionable flavor to the fruit, as may methyl anthranilate (grape flavor) or torula yeast, if they were to be used as deterrents.

Visual stimuli. SWD is known to be more attracted to dark (red or black) (http://swd.hort.oregonstate.edu/color_preference_spotted_wing_drosophila) rather than light colored objects. This information has been used to a limited extent for improving SWD traps for field monitoring (<http://www.greatlakesipm.com/spottedwingdrosophila.html>), but it also suggests that visual cues could be used to elicit response from a distance to a bait station where they may then be induced to feed on toxic baits, similar to the approach used with apple maggot flies (Hu et al. 1997), or to alight on a surface treated with an adhesive or toxicant that is effective upon contact, as used for some traps and stations for use against the Mediterranean and other tropical fruit flies (Epsky et al. 1999; Heath et al. 2009). Greenhouse tests supported by the NERIPM Urgent IPM proposal have not yet been conducted to test the principles of visual attractants and supernormal stimuli.

Phagostimulants. Phagostimulants are anticipated to greatly enhance insecticide efficacy. Several candidates include sucrose, artificial sweeteners, and molasses. Several methods have been used to quantify feeding responses of SWD to candidate phagostimulants, of which a simple choice test has been extremely efficient, modified from previous sensory studies of *D. melanogaster* (Gordesky-Gold et al. 2008). Twenty flies are placed in a 30 ml plastic container with 4 wells filled with 0.8% agar from a thermocycler microplate. Two wells contain a standard (usually 10% sucrose) and two contain the other candidate phagostimulant. The alternative

choices contain red and blue food dyes carefully calibrated to not elicit color preference. After 2 – 3 hours the flies are chilled and sorted based upon the color of the substance they had ingested (red, blue, or purple) and data analyzed to determine whether there is a statistically significant preference.

So far, 10% sucrose has been a very effective phagostimulant, which can be enhanced by the addition of certain fruit flavors. Other sweeteners do not appear to be effective, or require a high concentration to be competitive with sucrose. The practical question is: how much sugar would have to be added to a spray solution to make an effective phagostimulant bait spray? Laboratory tests of dried spray droplets containing varying quantities of sucrose combined with a food dye (to determine that flies ingested the spray residue) indicate that a concentration of approximately 0.1% sucrose (= 1,000 ppm) will be adequate to elicit a feeding response. This translates to 0.8 lb of sugar mixed with 100 gallons of spray solution, or 0.3 oz in a 3 gallon sprayer (RSC, unpublished data).

Toxicants. Available insecticides are currently being tested to determine their suitability for use in either baited sprays or with bait stations. So far, neonicotinoid insecticides, particularly dinotefuran (Venom) appear to very effective, killing flies within 2 h of feeding on bait containing ~100 ppm insecticide. These insecticides and spinosyns (Entrust or Delegate) probably will be the mainstay for sprayable baits, as they are registered for use on fruit crops. Additional tests are planned to determine whether strong synergism among products registered for use on fruit crops could allow lower over-all quantities of insecticides to be effective. Some additional experimental insecticides have shown promise for targeting *D. suzukii*, including boric acid and phosphites (used as fungicides on fruit crops), which may be useful in bait feeding stations.

The fact that insecticides will be evaluated in some cases with sucrose (either in a bait spray or in bait stations) will require great caution for avoiding non-target intoxication of pollinators, which will be present in strawberry and cane fruit crops at the time that the earliest ripening berries must be protected. Possible solutions include applying sucrose at a concentration to which bees do not respond, application with other ingredients that are deterrent to bees, or use of an active ingredient innocuous to bees. Although dipteran-specific insect growth regulators exist (e.g., cyromazine), use of growth regulators acting as chemosterilants (Moya et al. 2010) would allow adult flies to continue oviposition (of non-viable eggs) into fruit, thus continuing to cause fruit rots with introduced microorganisms (Phaff et al. 1956). An insecticide highly specific to SWD and with relatively rapid lethal action would be of great value to IPM efforts. *Bacillus thuringiensis* (Bt) endotoxins tend to have sub-ordinal insecticide specificity. A survey of Bts in Jordan found about 25% of the tested strains to be active against adults or larvae of *D. melanogaster* (Obeidat 2008), and so Bt toxins make good candidates.

As a new pest to the Northeast, SWD is not mentioned in any stakeholder-identified priorities. However, it is clearly the most economically important and destructive pest of raspberries and other cane fruits, day-neutral strawberries, and blueberries (especially late fruiting varieties), which are grown in all states in the Northeast, with extraordinary losses reported by growers. Conventional management practices rely on an array of products, among which methomyl, lambda-cyhalothrin, and malathion pose significant risks to applicators or beneficial organisms

(predators, parasites, and pollinators) found in these crops. Researchers involved in the SWD SCRI Project in the Northwest did not include behavioral manipulation approaches in their work, and so the work outlined in this project is complementary to work being supported elsewhere in the country. By using existing research farm plots, where untreated checks will not be disastrous (as they could be on commercial farms), the collaborators in this proposal will field test behavioral manipulation principles to improve efficiency and selectivity of SWD management. Growers are clearly desperate, and we anticipate immediate adoption of effective treatment methods. For example, within one week of determining that Surround (kaolin) was helpful in suppressing SWD, vineyard managers in CT had adopted this approach. Furthermore, this project represents a careful selection of experts within the U.S. to integrate their knowledge of mycology, insect pathology, and insect behavior to best combine new management tools. This project follows a logical progression for improving efficiency of existing tools (e.g., by combining spinosyns with a phagostimulant) that can be immediately be adopted by growers, and in exploring novel behavioral management methods for this pest (mass trapping and disease autodissemination) and biorational insecticides (*Bacillus thuringiensis* and products that interfere with carbohydrate metabolism) that will improve selectivity and reduce environmental impact over a longer period.

Objectives and Anticipated impacts

- 1) Determine effectiveness and refine bait formulation of insecticides for protecting fruit from damage by SWD, relative to conventional standard treatments
Potential impact: Rapid adoption of this technique is expected by growers; this should reduce the total amount of insecticide active ingredient used, thereby saving growers money, reducing total residues on fruits, and improving insecticide selectivity by differentially affecting flies.
- 2) Conduct bioassays of *Bacillus thuringiensis* isolates to identify strains active against SWD adults
Potential impact: Discovery of a Bt that has high toxicity to SWD will permit a new, highly selective insecticide to be registered through the IR-4 program.
- 3) Conduct laboratory selection of *Entomophthora muscae* to develop a strain with high virulence to SWD
Potential impact: A strain of E. muscae could be used in autodissemination stations to reduce overall populations of SWD in the field.
- 4) Determine the concentration of vinegar that may safely (regarding phytotoxicity) be used in attractant sprays to crops or surrounding vegetation
Potential impacts: Growers may be able to further enhance the efficiency of insecticide sprays by drawing SWD adults to where they will encounter insecticide residues, further decreasing the amount of insecticide required and potentially eliminating the need to apply insecticide to the crop itself.
- 5) Develop effective lure and trap systems and attract-and-kill stations, and determine optimum field placement of traps and attract-and-kill stations for mass trapping or annihilation of SWD adults
Potential impacts: Improved use of traps and bait stations, perhaps eliminating the need to apply insecticides to the fruit crops.

Methods

Objective 1) Determine effectiveness and refine bait formulation of insecticides for protecting fruit from damage by SWD, relative to conventional standard treatments.

Subobjective 1.1. (Year 1) Test best phagostimulant/insecticide combination. Year 1) Effective concentrations of insecticides will be combined with the best phagostimulant to formulate candidate mixtures. Likely mixtures are dinotefuran and/or spinetoram (Walsh et al. 2010) combined with sucrose. The rapidity in removal of SWD adults released into a greenhouse screen cage during winter months will provide a baseline evaluation of treatment efficacy to identify the best option for use in the field, without factoring in field aging of residues. Insecticide field efficacy will be compared with two complementary approaches. One is to spray small replicated plots on research farms, comparing conventional vs. baited sprays in a full season program to protect fruit (3 replicates per crop at each site). Field cages over these plants should not be necessary, as the sugar used as a phagostimulant with the insecticide is a poor attractant on its own and so the treatments should be statistically independent. We will evaluate the efficacy of these sprays by (1) determining the percent mortality of flies held in laboratory cages and exposed to field-collected foliage and fruit, for foliage collected at varying days after treatment, (2) the percent infestation of treated fruit exposed in cages to flies, and (3) the percent infestation of fruit harvested from the field plots, based on naturally occurring populations of SWD. Data will then be subjected to analysis of variance (AOV). Methods (1) and (2) will guarantee quantitative measurement of treatment efficacy if there are insufficient local populations of SWD. Crops to be tested are blueberries [NJ and RI], strawberries [CT], raspberries and grapes [NY], and the resulting percent fruit infestation will provide quantitative data on the efficacy of each candidate, compared with a standard spray program and untreated check. Products will be applied in these plots with a CO₂-pressurized research sprayer or a backpack hand-pumped sprayer, calibrated with a pressure-regulating controlled flow valve to permit precise dosing of plants.

Subobjective 1.2. (Year 2) Compare behavioral manipulation strategies. Alternatives to compare will be (1) bait spray applied to the crop, attractant plus bait applied to vegetation surrounding the crop (with (2) and without (3) deterrent being applied to the adjacent crop), and (4) conventional sprays to suppress SWD populations. The “push-pull” approach (method 2) will be tested in grapes and blueberries, because application of kaolin (Surround WP) as the deterrent is not expected to be objectionable to consumers on these crops. Because behavioral manipulation methods are expected to influence insects at a distance, large field plots with wild populations of SWD or caged plots with introduced populations are required to minimize interference among treatments. Caged plots will require releases of SWD adults, and so caged studies will take place at research farms operated by the co-PDs. Effectiveness of treatments will be assessed with regular monitoring of insect activity (using the Contech trap with a custom attractant) to follow adult SWD activity response to treatments, and fruit infestation. Trials in each crop and site will require a minimum of three replicates; trap catch and crop infestation data will be subjected to AOV.

Objective 2) Conduct bioassays of *Bacillus thuringiensis* isolates to identify strains active against SWD adults (Year 1 and 2)

Dr. Martin manages the USDA/ARS Insect Biocontrol Laboratory, Beltsville, MD, *B. thuringiensis* collection. This collection contains over 40,000 spore forming bacterial isolates, of which ~10,000 are likely Bt (Martin and Blackburn 2007). Although this is a daunting number of isolates, techniques are now available to use combinations of strains to efficiently identify those with activity (Martin and Blackburn 2007). Of this large collection, some 85 isolates from North America with known dipteran or dipteran/lepidopteran activity will be sent for bioassay to RSC. Fruit flies will be fed candidate Bts presented in a suspension containing 10% sucrose, and percent mortality noted 24 h later. Isolates with insecticidal activity will then be tested via a full dose-response protocol to identify slope and LD₅₀ via probit analysis (Finney 1947). The best candidate(s) will then be submitted for consideration for pesticide registration through the IR-4 Program for Registration of Biopesticides.

Objective 3) Conduct laboratory selection of *Entomophthora muscae* to develop a strain with high virulence to SWD (Years 1 – 2)

E. muscae is believed to form both asexual chlamydo-spores and sexual zygo-spores as thick-walled resting spores that overwinter (Roy et al. 2006). When the closely related *E. virulenta* is grown on artificial liquid or solid media the fungus undergoes sexual reproduction to form zygo-spores (Latgé et al. 1977). Germinated zygo-spores should express some degree of genetic variation characteristic of the previously occurring sexual recombination, which should provide an opportunity to screen for and select a strain virulent to SWD.

Resting spores and their associated conidia from *Drosophila* spp. (Fig. 1) are currently being isolated to start colonies *in vitro*, using 8% dextrose and 2% yeast extract in solid media (Latgé et al. 1977) to determine whether *E. muscae* infective to *Drosophila* spp. will form hyphal conjugation structures and zygo-spores. Additional field collected and repository isolates of *E. muscae* will be used to first infect house flies (*Musca domestica*, obtained from Carolina Biological), and the cadavers used in the infection method developed by Kramer and Steinkraus (1981) to test infectivity to SWD adults under conducive humidity and temperature conditions. Infectivity will be measured as the percentage of exposed flies that develop mycosis. Infectivity tests will be conducted with triplicate trials with groups of at least 20 flies per trial, and data subjected to AOV. If any SWD adults are found to be infected by *E. muscae*, as evident from the characteristic production of conidia through the intersegmental membranes of the abdomen, the strain will be maintained through asexual passage in other SWD and in house flies using the method of Kramer and Steinkraus (1981). The heads of freshly killed flies will be embedded into agar so that the body projects downward from an inverted Petri dish and the spores will be allowed to shoot over the next generation of flies to infect.

Autodissemination methods will be tested in caged greenhouse trials to determine whether this concept is adaptable for use in the field. House flies infected with a strain of *E. muscae* selected for virulence to SWD will be prepared as per normal procedure for maintaining the culture *in vivo* (Kramer and Steinkraus 1981), but the Petri dish containing sporulating house flies will be inverted over an attractant bait station, so that visiting SWD will be exposed to the spore shower. The percent mycosis will then be determined from flies visiting the station (identified by the presence of dye present in the bait). Improvements in autodissemination methods will be determined by enhancement in percent mycosis.

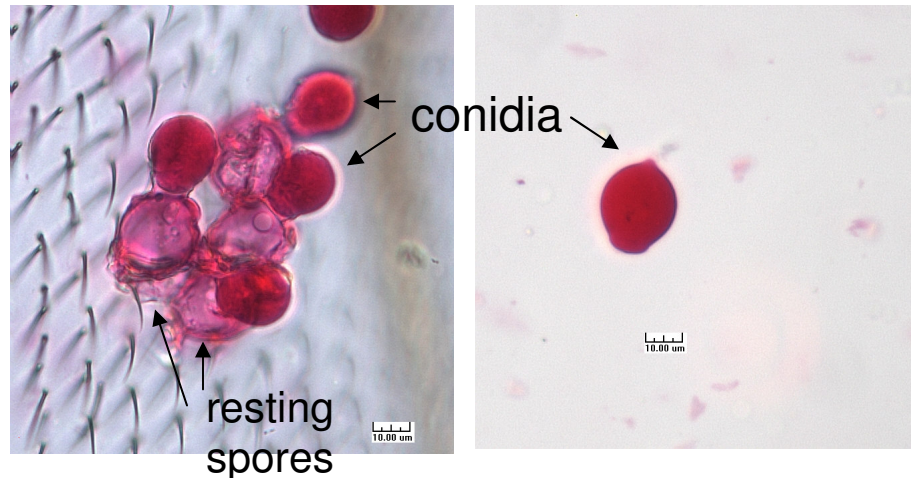


Fig. 1. Resting spores and conidia of *Entomophthora muscae* obtained from *Drosophila* spp. found in CT, 2012.

Objective 4) Determine the concentration of vinegar that may safely (regarding phytotoxicity) be used in attractant sprays to crops or surrounding vegetation (Year 1)

As noted earlier, vinegar is an important component of attractants for SWD, but acetic acid is a strong feeding deterrent. This suggests deployment of vinegar as an attractant may best be conducted close to, but not in a mixture with, a sugar-based phagostimulatory insecticide spray. However, vinegar also can be phytotoxic, as evident from its use as an organically acceptable herbicide (Miller 2007). Therefore, we need to determine whether vinegar will be practical as a sprayable attractant by finding (1) the concentration at which phytotoxicity is first observed on sprayed plants and (2) whether this concentration will attract SWD. Vinegar solutions will be applied at 1:2 dilution with water (a concentration known to cause injury to plants) and in further serial 1:2 dilutions in a randomized complete block design to weedy vegetation plots surrounding fruit crops. Vacuum samples of the vegetation 24 h post-spraying will determine whether there are significant differences among treatments regarding capture of any *Drosophila* adults. Vegetation will be assessed for injury using subjective rating assessment of chlorosis (a standard weed science approach), which, along with counts of captured *Drosophila*, will be subjected to AOV. The concentration at which no injury is observed will also be tested in a laboratory attraction assay to determine whether SWD behavior is influenced when vinegar is freshly applied at this concentration. These tests will be conducted with replicated multiple choice attraction assays arena modified from Becher et al. (2010), and numbers of flies caught in attractant traps subjected to AOV.

Objective 5) Develop effective lure and trap systems and attract-and-kill stations, and determine optimum field placement of traps and attract-and-kill stations for mass trapping or annihilation of SWD adults.

Subobjective 5.1. Develop effective lure and trap systems. A controlled release dispenser will be needed to provide optimized and long term release of any chemical attractants developed for SWD. There are many technologies available, and the selection of appropriate formulation and dispenser methods will be dependent on the volatility and desired release rates of identified chemical attractants. Any dispenser type will be validated in the laboratory using a combination of gravimetric (weight loss) and volatile collection techniques to determine the release rates of chemicals and also the changes in release rates and ratios of chemicals over time. Field tests will be conducted 1) to determine the relationship between release rate and SWD capture in traps, 2) changes in the attractiveness of lures with aging or over time, 3) the relative attractiveness of lures in the presence of fruit, and the effect of trap design on trap catch using a chemical lure.

The identification of chemical attractants will be conducted on other funds (PJL lab), and is expected to provide attractants for lure development late in the field season of 2012. Initial field tests of lures can be done late in 2012 to determine an optimum release rate with an initial dispenser type and the effect of lure aging on catches of flies. A standardized blend of a wine and a vinegar will be included in all experiments as a positive control, both to provide evidence of relative fly response and to repeatedly compare chemical lures with a standardized bait. Determining the relationship between attractiveness of a chemical lure and the presence of fruit as competing food and odor sources will be accomplished in a series of field tests in 2013 that repeatedly compares fly catches in traps with a wine/vinegar mix, a chemical lure, and a visual target (yellow sticky card) with no bait or lure, under a series of seasonal scenarios of no fruits, fruits, and aging fruits. In such a situation, it is assumed that increased impact of competing foods/odors would be seen as a shift in the ratio of flies captured with the bait and with the chemical lure compared to the unbaited visual target. There are numerous trap designs to be considered and there will not be time and resources to evaluate all of them. We will conduct a comparative study of basic design, with the assumption that numerous trap parameters might be further modified. Basic trap designs will include the modified McPhail trap developed for use in trapping tephritid flies, the clear plastic cup trap with holes in the side of the cup, the bottle trap with a single top entrance, and a sticky yellow panel trap. Because we will not be releasing SWD into crops, this experiment may be conducted in cooperating growers' fields. Traps will always be deployed as linear randomized complete block design experiments with at least 4 replicates per trial, and trap catches subjected to AOV.

Subobjective 5.2. Develop attract-and-kill stations. A disadvantage of mass trapping is the potential in high population densities for insects captured to interfere with the efficacy of the trap or the lure. Insects captured on a sticky surface will interfere with other insects being captured on that surface. Traps with containers can fill up, thereby limiting the numbers of insects captured. Captured insects can decompose, altering the odor at the trap site and potentially interfering with the attractiveness of the lure. Another approach is to use a toxicant on a visual target that is contacted by attracted insects, and immobilizing or killing attracted individuals. In most such designs, the insects then fall away or fly a short distance, leaving the station and its surface free for other insects to contact. Examples are the sugar/pesticide spheres for apple maggot flies (Hu et al 1997), pesticide treated stations developed for use against tropical fruit flies (Heath et al. 2009), and pesticide/attractant treated droplets or stations tested against pest moths (Charmillot et al. 2000; Camelo et al. 2007). Following the initial testing and optimization of a lure based on a chemical attractant from wine and vinegar, experiments will

evaluate fly response to a pesticide treated visual target that might be used as an attract and kill platform. Three experiments will be conducted during the 2014 field season, to look at fly response to simple adhesive coated targets to determine the effect of color, to evaluate the importance of shape and orientation, and to evaluate pesticide treatments to replace the adhesive. The color comparison will include black, white, red, yellow, and medium brown panels each baited with a lure. The experimental design will be as described for Subobjective 5.1. The shape and orientation comparison will include a vertical panel, a horizontal panel, a sphere, and a tube all of roughly equal surface areas, each baited with a lure. The evaluation of a pesticide treatment will use a video camera to evaluate the rate of contact of flies with a pesticide treated baited target in comparison to a baited target that is not treated with pesticide (PJL group).

Subobjective 5.3. Determine optimum field placement of traps and stations. Field experiments will be conducted to determine the effect of location of traps on SWD catch. Of primary interest is the location of the trap relative to a field with abundant fruit. A set of 5 traps will be placed well within a field or orchard with fruit in a transect that goes to the field edge and outside of the field. Traps will be 20 meters apart, with two inside the field or orchard, one on the edge or border, and two outside. This experiment will be conducted in at least three cropping systems: grape vineyards, cherry orchards, and raspberry fields (several states participating). An additional experiment will be conducted to compare SWD captured in traps placed at varied heights in a cherry orchard (WA). Trap catches will be subjected to AOV, and the experiment replicated in at least 3 fields per crop.

Timeline (The associated Subobjective is given in brackets, e.g. [1.2])

September 2012

- [1.1] Field tests of baited sprays in vineyards (CT and NY)
- [3] Acquire accessions of *E. muscae* from repositories (CT)
- [5.1] Test lures for traps (CT, NJ, NY, RI, WA)

October 2012 – May 2013

- [2] Complete initial laboratory combinatorial screening of Bt isolates (CT)
- [3] Grow *E. muscae in vitro*, conduct exposure assays with SWD (CT)

June 2013

- [1.1] Field tests of baited sprays in strawberries (CT)
- [4] Conduct field assessment of vinegar phytotoxicity (CT)
- [5.1] Test lures in strawberries (CT)

July 2013

- [1.1] Field tests of baited sprays in blueberries (NJ, RI)
- [5.1] Test lures in blueberries (NJ, RI)

August 2013

- [1.1] Field tests of baited sprays in raspberries (NY)
- [5.1] Test lures in raspberries (CT)

September 2013

- [1.2] “Push-pull” test in vineyards (CT and NY)
- [5.3] Optimize trap placement in vineyards (CT and NY)

October 2013 – May 2014

- [2] Complete laboratory probit trials for Bt isolates (CT)

[3] Complete selection procedures with *E. muscae* (CT)

[3] Conduct tests of *E. muscae* autodissemination in greenhouse (CT)

June 2014

[5.2] Test attract-and-kill in strawberries

July 2014

[1.2] “Push-pull” test in blueberries (NJ, RI)

[5.2] Test attract-and-kill in blueberries (NJ, RI)

[5.3] Optimize trap placement in blueberries (NJ, RI) and cherries (WA)

August 2014

[5.2] Test attract-and-kill in raspberries (CT, NY)

Evaluation Plans

Objective 1) Bait formulated insecticides for protecting fruit from damage by SWD will be considered effective if the level of damage is equivalent to or less than that observed for conventional standard treatments.

Objective 2) Strains of *Bacillus thuringiensis* will be considered to be toxic to SWD adults if the concentration required to kill them is similar to or less than the concentration used for other Bt products directed to control other agricultural pests.

Objective 3) Laboratory selection of *Entomophthora muscae* to develop a strain with high virulence to SWD will be considered a success if greater than 50% of SWD adults exposed to spores develop mycosis and die.

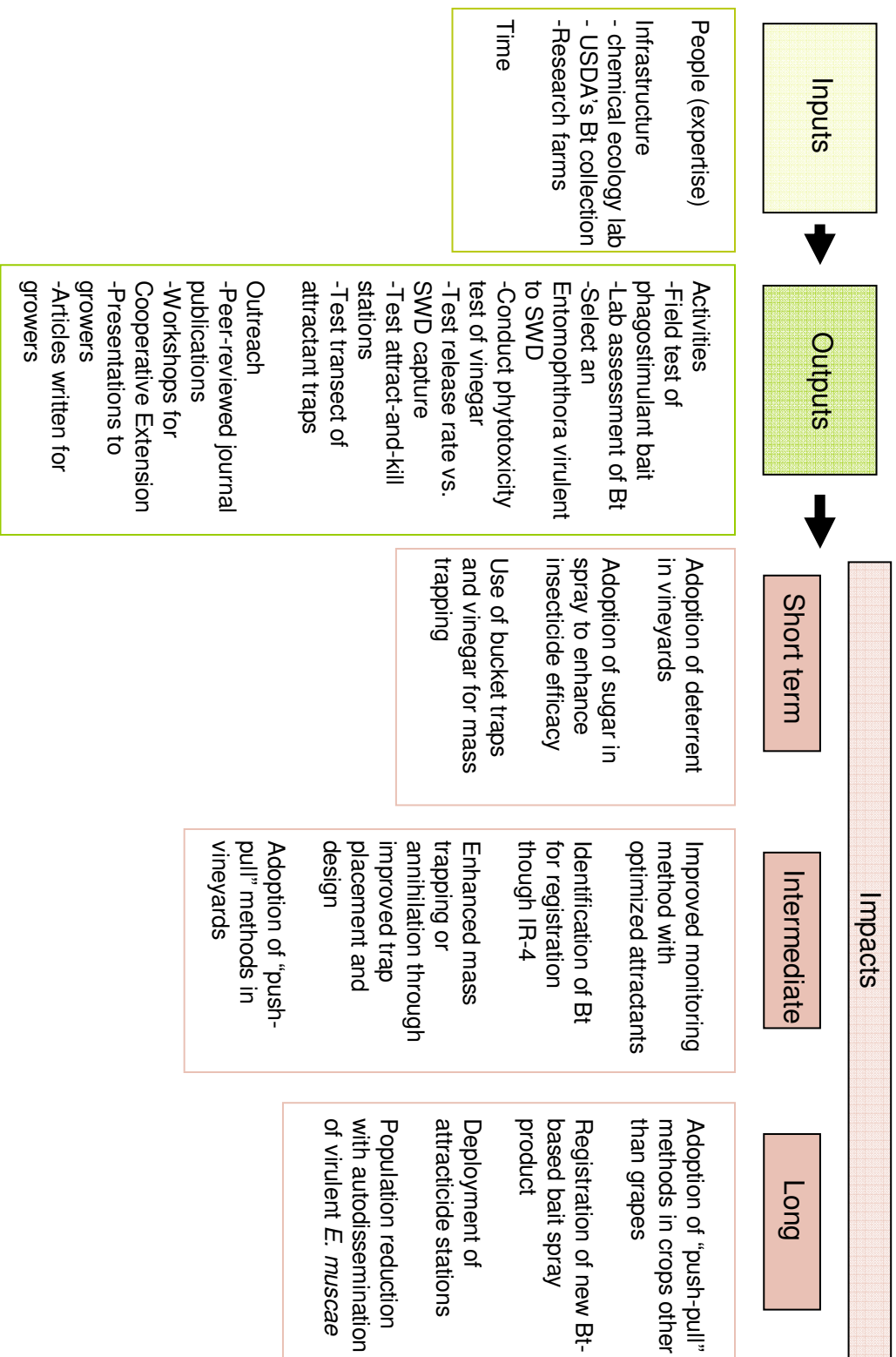
Objective 4) The objective to test vinegar will be considered accomplished when a concentration of vinegar is determined that (a) significantly attracts SWD and (b) causes no injury to sprayed foliage.

Objective 5) Development of effective lure and trap systems and attract-and-kill stations will be considered accomplished when these traps set in the field are shown to be highly competitive with nearby fruit (as determined by significant trap catches). Optimization of field placement of traps and attract-and-kill stations for mass trapping or annihilation of SWD adults will be considered complete when we have established placement guidelines that growers may then use in their fields.

Key Personnel

Richard S. Cowles (entomologist, CAES) is the project director, and will coordinate activity among the co-PDs, as well as having primary responsibility for testing management options and trapping in strawberries, and conducting bioassays of Bts. **DeWei Li** (mycologist, CAES) will specialize in working with *Entomophthora muscae*. **Peter Landolt** (entomologist, USDA, ARS, Wapato, WA), is our leader for identifying attractants for testing, for designing traps, and standardizing methods for testing traps. He will also be testing traps and attracticides in cherries grown in WA. **Cesar Rodriguez-Saona**, Rutgers Univ. (NJ), **Steven R. Alm**, Univ. Rhode Island, and **Gregory Loeb**, Cornell University (NY), are entomologists with expertise in IPM for small fruits in their respective states. They have access to research farm plots of blueberries, blueberries, and grapes, respectively. **Phyllis A. W. Martin**, (microbiologist, USDA, ARS, Insect Biocontrol Laboratory, Beltsville Agricultural Research Center, Beltsville, MD) will be collaborating through a material transfer agreement to provide RSC with Bt.

Situation : Current SWD management requires frequent sprays, often with broad spectrum insecticides, once fruits start to ripen.
Priorities : Improve efficacy of selective insecticides and biocontrol organisms by integrating their use with behavioral modification.



References

- Baker, T. C., J. Zhu, and K.-C. Chung, inventors; Iowa State University Research Foundation Inc., assignee. 2003. Fruit fly attractant compositions. U. S. Patent 6,543,181.
- Becher, P.G., Bengtsson, M., Hansson, B.S., Witzgall, P. 2010. Flying the fly: Long-range flight behavior of *Drosophila melanogaster* to attractive odors. *Journal of Chemical Ecology* 36: 599 – 607.
- Camelo, L. A., P. J. Landolt, and R. S. Zack. 2007. A kairomone based attract-and-kill system effective against alfalfa looper (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 100: 366 – 374.
- Carruthers, R. I., D. L. Haynes, and D. M. MacLeod. 1985. *Entomophthora muscae* (Entomophthorales: Entomophthoraceae) [sic] mycosis in the onion fly, *Delia antiqua* (Diptera: Anthomyiidae). *J. Invert. Pathol.* 45: 81 – 93.
- Charmillot, P.-J., D. Hofer, and D. Pasquier. 2000. Attract and kill: a new method for control of the codling moth *Cydia pomonella*. *Entomol. Exp. Appl.* 94: 211 – 216.
- Cowles, R. S. 2004. Manipulation of host finding and acceptance behaviours in insects: Importance to IPM. pp. 185 – 204. In: Koul, O., G. S. Dhaliwal, and G. W. Cuperus (eds.). *Integrated Pest Management: Potential, Constraints and Challenges*. CABI Publishing, Cambridge, MA. 329 pp.
- Ebeling, W. 1971. Sorptive dusts for pest control. *Annu. Rev. Entomol.* 16: 123-158.
- EPA. 1996. Ambient Levels and Noncancer Health Effects of Inhaled Crystalline and Amorphous Silica: Health Issue Assessment. National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC. 177 pp.
- Epsky, N. D., J. Hendricks, B. I. Katsoyannos, L. A. Vásquez, J. P. Ros, A. Zümreoglu, R. Pereira, A. Bakri, S. I. Seewooruthun, and R. R. Heath. 1999. Field evaluation of female-targeted trapping systems for *Ceratitis capitata* (Diptera: Tephritidae) in seven countries. *J. Econ. Entomol.* 92: 156 – 164.
- Finney, D. J. 1947. *Probit Analysis*. Cambridge Univ. Press. 256 pp.
- Goldstein, B. 1927. An *Empusa* disease of *Drosophila*. *Mycologia* 19: 97 – 109.
- Gordesky-Gold, B., N. Rivers, O. M. Ahmed, and PAS Breslin. 2008. *Drosophila melanogaster* prefers compounds perceived as sweet by humans. *Chem. Senses* 33: 301 – 309.
- Hauser, M. 2011. A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. *Pest Manag. Sci.* doi: 10.1002/ps.2265.

- Heath, R. R., A. Vazquez, E. Q. Schnell, J. Villareal, P. E. Kendra, and N. D. Epsky. 2009. Dynamics of pH modification of an acidic protein bait used for tropical fruit flies (Diptera: Tephritidae). *J. Econ. Entomol.* 102: 2371 – 2376.
- Hu, X. P., Kaknes, A., and R. Prokopy. 1997. Improved pesticide-treated wooden spheres for controlling apple maggot flies. *Fruit Notes* 62(2), Spring, 1997, pp. 3 – 5.
- Isaacs, R., N. Hahn, B. Tritten and C. Garcia. 2010. Spotted wing drosophila, a new invasive pest of Michigan fruit crops. *Mich. State Univ. Ext. Bull.* E-3140.
- Jensen, AB, L Thomsen, J Eilenberg. 2006. Value of host range, morphological, and genetic characteristics within the *Entomophthora muscae* species complex. *Mycological Research* 110(8): 941 – 950.
- Keller S. 1984. *Entomophthora muscae* als Artenkomplex. *Mitt Schweiz Entomol Ges* 57: 131 – 132.
- Keller, S. 1988. Arthropod-pathogenic Entomophthorales of Switzerland. I. *Conidiobolus*, *Entomophaga* and *Entomophthora*. *Sydowia* 40: 122 – 167.
- Kopp, A. and J. R. True. 2002. Evolution of male sexual characters in the oriental *Drosophila melanogaster*. *Evol. Develop.* 4: 278 – 291.
- Kramer, J. P., and D. C. Steinkraus. 1981. Culture of *Entomophthora muscae* in vivo and its infectivity for six species of muscoid flies. *Mycopath.* 76: 139 – 143.
- Landolt, P. J., T. Adams, and H. Rogg. 2011. 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.
- Latgé, J.-P., R. S. Roper and C. D. Madore. 1977. Media suitable for industrial production of *Entomophthora virulenta* Zygo spores. *Biotech. Bioengin.* 19: 1269 – 1284.
- Martin, PAW, and M. B. Blackburn. 2007. Using combinatorics to screen *Bacillus thuringiensis* isolates for toxicity against *Manduca sexta* and *Plutella xylostella*. *Biol. Control* 42: 226 – 232.
- Miller, T. W. 2007. Natural herbicides and amendments for weed control. pp 174–185, In: Rimando, A. M. and S. O. Duke. *Crop Protection Products for Organic Agriculture*. ACS Symposium Series, Vol. 947.
- Moya, P., S. Flores, I. Ayala, J. Sanchis, P. Montoya, and J. Primo. 2010. Evaluation of lufenuron as a chemosterilant against fruit flies of the genus *Anastrepha* (Diptera: Tephritidae). *Pest Manag. Sci.* 66: 657 – 663.

- Obeidat, M. 2008. Toxicity of local *Bacillus thuringiensis* isolates to *Drosophila melanogaster*. World J. Agric. Sci. 4: 161 – 167.
- Phaff, H. J., M. W. Miller, J. A. Recca, M. Shifrine, and E. M. Mrak. 1958. Yeasts found in the alimentary canal of *Drosophila*. Ecol. 37: 533 – 538.
- Pu, Z. L. and Liang, Z. Z. 1996. Insect Mycology. Anhui Science and Technology Press, Hefei, China 715 pp.
- Roy, H. E., D. C. Steinkraus, J. Eilenberg, A. E. Hajek, and J. K. Pell. 2006. Bizarre interactions and endgames: Entomopathogenic fungi and their arthropod hosts. Annu. Rev. Entomol. 51: 331 – 357.
- Shearer, P. W., H. Thistlewood, R. Van Steenwyk, V. Walton, S. Acheampong. 2011. *Drosophila suzukii*, a new pest of stone fruits in western North America. Presentation at IOBC Conference “*Drosophila suzukii*: new threat for European fruit production,” Trento, Italy, Dec. 11, 2011. (pdf of PowerPoint presentation)
- Steinkraus, D. C., and J. P. Kramer. 1988. *Entomophthora scatophagae* desc. ampl. (Zygomycetes: Entomophthorales), a fungal pathogen of the yellow dung fly, *Scatophaga stercoraria* (Diptera: Anthomyiidae). Mycotaxon 32: 105 – 113.
- Walsh, D. B., M. P. Bolda, R. E. Goodhue, A. J. Dreves, J. Lee, D. J. Bruck, V. M. Walton, S. D. O’Neal, and F. G. Zalom. 2010. *Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of ripening soft fruit expanding its geographical range and damage potential. http://extension.wsu.edu/swd/Documents/Drosophila_suzukii_Invasive_Pest_of_Ripening.pdf.
- Walton, V., J. Lee, D. Bruck, A. Dreves, P. Shearer, J. DeFrancesco, H. Rogg, L. Coop, and P. Jepson. 2010. http://extension.oregonstate.edu/umatilla/mf/sites/default/files/Spotted_Wing_Drosophila_Walton_2010.pdf
- Watson, D. W. and J. J. Petersen. 1993. Seasonal activity of *Entomophthora muscae* (Zygomycetes: Entomophthorales) in *Musca domestica* L. (Diptera: Muscidae) with reference to temperature and relative humidity. Biol. Control 3: 182 – 190.
- Zhu, J., K.-C. Park, and T. C. Baker. 2003. Identification of odors from overripe mango that attract vinegar flies, *Drosophila melanogaster*. J. Chem. Ecol. 29: 899 – 909.