COMPARATIVE ATTRACTIVENESS AND EFFICACY OF BAITS USED FOR CONTROL
OF WALNUT HUSK FLY

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ABSTRACT

In laboratory and field experiments, the responses of walnut husk flies to three bait substances (Mobait, molasses, and Nulure) were examined. In the laboratory, walnut husk flies demonstrated no differential attraction to or propensity to feed on the three baits tested against water (control). In a Hartley walnut orchard, flies were not differentially attracted to the bait substances sprayed on clear Plexiglas “pane” traps, although flies were attracted to these “pane” traps when they were baited with Superchargers (ammonium carbonate). Also in the Hartley orchard, spray trials indicated no differential control of walnut husk fly when each of the baits was sprayed in combination with pesticide. Furthermore, untreated control areas in this orchard had lower levels of husk fly damage at the end of the season than any of the areas sprayed with bait plus pesticide, or than areas sprayed with pesticide alone. At face value, these tests seem to indicate that the baits are ineffective in improving husk fly control compared to insecticide alone. However, because of difficulties with the field tests, we feel that the spray trial needs to be repeated with larger orchard treatment areas to prohibit fly movements between treatment areas. Also, new experiments using flies confined in sleeve cages in the field are needed to understand how flies react to encounters with baits in walnut trees.

OBJECTIVES

1) To compare attractiveness of three commonly-recommended bait substances (Nulure, Mobait, and molasses) to walnut husk fly in the field and under controlled conditions.

2) To determine whether or not walnut husk flies will feed on each of the three baits.

3) To compare efficacy of the different baits when sprayed in combination with pesticides in a naturally infested walnut orchard.

PROCEDURES

We developed a four-part approach to this problem; two different types of experiments were conducted in a naturally infested commercial walnut orchard, one experiment was conducted in the laboratory, and one preliminary experiment was carried out under semi-natural conditions in field sleeve cages. The two field experiments were developed to determine the attractiveness of the three bait substances to walnut husk fly and to compare efficacy of the baits when sprayed in combination with pesticides for husk fly control in a commercial orchard. The goal of the laboratory experiment was to investigate feeding responses of walnut husk flies after they
contacted the different baits. Finally, the preliminary sleeve cage experiment was developed to determine short-range attractancy of the three baits.

**Experiment 1:** Field tests were conducted in a husk fly-infested Hartley walnut orchard in East Biggs, Butte County (owner: Jon Bill). In the first field test, efficacy of the baits when sprayed in combination with pesticide was assessed. The orchard was divided into three blocks (replicates), each 20 rows wide by 14 trees long. Each block was further divided into 5 treatment areas. Three of the treatment areas in each block were 5 rows wide by 14 trees long. These areas received one of the three baits (Mobait, molasses, or Nulure) sprayed in combination with pesticide on every other row. The other two treatment areas were smaller and contained the positive and negative controls. The positive control areas, which received pesticide without bait sprayed on every row, were 5 rows wide by 10 trees long. The negative control areas were 4 rows wide by 4 trees long and received no treatment. One husk fly monitoring trap (Trece AM-NB with a Supercharger lure) was hung from a central tree in each control and treatment area (5 traps per block). Traps were checked for flies twice a week. All flies were removed and sexed, and females were squeezed to determine presence of eggs. Timing of control applications occurred according to the recommendations of Olson and Pickel (Walnut Husk Fly: Biology, Monitoring and Control Strategies video, 1996), i.e. spraying occurred within one week of the capture of female walnut husk flies which contained mature eggs. To determine extent of walnut husk fly damage in mid-season, 50 nuts per treatment area were sampled on August 6 and dissected in the laboratory. Eggs and/or larvae were noted. An additional sample of 100 nuts per treatment area was taken at harvest (September 22) to determine final extent of husk fly damage. Numbers of flies captured in the different blocks and numbers of damaged nuts were compared with ANOVA.

**Experiment 2:** The second field test was also conducted in the Butte County orchard. To determine relative attractiveness of the three baits and to avoid any confounding effects of visual stimuli (as might occur with yellow colored sticky traps), we created fifteen 30cm X 30cm clear Plexiglas traps. Each Plexiglas trap was coated on both sides with Stickem Special (Seabright Industries) which was roughed-up with a grooved trowel. Six ml of bait (either Mobait, molasses, or Nulure, formulated at recommended field concentrations) was sprayed on each side of 9 traps. Three other traps were positive control traps which each had a Supercharger lure hung on one side instead of bait. The remaining three traps were negative control traps which had no lure or bait, only Stickem. In each of the three control areas in the orchard (as designated in Experiment 1), we hung one of each of the 5 different Plexiglas traps using 5 different trees, making sure that each trap was not hanging in the same tree as the Trece monitoring trap from Experiment 1. The Plexiglas traps were checked for flies and resprayed with bait twice a week, and locations of the traps were re-randomized among the 5 trees once a week. Flies were removed from the traps and sexed. Counts of flies captured on each of the baited traps were log(x+1) transformed and analyzed with ANOVA.

**Experiment 3:** In the laboratory, wild flies, collected the previous year as pupae from naturally infested walnuts, were maintained in 23cm X 23cm Plexiglas and screen cages with food (hydrolyzed yeast and sugar) and water until between 4 and 16 days old. Sixteen to 24 hours prior to testing, food was removed from the cages. Testing began when 20 male and 20 female
walnut husk flies (except in one test when only 10 of each sex were available) were released into a clean 23cm X 23cm Plexiglas and screen cage with four lures hanging from the top of the cage. Each lure consisted of a 2cm X 2cm piece of filter paper onto which 50ul of test substance (Mobait, molasses, Nulure, or water) had been pipetted. For 40 min, flies were observed and landings on the different lures were recorded. If a fly attempted to feed on a lure after landing (i.e., put down its mouthparts), it was removed from the cage. Locations of lures were re-randomized every 10 min. A total of 130 flies of each sex were tested. Likelihood of feeding following landing on each of the three baits and the control (water) lure was assessed with a X² test.

Preliminary Experiment 4: A preliminary experiment was conducted to determine if fly behavior toward baits might be influenced by the presence of live walnut leaves and nuts, and by fly age or feeding status. In an unmanaged Payne walnut orchard in Newark, CA (Ardenwood Historic Farm, East Bay Regional Parks), cylindrical sleeve cages (1m long X 0.3m diameter) were constructed and hung over the ends of tree branches. Each sleeve contained a branch with approximately 30 leaves and 5 nearly mature walnuts, all of which had been washed with distilled water and allowed to air dry. For each cage, 4 additional leaves were picked from elsewhere on the tree, matched for size (all were approximately 30cm² area), washed with distilled water, and allowed to dry. On the top side, each leaf received 15 drops of a 25% concentration (in distilled water) of Mobait, molasses, or Nulure, or received 15 drops of distilled water. The leaves were gently agitated to allow the drops of liquid to roll around on the surface and were allowed to air dry so that leaves were tacky but not wet when tested. One leaf of each treatment was hung in each sleeve cage for a total of 4 test leaves per cage. The leaves were attached to the branches in the sleeve cage with fine wire so that each test leaf was no closer than 10cm to another test leaf. Into each cage, 5 male and 5 female walnut husk flies were released. In the first preliminary test, flies were 3-9 days old (reproductively immature) laboratory maintained wild flies (N=40) similar to the flies in Experiment 3 (food withheld for 24h). In the second preliminary test, flies were 15-18 days old (reproductively mature; N=40) and had been allowed to feed on hydrolyzed yeast and sugar until the time of testing. All flies were observed for 20 min. Flies landing on the top sides (bait or water sides) of test leaves were tallied and removed from the cages. Locations of test leaves relative to each other were re-randomized for each test, and test leaves were used for a maximum of 2h. Forty flies of each age group were tested. Counts of flies landing on the baits were analyzed with X².

RESULTS

Experiment 1: Trapping began on July 8. On the first day traps were checked, July 11, five female walnut husk flies were captured that contained mature eggs. The orchard was sprayed according to our block and treatment areas for the first time on July 13 (Fig. 1). Walnut husk fly population levels did not differ significantly among the treatment areas prior to spraying, according to trap captures from July 11 (ANOVA: F=0.52, df=4,14, p=0.72). Following the spraying, trap captures in all areas dropped dramatically, then slowly began to rise again over time. Over the two weeks following spraying, the molasses and the control areas appeared to develop higher fly populations than the other areas (Fig. 1). By July 29, this trend began to shift with the Mobait areas developing higher fly populations, and the pesticide alone and the Nulure
areas maintaining the lowest fly populations. On August 1, two females husk flies with mature eggs were captured in the orchard. An ANOVA on fly captures from August 1 detected no significant differences between fly population levels in the different treatment areas (F=1.79, df=4,14, p=0.21) despite apparent visual differences in average numbers of flies per trap (Fig. 1). The orchard was sprayed again on August 2, but treatment and block areas were inadvertently ignored, and the entire orchard was sprayed with a Mobait plus Diazinon mixture applied to every other row. Mid-season samples of 50 nuts per treatment area were taken and dissected on August 6. These samples indicated no significant differences in husk fly damage in the different treatment areas although the degree of damage ranged from 0 to 7.4% (ANOVA on damage: F=1.89, df=4,14, p=0.19). In the three weeks following the second spray, fly populations began to build back up, reaching the highest levels in the control and molasses areas (Fig. 1). Nevertheless, no significant differences were found in fly population levels in the different treatment areas according to trap captures from August 26 (ANOVA: F=1.00, df=4,14, p=0.45). On August 26, 42 females were captured which had mature eggs; the orchard was sprayed according to our treatment areas and blocks for the last time on August 28. Percent damage at harvest was assessed from samples of 100 nuts per treatment area. There were no significant differences between the different treatments in level of husk fly damage at harvest (F=0.21, df=4,14, p=0.93). Overall, the orchard sustained an average of 8.23% damage due to walnut husk flies.

Experiment 2:

Low numbers of flies were captured on the Plexiglas traps, regardless of bait or lure type. Nevertheless, a significant difference was detected in the number of flies captured per lure type (ANOVA: F=2.43, df=4,149, p=0.05) (Fig. 2). However, this difference occurred because the Supercharger-baited traps captured significantly more flies than any of the baits or the control, none of which differed significantly from each other.

Experiment 3:

In the laboratory, flies were not more likely to land on filter paper strips soaked in any of the baits than on filter paper soaked in water (Fig. 3). In addition, the flies that landed on the filter paper strips exhibited no statistically significant feeding preferences for any of the baits compared to water (X² = 5.177, df=3, p=0.16) although molasses was the most likely to be eaten, followed by Mobait, Nulure, and finally water (Fig. 4).

Preliminary Experiment 4:

In sleeve cages in the field, 3-9 day old starved flies landed on test leaves sprayed with baits or water significantly more than 15-18 day old fully fed flies (X² = 10.91, df=1, p=0.001). The young flies did not exhibit any strong preferences for any of the baits over the water. Four young flies landed on Mobait, five on molasses, two on Nulure, and seven on water.
CONCLUSIONS

Based on the field spray trial, we found no significant differences between fly populations or resulting husk fly damage when different bait plus insecticide mixtures were used. Neither did we find any difference between husk fly control between the use of bait plus insecticide on every other row and pesticide alone applied to every row. Perhaps the most disturbing finding, however, was the lack of significant difference between any of the treatment areas and the control (unsprayed) areas. We hesitate to conclude from this study that all of the baits are either equally effective or equally ineffective in control of walnut husk fly. In the future, two possible explanations need to be explored further. First, the individual treatment and control areas may have been too small so that flies moved freely between treatment areas, homogenizing the results. Further, the baits might have been so attractive that flies were effectively removed from the control areas which were not sprayed by flying into bait and pesticide blocks which were sprayed (but see results of Experiments 2 and 3). Second, the inadvertent whole orchard spray during mid-season may have suppressed fly populations in all areas sufficiently to erase any differences which may have otherwise occurred. We strongly feel that this orchard spray trial needs to be repeated using larger (albeit fewer, if the same size orchard is used) treatment areas and blocks.

The field trapping experiment, using Plexiglas traps sprayed with bait, seemed to indicate low overall attractiveness of the three bait substances. According to this experiment, flies are unlikely to fly any distance to reach bait sprays. In fact, the baits tested were significantly less attractive to walnut husk flies than Superchargers which contain ammonium carbonate. Although ammonia has long been known to be a powerful attractant, it is not a feeding stimulant and so holds little promise for use as a bait spray.

In the laboratory experiment and in preliminary sleeve cage tests, flies did not demonstrate any strong attraction to any of the baits nor any differential preference for feeding on any of the baits. Again, this seems to indicate that the baits should be relatively ineffective at controlling walnut husk fly when combined with insecticide and only sprayed on every other row. Nevertheless, our field trial seemed to indicate that flies might have been leaving control areas to feed on baits in other areas, thus reducing the fly population and damage levels in the unsprayed controls as well as the treated areas. One intriguing twist to this study occurred during the preliminary sleeve cage experiments which we carried out late in the summer: unfed young flies were more likely to encounter baits than older flies which were well fed. The problem with the preliminary sleeve cage experiment was that two variables were manipulated, age and feeding status. Thus, without further study, we cannot differentiate the effects of these two variables on the likelihood that flies will respond to baits. A possibility which was not adequately explored with the design of our Plexiglas trapping or laboratory feeding experiments is that the presence of bait substances increases general food foraging behavior in flies, thus increasing the probability that flies will contact and ingest bait plus insecticide mixtures, in spite of the fact that the baits are not terribly attractive substances. We plan to further refine and expand our approaches to these questions in 1998 through further sleeve cage tests and two field spray plots. In one spray plot, we will enlarge the individual treatment areas to try to reduce fly movements between areas. In a second spray plot, we plan to use a backpack sprayer to spray bait plus insecticide on individual trees, a method of husk fly control which some growers have used with success.
Figure 1. Average walnut husk fly trap captures in relation to treatment area and date (Butte County). (Control = unsprayed; Pesticide = malathion on every row; Molasses = molasses+malathion on every other row; Mobait = Mobait+malathion on every other row; Nulure = Nulure+malathion on every other row. Block spray = sprays according to our designated treatment areas; Overall spray = spray without regard to treatment areas.)
Figure 2. Average number of flies captured per type of Plexiglas trap per day.
Figure 3. Numbers of flies landing on each of the baits in the laboratory. (N=260 flies tested, but flies could land on baits more than once.)
Figure 4. Percent of flies landing on baits which also fed on the baits in the laboratory.