Testing an attracticide hollow fibre formulation for control of Codling Moth, *Cydia pomonella* (Lepidoptera: Tortricidae)

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ABSTRACT

Laboratory and field tests were conducted to evaluate the use of an experimental sprayable formulation of chopped hollow fibres loaded with codlemone and mixed with 1.0% esfenvalerate and an adhesive to control codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). Moths were not repelled by the addition of the insecticide to the adhesive and were rapidly killed following brief contact. A significantly greater proportion of male moths flew upwind and contacted individual fibres for a longer period of time when fibres had been aged $\geq$ 7 d versus fibres 0 – 7 days-old in flight tunnel tests. Field tests using sentinel fibres placed in 10.0 mg drops of adhesive on plastic disks stapled to the tree found that fibres were not touched until they had aged > 8 d. Conversely, moth mortality following a 3-s exposure to field-collected fibres deposited on the top of leaves was low in bioassays with fibres aged > 8 d. The deposition and adhesion of fibres within the apple canopy appear to be two major factors influencing the success of this approach. Fibres were found adhering to foliage, fruit, and bark within the orchard; however, visual recovery of fibres following each of the three applications was < 5.0%. Both the substrate and the positioning of the fibre on the substrate influenced fibre retention. The highest proportion of fibres was found initially on the upper surface of leaves and this position also had the highest level of fibre retention. Fibres on the underside of leaves or partially hanging off of a substrate were dislodged within two weeks.

Key words: sex pheromone, codling moth, attracticide, apple

INTRODUCTION

A variety of approaches have been developed that utilize the sex pheromone of codling moth, *Cydia pomonella* L., for its effective management in deciduous tree fruit and nut crops, including the application of hand applied dispensers (Charmillot and Pasquier 1992), sprayable microencapsulated materials (Charmillot and Pasquier 2001), widely-spaced aerosol emitters (Shorey and Gebers 1996), and paste droplets formulated with insecticides (Charmillot *et al.* 2000). Chopped hollow fibres loaded with codlemone have been used for codling moth both in hand-applied formulations (Cardé *et al.* 1977) and in a sprayable formulation in which the fibres were mixed with an adhesive (Moffitt and Westigard 1984). Fibres provided high levels of disruption throughout the season in these studies.

Hollow fibres have been widely used in aerial applications in cotton for management of pink bollworm, *Pectinophora gossyptella* (Saunders) (Baker *et al.* 1990). Cotton growers combined the use of the hollow fibres and synthetic pyrethroids to develop an attracticide formulation (Beasley and Henneberry 1984). Studies with pink bollworm showed that this attracticide approach had minimal effect on natural enemies (Butler and Las 1983) and had significant lethal and sublethal effects, which reduced the pest population (Floyd and

Crowder 1981). This attracticide approach has not been tested with codling moth. Herein are presented preliminary studies that evaluated the potential of this approach and the use of chopped fibres for communication disruption of codling moth.

MATERIALS AND METHODS

Laboratory test protocol. The response of male codling moths to an experimental formulation of black hollow Celcon fibres (200 µm i.d. x 15 mm) loaded with 15% codlemone diluted in hexane (Scentry Inc., Buckeye, AZ) was observed in a flight tunnel. Fibres were formulated to release 0.1 µg/h codlemone at 20 °C (Weatherston *et al*. 1985). The tunnel was 1.65 m long, 0.56 m wide and 0.56 m high and constructed from 6 mm thick acrylic sheeting. A blower was used to push air within the room (maintained at 22 – 24 °C and 50 – 60% RH) into a plenum, through a charcoal filter, and through a series of screens before passing into the working section of the tunnel. An identical blower was used on the opposite end to pull air through the tunnel. Power to the blowers was provided by two 12-volt battery chargers attached to 115-volt AC variable resistors. By carefully adjusting the speed of each blower, laminar airflow was created which passed through the tunnel at the rate of 0.13 m/sec (measured by movement of smoke). Exhaust was expelled to the outside of the building. Red lights installed above the working section of the tunnel provided enough light (4.3 lux) to make observations.

Insects were obtained as mature larvae inside corrugated cardboard strips from a laboratory colony reared on a soybean diet at the Yakima USDA Insectary (Toba and Howell 1991). Virgin male moths were collected daily and conditioned in constant light for 24 – 48 h at 21 °C and 60% RH. Prior to testing, moths were placed in complete darkness for 30 min.

Technical esfenvalerate (Dupont Agricultural Products, Wilmington, DE) was diluted in acetone and mixed with adhesive at a 1.0% wt/wt concentration. A single hollow fibre was placed on a 10.0 mg droplet of a polybutene adhesive (Biotac 100, Scentry Biologicals, Billings, MT) in the center of an 18.0 mm diameter plastic disk. The plastic disk with the fibre was placed on a small metal platform suspended 30 cm from the top of the flight tunnel at the air inlet end. Moths were released from a 30 cm high platform placed near the air outlet end of the tunnel. The number and duration of individual visits to the fibre were recorded for 7 min with an infrared motion detector coupled to a computer.

Attractiveness and toxicity of laboratory-aged fibers. Two types of tests were conducted in the flight tunnel to assess the attractiveness of individual fibres for male codling moths and the toxicity and possible repellency of adding an insecticide to the adhesive. In the first test, hollow fibres were aged for 7 d at 24 °C prior to testing. Ten replicates of 10 moths were flown to a fibre placed either in adhesive or in adhesive with insecticide. Treatments were run alternately for each replicate, n = 10. The attractiveness and toxicity of fibres placed on adhesive treated with 1.0% esfenvalerate and aged in a greenhouse were evaluated in the second test. Disks were collected after 0, 1, 4, 7, 14, 21, and 28 d and kept frozen at –10 °C. Five fibres from each age class were tested in the flight tunnel in a random order and each fibre was tested twice, n = 10 replicates. Six males were released simultaneously for each fibre and allowed to fly for 7 min. Moths were collected individually in vials at the end of each flight test and mortality was scored after 24 h at 24 °C.

Field test protocol. Field studies of fibres were conducted in 1992 and 2003. Three applications of fibres were made to a 0.3 ha (214 trees) 5-year-old 'Golden Delicious' block trained on a M-16 rootstock with central leader architecture on 5 May, 1 June, and 28 July 1992. The mean (SE) height of trees was 2.1 (0.1) m. A standard spinning cone applicator used for ground application of the fibre in field crops (Moffitt and Short 1982) was supplied by Scentry personnel and attached to a tractor. The tractor and sprayer were calibrated to deliver 100.0 g of fibres (15.0 g a.i.) in 6.0 L adhesive per hectare. The deposition and retention of fibres were evaluated following an application on 9 July 2003 in a 4.0-ha orchard of mixed apple cultivars. The orchard was treated with 250.0 g of a 10.0% a.i. fibre mixed with 4.7 L adhesive per hectare using a specialized tractor-pulled overhead applicator (Blue Line Manufacturing, Wenatchee, WA). The same adhesive and fibre were used in both years.

Attractiveness and toxicity of field-aged fibres. The attractiveness and toxicity of field-aged fibres were evaluated throughout the 1992 season. On each of the three spray dates one fibre was placed on a 10.0 mg adhesive drop in the center of each of 120 plastic disks that were stapled to the wooden posts of a wire deer fence situated > 50 m from the apple orchard. On each subsequent sampling date 10 disks without any moth scales were placed in the upper third of the apple orchard's canopy in a horizontal position. These sentinel fibres were left in the orchard for 5 – 7 d and then reexamined with a microscope for the presence of moth scales. In addition, on each sampling date 10 fibres deposited by the spray application on the upper surface of leaves were collected from the orchard and returned to the laboratory. Five 1 – 2 d-old chilled laboratory-reared moths were touched to each fibre using a suction hose for 3 s. Moth mortality was scored after 24 h at 24 ^{o}C.

Deposition and retention of fibres. Several studies were conducted during the season to assess the deposition and retention of fibres in the apple orchard. A trial was conducted on 12 September to estimate the number of fibres applied per hectare. Blank white celcon fibres (200 µm i.d. x 15 mm) were mixed with the adhesive and applied at the standard rate (100.0 g in 6.0 liters adhesive). Five dark blue tarps (2.92 m x 2.92 m) were placed in a row on a grassy strip. The sprayer was started 50 m away from the first tarp and once fibres had begun to be released from the spinning cone the tractor was driven forward at a speed of 4.0 km per h. The number of fibres deposited on each sheet was counted and used to estimate the number of fibres applied to the entire orchard (area equivalent to 426 tarps). Deposition of fibres within the canopy of the orchard was estimated following each spray application by visually examining 60 trees for fibres. Individual trees were inspected for 3 to 5 minutes from the ground. The retention of marked fibres within the canopy of the apple orchard was evaluated following the June application in 1992. Fifty-seven fibres were located on leaves and their location was marked with flagging. The retention of these fibres was checked after 2 and 7 wk.

One hundred and twenty-two fibres were located and marked with flagging one day after the application in 2003. The position of each fibre was recorded with respect to substrate and whether the fibre was in full contact with the substrate or if a portion of the fibre was detached from the substrate (overhanging). Their retention in the canopy was subsequently evaluated on 14 and 21 July and 22 August.

Statistical analyses. An unpaired t-test and analysis of variance on transformed data (square root [x+0.01]) were used to compare the attractiveness and toxicity of fibres placed in adhesive either with or without insecticide and to fibres aged from 0 – 28 d to cohorts of moths, respectively (Analytical Software 2000). Means in significant ANOVA's were separated with Fisher's LSD test, $P < 0.05$.

RESULTS

Attractiveness and toxicity of laboratory-aged fibers. No difference was found in the number of moth visits to fibres placed in either clean (mean ± SE = 13.4 ± 1.7) or insecticide-impregnated (17.4 ± 2.3) adhesive during the 7-minute bioassay in the flight tunnel ($t = 1.40$, df = 18, $P = 0.18$). Similarly, no difference was found in the duration of a moth visit between fibres placed in clean (1.9 ± 0.5) or insecticide-impregnated adhesive

(1.8 ± 0.4) (*t* = -0.09, df = 18, *P* = 0.93). Subsequent tests showed that the age of the fibre was a significant factor affecting moth contact and moth mortality (Table 1). Fibres placed in insecticide-impregnated adhesive and aged for < 7 d had significantly fewer moth contacts and reduced visitation time. No difference in either factor was found for fibres aged 14 – 28 d. A significantly greater proportion of moths per cohort were killed when flown to fibres aged 14 - 28 d versus < 1 d-old fibres. The highest moth mortality occurred with fibres aged 14 d (Table 1). The lack of a significant difference in moth mortality following exposure to fibres aged 4 – 7 d versus 21 – 28 d may have been due to a decline in the toxicity of the insecticide in the older drops.

Table 1

Influence of age on the attractiveness and toxicity of an individual hollow fibre loaded with 15% codlemone and placed on a 10.0 mg drop of adhesive treated with 1.0% esfenvalerate for male codling moths flown in a flight tunnel.

Age of fibre (d)[a]	Mean (SE) # of contacts[b]	Mean (SE) time (s) per source contact [b]	Mean (SE) proportion of dead moths[c]
0	2.4 (0.9) c	0.3 (2.1) b	0.29 (0.04)c
1	1.4 (0.7) c	0.2 (0.1) b	0.20 (0.05) c
4	1.9 (1.0) c	0.1 (0.03) b	0.50 (0.09) bc
7	5.7 (1.4) bc	0.3 (0.1) b	0.49 (0.05)bc
14	16.8 (3.5) a	1.4 (0.3) a	0.88 (0.04)a
21	11.8 (2.6) ab	0.9 (0.2) ab	0.68 (0.12) ab
28	19.1 (5.8) a	1.4 (0.5) a	0.67 (0.10) ab
Statistical analysis	*F* = 6.82; df = 6, 63; *P* < 0.0001	*F* = 3.93; df = 6, 46; *P* < 0.01	*F* = 9.82; df = 6, 46; *P* < 0.001

[a] Fibres were aged in a greenhouse maintained between 20 – 24 °C for up to 28 days.
[b] Ten cohorts of six moths were flown in the flight tunnel for 7 minutes for each fibre age class. The mean number of moth contacts and time per source visit per cohort were measured with an infrared motion detector hooked to a computer.
[c] Following each tests moths which contacted adhesive were collected and placed individually in vials. Mortality was scored after 24 h at 24 °C.

Attractiveness and toxicity of field-aged fibres. No moth scales were found on the sentinel fibres placed in the apple orchard during the first eight days after any of the three applications (Table 2). The proportion of fibres aged from 8 – 51 d visited by moths ranged from 0.43 – 0.85 during the season. Mortality of moths in the 3-s touch bioassay was > 85% for fibres collected on the day of the spray application. In general, fibres were initially sticky and associated with several milligrams of adhesive. Moth mortality dropped sharply with field aging of the fibres, however, 65 – 80% mortality occurred with 8 d and 5 d old fibres after the second and first applications, respectively. Moth mortality was much lower with 7 d-old fibres following the third application (Table 2). Moth mortality with field-collected fibres collected 2 – 7 wk after the application ranged from 0.0 – 30.0%.

Deposition and retention of fibres. The mean (SE) number of fibres counted per tarp was 32.9 (15.3). Extrapolating the deposition of fibres on the tarps to the area of the entire orchard (equivalent to 426 tarps) estimated 14,015 fibres were applied. Following the 5 May spray application a mean (SE) of 0.9 (0.3) fibres were sampled per tree. This first application was made a few days past full bloom and the growth of green foliage was limited. The mean density of fibres following the 1 June application when trees had abundant foliage increased to 2.5 (0.4) fibres per tree. However, fibre density following the third application on 28 July was somewhat lower, 1.5 (0.4) fibres per tree. The highest density of fibres found on a single tree during the season was 17. Extrapolating the mean density of fibres sampled per tree (1.5 – 2.5) multiplied by the number of trees in the block (214) suggests that only 2.3 – 3.8% of the estimated number of fibres sprayed in the orchard (14,015) were deposited on the trees.

Table 2

Proportion of sentinel hollow fibres placed in 10 mg adhesive on a plastic disk at various times following a spray application that contained moth scales and moth mortality following a 3-s touch to field-aged fibres deposited on the upper surface of apple leaves.

Date checked	Post-spray interval (d) fibre was in field	Proportion of fibres touched [a]	% moth mortality in touch bioassay [b]
5 May	0	--	100.0
10 May	1 - 5	0.00	80.0
17 May	5 - 12	0.85	22.0
1 June	0	--	96.0
9 June	2 - 8	0.00	65.0
16 June	8 - 15	0.45	30.0
23 June	15 - 22	0.85	10.0
30 June	22 - 29	0.60	12.0
7 July	29 - 36	0.40	18.0
14 July	36 - 43	0.55	6.0
22 July	43 - 51	0.40	0.0
28 July	0	--	86.0
4 August	1 - 7	0.00	26.0
11 August	7 - 14	0.75	14.0
19 August	14 - 22	0.43	8.0

[a] Positive visitation of codling moth to sentinel fibres was based on the microscopic detection of moth scales in the adhesive surrounding each sentinel fibre. Fibres and adhesive were placed in the center of plastic disks that were stapled horizontally in the upper third of the tree canopy and left in the field for 5 – 7 d.

[b] Moth mortality was assessed 24 h following a 3 s touch exposure to a field-collected fibre on the upper surface of a leaf. Five moths were tested per fibre and ten fibres were collected on each date.

Table 3

Retention of hollow fibres loaded with codlemone and mixed with an adhesive in the canopy of an apple orchard following a spray application on 9 July 2003.

Position of fibre	# fibres	% fibres lost after		
		4 d	11 d	43 d
Top of leaf	52	9.6	17.3	25.0
Top of leaf, overhanging	19	36.8	84.2	100.0
Bottom of leaf	9	55.6	55.6	100.0
Bottom of leaf, overhanging	16	68.8	75.0	100.0
Fruit	21	28.6	47.6	61.9
Bark	5	20.0	40.0	80.0
Total	122	28.7	49.2	59.8

Retention of fibres on apple trees was short-lived. Following the 1 June application in 1992, < 50% of marked fibres on leaves were retained on trees after 2 wk and approximately 10% were retained after 7 wk. The 2003 study showed that the retention of fibres is variable based on differences in their location and alignment on various substrates (Table 3). Following the 9 July application 58% of fibres were located on the top of leaves. Deposition of fibres on the bottom of leaves and on fruit was similar with about 20% each. Fibres deposited on the trunk and branches of trees accounted for < 5% of the total. A large proportion of fibres deposited initially on leaves were found overhanging the edge of the leaf. This was more common for fibres deposited on the underside of leaves with 64% of fibres overhanging. Retention of fibres was highest on the top of leaves with fruit being the second best. Fibres overhanging on leaves and all fibres deposited on the underside of leaves were lost within 43 d. In comparison, 60% and 80% of the fibres deposited on fruit and bark were loss within 6 wk, respectively. Only a quarter of the fibres deposited on the top of leaves and not overhanging were lost.

DISCUSSION

The experimental formulation of hollow fibres loaded with codlemone and mixed with an insecticide in this study was ineffective as an attracticide due to several factors including the emission rate of the fibre and the toxicity of the adhesive. The initial emission rate of codlemone from individual fibres was apparently too high to allow moth contact. Fibres had to be aged for > 7 d before male codling moths would contact fibres under both flight tunnel and field conditions. Moth mortality was high following brief contact with newly applied fibres but dropped rapidly with time. Modifications are needed to improve the performance of this attracticide approach.

The emission characteristics of sex pheromones from hollow fibres are well studied (Ashare *et al.* 1982). Fibres typically have an initial high release and then have a lower and fairly constant rate over an extended period of time. Previous studies with hollow fibres loaded with codlemone have shown that fibres can be long lived. Cardé *et al.* (1977) reported complete shutdown of lure-baited traps for 10 wk. Moffitt and Westigard (1984) reapplied fibres every 4 – 5 wk during the season. The emission rate of hollow fibres can be adjusted by modifying either the internal diameter of the fibre or by changing the length of the fibre (Ashare *et al.* 1982). Modifications of these factors could likely improve the use of fibres as an attracticide for codling moth.

Proper choice of an adhesive is critical in developing an effective attracticide. The viscosity of the adhesive affects both the application and the adhesion of the fibres. The polybutene adhesive Biotac has been widely used with hollow fibres (Beasley and Henneberry 1984, Moffitt and Westigard 1984) and is available in several formulations that differ in their viscosity and are appropriate for the range of temperatures experienced from early spring to late summer. Yet, fibres were generally associated with limited amounts of adhesive, < 1.0 mg; and were often poorly attached to the plant. In contrast, the initial laboratory studies placed fibres on large 10.0 mg drops of adhesive. This limited amount of adhesive associated with fibres under field conditions formed a dry film that was not effective in transferring a toxic dose of insecticide to codling moth adults. In comparison, the large drop of Biotac was toxic for several weeks in laboratory bioassays. The use of non-drying grease or a different type of adhesive instead of Biotac might extend the toxicity of the insecticide under field conditions.

Future improvements of the attracticide method for codling moth could include the use of a more concentrated insecticide dose. A 1.0% sticker formulation with permethrin and fenvalerate did not cause mortality of *P. gossypiella* while a 10.0% concentration was effective (Haynes and Baker 1986). Yet, formulations with only 0.1% concentrations of cyfluthrin killed 100% of codling moths when formulated in a castor oil-based paste (Lösel *et al.* 2000). One attract and kill paste formulation currently registered for control of codling moth contains 6.0% permethrin (Charmillot *et al.* 2000). These paste formulations remain effective against codling moth for at least 6 wk (Charmillot *et al.* 2000, Lösel et al 2000).

The impacts of an attracticide approach can include both lethal and sublethal effects such as the interference with mate location by males (Haynes and Baker 1986). While sublethal effects were not examined in our field studies, previous flight tunnel tests with codling moth found significant effects on male flight behaviours with concentrations of esfenvalerate as low as 0.04% (unpublished data). Further studies that can characterize the sublethal effects of the range of attracticide formulations for codling moth would be useful.

Depositing more fibres in the canopy would improve the effectiveness of this formulation both as an attracticide and for mating disruption of codling moth. The application methods used to apply fibres have included specialized and expensive ground and air equipment (Moffitt and Short 1982). Results reported here suggest that this approach is ineffective in placing a significant number of hollow fibres in the tree canopy. Fibres deposited in the apple tree canopy were primarily deposited in the middle of the upper leaf surface. This fibre position also appeared to be the most stable over time with nearly 75% of fibres retained after 6 wk. Unfortunately, the adhesion of fibres to bark or the underside of leaves was low and short-lived. Increasing the number of fibres sprayed per hectare is one approach that could be used to increase the density of deposited fibres. Ground applications in orchards with larger trees or denser canopies or perhaps the use of aerial applications might improve the deposition rates of hollow fibres and needs to be further examined.

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