Improved Deposition and Performance of a Microencapsulated Sex Pheromone Formulation for Codling Moth (Lepidoptera: Tortricidae) with a Low Volume Application

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ABSTRACT

Experiments were conducted to evaluate the deposition pattern and effectiveness in disrupting male orientation to virgin female-baited traps of a microencapsulated sex pheromone formulation for codling moth, *Cydia pomonella* (L.) in apple, *Malus domestica* Bordhausen. The efficacy of two application techniques was evaluated in field trials with the microencapsulated formulation Checkmate® CM-F: a high volume (926 liters per ha) application with an air blast sprayer and a low volume (46 liters per ha) application with a custom-built vertical boom sprayer. These treatments were compared to an unsprayed control and a control treatment where the formulation was applied directly on the ground within the plots. Disruption of virgin female-baited traps was significantly greater in the low volume versus the air blast application and versus the two types of control plots. Levels of disruption in the air blast-sprayed plots were not different from untreated plots or in plots where the sprayable sex pheromone was applied directly on the ground. A significant increase in the proportion of traps catching moths occurred in week 4. A significant interaction occurred among the effects of spray method, tree canopy position, and leaf surface on microcapsule deposition. This interaction was likely due to the low rate of deposition of microcapsules on the undersides of leaves in the lower canopy with the low volume sprayer. The low volume sprayer deposited significantly more microcapsules in the upper canopy than the air blast sprayer. Significantly more microcapsules were deposited on the underside versus the top of leaves in the upper canopy with the air blast but not with the low volume sprayer.

Key Words: sex pheromone, mating disruption, apple, pest management

INTRODUCTION

Various techniques have been developed to achieve mating disruption of codling moth, *Cydia pomonella* (L.), by treating orchards with controlled release devices (Vickers and Rothschild 1991). The use of sex pheromones in the western U.S. has been adopted rapidly since 1991 and is used on nearly 40,000 ha in Washington State alone (Brunner *et al.* 2002). Various hand-applied dispensers registered for codling moth have accounted for > 90% of this treated acreage (Thomson *et al.* 2000). Concurrently, microencapsulated sprayable formulations have been tested for codling moth (Knight 2000), but have not yet been widely adopted.

The ease of applying microcapsules with conventional equipment is a major factor generating grower's interest in sprayables (Campion 1976, Doane 1999). Sprayable sex pheromone formulations allow pest managers to treat crops with tall canopies, such as walnuts, and they increase grower's flexibility in adjusting application rates and timings during the season. In addition, microcapsules can be tank-mixed with other pesticides and can easily be included within a grower's inte-

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grated control program.

Conversely, a major limitation affecting the adoption of sprayable pheromone formulations has been their relatively short residual activity (Färbert *et al.* 1997). The emission profile from microcapsules typically exhibits a large initial burst followed by a sharp decline (Hall and Marrs 1989). Microcapsules for codling moth have been applied every four weeks during the growing season, but significant disruption of moth catches may occur for only one to two weeks (Knight 2000). Any efforts to extend the current activity of microcapsules will speed the adoption of this technology for codling moth management.

A large number of factors are likely involved in the successful use of microcapsules, including their emission profile over time, their structural integrity, the chemical stability of the sex pheromone, and density initially deposited and retained over time in the active area of the crop.

Surprisingly little is known about how these factors affect the performance of sprayable formulations for codling moth. The addition of antioxidant and UV stabilizers has reportedly improved the chemical stability of codlemone within a sprayable formulation (Eng *et al.* 2003). Laboratory studies have shown that differences in the epicuticular wax layer and the degree of pubescence of various plant tissues can affect microcapsule deposition rates in apple (Waldstein and Gut 2003, Knight *et al.* 2004). In addition, water has been shown to be a major factor causing dislodgement of microcapsules from leaves (Knight *et al.* 2004). Other factors, such as wind, abrasion, or other environmental factors impacting microcapsule retention under field conditions have not been investigated.

Adjusting one or more application parameters to increase either the deposition and/or retention of microcapsules over time may be a useful approach to extend the efficacy of sprayables for codling moth. Increasing the rate of application would likely also increase the density of microcapsules deposited in the canopy, but this approach is restricted by the comparable costs of alternative pest management tactics. Applying the same total rate of sex pheromone per season using more frequent applications of low rates of sprayables has been an effective compromise for some pest / crop systems (Polavarapu *et al.* 2001), but have not been successful in limited tests with codling moth (Hull *et al.* 2004). An additional parameter that may improve the performance of sprayables over time could be the refinement of the application technique.

Many pesticides including sprayables are applied in tree crops as concentrated sprays in spray volumes of 500-1,000 liters/ha using high velocity, air blast sprayers (Barnett *et al.* 1991). The use of these concentrated sprays can reduce application costs and avoid excessive leaf run-off as well as spray drift out of the orchard. Various factors on the sprayer are adjusted to generate a uniform spray coverage within the canopy, including tractor speed, spray velocity, and nozzle number, orifice size, and orientation (Byers *et al.* 1984). Pesticides are typically not applied with spray volumes <500 liters/ha in pome fruit orchards due to the difficulty in attaining good spray coverage (Byers *et al*. 1984). Similarly, microcapsule formulations have not been applied in tree fruits in spray volumes <500 liters/ha because they are thought to achieve mating disruption by camouflaging the calling of virgin females with a uniform 'fog' of sex pheromone (Doane 1999). Herein, we examine the influence of two spray application techniques on the pattern of deposition of microcapsules and their efficacy in disrupting the sexual communication of codling moth in apple, *Malus domestica* Bordhausen.

MATERIALS AND METHODS

Studies were conducted to compare the efficacy of applications of Checkmate® CM-F (Suterra LLC, Bend, OR), a microencapsulated sex pheromone, with either an air blast or a low volume sprayer in a 35 yr-old "Red Delicious' apple orchard situated near Naches Heights, WA from 10 July to 08 August 2000. Twelve 0.25 ha plots separated by 75 m were established within the orchard and randomly assigned one of four treatments: air blast application of sex pheromone; air blast application of water as a control; low volume application of sex pheromone; and a low volume application of sex pheromone directly on the ground as a second control. The latter treatment was included to experimentally assess the impact of microcapsules that are not deposited on the foliage following a canopy spray application. Plots consisted of 48 trees (6.8 x 8.0 m tree spacing). Mean $\pm$ SE tree height was 4.3 $\pm$ 0.1 m. Sex pheromone–treated plots were sprayed with a 50:50 mixture of microcapsules containing 14.3% codlemone and microcapsules formulated with 0.50% of a fluorescent material "Dye-Lite" (Tracer Products, Westbury, NY). Codlemone was applied in all plots at a rate of 49.0 g a.i. per ha. A Victair Mistifier (H. F. Hauff Co., Yakima, WA) sprayer with a 100-liter tank pulled by an all-terrain vehicle (ATV) was used to apply the air blast application of sex pheromone and of water alone. Seven spray nozzles angled at 45 ^{o} and positioned on the sprayer at heights from 1.1 to 1.7 m applied 10.4 liters per minute at 686 kilopascals. Plots were sprayed at a rate of 926 L/ha. The low volume sprayer consisted of a 95-liter polyolefin tank mounted on an ATV. The sprayer was rigged with an adjustable vertical spray boom outfitted with two flat fan nozzles. Nozzles were positioned on the boom at a height of 3.1 m and angled upward at a 45^{o} angle. The two nozzles together deliver a spray volume of 2.36 liters per minute at 18 kilopascals. Plots were sprayed at a rate of 46 L/ha. A horizontal boom attached to the ATV at a height of 1.0 m was used to apply the ground application of sex pheromone and fluorescent materials (49.0 g a.i. per ha) with the same nozzles at the same low volume rate used in the canopy.

The density of fluorescent microcapsules on the ground was estimated by placing five plastic cards (18 x 35 cm) beneath the tree canopy prior to the spray application. Cards were collected < 2 h after sprays were applied, returned to the laboratory, and the number of microcapsules was counted under UV illumination (Black-Ray Long Wave Ultraviolet Lamp, Ultra-Violet Products, Inc., San Gabriel, CA). Cards placed in plots where the material was sprayed directly on the ground were subsampled. Cards were first subdivided into ten 7 x 9 cm squares and the number of microcapsules was counted in one randomly selected square from each card.

The density of fluorescent microcapsules per leaf in the canopy was sampled in both the air blast and low volume-treated plots by collecting five leaves from 10 trees in each replicate from both low (2.2 m) and high (3.2 m) positions in the canopies. Leaves were bagged and microcapsules on both the top and bottom leaf surfaces were counted under UV light in the laboratory.

The efficacy of treatments in disrupting codling moth was evaluated with the use of virgin female-baited traps. Ten wing-shaped sticky traps (Pherocon 1CP, Trécé Inc., Salinas, CA) were placed in each plot spaced approximately 10 m apart and 10 m from the edge. Traps were baited with two virgin female codling moths (< 3 d-old and maintained at 5.0 $\pm$ 0.5 ^{o}C) placed inside a screened PVC cage (4.2 cm [O.D.] x 5.5 cm) hung inside from the top of each trap. Traps were checked and females were replaced every three to four days. The proportion of traps not catching any male moths was used as a measure of mating disruption within each treatment.

Data Analysis. A repeated-measures analysis of variance (ANOVA) was used to compare the proportion of virgin female-baited traps catching male moths with treatment as the between subject factor and week as the within subject factor (Analytical Software 2000). These proportional data were transformed prior to analysis with arcsine (square root [x]). A three-way ANOVA was used to evaluate the density of microcapsules on leaves with spray application, leaf surface (top or bottom surfaces), and canopy height (low and high) as the main effects. Significant means in all ANOVA's were separated with a least significant difference test, $P < 0.05$. The distribution of microcapsule density between each spray method was compared with a Kolmogorov-Smirnov test (Analytical Software 2000).

RESULTS

Significant differences in disruption of male capture in female-baited traps were found among plots sprayed either with an air blast or a low volume sprayer, plots where the sex pheromone was sprayed on the ground, and unsprayed plots ($F = 6.72$; df = 3, 8; $P < 0.05$) (Table 1). Significant differences were also found in levels of disruption among weeks ($F = 30.84$; df = 3, 9; $P < 0.001$) (Table 1). The interaction of treatment and time was not significant ($P = 0.42$). Disruption of sexual communication was significantly higher in the low volume-treated plots than the other treatments. No difference in the male capture in female-baited traps occurred in plots treated with either the air blast sprayer, applying the sex pheromone directly on the ground, or the application of only water. The proportion of traps catching moths among treatments was significantly lower during the first three weeks of the study versus week four (Table 1).

The density of microcapsules deposited on the ground beneath the trees varied among the three sex pheromone treatments ($F = 78.20$; df = 2, 6; $P < 0.001$). The highest mean $\pm$ SE density of microcapsules was in plots where the sex pheromone was sprayed directly on the ground, 4.5 ± 0.8 per 10 cm^2 and was significantly different from the microcapsule densities on the ground in the canopy-treated plots sprayed with either an air blast, 0.05 ± 0.02 per 10 cm^2 or a low volume sprayer, 0.14 ± 0.05 per 10 cm^2. The density of microcapsules on the ground was not significantly different between the two canopy spray treatments.

The mean density of microcapsules deposited in the lower and upper tree canopy and on the top and bottom surfaces of leaves varied with both spray methods (Fig. 1). The significant three-way interaction for canopy position * leaf position * spray method ($F = 7.61$; df = 1, 16; $P < 0.05$) appears to be the result of low microcapsule deposition on the lower leaf sur-

Table 1.

Mean $\pm$ SE proportion of virgin female-baited traps catching male codling moths in plots treated with microencapsulated sex pheromone (49 g a.i. per ha) applied with either an air blast or low volume sprayer in the canopy or a low volume sprayer on the ground versus water control in replicated 0.25 ha apple plots, July – August 2000.[1]

No. weeks post-spray	Proportion of traps catching moths				Overall week means
	Water control	Air blast canopy	Low volume canopy	Low volume ground	
1	0.30 (0.06)	0.13 (0.07)	0.03 (0.03)	0.30 (0.15)	0.19 (0.05)c
2	0.43 (0.07)	0.43 (0.17)	0.23 (0.03)	0.53 (0.09)	0.41 (0.06)b
3	0.13 (0.13)	0.23 (0.07)	0.03 (0.03)	0.23 (0.03)	0.15 (0.04)c
4	0.90 (0.10)	0.83 (0.03)	0.63 (0.03)	0.70 (0.12)	0.77 (0.05)a
Overall treatment means	0.44 (0.07)a	0.41 (0.09)a	0.23 (0.08)b	0.44 (0.09)a	

[1] Column and row overall means followed by a different letter were significantly different, repeated measures analysis of variance, $P < 0.05$, LSD.

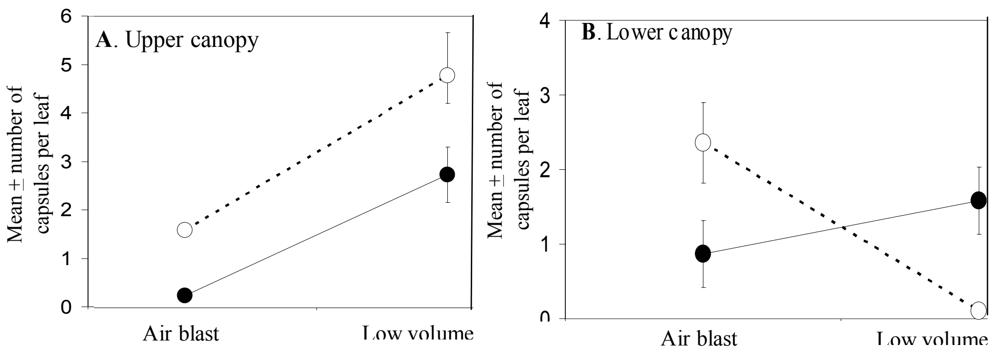

Figure 1. The mean ± SE number of microcapsules deposited on the top (●—●) or bottom (○--○) surface of leaves in the upper (A) and lower canopy (B) of apple trees sprayed with either an air blast or low volume sprayer.

face in the bottom of the canopy when applied with the low volume sprayer (open symbols Fig. 1B). We conducted one-way ANOVA on simple effects means to compare spray methods and to compare leaf surfaces in only the top of the canopy. A significantly greater number of microcapsules were deposited per leaf with the low volume (mean ± SE = 7.5 ± 1.4) versus the air blast (mean ± SE = 3.0 ± 0.7) application in the upper canopy ($F = 16.40$; df = 1, 4; $P < 0.05$). The difference in microcapsule density was significant between upper and lower leaf surfaces for the air blast sprayer ($F = 214.02$; df = 1, 4; $P < 0.0001$) with more microcapsules on the lower leaf surface, but not with the low volume sprayer (F=3.69; df = 1,4; $P = 0.13$).

The overall distribution of microcapsule densities per leaf in the canopy did not differ significantly between spray methods (K-S statistic = 0.10, $P = 0.09$) (Fig. 2). The highest microcapsule density per leaf in the plots treated with the low volume and air blast sprayer ranged up to 116 and 17 microcapsules per leaf, respectively. Five percent of the leaves sampled in the low volume-treated plots had > 20 microcapsules and these were all in the upper canopy.

DISCUSSION

Spray application method was found to have a significant effect on the efficacy of Checkmate® CM-F for codling moth. An air blast application did not increase the disruption of virgin female-baited traps more than an untreated check or when the material was sprayed directly on the ground. This result is consistent with the poor performance of this formulation in a series of grower field trials using air blast sprayers conducted from 2001-2003 (A. L. K., unpublished data). In contrast, a low volume application to the canopy provided significant levels of disruption during a four-week trial.

The difference in efficacy observed between spray methods was associated with differences in microcapsule deposition. The low volume application deposited significantly more capsules than the air blast spray in the upper canopy. The sexual activity of codling moth is generally restricted to the upper canopy and mating disruption is enhanced when sex pheromone dispensers are placed in the upper versus in the lower canopy (Weissling and Knight 1995). The low volume application also treated 5.0% of leaves with more than 20 microcapsules. The potential for these leaves to serve as attractive point sources and enhance "false-trail following" by male codling moths has not been explored. In general, the emission rate of sex pheromone from individual or clumps of microcapsules has been thought to be too low to create an attractive point source (Sanders

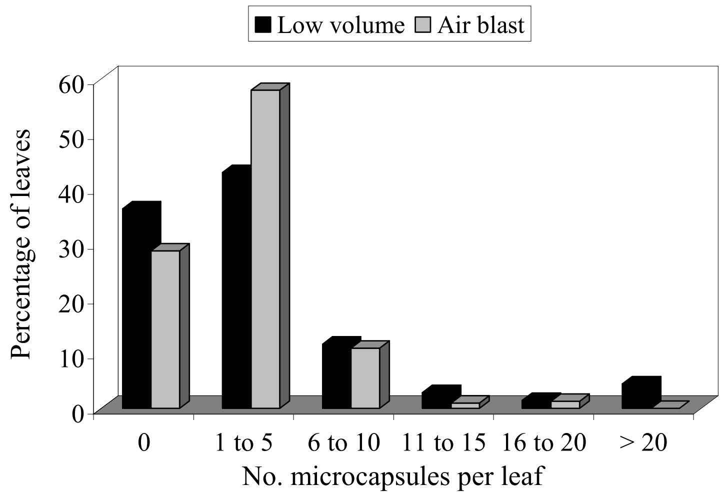

Figure 2. Frequency distribution of microcapsule density per leaf in plots sprayed with either an air blast or low volume sprayer.

1997). Instead, microcapsule formulations are thought to achieve mating disruption by camouflaging the calling of virgin females with a uniform 'fog' of sex pheromone (Doane 1999). However, the mean diameter of the Checkmate® CM-F is approximately 100 µm, which is much larger than many of the formulations (< 5 µm) used in earlier studies (Bakan 1980). Additional research will determine if leaves with variable numbers of microcapsules are attractive to codling moth and whether the low volume application of microcapsules may increase its effectiveness by enhancing the role of "false-trail following".

Factors influencing the deposition of microcapsules due to differences in spray practices are not understood. The two canopy spray methods used in our trials differed in several ways including water volume (20-fold difference) and spray pressure (40-fold difference). However, it is not clear how these factors influence the deposition of microcapsules on foliage. Previous studies have found that the structural integrity of these microcapsules is not impaired by high velocity spray applications (T. E. L., unpublished data). Interestingly, no difference was found in the deposition of microcapsules on the ground beneath trees with either canopy spray application method. The addition of adjuvants could perhaps improve the deposition of microcapsules on leaves with either spray method. Laboratory tests have found that a latex sticker significantly increased deposition and retention of microcapsules on dipped apple leaves before and after simulated rainfall (Knight *et al.* 2004).

The height and angle of the spray nozzle may also affect the deposition pattern within the canopy, i.e., the low deposition of microcapsules on the underside of leaves in the lower canopy in plots treated with the low volume sprayer. Laboratory studies have found that significantly more microcapsules are deposited on the underside versus the top of apple leaves with dipped leaves (Knight *et al.* 2004). This pattern was clearly seen in the upper canopy with the air blast but not with the low volume spray application. Lowering the vertical boom of the low volume sprayer and aiming the spray stream upwards into the canopy would likely increase the deposition of microcapsules on the underside of leaves. Microcapsules deposited on the underside of leaves typically have greater longevity due to shading effects (Hall and Marrs 1989). This may be especially important with conjugated dienes such as codlemone due to their sensitivity to isomerization and oxidation (Millar 1995). The addition of UV stabilizers has significantly extended the longevity of the Checkmate® CM-F microcapsules; however, the effects of shading provided by the undersurface of apple, pear, or walnut leaves has not been reported (Eng *et al.* 2003).

The compatibility of microencapsulated sex pheromone products with other pesticides has been one factor used to promote their use (Doane 1999). Air blast sprayers apply materials at very high velocities (150 - 300 km/h) to generate a uniform coverage of small droplets throughout the canopy. Most pesticides during the season are applied as concentrated sprays (< 1,000 liters per ha) due to significant reductions in spray costs versus dilute applications (Barnett *et al.* 1991). Yet, this study suggests that applying a sprayable sex pheromone formulation for codling moth is not effective with these standard spraying methods. Difficulties in spraying orchards late in the season with standard equipment could be an important factor limiting the adoption of sprayable pheromone in apple. The low volume sprayer pulled by an ATV allowed orchard rows with a closed canopy to be sprayed without dislodging fruit. The density and deposition patterns of microcapsules should be evaluated with the use of alternative spray methods, such as aircraft, handgun sprayers, or herbicide sprayers. Further reductions in spray volume with these various methods should also be considered.

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REFERENCES

Analytical Software. 2000. Statistix 7. Tallahassee, FL.

Bakan, J.A. 1980. Microencapsulation using coacervation/phase separation techniques, pp. 83-105. *In* A. F.Kydonieus, ed., Controlled release technology: methods, theory, and applications. CRC Press, Boca Raton, FL.

Barnett, W.W., W.J. Bentley, R.S. Bethell, C. Pickel, P.W. Weddle, and F.G. Zalom. 1991. Managing pests in apples and pears, pp. 21-53. *In* M. L. Flint, ed., Integrated pest management for apples and pears. Publication 3340. University of California Press. Oakland, CA.

Brunner, J., S. Welter, C. Calkins, R. Hilton, E. Beers, J. Dunley, T. Unruh, A. Knight, and R.Van Steenwyk. 2002. Mating disruption of codling moth: a perspective from the Western United States. International Organization of Biological Control Western Palaeartic Regional Section Bulletin 25(9): 11-21.

Byers, R.E., C.G. Lyons, K.S. Yoder, R.L. Horsburgh, J.A. Barden, and S.J. Donohue. 1984. Effects of apple tree size and canopy density on spray chemical deposit. Horticultural Science 19: 93-94.

Campion, D.G. 1976. Sex pheromones for the control of lepidopteran pests using microencapsulated and dispenser techniques. Pesticide Science 7: 636.

Doane, C.C. 1999. Controlled-release devices for pheromones, pp. 295-317. *In* H. B. Scher, ed., Controlled-release delivery systems for pesticides. Marcel Dekker, Inc., New York.

Eng, J.A., E. Holmes, T. Larsen, S. Stadlmann, and K. Ketner. 2003. Effects of sunlight on encapsulated sprayable codling moth pheromone, p. 9. *In* Proceedings 77th Western Orchard Pest and Disease Management Conference, 15-17 January 2003, Portland, OR. Washington State University, Wenatchee, WA.

Färbert, P., U.T. Koch, A. Färbert, and R.T. Staten. 1997. Measuring pheromone concentrations in cotton fields with the EAG method, pp. 347-358. *In* R. T. Carde and A. K. Minks, (eds.), Insect pheromone research: new directions. Chapman & Hall, New York.

Hall, D.R. and G.J. Marrs. 1989. Microcapsules. pp. 199- 248. *In* A. R. Jutsum and R. F. S. Gordon, (eds.), Insect pheromones in plant protection. John Wiley and Sons Ltd., New York.

Hull, L.A., C. Myers, and G. Krawazyk. 2004. Low rate-frequent applications of sprayable pheromone for oriental fruit moth and codling moth mating disruption in Pennsylvania, p. 5. *In* Proceedings 78th Western Orchard Pest and Disease Management Conference, 14-16 January 2004, Portland, OR. Washington State University, Wenatchee, WA.

Knight, A.L. 2000. Applying sex pheromones - from puffers to air-blast sprayers. Proceedings of the Washington Horticultural Association 96: 161-163.

Knight, A.L., T.E. Larsen, and K.C. Ketner. 2004. Rainfastness of a microencapsulated sex pheromone formulation for codling moth (Lepidoptera: Tortricidae). Journal of Economic Entomology 97: 1987-1992.

Millar, J.G. 1995. Degradation and stabilization of E8,E10-dodecadienol, the major component of the sex pheromone of the codling moth (Lepidoptera: Tortricidae). Journal of Economic Entomology 88: 1425-1432.

Polavarapu, S., G. Lonergan, H. Peng, and K. Neilsen. 2001. Potential for mating disruption of *Sparganothis sulfureana* (Lepidoptera: Tortricidae) in cranberries. Journal of Economic Entomology 94: 658-665.

Sanders, C.J. 1997. Mechanisms of mating disruption in moths, pp. 333-346. *In* R. T. Carde and A. K. Minks, (eds.), Insect pheromone research new directions. Chapman & Hall, New York.

Thomson, D., J. Brunner, L. Gut, G. Judd, and A. Knight. 2000. Ten years implementing codling moth mating disruption in the orchards of Washington and British Columbia. Starting right and managing for success. International Organization of Biological Control Western Palaeartic Regional Section Bulletin 24 (2): 23-30.

Vickers, R.A. and G.H. Rothschild. 1991. Use of sex pheromones for control of codling moth, pp. 339-354. *In* L. P. S. van der Geest and H. H. Evenhuis, (eds.), Tortricid pests: their biology, natural enemies and control. Elsevier, Amsterdam, The Netherlands.

Waldstein, D.E. and L.J. Gut. 2003. Comparison of microcapsule density with various apple tissues and formulations of oriental fruit moth (Lepidoptera: Tortricidae) sprayable pheromone. Journal of Economic Entomology 96: 58-63.

Weissling, T.J. and A.L. Knight. 1995. Vertical distribution of codling moth adults in pheromone-treated and untreated plots. Entomologia Experimentalis et Applicata 77: 271-275.