Field testing of synthetic attractants for male *Grapholita libertina* (Lepidoptera: Tortricidae)[1]

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Abstract—In an effort to develop an efficient monitoring method for the lingonberry fruitworm, *Grapholita libertina* Heinrich, an economically important pest of lingonberries, *Vaccinium vitis-idaea* L. var. *minus* Lodd. (Ericaceae), in Newfoundland, various known sex attractants for other species of the genus *Grapholita* Treitschke were evaluated for their ability to attract *G. libertina* moths in field trials in eastern Newfoundland. The chemicals tested were (*E,E*)-8,10-dodecadien-1-ol acetate (*EE*8,10-12:Ac), (*E*)-8-dodecen-1-ol acetate (*E*8-12:Ac), (*Z*)-8-dodecen-1-ol acetate (*Z*8-12:Ac), and (*Z*)-8-dodecen-1-ol (*Z*8-12:OH). Field trials in 1996 showed highest attraction to *E*8-12:Ac, with *Z*8-12:Ac and *Z*8-12:OH also being attractive. Trials in 1997 demonstrated that a blend of *E*8-12:Ac, *Z*8-12:Ac, and *Z*8-12:OH at a ratio of 85:10:5, respectively, was attractive to *G. libertina* males. All chemicals captured significantly more moths than did controls and all moths examined were males indicating these chemicals may be constituents of the naturally occurring female sex pheromone.

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Résumé—Dans le cadre de recherches d'une méthode efficace pour étudier la tordeuse de la lingonne, *Grapholita libertina* Heinrich, un important ravageur de la lingonne, *Vaccinium vitis-idaea* L. var. *minus* Lodd. (Ericaceae) à Terre-Neuve, diverses substances connues pour leur pouvoir d'attraction sexuelle chez plusieurs espèces du genre *Grapholita* Treitschke ont été testées pour leur capacité d'attirer des *G. libertina* au cours d'expériences sur le terrain dans l'est de Terre-Neuve. Les substances suivantes ont servi au cours des tests : l'acétate de (*E,E*)-8,10-dodécadién-1-ol (*EE*8,10-12:Ac), l'acétate de (*E*)-8-dodécén-1-ol (*E*8-12:Ac), l'acétate de (*Z*)-8-dodécén-1-ol (*Z*8-12:Ac) et le (*Z*)-8-dodécén-1-ol (*Z*8-12:OH). Les tests en nature en 1996 ont démontré que c'est le *E*8-12:Ac qui est le plus attirant et que le *Z*8-12:Ac et le *Z*8-12:OH ont aussi un pouvoir d'attraction. Les tests de 1997 ont montré qu'un mélange de *E*8-12:Ac, de *Z*8-12:Ac et de *Z*8-12:OH, dans des proportions de 85:10:5 respectivement, est efficace pour attirer des mâles de *G. libertina* Toutes les substances testées attirent significativement plus de tordeuses

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que des produits témoins et les spécimens capturés sont toujours des mâles, ce qui semble indiquer que ces substances entrent probablement dans la composition de la phéromone sexuelle naturelle de la femelle.

[Traduit par la Rédaction]

Introduction

The lingonberry, *Vaccinium vitis-idaea* L. var. *minus* Lodd. (Ericaceae), is a low-growing, evergreen shrub that produces edible berries which ripen in mid-September in Newfoundland and Labrador (Morris *et al.* 1988). It occurs on rocky and dry peaty soils, wet heaths, barrens, and coastal headlands (Ryan 1978). Larvae of *Grapholita libertina* Heinrich (Lepidoptera: Tortricidae), the lingonberry fruitworm, feed within the lingonberry fruit. In Canada, this fruitworm occurs in Newfoundland and Labrador, Nova Scotia, and British Columbia (Morris *et al.* 1988), and causes direct loss in berry production and diminished product quality.

In Newfoundland, lingonberries are currently harvested commercially and are an economically important export to Scandinavia and other countries (Hendrickson 1997). The Newfoundland Department of Forest Resources and Agri-Foods and Agriculture and Agri-Food Canada have been evaluating a number of European domesticated varieties since 1991 for potential development of a U-Pick market (Penney *et al.* 1996). Two local estate wineries produce a wide range of fruit wines, including lingonberry wine (Gamble 2002), which are exported world-wide. To facilitate commercial cultivation of the lingonberry, a means of monitoring and managing *G. libertina* is needed. Development of a sex attractant for this species would allow for establishment of an integrated pest management program, wherein populations potentially could be monitored and controlled with minimal use of insecticides. Identification of pheromone analogues, chemicals which elicit a similar behavioral response to the pheromone, may produce suitable sex attractants for monitoring insects, with much less cost and effort than identification of the specific chemical structure of an insect sex pheromone (Ando *et al.* 1977). Closely related insect taxa may share similarity in pheromone structure, and this homology is particularly notable in the Lepidoptera (Ando *et al.* 1977). Within the genus *Grapholita* Treitschke, it has been shown that attractants for different species may vary by just a single carbon, or by *cis–trans* isomerism (Mayer and McLaughlin 1993; Arn *et al.* 1992). Pheromones of the lesser appleworm, *Grapholita prunivora* Walsh, and the Oriental fruit moth, *Grapholita molesta* Busck, have been isolated and as many as 15 other species in this genus have shown attraction to certain compounds (Arn *et al.* 1992). Most of these compounds and pheromone components are unsaturated 12-chain alcohols with or without an acetate.

Previous research has found that *G. libertina* is difficult to rear under laboratory conditions, with adults failing to emerge following overwintering. As a result, virgin females could not be obtained to pursue pheromone gland or effluvial extract analysis. However, the similarity in the structure of attractant compounds within the genus suggests that *G. libertina* may have a similar attractant. Our objective was to identify a synthetic attractant for monitoring wild *G. libertina* populations by field testing attractant compounds known from other species of the genus *Grapholita*.

Materials and methods

Study sites

Attractant trapping was carried out in wild lingonberry stands in the eastern part of the island of Newfoundland, Canada, during 1996–1997. All sites were exposed headlands, adjacent to the ocean. This area is within the Boreal Shield ecozone and the Maritime Barrens ecoregion, which has characteristically cool, foggy summers and short moderate winters, with a mean annual temperature of 5.5°C (Ecological Stratification Working Group 1995). The study sites comprise a mixture of heaths of the genera *Empetrum* L. (Empetraceae) and *Kalmia* L. (Ericaceae), with a carpet of low growing vegetation (*Empetrum nigrum* L., *Vaccinium vitis-idaea*, and *Potentilla tridentata* Aiton (Rosaceae), and lichens of the genus *Cladonia* Wigg (Cladoniaceae), punctuated by thickets of shrubs in more sheltered areas [*Kalmia angustifolium* L., *Vaccinium angustifolium* Aiton, and *Ledum groenlandicum* Oeder (Ericaceae)] (Meades 1983).

During 1996, trials took place at three sites where lingonberries were plentiful: Little Catalina (48°34′N, 53°02′W), Freshwater (47°45′N, 53°14′W), and Pouch Cove (47°46′N, 52°47′W). In 1997, field tests at the Pouch Cove and Freshwater sites were discontinued because they had low catches of *G. libertina* in 1996, and two new sites, Bryant's Cove (47°41′N, 53°11′W) and Chance Cove (47°38′N, 54°50′W), were selected instead.

Chemicals

Four compounds, which constituted the major attractant compounds of *Grapholita* spp., were studied in 1996: (*E,E*)-8,10-dodecadien-1-ol acetate (*EE*8,10-12:Ac), (*E*)-8-dodecen-1-ol acetate (*E*8-12:Ac), (*Z*)-8-dodecen-1-ol acetate (*Z*8-12:Ac), and (*Z*)-8-dodecen-1-ol (*Z*8-12:OH) (+99% purity, Bedoukian Research Inc, Danbury, Connecticut) (Arn 1999). Acetone was used as a solvent for all compounds and acetone blanks (*i.e.*, no attractive compound added) were used as controls. Lures were prepared by pipetting attractant solutions onto rubber septum dispensers. Septa were prepared by Research Productivity Council Laboratories, Fredericton, New Brunswick, Canada. In 1996, each compound was tested at concentrations of 0.01, 0.1, 1, and 10 mg/mL of acetone solvent per septum.

The three most attractive compounds from the 1996 season were blended and tested in 1997 field trials. Blend ratios were selected based on typical ratios of other lepidopteran and tortricid pests (Mayer and McLaughlin 1993). These blends were at ratios of 85:10:5, 90:7:3, and 94:4:2 (*E*8-12:Ac : *Z*8-12:Ac : *Z*8-12:OH), and all were at a concentration of 1 mg/mL.

Field trials

Pherocon® 1C impaction traps were used at all sites in 1996 and 1997. Each trap was baited with a single rubber septum, placed centrally, and held in place with an insect pin. Rubber septa were not replaced during the field season unless they were lost or damaged. Traps were suspended by wire from wooden stakes at 5–10 cm above ground. In each study site, traps baited with different lures were setup in a randomized grid, within which traps were spaced 20 m apart.

1996

Each of the four compounds was present in six traps at each site. Of these six traps, three contained a lure at 10 mg/mL and the other three traps contained a lure at 0.01, 0.1, or 1 mg/mL. Two control (blank) traps, containing only acetone solvent, were present at each site. This resulted in 26 traps at each site: six traps for each of the four compounds tested and two controls.

1997

Traps were again setup in a randomized grid. Traps were baited with 1 mg/mL of the three most attractive compounds from 1996, the three types of blended lures (85:10:5, 90:7:3, and 94:4:2) and acetone blanks. Each lure and acetone control was replicated three times at each site, for a total of 21 traps, testing pure compounds, blends, and controls.

Sampling regime

Traps were checked twice weekly. At each check, the number of *G. libertina* in each trap was recorded and traps were advanced by one position through the grid to minimize any position or edge effects. When trap catch exceeded 50 moths, or when the sticky surface of the trap was congested with debris, the trap bottom was changed. All *G. libertina* moths captured were counted and a random sample of 100 moths were sexed from among all traps collected each year. Sexing was conducted by excising the abdominal tip, clearing with potassium hydroxide, and comparing with preserved specimens. Voucher specimens of *G. libertina* were sent to the Canadian National Collection of Insects at the Eastern Cereal and Oilseed Research Centre (Agriculture and Agri-Food Canada) for confirmation.

In 1996, trapping began on 24 June and continued until the end of the flight season, 12 August in Pouch Cove and 19 August in Freshwater and Little Catalina. Trapping in 1997 began on 30 June and continued until 18 August in Bryant's Cove and Chance Cove and 25 August in Little Catalina.

Data analysis

The basic data unit was the mean trap catch per compound. In all trials, trap catches were standardized by subtracting the average control trap catches from each experimental trap catch. In 1996, the three replicates of the 10-mg/mL concentrations were averaged for comparison with other concentrations. Owing to the high number of zero values obtained, all catches were square-root transformed ($\sqrt{0.5+x}$), before being subjected to ANOVA with unequal sample size (Sokal and Rohlf 1995). Effects of compound, concentration, and site during the 1996 season were analyzed by three-way ANOVA. Where an ANOVA was significant, the Fisher's least significant difference (LSD) test (Sokal and Rohlf 1995) was used to separate means ($P < 0.05$). In 1997, the effect of compound and site were evaluated by two-way ANOVA and means again separated by Fisher's LSD (Sokal and Rohlf 1995). MINITAB® was used for all ANOVA calculations (Ryan *et al.* 1994).

Results

1996

All randomly examined moths from traps were male. The *E*8-12:Ac attracted the most moths at all sites ($F_{3,16} = 9.08$, $P < 0.05$; Tables 1, 2), followed by *Z*8-12:Ac, *Z*8-12:OH, and *EE*8,10-12:Ac, which also caught significantly more than the controls. Moth capture was variable between sites ($F_{2,16} = 157.89$, $P < 0.05$; Tables 1, 2). Total trap captures were greater at Little Catalina than at Pouch Cove or Freshwater. Within each site the ranking of compounds was similar (with the exception of a relatively higher *Z*8-12:Ac capture in Pouch Cove; Table 1).

The effects of compound concentration were also significant ($F_{3,16} = 6.98$, $P < 0.05$; Tables 1, 2). Catch rates were generally greater at the 1- and 10-mg/mL concentrations,

TABLE 1. Mean capture of *Grapholita libertina* in pheromone traps at three sites in eastern Newfoundland in 1996.

		Little Catalina		Pouch Cove		Freshwater		All sites	
Treatment	Concentration (mg)	*n**	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE
*E*8–12:Ac	0.01	17	6.91±2.77*a*	17	0.06±0.06*a*	17	0.12±0.12*a*	51	2.36±1.01*a*
	0.1	17	20.47±7.39*b*	17	0.88±0.33*b*	17	0.21±0.18*a*	51	7.19±2.76*b*
	1	17	8.85±3.64*a*	17	0.41±0.26*b*	17	1.56±0.90*b*	51	3.61±1.32*ac*
	10	17	14.76±4.24*ab*	17	0.86±0.54*b*	17	1.08±0.32*b*	51	5.57±1.67*bc*
	Mean (all concentrations)	68	12.75±2.46*b*	68	0.55±0.17*b*	68	0.74±0.25*b*	51	4.68±0.91*b*
*Z*8–12:Ac	0.01	17	3.62±1.29*a*	17	0.35±0.17*a*	17	1.09±0.58*a*	51	1.69±0.51*a*
	0.1	17	3.32±2.42*a*	17	0.53±0.31*ab*	17	0.47±0.20*a*	51	1.44±0.82*a*
	1	17	10.32±6.42*ab*	17	1.53±0.69*b*	17	0.06±0.06*b*	51	3.97±2.20*ab*
	10	17	11.07±2.89*b*	17	0.83±0.26*b*	17	0.61±0.19*a*	51	4.17±1.17*b*
	Mean (all concentrations)	68	7.08±1.91*c*	68	0.81±0.21*b*	68	0.56±0.17*b*	51	2.82±0.67*c*
*Z*8–12:OH	0.01	17	2.62±0.64*a*	17	0.53±0.26*a*	17	0.06±0.06*ab*	51	1.07±0.28*a*
	0.1	17	1.47±0.59*a*	17	0.06±0.06*b*	17	0.12±0.08*a*	51	0.55±0.22*b*
	1	17	8.91±2.39*b*	17	0.06±0.06*b*	17	0.00±0.00*b*	51	2.99±0.98*c*
	10	17	6.63±1.94*b*	17	0.29±0.14*a*	17	0.18±0.10*a*	51	2.37±0.77*c*
	Mean (all concentrations)	68	4.91±0.87*c*	68	0.24±0.08*a*	68	0.09±0.04*a*	51	1.74±0.30*d*
*EE*8,10–12:Ac	0.01	17	1.21±0.35*a*	17	0.29±0.14*a*	17	0.00±0.00*a*	51	0.50±0.14*a*
	0.1	17	1.15±0.53*a*	17	0.24±0.18*a*	17	0.35±0.24*b*	51	0.58±0.21*a*
	1	17	5.00±1.06*b*	17	0.06±0.06*b*	17	0.09±0.06*b*	51	1.72±0.48*b*
	10	17	1.57±0.34*a*	17	0.14±0.06*ab*	17	0.18±0.07*b*	51	0.63±0.15*a*
	Mean (all concentrations)	68	2.23±0.37*a*	17	0.18±0.06*a*	68	0.15±0.07*a*	51	0.86±0.14*a*
	Overall mean ± SE	68	6.74±0.84*b*	68	0.45±0.12*a*	17	0.38±0.08*a*	51	2.52±0.30*c*

NOTE: Mean captures for concentrations of compounds from the same site followed by the same letter do not differ (Fisher's LSD test, $P < 0.05$). Mean captures for compounds across all concentrations at the same site followed by the same letter do not differ (Fisher's LSD test, $P < 0.05$). Overall mean captures followed by the same letter do not differ (Fisher's LSD test, $P < 0.05$).

* *n* refers to the number of measurements or days that traps were checked.

TABLE 2. Analysis of variance of captures in pheromone traps of *Grapholita libertina* by site, treatment, and concentration in 1996 and by site and treatment in 1997 at five sites in eastern Newfoundland.

	1996			1997		
Source	df	*F*	*P*	df	*F*	*P*
Site	2	157.89	0	2	3.32	0.04
Treatment	3	9.08	0	5	4.91	0
Concentration	3	6.98	0	—	—	—
Site × treatment	6	2.37	0.03	10	0.49	0.9
Site × concentration	6	2.85	0.01	—	—	—
Treatment × concentration	9	2.12	0.03	—	—	—
Site × treatment × concentration	18	2.22	0	—	—	—

with the exception of the 0.1-mg/mL concentration of *E*8-12:Ac, which captured significantly more moths than other concentrations tested (Table 1). Significant interactions were also noted between site, treatment, and concentration effects (Table 2).

1997

All randomly examined moths from traps were male. Moth captures were significantly different between sites ($F_{2,13}$ = 3.32, P < 0.05; Tables 2, 3). Total trap captures were lower at Bryant's Cove than in Little Catalina or Chance Cove. Some variation was evident in the ranking of compounds between sites, although differences were not different (P < 0.05; Table 3). The 90:7:3 blend ranked highest in Bryant's Cove and the 94:4:2 blend ranked highest in Chance Cove, whereas the 85:10:5 blend ranked highest in Little Catalina and across all sites. No site × treatment interaction was noted ($F_{10,13}$ = 0.49, P = 0.90; Table 2).

With the unblended compounds, *Z*8-12:Ac was the most attractive ($F_{5,13}$ = 4.91, P < 0.05; Table 3), followed by *E*8-12:Ac, which was more attractive than *Z*8-12:OH. All compounds captured significantly more than the control traps. With the blends, 85:10:5 captured significantly more than the control, *Z*8-12:OH, or *E*8-12:Ac lures (Table 3), followed by *Z*8-12:Ac and the other two blends. All three blends and *Z*8-12:Ac captured significantly more than the control and *Z*8-12:OH lures. The *Z*8-12:Ac, 90:07:03 and 94:04:02, lures were not significantly different from the *E*8-12:Ac lure. Overall, the blends were not significantly different from one another or from *Z*8-12:Ac.

Trap captures by each compound varied between years (Tables 1, 3). *E*8-12:Ac caught relatively more moths than *Z*8-12:Ac in 1996 ($F_{3,16}$ = 9.08, P < 0.05), whereas *Z*8-12:Ac caught relatively more moths than *E*8-12:Ac in 1997 ($F_{5,13}$ = 4.91, P < 0.05). *Z*8-12:OH caught fewer moths than *Z*8-12:Ac or *E*8-12:Ac in both years.

Discussion

The fact the *E*8-12:Ac was the most attractive compound in 1996, and *Z*8-12:Ac in 1997, cannot be readily explained. Similar effects have been shown in the spruce seed moth, *Cydia strobilella* L. (Lepidoptera: Tortricidae), in which the most attractive pheromone blends differed from year to year (Grant *et al.* 1989). It is possible that by selecting the 1-mg/mL concentration in 1997, the attraction to *Z*8-12:Ac was enhanced, whereas the performance of the other compounds was diminished. In *G. molesta*, Baker *et al.* (1981) found that concentrations of pheromone of 1 mg/mL and higher decrease the efficiency of trapping because moths terminate their upwind flight prior to reaching the source. In other words, unusually high concentrations make the insect behave as

TABLE 3. Mean captures of *Grapholita libertina* in pheromone traps baited with three components in 1997 at three sites in eastern Newfoundland.

Ratio of components			Little Catalina		Bryant's Cove		Chance Cove		All Sites	
*E*8–12:Ac	*Z*8–12:Ac	*Z*8–12:OH	*n**	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE
85	10	5	14	7.24±1.94*a*	14	2.95±1.60*a*	14	2.88±1.17*a*	42	4.36±1.06*a*
90	7	3	14	5.10±2.47*ab*	14	3.24±1.59*a*	14	1.53±0.78*ab*	42	3.29±1.01*a*
94	4	2	14	4.38±2.12*ab*	14	1.45±0.56*a*	14	3.81±1.53*ac*	42	3.22±0.89*a*
100	0	0	14	1.95±0.78*bc*	14	0.03±0.20*b*	14	1.31±0.81*ab*	42	1.10±0.39*b*
0	100	0	14	2.88±1.17*b*	14	1.95±1.65*a*	14	6.10±3.28*ac*	42	3.65±1.28*a*
0	0	100	14	0.81±0.50*c*	14	0.01±0.10*b*	14	0.38±0.46*b*	42	0.29±0.15*c*
Overall site (mean ± SE)			14	3.71±1.52*a*	14	1.61±0.92*a*	14	2.67±1.27*a*	42	2.70±0.80*a*

NOTE: Means with different ratios of components or from the same site followed by the same letter do not differ (Fisher's LSD test, $P < 0.05$).

* *n* refers to the number of measurements or days that traps were checked.

though it is closer to the source than it actually is, suppressing source-oriented behavior. This may explain the decrease in efficiency of *E*8-12:Ac, particularly if its optimal concentration is closer to 0.1 than 1 mg/mL. *Z*8-12:Ac was as attractive as the *E*8-12:Ac at 1 mg/mL in 1996, and significantly more attractive in 1997. However, this does not explain the low catch rate of *Z*8-12:OH in 1997, since 1 mg/mL was the most attractive concentration in 1996.

The lower trap catches observed in 1997 perhaps indicate a population decline or environmental and extrinsic factors between years affecting catch rates. In Little Catalina it is possible that trapping in 1996 caused a subsequent reduction in the population, but this is unlikely as the study site is surrounded by large lingonberry-rich barrens from which *G. libertina* could emigrate. Variability in abundance noted between sites may also be due to differences in the abundance of lingonberries. Lingonberries were more plentiful at Little Catalina than at the other sites and should have been able to support a larger population of *G. libertina*. Unfortunately, no accurate numbers are available on lingonberry abundance at the sites during 1996 and 1997.

Concentrations had a significant effect on the trap catches, with higher concentrations of each compound capturing greater numbers of moths. In 1996, traps baited with 0.1 mg/mL of *E*8-12:Ac captured more moths than traps baited with 10 mg/mL, but catches were not significantly greater. Other studies have shown that a dosage-dependant relationship exists between concentrations of attractant substances and moth trapping ability (Baker *et al.* 1981; Roelofs and Cardé 1974). Walker and Welter (1999) showed that trap captures of *Argyrotaenia citrana* Fernald (Lepidoptera: Tortricidae) were significantly higher in traps containing higher dose lures. Daily trap captures of *Croesia curvalana* Kearfott (Lepidoptera: Tortricidae) in Unitraps™ have been shown to increase up to lure dosages of 0.03% and decrease at higher concentrations (Polavarapu and Seabrook 1992). In *G. molesta*, a dosage-dependant relationship is present between pheromone concentration and male upwind flight, and wing fanning and hair-penciling at the odor source (Baker *et al.* 1981).

Because the 85:10:5 blend of *E*8-12:Ac : *Z*8-12:Ac : *Z*8-12:OH, respectively ranked the highest among the blends, it can be recommended as an appropriate synthetic attractant for *G. libertina*. However, the lack of significant differences between the blends suggests that the ideal ratio may not be 85:10:5. The high capture rate of *Z*8-12:Ac relative to the blends also suggests that *Z*8-12:Ac may appropriately be listed as the major component in a synthetic attractant for *G. libertina*. As well, adult capture in 1997 increased as the ratio of *E*8-12:Ac decreased and the ratio of *Z*8-12:Ac increased. The natural pheromone is probably similar to that of *G. molesta*, which is an optimized 74:4:22 blend of *Z*8-12:Ac : *E*8-12:Ac : *Z*8-12:OH, respectively (Arn *et al.* 1992).

The specific composition of the *G. libertina* sex pheromone is unknown, and further refinement and ratio testing might produce a more efficacious synthetic attractant. For the purposes of this study, however, the 85:10:5 blend proved an effective field attractant, capturing 25% of the total catch in 1997. It was the most attractive of the top four lures, making it the best choice of the synthetic lures tested.

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