

# Rapid Measure of Sex Pheromone Emission from Plastic Rope Dispensers: Example of Utility in Sexual Communication Disruption of the Diamondback Moth, *Plutella xylostella*<sup>1</sup>

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An easy and reliable method to measure the emissions from plastic dispensers, which is adaptable to other dispensers and substrates, is reported. A small bed of the adsorbent Super-Q7<sup>®</sup> was found to adsorb large quantities of all of the sex pheromone components tested and, concomitantly, to enable enough air flow to approximate air speeds over the dispensers that may be encountered in nature. Some of the advantages of this method over others are its ease of use and accuracy and the direct measures of emissions. *E.g.* measurements showed that Z11-16:Al, one of the constituents of a rope lot, was emitted at a rate inconsistent with evaporative processes; rather, it was indicative of degradation processes. Brief reference is made of the utility of the timely measurements of emission rates in field experiments to control the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), in commercially grown cabbage in 1996.

KEY WORDS: Sex pheromones: components, emission, half-life, rope dispensers; *Plutella xylostella*; diamondback moth; models; measurement techniques.

## INTRODUCTION

For some time now, entomologists have deployed sex pheromones in fields to disrupt insect communication and mating. In recent large-scale field trials sex pheromone communication and mating of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), was disrupted in commercial cabbage fields in central and north central Florida by use of Shin-Etsu plastic rope dispensers formulated with (Z)-11-hexadecenal (Z11-16:Al) and (Z)-11-hexadecenyl acetate (Z11-16:Ac) (11,13). Variants of this dispenser have been assayed for disruption of mating and sexual communication that combine the above components with others appropriate for beet armyworms, *Spodoptera exigua* (Hübner), and cabbage loopers, *Trichoplusia ni* (Hübner) (13,14). These and many other experiments demonstrate the merit of commercially prepared sex pheromone dispensers in disrupting mating and communication in field-scale experiments, but some useful measures are missing.

Missing from many of these experiments were accurate measures of the chemical constituents that are released and their respective release rates, which are important to

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maintain effective component ratios (12). Thus, if the release rates of the different components change during a treatment interval, then so will the ratio of the compounds that are released. Hence, while it is as important to know what pheromones are deployed, it is perhaps more important on a scientific, practical and economic basis to know the rate at which they are released. Furthermore, where the disruptant chemical is unstable, such as (*Z,E*)-9,12-tetradecadienyl acetate (ZETA) or ultraviolet-sensitive aldehydes, a measure of exactly what is emitted becomes even more crucial. Thus, when one or both of the active components of these ropes are unstable, modeled emission rates may not be useful.

What is established from the preceding is the need for a timely, simple and accurate measure of the release rates so that an operator or scientist can simply and quickly re-establish effective treatment levels and/or ratios during the treatment. For this reason we developed, and report herein, an easy, reliable and direct measure of Z11-16:Al and Z11-16:Ac emission from a newly manufactured commercial formulation of the Shin-Etsu dispenser. It is an airflow method that is easily adaptable for plastic, rubber or any other type of substrate and, as noted above, is especially germane to the measures of unstable sex pheromone components such as ZETA, a sex pheromone component of several economically important insects.

We exemplify the value and utility of these quantitative measures in estimating the total emission, the emission rates and the ratio change of the components in experiments in which large numbers of the dispensers were deployed in Florida cabbage fields to control diamondback moth in the spring of 1996. Retrospective analyses of pheromone emission from similar dispensers that were deployed in the same fields in 1995 are provided as further examples and to demonstrate some potential for error in the measurements. The examples are not meant to be statements on the effectiveness of the various treatments.

## MATERIALS AND METHODS

**Field Tests Dispensers** Experiments to control diamondback moth populations in cabbage by disruption of sexual communication were conducted in the 1995 winter-spring growing season in fields located near Bunnell, Florida (USA). Pheromones were dispensed from plastic tubes developed by Shin-Etsu Chemical Co., Ltd., Tokyo, Japan, commonly referred to as the Yoto-con-S<sup>®</sup> 'rope' dispenser. The pheromones are released through the walls of the dispenser. The physical properties of the formulation stabilizer are proprietary.

**Rope Samples** All rope samples, except where noted, were stored in their original hermetically sealed containers in cold storage maintained at 6°C. The diamondback moth-only pheromone blend that was deployed in field trials in 1996, Lot 80101, the focus of this report, was received immediately after formulation in January 1996.

Other formulations were also investigated to compare emission rates and effects of storage on the dispensers. Lots 51201 and 69025 received in 1995, were formulated to contain a 50:50 ratio of Z11-16:Al and Z11-16:Ac. According to the manufacturer, each 20-cm segment of these diamondback moth-only formulations contained a total of 56.5 mg of pheromone components. Formulation Lot 69025 was received fresh from the manufacturer in January 1995; Lot 51201 was received in February 1994 and used in spring 1995. Formulation Lot 69024 received from the manufacturer in January 1995 was formulated with components for both cabbage loopers and diamondback moths. Each 20-cm-long rope

of Lot 69024 was formulated to contain a total of 81.3 mg active ingredients: (a) cabbage looper related compounds, Z7-12:Ac (24.3 mg) and Z7-12:OH (0.5 mg); (b) diamondback moth compounds, Z11-16:Al (27.7 mg), Z11-16:Ac (28.2 mg) and Z11-16:OH (0.6 mg).

**Air Sampling of Rope Emissions** Six to eight ropes were folded at the crimped section within the glass sampling device and the emissions collected for 8 h, although this collection interval can be reduced significantly for all but the most depleted rope samples. Prior to measurement, the rope samples were inspected to insure that the ropes were cut at the center of the seals. For the 1996 sampling, different ropes were used for each sample to estimate sampling error, manufacturing differences and other associated emission characteristic differences. All results of emission analysis were calculated in terms of emission/rope/h. For comparison with other lots, collections were made of the volatile emissions from three Lots of rope samples from the 1995 season. Some of the samples that were used in 1995 field trials were taken from the field and stored separately in plastic bags under refrigeration.

**Emission Analysis Filters** The filters were purchased from Analytical Research Systems, Inc. (Gainesville, FL, USA). They were fabricated from capillary glass (Pyrex<sup>®</sup>) and were 12.5 cm long, 6 mm OD and 3 mm ID. The volatile compounds were collected on a 2-cm-long filter bed of Super Q (Alltech Associates, Deerfield, IL, USA), supported one-third of the way from one end on a stainless steel screen and packed at the upper end with glass wool (3,4). In practice, air was drawn in from the end with the screen. Two filters were always connected in tandem with the front end of the front filter connected to the rear of a glass device usually used to dispense pheromones (8). As a final precaution against leaks, Teflon<sup>®</sup> tape was wrapped around each joint.

**Vacuum Pump System** A portable battery-powered pump capable of supplying 18.8 l/min (Air Cadet, Model 7530-25, Cole-Parmer Instrument Co., Niles, IL, USA) was used as the vacuum source for a paired system. Rotameter flow meters (Gilmont Instruments, Barrington, IL, USA) monitored the air flow in the system, but are not in calibration during vacuum operation, serving mainly to ensure that there was a steady airflow. Therefore, the amount of vacuum through the filters, meters and pump was measured by attaching the filters to the top of a glass soap bubble burette and adjusting the flow manually.

The dispensers were placed within glass tubes used in electrophysiological studies to dispense pheromone, but serving here only as a means to confine the rope samples (8). The inside of the tube was 15 mm and the nozzle diameter was 6 mm. The air flow through the filters was maintained at 333 ml/min throughout the collection periods although measures at 600 ml/min were achievable with the filter bed described. The airspeed over the dispensers within the glass tube was calculated to be 3 cm/sec; at the nozzle, the airspeed was calculated to be 20 cm/sec with the stated volume flow rate. The orientation of the dispensers in the glass tube ensured turbulence. The temperature in the laboratory during the collections was maintained at 24±2°C throughout.

**Analytical Methods** Adsorbed sex pheromones were eluted from the filtrant by washing the filter bed with three or four 100- $\mu$ l aliquots of methylene chloride (GC/GC-MS Grade,

Burdick & Jackson, Muskegon, MI, USA) into 3-ml chromatography microvials (Kontes #422570-0000), ending usually with  $\sim 300 \mu\text{l}$  final volume. After elution, a quantitative standard of  $20 \mu\text{g}$  tridecanyl acetate was added to the sample and thoroughly mixed by cycling three or four times back and forth through the tip of a Pasteur pipette.

**Filter and Filter Elution Efficiency** A few experiments using measured amounts of Z11-16:Al and internal standards gave no evidence that significant amounts of Z11-16:Al break down on Super-Q. Two procedures were used to ensure that adsorbed sex pheromone was completely extracted and to ensure that breakthrough had not occurred. The first was to add an internal standard of  $20 \mu\text{g}$  Z7-12:Ac to the top of the filter bed before elution and compare its quantity with another standard added to the eluate after extraction. The front filter was eluted a second time and analyzed as above until experience showed that only traces of the pheromone components remained after the first elution. Likewise, the back filter was eluted and standards added in the same manner as for the front filter and analyzed. In practice, the first extraction was always complete and there was no evidence of breakthrough into the back filter.

**Gas Chromatography** The samples were analyzed on a Hewlett-Packard (HP) Model 5890 gas chromatograph (glc) with a fused silica capillary column (HP,  $12 \text{ m} \times 0.32 \text{ mm}$  ID; a  $0.25\text{-}\mu\text{m}$ -thick bonded stationary phase, 5% phenyl methyl silicone). Injections were made in the splitless mode and the injector was purged after 30 sec. The column was operated under the following conditions: the oven temperature program began at  $50^\circ\text{C}$  for 1 min and then was programmed to increase at  $10^\circ\text{C}/\text{min}$  to  $250^\circ\text{C}$ ; injector,  $170^\circ\text{C}$ ; FID detector,  $300^\circ\text{C}$ ; helium carrier was constant at 9.1 psi. The linear flow velocity was  $32 \text{ cm}/\text{sec}$ . At least four injections were made of each sample. The above procedures follow in principle those employed in other studies (7).

**Statistics** Samples of volatile emissions were collected from ropes of Lot 80101 of nine different ages during the 1996 field treatment. An initial reading, at time = 0, was obtained from a newly opened hermetically sealed sample. Four different glc injections were made from each sample collection. The coefficients of the exponential decay curves for the emission rates were computed by SAS (SAS Inst. Inc., Cary, NC, USA) PROC NLIN non-linear methods (2).

## RESULTS

**System** System control experiments run without dispensers showed no observable peaks eluting at the same retention times as the pheromone components and internal and external standards. This system check eliminated the necessity of a source of clean air and, at the same time, enabled us to use a simple open system as described in Materials and Methods.

**Dispenser Emission** The release of two sex pheromone components from Lot 80101 rope dispensers during the cabbage-growing season of 1996 was expected *a priori* to be that typical of passive processes, fitting the common exponential decay curve:

$$Y_t = ae^{-bX} \quad (1)$$

where, for the present application,  $Y_t$  is the emission rate at time = t, and a is the emission rate at time = 0. The release of both components fits such a curve (Table 1; Fig. 1). The exponent, b, the slope, of Eq. 1 was estimated by an iterative process in SAS (PROC NLIN). The half life,  $t_{\frac{1}{2}}$ , is simply:

$$t_{\frac{1}{2}} = \ln 2 / b \quad (2)$$

where b is the slope of Eq. 1. Rearranging the above yields the more common and useful half-life equation,

$$Y_t = ae^{-(t)(1/t_{\frac{1}{2}})(\ln 2)}. \quad (3)$$

TABLE 1. Regression parameters and estimates of emission half-life for diamondback moth formulation from rope Lot 80101<sup>z</sup> that was deployed for the 1996 growing season

	Parameter <sup>y</sup>	Estimate	Standard error of estimate	95% Confidence intervals
Z11-16:Al	a	4.201	0.108	3.982–4.421
	$t_{\frac{1}{2}}$	19.3		17.3–21.2
Z11-16:Ac	a	0.4321	0.018	-0.396–0.469
	$t_{\frac{1}{2}}$	41.8		37.2–46.3

<sup>z</sup>Combination cabbage looper plus diamondback moth disruptant formulation. Analysis retrospective, no original samples to analyze. (Iron oxide formulation: color burnt orange)

<sup>y</sup>Values are for eq. (1), where a is the emission rate at t = 0,  $\mu\text{g}/\text{rope}/\text{h}$ ; t = days.

The small error of estimate of the release rate regression, 0.108 for Z11-16:Al and 0.018 for Z11-16:Ac, results in narrow confidence limit bands (Fig. 1, inset). Consequently, there is a high level of confidence for the estimate of the half-life. As a result of this differential in evaporation rates, the proportion of the two components that are emitted changes constantly over time. For manufacturing Lot 80101, the release rate of Z11-16:Al was  $\sim 9.7$  times higher than that of Z11-16:Ac as would be expected from differences in the molecular weights (Table 1; Fig. 1). However, note that this expected emission ratio changes significantly during the measurement interval. For example, the ratio of the two components changes from  $\sim 8:1$  (Z11-16:Al: Z11-16:Ac) by day 7 to  $\sim 2:1$  by day 40.

There is another result manifest from the above data and methodology. Only insignificant deviations exist between any six individual ropes, because each sample measured from the field was from a different set of six or eight ropes and the error terms remained small. Although all measures of the emission were made at  $24 \pm 2^\circ\text{C}$  in the laboratory, the ropes had been continuously exposed to sunlight and other ambient weather conditions throughout the growing season, during which the temperature varied from  $\sim 1\text{--}30^\circ\text{C}$ . The small errors also suggest that the environmental elements to which the ropes are exposed in the field fail to degrade the physico-chemical release characteristics of the ropes.

To extend the analysis over other rope lots and formulations, we made a retrospective analysis of rope samples that had been used in 1995 field plot trials (14). The measures of sex pheromone components that were emitted from three of these rope lots are given in Table 2. Even though the samples had been stored individually in plastic bags, some cross-contamination appears to have occurred. Analysis revealed an initial release of  $0.44 \pm 0.01$

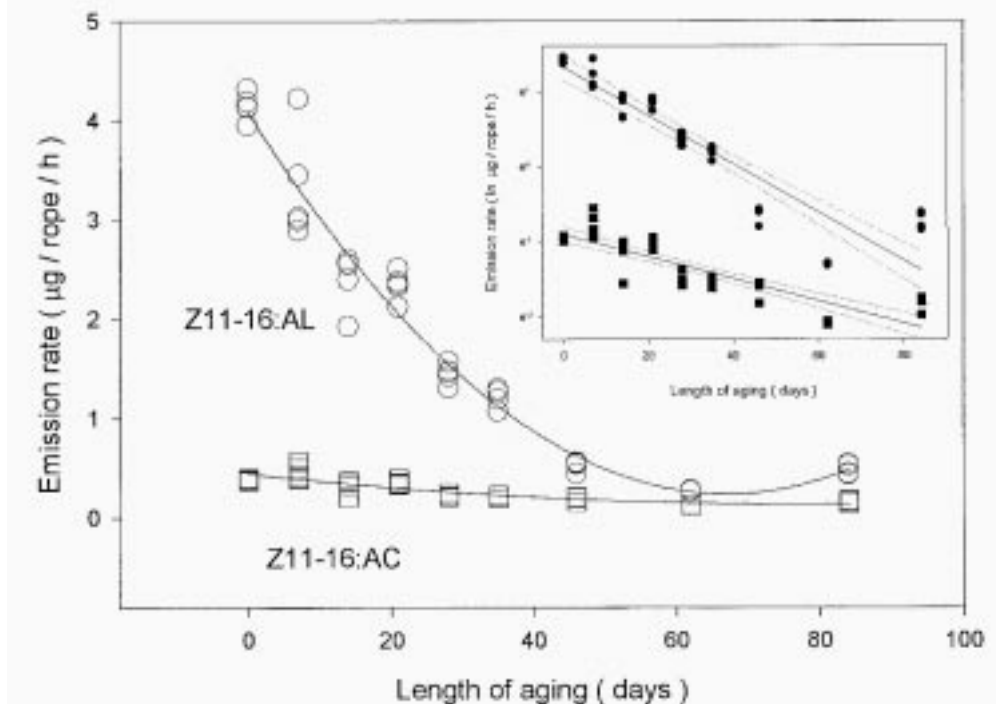


Fig. 1. Emission of Z11-16:Al and Z11-16:Ac from Shin-Etsu plastic rope samples (Lot 80101). Inset is a semilogarithmic plot of the natural logarithm of the same data to illustrate the linearity of the regression and the fiducial limits.

$\mu\text{g}/\text{rope}/\text{h}$  of a compound that eluted at the same retention time as a Z7-12:OH standard from a sample of Lot 51201 that had remained hermetically sealed during storage. We reiterate that the identity of Z7-12:OH is based on only very limited analytical chemical evidence. The likelihood that contamination occurred during storage was reinforced because Z7-12:Ac was not detected in the emissions from the sealed sample, but was found in the bagged and stored samples. So, while these results are unsettling, they serve as a warning for future studies that sample storage practices that are less than meticulous may result in cross-contamination of samples.

There was no significant field population of diamondback moths during much of the 1996 growing season. Thus, an estimate of the efficacy of the pheromone treatment on mating disruption, field trap captures, or larval populations – all of which are used commonly as measures of treatment efficacy – is difficult and beyond the scope of this report. We simply used the half-life equation (Eq. 3) to estimate the total milligrams of Z11-16:Al that are released in a hectare within one day. Of course, this is not a measure of the airborne concentration but is, rather, an easily obtained measure that should correlate in some manner with mating or trap captures. During the course of the 1996 field trials, trap captures of males and larval populations began increasing about the time the emission of Z11-16:Al had declined to  $\sim 100$  mg/ha/day.

TABLE 2. Regression parameters and estimates of emission half-lives for several different lots of rope deployed in 1995

Pheromone emitted	Emission Parameters and Regression Estimates <sup>z</sup>				
	a	b	r <sup>2</sup>	t $\frac{1}{2}$	t $\frac{1}{2}$ ± 95% confidence interval
<b>Pheromone Rope Lot 69025<sup>y</sup></b>					
Z7-12:OH	emission linear; mean = ~50 ng/rope/h				
Z7-12:Ac	emission linear; mean = ~50 ng/rope/h				
Z11-16:Al	0.158	-0.018	0.749	38.8	33.3–44.3
Z11-16:AC	emission linear; mean = ~50 ng/rope/h				
<b>Pheromone Rope Lot 69024<sup>x</sup></b>					
Z7-12:OH	emission linear; mean = ~90 ng/rope/h				
Z7-12:Ac	10.369	-0.019	0.933	37.4	34.7–40.4
Z11-16:Al	1.941	-0.016	0.645	42.7	35.8–49.6
Z11-16:AC	emission linear; mean = ~54 ng/rope/h				
<b>Pheromone Rope Lot 51201<sup>w</sup></b>					
Z7-12:OH	0.386	-0.013	0.724	51.3	42.5–60.1
Z7-12:Ac	not present in original sample – see text				
Z11-16:Al	2.876	-0.026	0.984	26.7	25.3–28.1
Z11-16:AC	0.3869	-0.013	0.809	77.0	63.5–91.5

<sup>z</sup> Values are for eq. (1), where a is the emission rate at t = 0,  $\mu\text{g}/\text{rope}/\text{h}$ ; t = days.

<sup>y</sup> Diamondback moth mating disruptant formulation. Analysis retrospective, no original sealed samples remain. (Iron oxide formulation: color, burnt orange)

<sup>x</sup> Combination cabbage looper plus diamondback moth mating disruptant formulation. Analysis retrospective, no original samples to analyze. (Iron oxide formulation: color, burnt orange)

<sup>w</sup> Diamondback moth mating disruptant formulation. Original sealed sample emits a material eluting at the same retention time as a Z7-12:OH standard at  $21.8 \pm 0.4$  ng/rope/h. (Titanium chloride formulation: color, white)

## DISCUSSION AND CONCLUSIONS

We have described a simple and accurate method of measuring the emissions from Shin-Etsu hollow tube dispensers that is similar to methods developed to collect plant volatiles (3). The present method differs from other measures of sex pheromone emission in the small size of the Super-Q filter bed, which also adsorbs a large amount of sex pheromone, does not allow chemical breakthrough and, especially, enables a realistic airspeed to be drawn over the dispensers. Furthermore, the adsorbed sex pheromones are completely desorbed from the filter bed with small amounts of solvent, which translates into significant glc sensitivity without reduction in sample volume. Recent unpublished studies demonstrate that even smaller filter beds are as good as the one described. A disadvantage of our procedure is that it measures the behavior of the dispenser after exposure in the field and the results may only approximate those releases.

Why not use a model to estimate the release rates? Model emission rates are useful and a few have been reported recently (1,6,17, cf 9,10,15,16). However, model coefficients have to be re-measured when the surface area or any other of the physical or chemical parameters of the model is changed through formulation or manufacture. This is especially true when the constituent compounds are unstable or are sequestered within the walls of the

dispenser. The present study of Lot 80101 is noteworthy in this respect. If all of the losses were due to evaporation, the half-lives of the two components should maintain a constant ratio. Because they do not, we hypothesize that the emission of Z11-16:Al diminishes through combined emission, degradation and/or polymerization. Another variable that has not been extensively investigated is the shelf life of the dosed dispenser. Furthermore, let us consider yet another applicable notion. Even if the model accurately predicts the emission rate of the complex interactions between air velocity and temperature (17), the emission rates at the temperature and wind speed conditions under which the insects behave are highly variable. So, for any of the above noted models, the release rate would not appear to vary by much over 2–4 times. Now, consider that the sensitivity curve of antennal olfactory sex pheromone specialist receptor neurons and intensity coding are logarithmic functions (5). A difference of between 2 and 4 on a logarithmic scale is not large compared with the other imponderables within the habitat, and probably would not be important to mating or communication disruption at levels considerably above the threshold. However, such a difference would be important when the treatment begins to approach the threshold of effectiveness. That is where the usefulness of the timely, simple and rapid measure that is the subject of this report is important.

What practical example can be drawn from the data on hand and how can it be used? We have discussed certain inadequacies of models when critical parameters change with formulation and chemical degradation of a component occurs as appears to be the case with Lot 80101. Perhaps a simple, but direct, measure may be more useful. For example, during field trials in 1996, we observed that when laboratory measures estimated emission of less than 100 mg/ha/day, ‘communication disruption’, or reduction in trapping, failed. If this estimate was correlated with a threshold of effectiveness in the field, the amount and number of ropes required to restore the treatment to an effective level can be estimated easily from laboratory measurements. This would enable an operator simply to add to the original treatment, and not to replace it in its entirety.

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