A LABORATORY EVALUATION AND COMPARISON OF RELEASE RATES OF MATING PHEROMONES FOR ORIENTAL FRUIT MOTH (*GRAPHOLITA MOLESTA*), PINK BOLLWORM (*PECTINOPHORA GOSSYPEILA*) AND GYPSY MOTH (*LYMANTRIA DISPAR*).

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LIST OF ABBREVIATIONS

IPM	Integrated Pest Management
MD	Mating Disruption
OFM	Oriental Fruit Moth
PBW	Pink Bollworm
GM	Gypsy Moth
STS	Slow-the-Spread
GC	Gas Chromatography
°C	Celsius
MTD	Methyl tridecanoate
FID	Flame Ionization Detector
PVA	Polyvinyl Alcohol
OH	Alcohol
OAc	Acetate
g	gram
mg	milligram
µg	microgram
min	minute

ABSTRACT

A LABORATORY EVALUATION AND COMPARISON OF RELEASE RATES OF MATING PHEROMONES FOR ORIENTAL FRUIT MOTH (GRAPHOLITA MOLESTA), PINK BOLLWORM (PECTINOPHORA GOSSYPEILA) AND GYPSY MOTH (LYMANTRIA DISPAR).

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There has been an increasing interest in more environmentally friendly alternatives to the use of pesticides in agriculture, due to health and environmental concerns. The controlled release of pheromones can be used as part of an Integrated Pest Management Program (IPM) to minimize the quantity of pesticides used, and there have been a number of methods developed to release sex pheromones for mating disruption of insect pests. One of these methods uses wax emulsions as carriers for pheromones that are sprayed on forests and agricultural crops to manage insect pest populations.

This research examined the effects of emulsion formulation variables on pheromone release rates for Oriental fruit moth, (*Grapholita molesta),* gypsy moth, *(Lymantria dispar),* and pink bollworm, *(Pectinophora gossypeila)*. The effects of the wax type, the emulsifier concentration, the addition of a thickener, the pheromone volatility, and the emulsion consistency were examined in this research. Additionally, a commercial gypsy moth product, Hercon® Disrupt® II, was evaluated and compared to wax emulsions made in this research. All emulsion samples were subjected to the same flow cell conditions: 5 g of emulsion (containing 190 mg of pheromone) was tested at

30ºC, with an air flow of 0.5 L/min. The released pheromone was trapped and quantified by gas chromatography.

The results indicated that emulsions made from paraffin wax released pheromone at a higher rate than emulsions made from microcrystalline wax. Increasing the amount of emulsifier from 2% to 4% had no significant effect on release rates. Similarly, the amount of thickener had no significant effect on release rates. Interestingly, the emulsion consistency had a direct correlation to pheromone release rate; a poorly emulsified emulsion resulted in higher releases rates than the well emulsified emulsion. Finally, the Hercon® Disrupt® II flake dispensers had similar release rates to paraffin wax emulsions. Pheromone volatility also played a vital role in emulsion formulations and release rates. Overall, the Oriental fruit moth emulsion had the highest release rate, with an average release of 0.9 mg/day for each of the emulsion formulations. The pink bollworm had the second highest average release of 0.08 mg/day, while gypsy moth had the lowest average release of 0.02 mg/day.

In summary, wax emulsions were used to provide a sustained release of insect pheromones. The release rate was modified by the type of wax used in the formulation. Other formulation variables tested, such as the amount of emulsifier and the use of a thickener, did not significantly affect the pheromone release rates.

INTRODUCTION

History of Pesticides

Pesticides have been used for insect control for centuries. Around 1000 B.C., Homer referred to the use of sulfur to fumigate homes (Shepard, 1951). By 900 A.D., the Chinese were using arsenic to control garden pests (Shepard, 1951). As early as 1848, Rotenone was used as an insecticide (Mrak, 1969). In the late nineteenth century, pesticides such as arsenic, pyrethrum, lime sulfur, and mercuric chloride were used (Shepard, 1951). The first synthetic organochlorine insecticide, DDT (dichlorodiphenyl-trichloroethane), synthesized in Switzerland in 1939, was very effective and used extensively to control insects which carried diseases like typhus and malaria and was extensively used as an agricultural insecticide for control of coddling moth, corn earworm, cotton bollworm and tobacco budworms (Ordish, 1976). All organochlorines are relatively insoluble in water, persist in soils and aquatic sediments, can bioconcentrate in the tissues of invertebrates and vertebrates from their food, move up trophic chains, and affect top predators (EPA, 2007). These properties of persistence and bioaccumulation led eventually to the withdrawal of registration and use of organochlorine insecticides from 1973 to the late 1990s in industrialized nations, although they continued to be used in some developing countries (EPA, 2007).

The adverse effects of pesticides on humans and wildlife have fostered the goal to reduce their use. An alternative approach for insect control is integrated pest management (IPM), first proposed in 1959 (Stern et al., 1959). IPM combines minimal

use of the least harmful pesticides, integrated with biological and cultural methods of minimizing pest losses (Jones et al., 2009). Pesticides are only used when threshold levels of pests have been identified.

Integrated Pest Management

Stern et al. (1959) proposed several components that are considered crucial for the integration of pesticides and biological control. These components are the recognition of ecosystem-level interaction between pests and their natural enemies; methods of sampling and prediction of pest occurrence; enhancement of the benefits of natural enemies through importation, augmentation or conservation; and understanding the effects of pesticides on natural enemies and how to mitigate those effects through ecological and physiological selectivity. IPM has become extremely useful in the development of more sustainable strategies for pest management and is growing in interest to scholars and the public alike. IPM is an effective and environmentally sensitive approach to pest management that relies on a combination of common-sense practices. IPM programs use current, comprehensive information on the life cycles of pests and their interaction with the environment. This information, in combination with available pest management methods, is used to manage damage by the most economical means, and with the least possible hazard to people, property and the environment.

Mating Disruption

Mating disruption (MD) involves the use of sex pheromones that evoke a specific mating behavior response in individuals of the opposite sex of the same species (Ahmed,

1993). One mechanism for MD is false trail following, where high levels of female insect sex pheromone are used to disrupt the male insect's sensory ability to locate and therefore mate with females (EPPO Bulletin, 2008). The prevention or delay of mating can have significant impairment of breeding success with commensurate benefits in reducing subsequent crop damage (EPPO Bulletin, 2008). Early mating disruption trials took place in Australia, and Oriental fruit moth was one of the first insects for which MD was developed (Rothschild, 1975, 1979). MD is beneficial in IPM because pheromones are species specific, have low environmental impacts, and are more sustainable than broad-spectrum tactics, with no current evidence of resistance which may occur with insecticides (Rechcigle, 2000).

Oriental Fruit Moth *(Grapholita molesta)*

Oriental Fruit Moth (OFM) *Grapholita molesta* (Busck) is a key pest of stone fruit worldwide (Rothschild and Vickers, 1991), a primary pest of peaches (Rice and Kirsch, 1990), and recently it has emerged as a key pest of apples in the eastern U.S. (Hull et al., 2001) and other parts of the globe. OFM was first introduced into the U.S. on nursery stock from Japan between 1913 and 1916, and is a serious stone fruit pest in the mid-Atlantic area (Rothschild and Vickers, 1991). In North Carolina, OFM was first recorded as causing serious damage to apples in 1998, when many orchards were found to be infested with live larvae at harvest (Walgenbach et al., 1999). The larva attacks the fruit as well as the terminal shoots of peaches and apples (Chapman and Lienk, 1971).

Oriental fruit moth completes four generations per year in North Carolina and there are overlapping generations late in the season (Kovanci et al., 2004). Feeding by

first-generation larvae causes damage to sprouting shoot growth in the early spring and early summer, whereas internal feeding by larvae in later summer generations destroys fruit (Rothschild and Vickers, 1991). Continuous egg-laying can occur from mid-July to October (Walgenbach et al., 2000). Repeated season long insecticide applications are needed to manage OFM because this species can be found in various stages during the entire growing season (Rothschild and Vickers, 1991). Additionally, implementation of the Food Quality Protection Act (FQPA) has eliminated or restricted the use of many organophosphate insecticides commonly used for control of OFM (Kovanci et al., 2004). OFM can cause up to 50% of apple crop loss, and prolonged use of broad-spectrum insecticides, as a main control tactic, has resulted in the occurrence of organophosphate-resistant OFM populations (Kanga et al., 2003). These factors have instigated the application of alternative, non-insecticidal methods for OFM management techniques.

The possibility of using mating disruption for OFM was successfully demonstrated three decades ago, using hand-applied hollow fiber dispensers of pheromone (Carde et al., 1977).

Pink bollworm *(Pectinophora gossypiella)*

There are as many as 1,326 insect species associated with cotton (Hargreaves, 1948). However, pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) was recognized worldwide as the most injurious (Ingram, 1994). PBW was first described by W.W. Saunders as *Depressaria gossypiella* in 1843 from specimens found damaging cotton in India in 1842 (Naragjo, 2002). PBW has been recorded in nearly all cotton-growing countries of the world and is a key pest in many of these areas. PBW infests

buds and flowers early in the season without significant damage. However, later in the season, larvae feeding on fruit/bolls may result in high yield losses (Lykoruessis et al., 2004). The control of PBW with contact pesticides is problematic because the larvae are often protected within the plant, the females' preference to oviposit on sheltered places, and the short period from egg hatching until the larvae enter the bolls (Lykoruessis et al., 2004). Control of PBW populations is attempted, therefore, with a series of insecticide cover sprays, which are not always effective and may lead to adverse effects such as outbreaks of secondary pests (Lykoruessis et al., 2004).

Efforts to develop more effective and environmentally friendly methods to control the insect led to studies to identify the PBW sex pheromone for use in MD.

Gypsy Moth *(Lymantria dispar)*

Lymantria dispar (Linnaeus), gypsy moth (GM), is a leaf-feeding (*Lepidoptera)* that poses a serious threat to many forest and ornamental trees. GM is one of North America's most devastating forest pests. The species originally evolved in Europe and Asia, and has existed there for thousands of years (McManus, 1987). The gypsy moth accidentally escaped the Massachusetts laboratory of Etienne Leopold Trouvelot who was hoping to create a more robust variety of silk moth (McManus, 1987). Attempts to eradicate the GM ultimately failed, and the range of the GM has continued to spread.

GM has only one generation per year. GM populations will go through cycles in which the populations will increase for several years then decline, and then increase again (McManus, 1987). Area-wide outbreaks can occur for up to ten years, but generally population densities in localized areas remain high for two to three years (McManus,

1987). The larva or caterpillar causes damage by eating the leaves of trees in the spring. When populations reach outbreak proportions, the caterpillars can completely defoliate host trees over wide geographic areas. Consistent, repeated defoliation over several years often leads to tree stress and death. GM has a wide host-range, which includes, but is not limited to, oaks, poplar, beech, willow, and birch (McManus, 1987). The ecological and economic impact of GM is a serious concern. GM defoliation can change the species diversity of under story growth, thus resulting in specific changes of fauna or flora (McManus, 1987). GM defoliation may predispose trees to attack by opportunistic insects or disease.

OBJECTIVE

The objective of this research was to determine the effects of wax emulsion formulations on pheromone release rates for Oriental fruit moth (OFM) (*Grapholita molesta),* gypsy moth (GM) *(Lymantria dispar),* and pink bollworm (PBW) *(Pectinophora gossypeila)*. It was hypothesized that the amount of pheromone released from wax emulsions would decease by thickening the wax emulsion, increasing the amount of emulsifier, and by using microcrystalline wax. To test this hypothesis, a total of five different formulations were evaluated to determine the effect of the wax, the emulsifier concentration, the addition of a thickener, the pheromone volatility, and the emulsion consistency. All formulations contained wax, water, oil, emulsifier, preservative, and pheromone. Each emulsion was subjected to the same flow cell conditions. A total of 5 g of emulsion (containing 190mg of pheromone) was placed in each flow cell at 30ºC, with an airflow rate of 0.5 L/min. The quantity of pheromone released was determined by gas chromatography. The gypsy moth paraffin wax emulsion was also compared to commercial Hercon® Disrupt® II flakes.

MATERIALS AND METHODS

There has been an increasing interest in more environmentally friendly alternatives to the use of pesticides in agriculture. Paraffin wax, in both solid and emulsified form, has been demonstrated to provide a good matrix for dispensing volatile compounds (Atterholt, 1996). One method uses wax emulsions as carriers for pheromones (Atterholt, 1996; Rice et al. 1997; Atterholt et al., 1998; Meissner et al., 2000; de Lame, 2003). These dispensers are biodegradable, inexpensive, and easy to produce (Stelinski et al., 2005).

Paraffin Wax and Microcrystalline Wax

Paraffin wax is primarily composed of unbranched alkanes, with the number of carbons ranging from 18 to 24 (Mansoori, G. A. et al., 2003). Paraffin wax is hard and brittle at room temperature, with a melting point range of 47˚ C to 64˚ C. In contrast, microcrystalline wax contains a higher percentage of branched and cycloalkanes, with the number of carbons typically ranging from 41-50 (HP Wax, 2009). Microcrystalline wax is soft and malleable at room temperature, with a higher melting point range of 71˚ C to 89˚ C (CRC Handbook of Chemistry and Physics, 1991). Microcrystalline wax is a "stickier" wax, and has better adhesion to tree bark when applied as a carrier for the controlled release of pheromone for mating disruption (de Lame, 2003).

OFM

The primary component of the female-released OFM sex pheromone is Z-8-dodecenyl acetate (Z8-12:OAc)(Roelofs, 1969). The molecular formula is $C_{14}H_{26}O_2$ and the molecular weight is 226.20 g/mole. OFM pheromone chemical structure is shown below in Figure 1. There are also two secondary components, the E isomer (E8-12:OAc) and the alcohol (Z8-12:OH) of the above acetate (Carde et al., 1979).

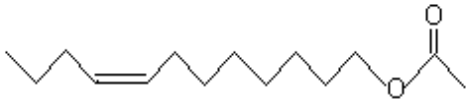
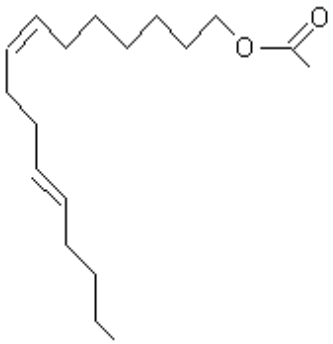


Figure 1. Oriental fruit moth pheromone structure. IUPAC name: (Z)-dodec-8-en-1-yl acetate.

PBW

The primary components of the sex pheromone for PBW are (7-Z, 11-E)-hexadeca-7-dien-1-yl acetate and (7-Z, 11-Z)-hexadeca-7,dien-1-yl acetate, which were identified by Hummel et al. 1973. The molecular formulae for both compounds are $C_{18}H_{30}O_2$ and the molecular weight is 278.23 g/mole. PBW pheromone chemical structures are shown in Figure 2.

a.



b.

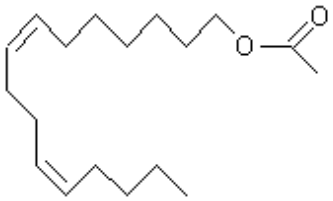


Figure 2. (a) Pink bollworm pheromone cis-structure., IUPAC name: (7Z, 11E)-hexadeca-7,dien-1-yl acetate. (b) Pink bollworm pheromone trans–structure, IUPAC name: (7Z, 11Z)-hexadeca-7,dien-1-yl acetate.

GM

The main sex pheromone component of the GM is (7R, 8S)-2-methyl-7,8-epoxyoctadecane (Miller, 1977). The molecular formula is $C_{19}H_{37}O$ and the molecular weight is 281.29 g/mole. The GM pheromone chemical structure is shown below in Figure 3.

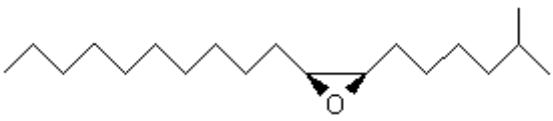


Figure 3. Gypsy moth pheromone structure. IUPAC name: (7R, 8S)-7, 8-epoxy-2-methyloctadecane.

Pheromone/ paraffin mixtures

Emulsions were prepared from paraffin or microcrystalline wax, canola oil, Span 60® (sorbitan monostearate), a preservative, water, and pheromone. The differences between emulsions were the type of wax used, the amount of the emulsifying agent (Span 60®), and the addition of 1% of thickener. The wax emulsions were made using canola oil (4.8%), paraffin wax (30%), water (59.4%), pheromone (3.8%), Span 60® (2% or 4%) and a preservative (0.02%) (Atterholt, 1996). In situations where the amount of any ingredient was modified, the amount of water was increased or decreased by those same amounts. The wax emulsion was prepared by first melting the wax on a hot plate. Then Span 60®, the preservative, and canola oil were added, after which the pheromones were then added to the wax blend. The wax mixture was emulsified with the water using a high sheer vortex mixer. Samples (5 g) of wax emulsions were placed in flow cells to measure the pheromone release-rates.

Thickener

A proprietary thickener was used to adjust the viscosity of the emulsion.

Hercon® Disrupt® II Flakes

The Hercon® Disrupt® II Gypsy Moth commercial pheromone product is composed of laminated PVA (3mm long x 1 mm wide x 0.5mm thick), containing 17.9% (m/m) racemic disparlure formulated by Hercon® Environmental Corporation, Emigsville, Pa, USA. Hercon® Disrupt® II was designed as a controlled release dispenser to be aerially applied (Tcheslavskaia et al., 2005). It has been used by the USDA Forest

Service in the Slow-the-Spread (STS) of the gypsy moth (Tcheslavskaia et al., 2005). The Slow-the-Spread program is a combined state and federal effort to slow the rate of gypsy moth population expansion in the United States (Tcheslavskaia et al., 2005).

Flow cell environmental chamber

The flow cell methods were adapted from Atterholt, 1996. A flow cell environmental chamber was used to measure the effects of formulation on the pheromone release-rate of different formulations. The environmental chamber contained 21 flow cells, with the ability to control temperature and air flow.

The flow cells were machined from aluminum with an 8.3 cm diameter and a 5.4 cm depth (Gore, 2000). An aluminum lid was bolted to the top of each flow cell and sealed with a Teflon gasket. Each cell was placed in an oven maintained at a constant temperature during the experiment. A compressed air line ran into a manifold that split the air flow to the 21 flow cells. There was one flow meter for each of the 21 flow cells. The air stream entered the flow cells and was distributed over the surface of the carrier material after passing through a sintered metal diffuser. The released pheromone was captured from the air stream via a trap of Super Q® resin. Pheromone captured by these traps was periodically eluted with ethanol or hexane for quantification.

Five grams of these emulsions were placed in triplicate aluminum flow cells for 3 to 6 months. A glass trap was placed on each flow cell to collect the volatile pheromones released. Each glass trap contained a packed layer of silane-treated glass wool, a layer of Super Q® (Alltech, Deerfield, IL) (a divinylbenzene polymer adsorbent) and another layer of silane-treated glass wool. Each trap was removed from the flow cell apparatus

once a week and rinsed with 2-4 mL of ethanol or hexane to remove any pheromone attached to the Super Q® material.

Quantification by gas chromatography

The gas chromatography methods used were modified from Atterholt, 1999. The amount of pheromone in samples was measured using a gas chromatograph (GC) (Hewlett-Packard Model 5890, Palo Alto, CA or Shimadzu Class VP, Columbia, MD) equipped with a flame-ionization detector (FID) and a capillary column (DB-5, 30 m X 0.25 mm X 0.25 μ, J&W Scientific, Folsom, CA). The GC program that was used for OFM pheromone analysis had the injector port and detector heated to 225˚C and 250˚C, respectively, with the oven heated to an initial temperature of 150˚C. After injecting a sample, the oven temperature was held at 150˚C for 2 min, and then heated to 225˚C at a rate of 7˚C/min. For GM pheromone, there were two programs used. For the ethanol solvent, the injector port and detector were heated to 225˚C and 250˚C, respectively, with the oven temperature heated to 150˚C. After injecting a sample, the oven temperature was heated to 225˚C at a rate of 8˚C/min, and then held at the top temperature for 4 minutes. For the hexane solvent, the injector port and detector were heated to 225˚C and 250˚C, respectively, with an initial oven temperature of 160˚C. After injecting a sample, the oven was heated to 230˚C at a rate of 9˚C/min and held for 4 minutes. For PBW the injector port and detector were heated to 225˚C and 250˚C, respectively, with an initial oven temperature of 150˚C. After injecting a sample, the oven temperature was increased to 225˚C at a rate of 6˚C/min and held for 5 minutes.

An internal standard of methyl tridecanoate (MTD) was then added to each sample and analyzed using a gas chromatograph (Hewlett-Packard Model 5890 series II, Palo Alto, CA). Graphs were used to compare the pheromone released from the different formulations.

Response Factor

To quantify the amounts of pheromone released, standard solutions of analyte and MTD were prepared and used to make calibration curves. The concentration of the analyte and internal standard solutions ranged from 40-1000 μg/g. The peak area was plotted against the concentration to obtain a GC calibration curve. The slopes of the regression lines represent the relative sensitivity of the FID detector to the analyte and internal standard and are thus termed the response factors.

Calculations

The calculation using the comparison of the response factors to quantify amounts of pheromones is shown below, with PH=analyte of interest and IS= internal standard.

X[mg]PH=[Peak Area]PH/[Peak Area]IS x IS (mg) x [Slope]IS/[Slope]PH

RESULTS and DISCUSSION

Effects of Formulation on Pheromone Released

Effect of Wax

Two different types of wax, paraffin and microcrystalline, were tested to determine the effect of the wax on the amount of pheromone released. Separate emulsions were made from paraffin and microcrystalline wax for OFM, GM and PBW pheromones. Figures 4 through 6 show the cumulative release of each pheromone from paraffin and microcrystalline wax emulsions. The average daily amount released and the cumulative pheromone released are shown in Tables 1 and 2.

As shown in figures 4 through 6, the type of wax had a significant effect on the cumulative pheromone released for each of the three pheromones tested. In each case, more pheromone was released from the paraffin wax emulsion than from the microcrystalline wax emulsion. The physical differences between the paraffin and microcrystalline wax plays a role in the release of pheromone. This may be due to a higher solubility of pheromone in the microcrystalline wax.

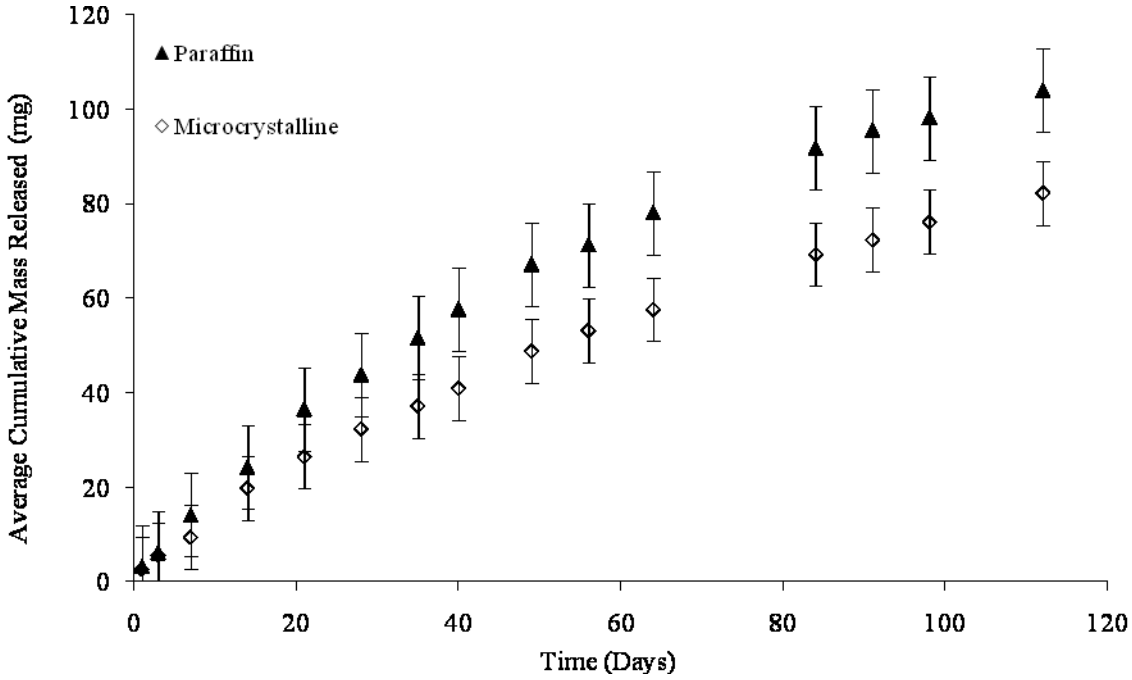


Figure 4. Average cumulative OFM pheromone released (mean ± SEM) from paraffin and microcrystalline wax emulsions at 30ºC. The air flow was 0.5 L/min. There were 190 mg pheromone present in each 5 g sample of emulsion.

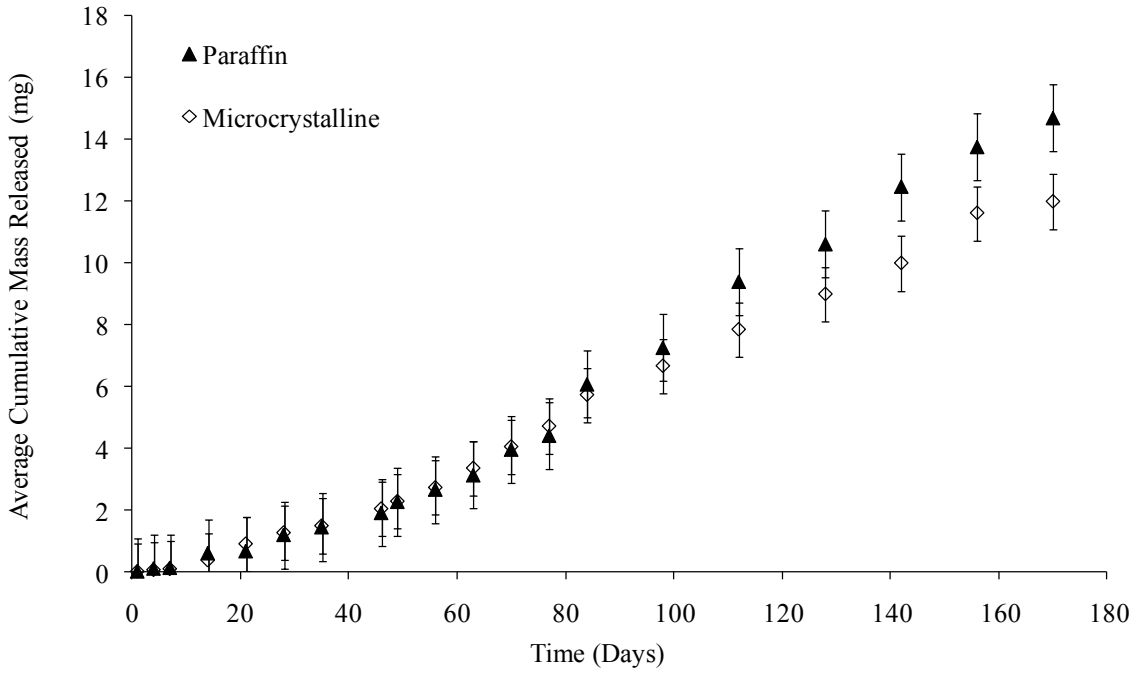


Figure 5. Average cumulative PBW pheromone released (mean ± SEM) from paraffin and microcrystalline wax emulsions at 30ºC, with an air flow of 0.5 L/min. There were 190 mg pheromone present in each 5 g sample of emulsion.

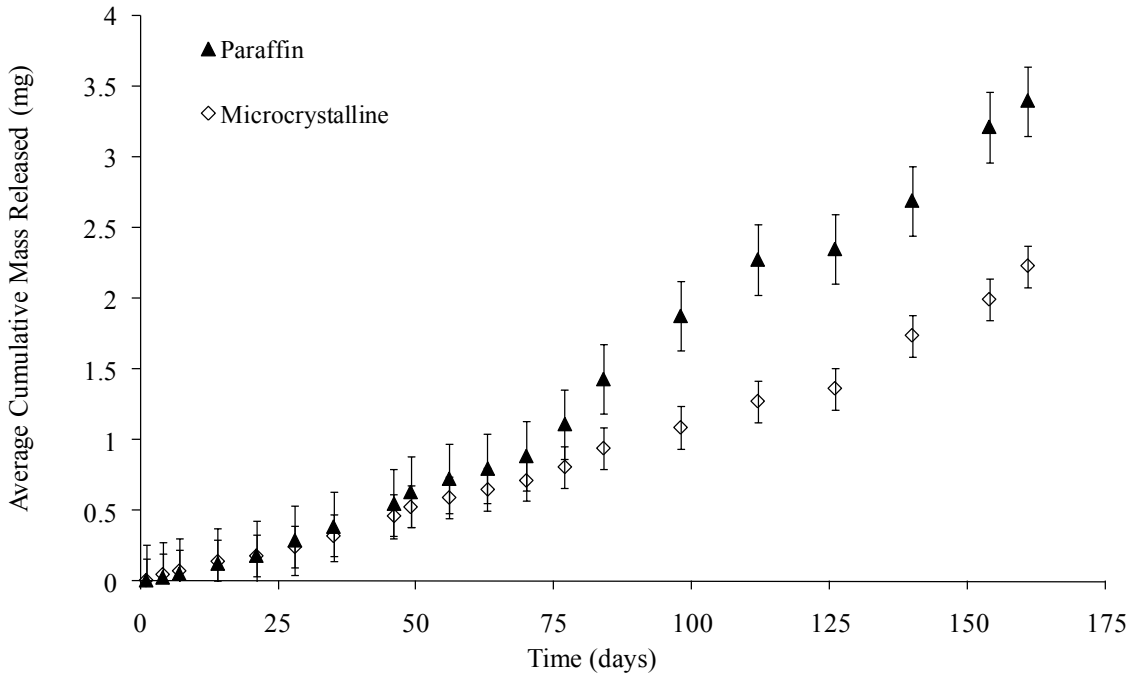


Figure 6. Average cumulative GM pheromone released (mean ± SEM) from paraffin and microcrystalline wax emulsions at 30ºC, with an air flow of 0.5 L/min. There were 190 mg pheromone present in each 5 g sample of emulsion.

Table 1. Average daily pheromone released (mg/day) from paraffin and microcrystalline wax emulsions.

Pheromone	***Release from Paraffin Wax Emulsion (mg/day)***	***Release from Microcrystalline Wax Emulsion (mg/day)***
OFM	**0.9**	**0.7**
GM	**0.02**	**0.01**
PBW	**0.09**	**0.07**

Table 2. Average cumulative pheromone released (mg) for 112 days from paraffin and microcrystalline wax emulsions.

Pheromone	***Release from Paraffin Wax Emulsion (mg)*** **± Std. Error**	***Release from Microcrystalline Wax Emulsion (mg)*** **± Std. Error**
OFM	**104 ± 15.8**	**82 ± 9.3**
GM	**2.3 ± 0.29**	**1.2 ± 0.12**
PBW	**9.4 ± 1.0**	**7.8 ± 1.0**

Effects of Emulsifier Concentration

In addition to measuring the effect of the wax on pheromone release rates, the effects of the emulsifier concentration was also examined. Two emulsions were made from paraffin wax for each of the three pheromones tested, OFM, GM, and PBW. One emulsion for each pheromone contained 2% Span 60®, and the other contained 4% Span 60®. All emulsions were exposed to the same conditions, regarding temperature (30°C), time, and air flow (0.5 L/min).

Span 60® is a nonionic surfactant with excellent emulsifying, dispersing and wetting properties. Span 60® (sorbitan monostearate) is an ester of sorbitol and stearic acid and is known as a synthetic wax. The sorbitol end of the molecule is very soluble in water and the stearic acid end is soluble in fats. These properties make the molecule very good at making emulsions of oil and water, and Span 60® is a good emulsifier for the pheromones tested in this research.

Increasing the amount of Span 60® in paraffin wax emulsions from 2% to 4% did not have a significant effect on the amount of pheromone released, as shown in Figures 7 through 9. The average and cumulative pheromone released are shown in Tables 3 and 4.

The amount of emulsifier required depends on the particular pheromone being dispersed and the concentration of the pheromone in the emulsion (Atterholt, 1996). The amount of emulsifier does have an effect on the viscosity of the emulsions. However, the amount of emulsifier tested in this set of experiments did not have a significant affect the amount of pheromone released.

Table 3. Average daily pheromone released (mg/day) from paraffin wax emulsions with 2% Span 60® and 4% Span 60®.

Pheromone	*Average Pheromone Released from Paraffin Wax Emulsion (mg/day)*	
	2% Span 60®	**4% Span 60®**
OFM	**0.9**	**1**
PBW	**0.09**	**0.08**
GM	**0.02**	**0.02**

Table 4. Average cumulative pheromone released (mg) for 112 days from paraffin wax emulsions with 2% Span 60® and 4% Span 60®.

Pheromone	*Pheromone Released from Paraffin Wax Emulsion (mg)*	
	2% Span 60® ± Std. Error	**4% Span 60® ± Std. Error**
OFM	**104 ± 15.8**	**120 ± 9.09**
PBW	**9.4 ± 1.0**	**9 ± 1.1**
GM	**2.3 ± 0.29**	**2.0 ± 0.36**

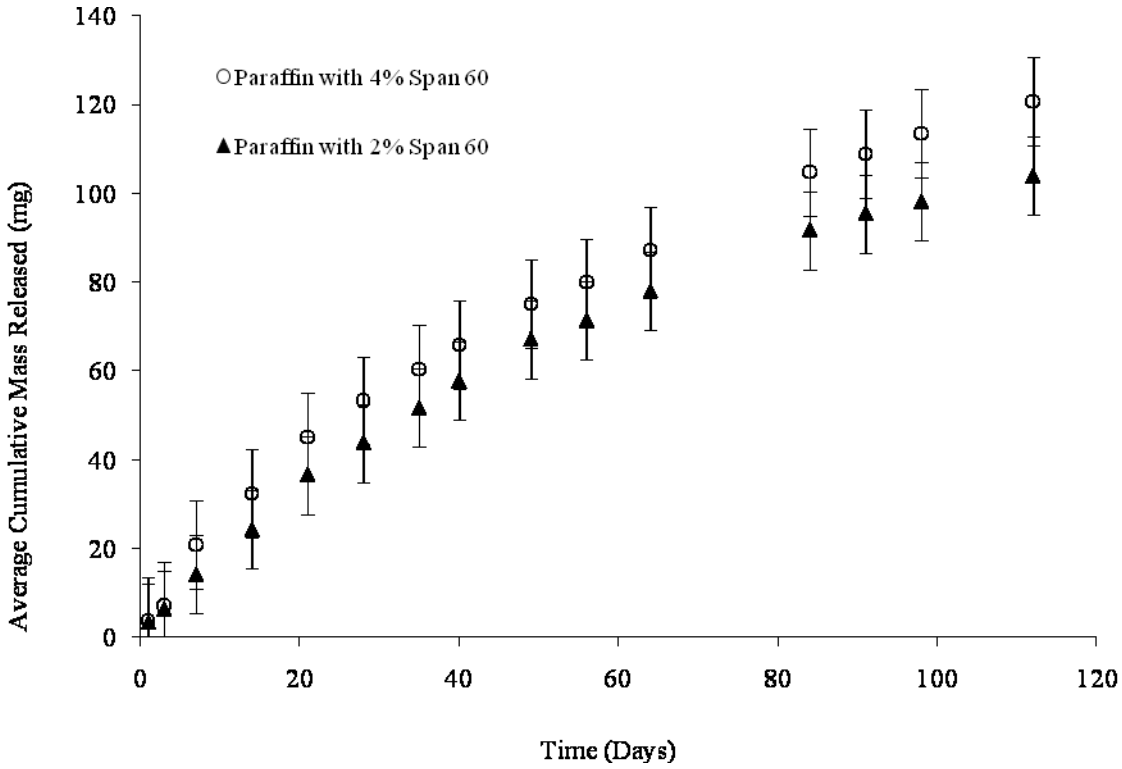


Figure 7. Average cumulative OFM released (mean ± SEM) from paraffin wax emulsions with 2% Span 60® and 4% Span 60®. The temperature was 30ºC and the air flow was 0.5 L/min. There were 190 mg pheromone present in each 5 g sample of emulsion.

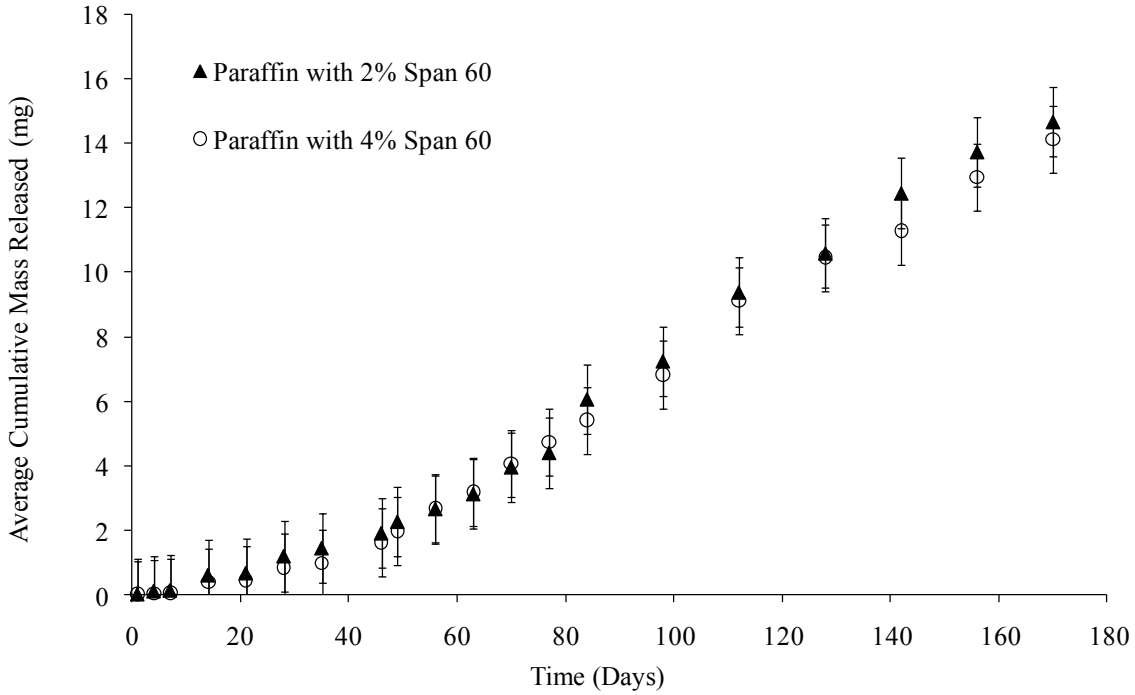


Figure 8. Average cumulative PBW pheromone released (mean ± SEM) from paraffin wax emulsions with 2% and 4% Span 60®. The temperature was 30ºC and the air flow was 0.5 L/min. There was a total of 190 mg pheromone present in each 5g sample of emulsion.

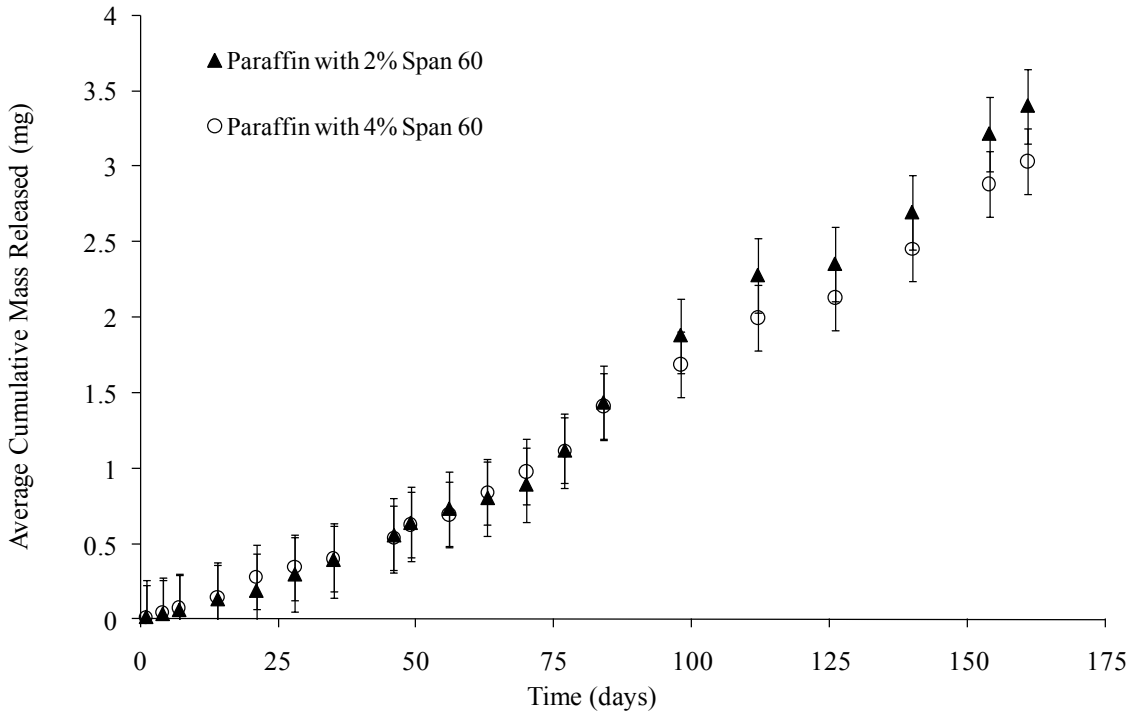


Figure 9. Average cumulative GM released (mean ± SEM) from paraffin wax emulsions with 2% and 4% Span 60®. The temperature was 30ºC and air flow is 0.5 L/min. There was 190 mg pheromone present in each 5g sample of emulsion.

Effects of Thickener

A proprietary thickener was tested to determine whether it had an effect on pheromone release rates from wax emulsions. This thickener was added prior to mixing the emulsion. The thickener caused an increase in the viscosity of the emulsion. However, as shown in Figure 10, it had no significant effect on the pheromone release rate. The average amount released from both emulsions was 0.02 mg/day with total amount released 3.4 mg.

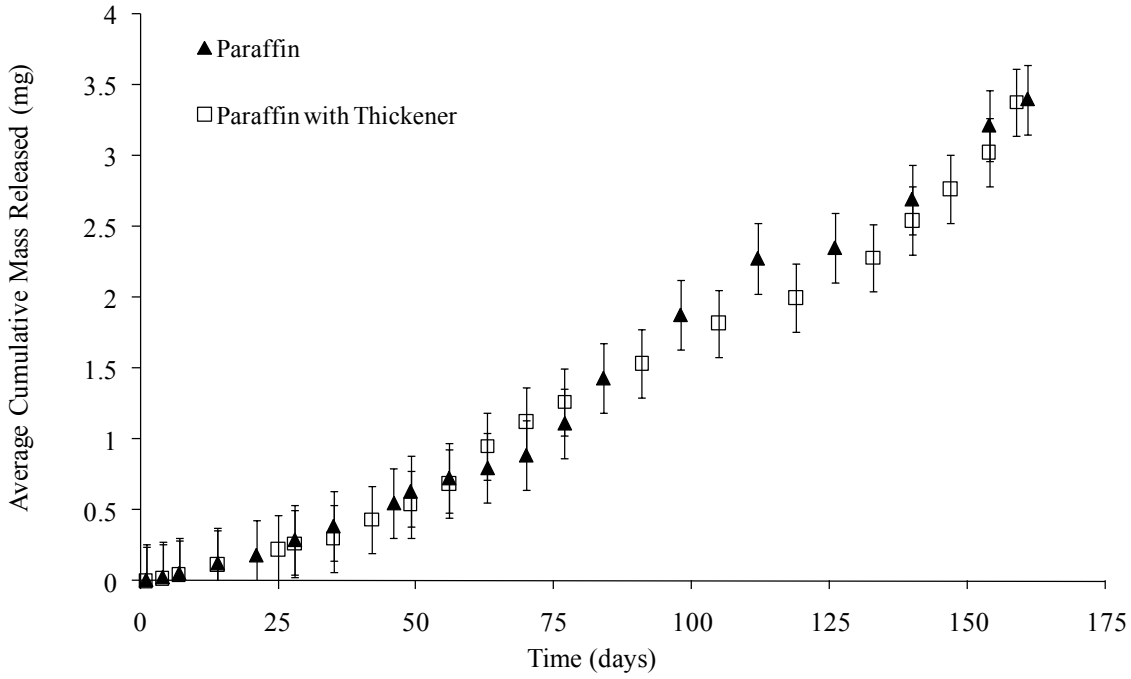


Figure 10. Average cumulative GM pheromone released (mean ± SEM) from paraffin and paraffin wax emulsions with an emulsion thickener. The temperature was 30ºC and the air flow was 0.5 L/min. There was a total of 190 mg pheromone present in each 5 g sample of emulsion.

Effect of Pheromone Volatility

Figure 11 shows a comparison of the pheromone released from paraffin wax emulsions for each of the three pheromones examined in this research and Table 5 shows a comparison of the molecular weights and cumulative amounts released for OFM, GM and PBW pheromones. Pheromone release rates are related to the molecular weights and molecular interactions. Compounds with lower molecular weights and weaker intermolecular interactions would be expected to have higher volatilities and thus higher release rates. OFM pheromone has a lower molecular weight than either PBW or GM pheromones, which explains its higher release rate. A total of 109 mg OFM pheromone was released over 112 days**,** compared to a total of 15 mg PBW pheromone and 3.4 mg GM pheromone released over the same time period. GM and PBW have higher molecular weights, and thus lower release rates.

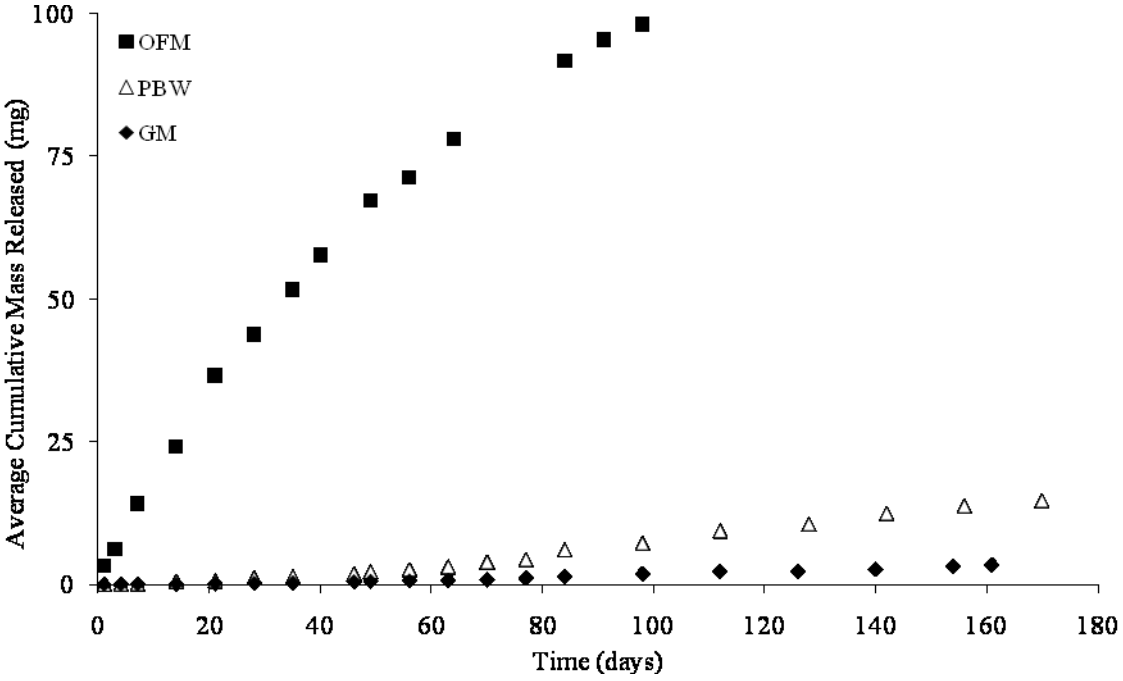


Figure 11. Average cumulative OFM, PBW, and GM pheromones released (mean ± SEM) from paraffin wax emulsions. The temperature was 30ºC and the air flow was 0.5 L/min. There was a total of 190 mg pheromone present in each 5 g sample of emulsion.

Table 5. Total pheromone released (mg) for 112 days and molecular weights (g/mole) for OFM, GM, and PBW pheromones.

Pheromone		***Release from Paraffin Emulsion***
	Molecular Weight (g/mole)	**Total Amount Released (mg) ± Std Error**
OFM	**226.20**	**104 ± 15.8**
PBW	**278.23**	**15 ± 1.0**
GM	**281.29**	**3.4 ± 0.29**

Effect of Emulsion Consistency

In preparing emulsions for OFM, one of the microcrystalline wax emulsions did not have a smooth consistency, which resulted in a poorly emulsified emulsion that contained small chunks of wax that resembled course ground meal. Figure 12 shows the average cumulative amount released from both emulsions. The poorly emulsified microcrystalline wax emulsion released an average of 109 mg OFM pheromone over 112 days, with an average release of 0.98 mg/day. The well emulsified microcrystalline wax emulsion released an average of 82 mg over 112 days, with an average release of 0.73 mg/day. The poorly emulsified mixture may have released more pheromone because the pheromone was not well dispersed and not held as well in the emulsion mixture. Also, the phase separation may have contributed to a higher pheromone release rate.

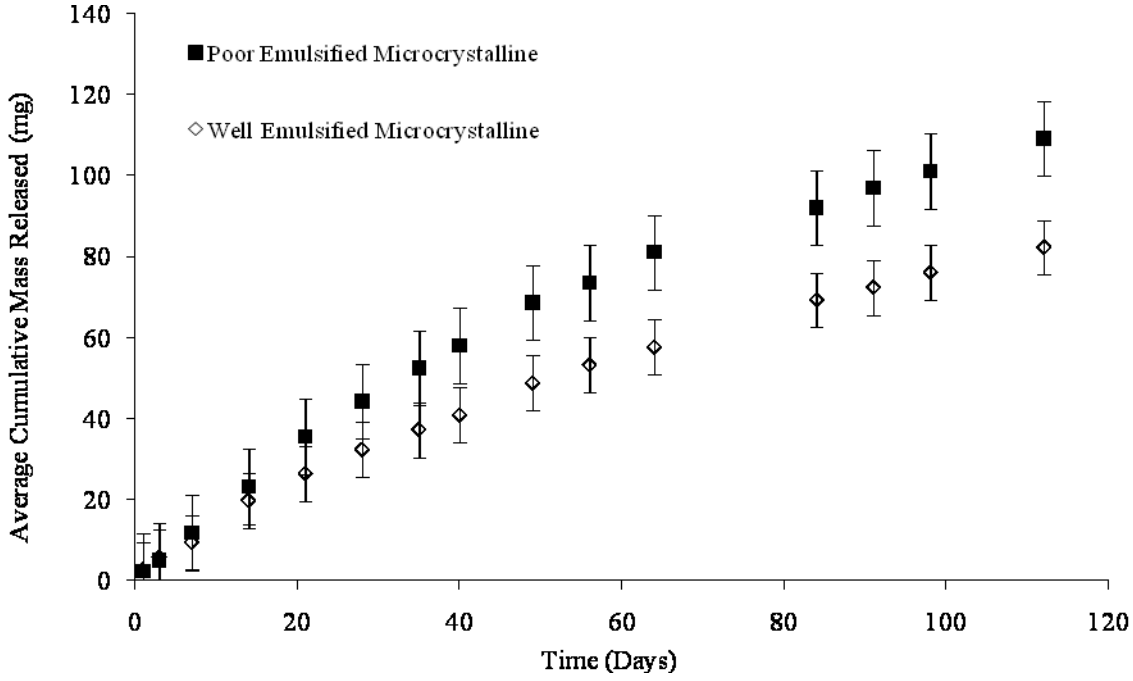


Figure 12. Average cumulative OFM released (mean ± SEM) from well emusified and poorly emusified microcrystalline wax emulsions. The temperature was held at 30ºC and the air flow was 0.5 L/min. There was a total of 190 mg pheromone present in each 5 g sample of emulsion.

Comparison of Gypsy Moth Paraffin Wax Emulsion and Hercon® Disrupt® II Flakes

An experiment was conducted with a commercial product (Hercon® Disrupt® II) to compare the GM release rate with that measured from the pheromone wax emulsions. An average of 5 g of Hercon® Disrupt® II flakes was placed in each flow cell, with a total of 895 mg of pheromone per flow cell. A total of 5 g paraffin wax emulsion, containing 190 mg of pheromone (ca. 1/5th the amount of pheromone), was placed in another set of flow cells. The Hercon® Disrupt® II product was subjected to the same flow cell conditions as the other emulsions. Although the Hercon product had nearly 5 times the amount of pheromone of the wax emulsion, after 60 days it released only 80 percent more than the emulsion (Figure 13). The cumulative amount of GM pheromone released from Hercon® Disrupt® II flakes was 1.7 mg, and the cumulative amount released from the paraffin emulsions was 0.9 mg. Thus, the paraffin emulsion seems to provide more efficient controlled release of the GM pheromone, since a similar release rate is achieved with a much smaller quantity of pheromone.

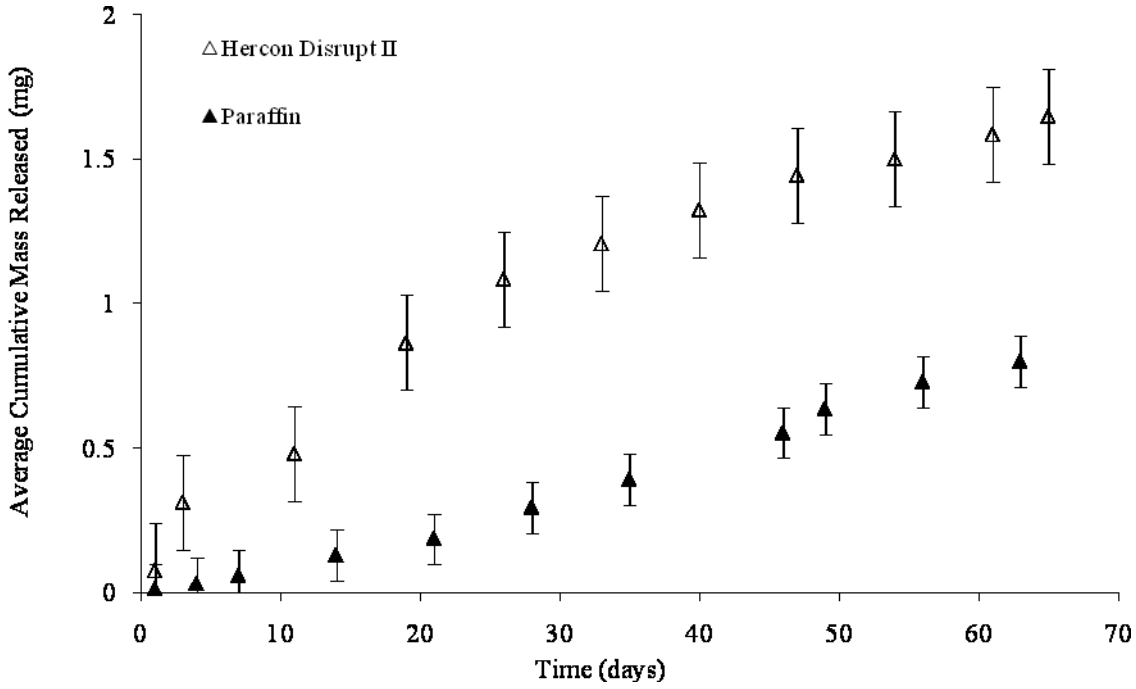


Figure 13. Average cumulative GM released (mean ± SEM) from paraffin wax emulsions and commercial Hercon® Disrupt® II flakes. The temperature was 30ºC and the air flow was 0.5L/min. There was a total of 190 mg pheromone present in each 5g sample of emulsion.

CONCLUSIONS

Emulsion formulations were varied with respect to the particular pheromone, the type of wax, the amount of emulsifier, and a thickening agent. Overall, emulsions made from paraffin wax had higher pheromone release rates than emulsions made from microcrystalline wax. Increasing the Span 60® emulsifier concentration in paraffin wax emulsions from 2% to 4% did not have a significant effect on the amount of pheromone released. The presence of an emulsion thickener increased the viscosity of the emulsion but had no significant effect on the pheromone release rate. However, the quality of the emulsion had a significant effect on the pheromone release rate. The poorly emulsified microcrystalline wax emulsion released more pheromone than the well emulsified microcrystalline wax emulsion. The commercial (Hercon® Disrupt® II) was compared to the GM paraffin emulsion, and while the wax emulsion released 0.8 mg less product than the commercial standard, the actual release rate based on percent pheromone for each product was 2.5 times greater.

Emulsions can be custom designed with different physical properties for different applications. The emulsion could be designed with different viscosities for different spray applicators, and the thickener and emulsifier did not have a significant effect on the pheromone release rates. Also, different types of wax could be used to modify either the pheromone release rates or other physical properties of the emulsion. Using wax as a pheromone carrier provides the flexibility to custom design controlled release carriers for different insects, crops, climates, or different types of spray applicators.

FUTURE STUDIES

Future studies could be conducted to evaluate other waxes, such as soy wax, as carriers for the controlled release of pheromones. Soy wax retains fragrance oils in candles well, and thus might also retain pheromone molecules well. The properties of soy wax are similar to paraffin and microcrystalline wax. However the melting point is slightly lower (43˚C-80˚C), and the wax contains double bonds that might help solubilize the pheromone better, possibly resulting in slower release rates.

Future studies could also be conducted to evaluate the effect of other formulation variables, such as other emulsifiers or additives, on the pheromone release rate. Emulsifiers other than Span 60® might be more effective for some pheromones and waxes.

Additionally, studies could be conducted to determine the effect of formulation variables on the viscosity of the emulsions. The viscosity is an important property that has an effect on storage stability and the type of spray equipment that can be used to apply the wax emulsions in commercial crops and forests for insect pest management. By measuring and controlling the viscosity, the formulations can be custom designed for the particular application.

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