Development of a Pheromone-based Monitoring Sampling System for *Dendrolimus superans sibircus*, the Siberian Moth

Final Report

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U.S. Cooperating Institutions and Principal Investigators:

Iral Ragenovich	USDA Forest Service
Victor Mastro	USDA Animal and Plant Health Inspection Agency
Jerome Klun	USDA Agriculture Research Service

Collaborating Country: Russia
Russian Cooperating Institution and Principal Investigator:

Yuri Baranchikov	Russian Academy of Sciences, Siberian Branch Sukachev Institute of Forests

Abstract

Previous experience has shown that early detection of introduced and potentially invasive species can prevent significant impacts to economics and native ecosystems. A sex-attractant-based monitoring and detection system was developed for Siberian moth, *Dendrolimus superans sibericus.* All field experiments were conducted in South Central Siberia in Russia against the larch race of the Siberian moth. An effective trap for all population levels was the modified gypsy moth milk carton trap equipped with an insecticide strip. Sticky traps, both the Wing trap and the I.N.R.A. delta trap were equally effective only in situations of very low populations and where traps could be visited frequently to change or renew the sticky surface. Traps baited with synthetic mixtures of C_{12} straight chain aldehydes, alcohols, and acetates alone and in combination were tested. Traps baited with a 1:1 blend of aldehydes (Z,E-5-7-dodecadienal+monoene aldehydes), and alcohols (Z,E-5,7-dodecadien-1-ol+monoene alcohols) captured as many males as traps baited with virgin females. Further testing of the monenes and the dienes, and some of the binary compounds show that the both the alcohol (ad) and aldehyde (cd) diene components appear to be critical. The addition of a monene to the blend containing both dienes did not increase moth captures.

Preliminary testing of the aldehyde and alcohol combinations was begun against the fir race of the Siberian moth. Additional information on the diel attractiveness and moth age-effects of the female were studied.

Based on this work, the combination of the modified gypsy moth milk carton trap, in conjunction with an insecticide strip, and baited with the synthetic mixture of aldehydes and alcohols can be used to monitor endemic Siberian moth populations in Asia, and for surveillance and detection of the moth in countries where it may become accidentally introduced. Further testing should be done to confirm that the same combination of aldehydes and alcohols is attractive to the fir race of the Siberian moth and across the range of the moth (from the Urals to the Russian Far East).

To date, three papers, three presentations at meeting appearing as proceeding abstracts, and a poster presented at three separate meetings have resulted from this study. One PhD thesis, at Sukachev Institute, Russian Academy of Sciences, and three diploma studies for students at Krasnoyarsk State University were supported by this work.

Development of a Pheromone-based Monitoring Sampling System for *Dendrolimus superans sibircus*, the Siberian Moth

Introduction

Introduced organisms often cause significant ecological and economic damage to native forests; and as long as there is trade and travel between countries, the potential for introducing non-native species exists. Previous experience with the Asian gypsy moth, the Asian long-horned beetle, chestnut blight and Dutch elm disease have demonstrated how easily insects and diseases from other countries can be introduced into the United States; how difficult and expensive they are to eradicate once they have become established; and the significance of potential for long – term impacts. Pheromone traps are one of the most effective and efficient tools for detecting early introductions of some insects. They can also be used for monitoring insect populations in their native countries; and are a valuable tool for monitoring success of control practices that must be implemented when introductions do occur.

The Siberian moth, *Dendrolimus superans sibircus*, is considered one of the most destructive conifer defoliators in northern Asia. Within the native range of the Siberian moth, outbreaks frequently occur in larch, fir, and pine forests. There are two races of the Siberian moth – the larch race, and the fir race. Because of similarities in forest types and forest species, this insect has the potential to cause significant damage should it be introduced into North America.

The objectives of this project were to:

1. Identify and develop a pheromone –based attractant for *Dendrolimus superans sibircus*,

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2. Develop a trapping/sampling system that could be used to detect introductions into the United States, and

3. Develop at trapping/sampling system that could be used to monitor native populations for occurrence and potential outbreaks.

Research Design and Methodology

This study had both laboratory and field components, and consisted of three years of research. The chronology of the research was as follows:

1) During the first year we tested trap designs for trapping efficiency and effectiveness. Also, sex gland and pheromone extracts were collected from virgin females and analyzed, pheromone components were

identified and a laboratory procedure to synthesize the three components was developed..

2) During the second year additional trap configurations were tested. The daily attractiveness of females was determined. Traps baited with various combinations of the synthesized aldehydes/alcohols/acetates that were determined to be the primary components of the Siberian moth pheromone were evaluated by monitoring their attractiveness to male moths.

3) In the third year, using results of the previous year, additional tests using traps baited with combinations of the monoene and diene alcohol and aldehyde components were conducted on the larch race. A field dose response test was also conducted on natural populations. Preliminary testing of the monoene and diene alcohol and aldehyde components was repeated on the fir race in northern Siberia.

The laboratory phase included rearing Siberian moth larvae in the laboratory in Russia, and taking pheromone extracts from emerging females. Pheromone extracts were then sent to the ARS laboratory in Beltsville, Maryland for analysis and development of a procedure for synthetic pheromone production.

The field phase was conducted in Russia, where there was a current Siberian Moth outbreak near the village of Ozerny, Shira Region, Republic of Khakassiya, Siberia, Russia.in South Central Siberia. As a result, most of the initial work was done with the larch race of the Siberian moth. Trap designs and combinations of synthetic pheromones were tested in the field**.** During the third year of the study, preliminary work with the fir race in northern Siberia, was included.

Pheromone Extraction and Identification

Larvae were collected each spring and reared in the laboratory in Krasnoyarsk. Sex of each pupa was determined, and as virgin female moths emerged, sex pheromone glands associated with the female ovipositors were extracted with heptane. Some of the females each year were used as the controls in the field tests. Extractions were hand-carried to the ARS lab in Beltsville, MD where they were prepared and analyzed on polar and nonpolar capillary columns using gas chromatography procedures described by Klun, et.al. (1980) (cited in Klun, et. al. 2000)

In 1997, all larvae were collected in Tuva and represented the larch race of the Siberian moth. Approximately 160 extractions were made. There was insufficient amounts of compounds in the extracts to allow conclusive chemical identification; however, analysis indicated that the extracts contained pheromonal components similar to those previously identified from other species within the genus *Dendrolimus*. These included conjugated C-12 chain length dienyl alcohols, aldehydes, and acetates.

In 1998, additional extracts were made. Extractions were to be made on various aged females (2,3,5,7, and 9 days old) to determine how the make up of the pheromone changes over time, with a minimum of 10 extractions. Extractions were made between 6:00 p.m. and 10:00 p.m. rather than between 11pm and 2am. When analyzed, all of the extracts were blank, possibly because of extraction timing.

In 1999, larvae were again collected in Tuva and reared in the laboratory and the extraction process to determine pheromone changes over time was repeated. This time, extractions were made between 11 p.m. and 2 a.m. Because of the high parasitism rate, there was a lower number of emerging adults than anticipated. Extractions were placed in vials and transported to the U.S. Extracts were placed in vials with caaps with resin plugs. Because of faulty containers (vials and caps) most extracts were lost.

Analysis of the extracts indicated the presence of trace amounts of 12-carbon chain length pheromone-like components. Using this and information on pheromone components previously identified for four other species in the genus *Dendrolimus*, it was determined that the Siberian moth might use one or more of the identified dienes as pheromone. As a result, *Z,E-5,7*-dodecadienal, *Z,E-5,7*-dodecadien-1-ol, and *Z,E-5,7*-dodecadienyl acetate were synthesized according to synthesis processes described in detail in Klun, et.al, (2000).

Trap Design and Testing

Very little work on trapping Siberian moth has been done. The Siberian moth is a relatively large moth. One of the first tasks was to test a variety of traps to determine their efficiency and effectiveness in trapping these moths. Several types of sticky traps baited with a sex attractant are commonly used for detecting and monitoring populations of Lepidoptera, such as gypsy moth, Douglas-fir tussock moth, and western spruce budworm. A delta-type trap is commonly used in monitoring and detection programs for these insects. These moths, however, are much smaller that the Siberian moth. The first year, two types of sticky traps were tested – a large delta trap (INRA) and a wing trap (Trece, Inc.). The INRA trap is a delta trap that is open on both ends, with one sticky surface (a bottom panel) that can be periodically changed. The wing trap has two surfaces - a top and a bottom - and is open all around the sides. Both surfaces are sticky. Since no sex attractant had been identified at this point, live virgin females were placed in rigid plastic screen cages that were placed inside the traps and used as the attractant source. Baited and control (no attractant source) traps were placed in a line and positioned approximately 1.5 m above the ground and 100 m apart. This was replicated four times. Traps were checked each morning, when the sticky panels were changed and the captured moths counted.

During the second year a non-sticky trap was added to the test. This trap was constructed from two standard green USDA gypsy moth milk carton traps with enlarged entrance holes. This was referred to as the "Otis Live-Catch Trap". The trap was fitted with a plastic funnel in order to keep captured moths from escaping. All traps were baited with 2-day old virgin females in a plastic mesh cages. Again the traps were placed in a line at approximately 1.5 m above the ground and with 100 m between traps. Traps were placed in an alternating A-B-C sequence, and replicated 5 times, and monitored for three consecutive days. The traps were checked daily; the sticky panels were changed in the sticky traps, and the captured moths counted. At that time each cage with a female was also rotated to the next trap position in the line.

For statistical analysis, the daily catch of each trap was considered an independent variable. Numbers of captured moths were transformed by $\log_{10}$ (x+1) to insure equal variance before analysis of variance by ANOVA. Significant differences in mean trap catches were identified by the least significant difference test (LSD) at *a*=0.05.

Field Tests of Pheromone Components

During the first year of the study three potential sex attractant components were identified and synthesized. This was based on a common chemical theme in the genus involving 12-carbon chain length aldehyde, alcohol, or esters having *Z,E-5,7* diene unsaturation in the carbon chain. These three components – alcohols (OH), aldehydes (Ald), and acetates (OAc) – were prepared separately. See Klun, et.al. (2000) for a detailed description of the synthesis and reduction process for preparing the components. The compounds were loaded into rubber septa, either singly or in combination at a rate of 100 micrograms of each component, in order to determine which compound or combination of compounds might be the attractant source. The following combinations were tested where A, B, and C represented the three components: A, B, C, A+B, A+C, B+C, and A+B+C. A virgin female in a cage served as the control for a total of 8 treatments per replication, in a complete randomized block design. The full compliment of treatments was tested in the Otis modified gypsy moth milk carton trap with an insecticide strip. The septum was suspended on a wire at the center of the trap and in front of the openings. Moths entering the trap were killed by an insecticide strip (Vapona©) placed in the bottom of the trap. Virgin females were placed in cages in the Otis Live Catch Trap that was fitted with the inverted cone. The cone allowed males to enter the trap, but then prevented their leaving. The first night, traps were baited with four-day old virgin females. The second night these females were replaced with two-day old virgin females, which then were used for the duration of the study. The treatments were replicated 6 times. Traps were monitored for 13 consecutive nights from July 16 to July 28, during the adult flight of the Siberian moth. Traps were checked at least once a day, and moths counted and removed.

Preliminary analysis indicated that most of the treatments exhibited similar variability among the number of insects captured, but other treatments exhibited a larger variability. The error varience was partitioned into 4 groups to improve the precision of the analysis of varience. Analysis and subsequent mean comparisons were made using SAS Proc Mixed (see Klun, et. al, 2000).

During 1999, an additional 4 studies were conducted. The results of the empirical testing showed that a synthetic alcohol/acetate mixture containing 5,7-dodecadienal, 5,7-dodecadienol, and 5dodecenal, 7-dodecenol and 5-dodecenal is a sex attractant for Siberian moth males. However, it was not known which of these might be the biologically active ingredient(s). During the third year, synthetic formulations of these components were prepared at Howard and Morgan State Universities and nine combinations were tested in the field in Siberia. These nine combinations were:

[ad+bm] + [cd+dm] = control of aldehyde and alcohol
ad + [cd+dm]
bm + [cd+dm]
cd + [ad+bm]
dm + [ad+bm]
ad + cd
ad + dm
bm + cd
bm + dm

where: ad = alcohol diene
bm = alcohol monoene
cd = aldehyde diene
dm = aldehyde monoene

Each of the above 9 treatments plus virgin females as controls constituted this test. The combination of components were placed in rubber septa, which were suspended in the Otis modified gypsy moth milk carton traps with an insecticide strip in the bottom of the trap as before; and the females were placed in the Otis –Live-Catch-Traps. Both fir and larch females were used as controls. The treatments were replicated 6 times. These treatments were monitored for 12 days from July 10 through July 21, 1999. Traps were located in one long line perpendicular to the prevailing wind direction off of the lake. Each trap was rotated forward to the next position each day to account for differences of populations within the stand.

The above combinations were monitored for three days. Using preliminary results, a second line of combinations testing the binary monoenes was installed. Combinations selected for testing were:

[ad+bm] + [cd+dm] = control of aldehyde and alcohol
ad + cd
ad + [cd+bm]
ad + [cd+bm1]
ad + [cd+bm2]
ad + [cd+dm]
ad + [cd+dm1]
ad + [cd+dm2]

The same trapping design, incorporating 8 treatments plus the virgin females, and replicated 6 times, was used. As with the previous test, traps were placed in a long line perpendicular to the prevailing winds and each day the traps were rotated forward to the next location to account for differences in populations in the stands. Traps were monitored for 10 days, from July 12 through July 21.

A small dose rate study using 10 mmg and 100 mmg was also done. Traps were placed in a line similar to the previous line. The 10 and 100 mmg rates were alternately placed along the line and replicated 6 times.

In a fourth study the synthetic compounds were tested against the northern or fir race of the Siberian moth. The possibility existed that the fir race might respond to different components; therefore, the first combinations of blends of alcohols and aldehydes were repeated in the north. Fir females were used as the control. This study was replicated 3 times and conducted for 9 days, from July 19 to July 26. This test occurred towards the end of the flight season, and as a result, very few moths were captured.

Larval Rearing and Establishment of a Laboratory Colony
Because of the difficulty of collecting wild larvae in the field, rearing them in the laboratory, and extracting and transporting the pheromone extracts; and because it would be desirable to test male moth responses to pheromone components in a wind tunnel, several attempts were made establish a Siberian moth colony in the United States at the APHIS Otis Plant Protection Quarantine Facility. Siberian moth has a complicated life cycle lasting from one to four years. As a result, there can be various instars of larvae, as well as pupae, adults, and egg masses present all during the same time. In 1997, 234 third-instar, summer diapause larvae, and 134 eggs of the larch race were brought to the U.S. Very few of the larvae pupated, and, of those that did, development was staggered and the opportunity for successfully establishing a colony was low.

Larvae were reared in Krasnoyarsk as well. About 3,500 larvae from the larch race in Tuva were brought to the laboratory. An attempt was made to maintain the colony on fir foliage. Only 12 larvae were reared through to pupation and of those there were 3 females and 7 males. These mated easily, but the hatched larvae refused to feed on fir needles.

Larvae were again collected in the spring of 1999. Approximately 1000 larvae were collected from the Tuva and Black Lake Regions and carried by courier to the U.S. These were also from the larch race. The populations were heavily parasitized, and none completed development through to the adult stage. Larvae were parasitized by a Diptera identified as *Masicera sphingivora* Robineau-Desvoidy. Rearing larch race larvae proved to be difficult, especially if they were in the summer diapause state and actively feeding over the winter, when no fresh larch foliage was available.

Approximately 4000 4^{th} and 5^{th} instar larvae were collected from larch forests in Tuva in the early spring, and reared in the lab in Krasnoyarsk. In addition, about 380 larvae of the fir race were collected in the Altay Mountains. There was a high degree of parasitism: 15 females were reared from the larch race, and 45 from the fir race. Some of these females were used for extractions, and the remaining females were used as controls in the field studies.

Female Attractiveness Study
A separate study was conducted to determine the diel periodicity of attractiveness of males to females. Thirty-two Otis-Live-Catch Traps, each baited with a virgin female in a plastic net cage. Females were from 1 to 7 days old, and there were from 2 to 7 females in each age group. Traps were placed 1.5 meters from the ground and 50 feet apart. They were checked daily from July 14 to July 26, and were monitored hourly during July 14-16. The dynamics of changes in female attractiveness (S) with their age (T) for each female was approximated and calculated by the least squares method using the data for individual females.

Results

Pheromone Extraction and Identification *- A process was developed whereby an effective synthethic sex attractant for Siberian moth can be produced.*

Attempts were made to obtain pheromone extractions from females three times. During the first attempt there was very little pheromone in the vials. Combined and individual heptane extracts of the ovipositors were analyzed by gas chromatography. The quantity was insufficient to allow conclusive chemical identifications. However, analysis indicated that the extracts contained trace amounts of 12-carbon chain length pheromone-like compounds. Pheromonal components previously identified in four other species of the genus *Dendrolimus* had a common chemical theme involving 12-carbon chain length aldehyde, alcohol, or esters having Z,E-5-7 diene unsaturation in the carbon chain. Based on this information, it was believed that the female Siberian moth might also use one or more of these dienes as pheromone and a method was developed to synthesize Z,E-5-7-dodecadienal, Z,E-5-7 - dodecadien-1-ol, and Z,E-5-7 -dodecadienyl acetate. The process for synthesizing a sex attractant for the Siberian moth is described in detail in Klun, et. al, (2000).

During the second year, additional pheromone and ovipositor extractions were made from virgin females (2,3,5,7, and 9 days old) to determine how the make up of the pheromone changes over time. The extractions contained no pheromone components, and on examination, it was found that the assistant had misunderstood the instructions and had made the extractions between 6 pm and 10 pm, rather than between 11 pm and 2 am. As a result the females were not actively calling and may not have been producing pheromone at the time the extracts were made.

The third year, attempts were once again made to obtain extracts from virgin females using the same scheme as the previous year – to take extracts from various aged females to determine changes in the pheromone over time. In addition, it was planned to take these extracts from both larch and fir race females, in order to determine if there were differences between the races. Due to the high parasitism in the larvae, a limited number of females were available for extractions. Extractions were made in the same manner as previously except the extractions were made between 11pm and 2 am. The vials provided were faulty, and we were not able to analyze the samples upon return to U.S.

Further tests on the vials showed that 50 microliters of heptane evaporated through the cap after 24 hours at room temperature and after 30 hours in the refrigerator. Once it was determined that the vials were faulty, a switch was made to ones with solid caps. However, these contained extracts only from the 2, 4, and 6 day old fir females. Both full and empty vials were carried to the U.S., in the event that some pheromone could be salvaged from the empty vials.

Since so little pheromone was obtained in the extractions, there is a question as to whether the extractions were actually capturing the pheromone gland. A more detailed study of the female genitalia morphology may be needed to determine if a different extraction method may need to be used.

Potential future efforts:

- *-research the morphology of the female genitalia to determine appropriate method for extraction.*
- *-extract and chemically describe the actual Siberian moth pheromone.*

Trap Design and Testing- *A modified gypsy moth milk carton trap is the best overall trap for Siberian moth, for use in both low or high populations; although a sticky trap could be used in very low populations.*

Finding a trap for this moth presented some interesting challenges. The moth is fairly large, and its size needed to be taken into account.

Two sticky traps were tested – the Wing trap and the INRA delta trap. In addition, a modified gypsy moth milk carton trap was tested. Studies showed that at low populations, the three traps were equally effective at catching male moths, with an average of 3.1, 2.8, and 3.3 males per trap per night for the wing, delta, and milk carton traps, respectively.

At higher densities, we found that the sticky traps could not capture and retain all of the moths that visited them in one night. The maximum numbers of moths trapped in one night were 6 for the wing trap and 5 for the delta trap. The moths are fairly large, and once caught in the traps, would beat their wings, coating the sticky surface with scales, and rendering it ineffective for capturing additional moths that may visit the trap. In comparison, the milk carton traps caught a maximum of 115 moths per night, with plenty of space still in the trap (see Baranchikov, Klun, Kucera, et. al., 1999).

The sticky traps could be used for monitoring very low populations, such as detecting populations at sites where studies indicate an introduction could occur. In these applications, the presence of even one adult male in a trap provides the evidence that an introduction has occurred. However, the traps would need to be checked on a regular basis and the sticky surfaces exchanged, if needed. If frequent visits to the traps are not possible, or if monitoring the distribution and population density is desired, then, the modified gypsy moth milk carton trap with the insecticide strip is the recommended trap.

The modified Otis Live Catch Trap with the funnel, works well if it is desirable to keep moths alive or if the pesticide strip cannot be used..

Potential future efforts:

- *Additional trap development is not necessary.*

Field Tests of Pheromone Components **–** *The combination of synthetic aldehydes and alcohols were as effective as the virgin females at attracting male moths. The aldehyde and alcohol dienes are the critical components for attracting male moths.*

The mean responses of Siberian moth males to traps baited with virgin females and synthetic chemicals is shown in Table 1. Results show a 1:1 mixture of the Ald:OH was as attractive as the virgin females and statistically not different than the response to virgin females.

When acetate (OAc) was added to the Ald:OH, captures were significantly reduced when compared to the Ald:OH alone. Thus, the OAc was a deterrent when added to the Ald:OH. By itself, the Ald was moderately attractive, while the OH or the OAc, either alone or in combination with each other, did not attract males. These findings are discussed in more detail in Klun, et.al. (2000).

Table 1: Response of Siberian moth males to virgin females and 100 micrograms of aldehydes (Ald), alcohols (OH), and acetates (OAc), alone or in combination in traps. Means followed by the same letter are not significantly different (P>F<0.05).

Treatment	Mean captures/trap/night	Mean Std Error
A. Virgin female	3.21a	1.08
B. Ald	0.44b	0.13
C. OH	0.00c	0.01
D. OAc	0.00c	0.01
E. Ald+OAc	0.04c	0.01
F. Ald+OH	3.31a	0.54
G. OH+OAc	0.00c	0.01
H. Ald+OH+OAc	0.03c	0.01

Based on this information, it was determined that the synthetic preparation of Ald + OH was and effective sex attractant for the male Siberian moth. The following year, additional tests comparing various combinations of the monoenes and dienes for this mixture (Ald + OH) was conducted in order to more specifically identify the attractive components. The aldehyde and the alcohol contained both monoene and diene components. The following year in Test 1, various combinations of these aldehyde and alcohol monoenes and dienes were compared to the standard Ald+OH mixture. These mixtures are identified in Table 2, Mixtures for Test 1. Larch and fir virgin females were also used in Test 1, as well as an empty trap.

Table 2. Treatments evaluated in Tests 1 and 2, Black Lake

Treatment	**Mixtures, Test 1**	**Mixtures, Test 2**
1	(ad + bm)+(cd + dm) *(Ald + OH)*	(ad + bm)+(cd + dm) *(Ald + OH)*
2	ad + cd + dm	ad + cd
3	bm + cd + dm	ad + cd + bm
4	ad + cd + bm	ad + cd + bm1
5	ad + dm + bm	ad + cd + bm2
6	ad + cd	nothing under #6
7	ad + dm	ad + cd + dm
8	bm + cd	ad + cd + dm1
9	bm + dm	ad + cd + dm2
10	empty trap	--
11	fir female	fir female
12	larch female	larch female

ad = diene alcohol; bm, bm1, bm2 = monoene alcohol
cd = diene aldehyde; dm, dm1, dm2 = monoene aldehyde

After three days of monitoring traps in Test 1, preliminary moth captures indicated that the treatment containing both dienes appeared to be important. Therefore, an additional test that evaluated some of the binary monoenes was also conducted to determine if there was any additional effect of these individual components. Table 2 shows the different combinations of components that were used in both Tests 1 and 2.

Results of analysis (Sawyer, 2000) show that in Test 1, there was no significant differences in moth capture between traps containing the original Ald + OH compound(treatment 1) and those traps baited with blends containing two diene components, either with or without the addition of monoene components (treatments 2, 4, and 6). See Table 3 for the mean number of moths captured (and standard deviation) per trap per day for each treatment in Test 1. There was also no significant differences in moth capture between these treatments and traps containing a “larch” female. Treatments 1, 2, 4, 6, and the larch females all caught significantly more moths than empty traps, traps baited with “fir” females, and traps containing pheromone blends having only one, or no diene components. Thus, the presence of both the alcohol and aldehyde diene components appears to be critical.

Table 3. Mean number (and SD) of male Siberian moths captured per trap per day in each treatment of Test 1 and Test 2. See Table 1 for treatment descriptions, which differ in the two tests. A dash indicates a treatment number that was not assigned.

Test	Treatment	N	Mean	SD
1	1	72	1.778	3.701
1	2	72	1.250	3.084
1	3	72	0	na
1	4	72	1.278	3.436
1	5	72	0	na
1	6	72	0.778	1.594
1	7	72	0.042	0.262
1	8	72	0.014	0.118
1	9	72	0.056	0.231
1	10 (empty)	48	0.063	0.245
1	11 (fir F)	17	0.176	0.529
1	12 (larch F)	9	2.222	3.193
2	1	60	0.650	1.376
2	2	60	0.333	0.914
2	3	60	0.467	0.853
2	4	60	0.450	1.080
2	5	60	0.267	0.710
2	6	--	--	--
2	7	60	0.467	1.112
2	8	60	0.417	0.996
2	9	60	0.300	0.869
2	10	--	--	--
2	11 (fir F)	24	2.542	2.484
2	12 (larch F)	3	0.333	0.577

In Test 2, all of the pheromone component blends captured as many moths as did the original Ald + OH mixture. All of these treatments contained both diene components. Capture was not significantly affected, regardless of which monoene component was added to the blend of the two dienes. In this Test, all treatments, including the original Ald + OH blend, caught significantly fewer male moths than the "fir" females. There were not enough "larch" females to adequately test their attractiveness in Test 2. Why "fir" females did so well in Test 1, and so poorly in Test 1 is not known.

In both tests, male moth capture decreased over time. This could be a function of loss of attractiveness of the synthetic pheromone bait, or decreased numbers of male moths as the flight season ended, or both.

An interesting side note is that traps captured a fair number of other moths. The predominant species captured was a poplar defoliator, *Gastropacha popalifolia* (Lasiocampidae). Although no specifc test or analysis was doine, in Test 1, treatments 3,5, and 9, (all containing an alcohol monoene and aldehyde monoene combination) appear to hold some degree of attractiveness to this other moth. There was no similar consistency in Test 2.

A concentration test comparing traps baited with 10 mmg and 100 mmg of Ald:OH mixture was conducted. No statistical analysis has been done on this data, however, results show that

The same components used in Test 1 were tested against the northern fir race. However, traps were placed fairly late in the flight period, and too few moths were captured to draw any statistical conclusions.

Potential future efforts:

- *Test the original combinations of Ald, OH, and OAc to determine if the same combination of Ald:OH is as attractive to the fir race of the Siberian moth as to the larch race – or if there is another component or combination of components that is more attractive.*
- *Determine the longevity of different attractant dispensers for synthetic pheromones.*
- *Determine if the Ald:OH combination is equally attractive to Siberian moth males across its entire range of the moth, from the Urals to the Siberian far east.*

Establishment of a Laboratory Colony*- Siberian moth larvae collected in the spring were easily raised through to adults in the lab. However, It is extremely difficult to get a colony of Siberian moth established from the summer diapause larvae of the larch race, because of the variability of the life cycle and lack of food source during the winter. The fir race may be easier to rear.*

The timing for transporting Siberian moth became a critical component when attempting to establish a colony in the U.S.D.A. APHIS quarantine facility. Initially pupae were collected, but adults began to emerge before they could be transported. Older larvae can have a high degree of parasitism. Larvae collected early in the spring readily pupate, if they are not parasitized, and emerge as adults and mate. Larvae reared in the lab, were reared on their host foliage, i.e. larvae collected on larch were fed larch.. Larvae emerging from the eggs of larch race adults would not feed on fir foliage.

Potential future efforts:

- *-Continue to try to get a laboratory colony established in the U.S. using individuals from the fir race*
- *-Use extracts and/or airborne volatiles from females to determine chemical composition of the pheromone*
- *-Use individuals raised in the laboratory colony to further determine to various attractants using wind tunnel and electroantenagram tests.*
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Female Attractiveness Study – Although not part of the original proposal, a study on the periodicity of female attractiveness, and how female age effects attractivness, was conducted. During this study, individual females were attractive as long as 14 days after ecolsin, with an average of 9 days. All females were alive at the end of the study. It was observed that for each individual female there was significant decrease in attractiveness with age. A significant correlation between the calculated starting attractiveness and the attractiveness decreasing rate was found. The higher the starting level of attractiveness, the faster it decreased with the females age (See Baranchikov, Klun, Mastro, et.al,1999)..

Discussion

The Siberian moth, *Dendrolimus superans sibericus,* is one of the few forest defoliators capable of causing damage to millions of acres during one episodic event. Its impacts on conifer forests of Russia, which are becoming more important ecologically and economically, can only increase in the future. The ability to manage any pest is dependent on systems composed of a number of interlinking and interactive components. A key to a management system is a tool for evaluating the distribution, density, and population dynamics of the target species. Development of an attractant and trap, which was accomplished under this project, has provided a basis for this component. These developments also provide the United States and other countries, which have “at risk” conifer forests, a tool for detecting introductions of this potentially devastating pest. If introductions occur, it also provides a monitoring tool to assess populations and the impacts of any eradication or control efforts.

A complete description of the pheromone system used by various races of *D. superans*, and other *Dendrolimus* species would potentially provide the basis for developing even better monitoring tools. With development of these tools, a research effort should be initiated to relate trap catches to population distribution and density. This would facilitate development of a management strategy rather than solely response to outbreak populations and damage. Development would potentially reduce damage, decrease cost and pesticide use and ecologically protect the conifer ecosystem in Russia.

A better description of the mating behavior and the semiochemoicals involved would also provide a basis for the development of target specific control techniques including mating disruption, mass trapping, and devices for auto dissemination of pathogens.

Other Benefits

Publications:

Baranchikov Yuri N., V.M. Pet'ko, V.G Sukhovol'skiy, I.R. Ragenovich, J. Klun, V. Mastro. 1999. Daily and age-connected dynamics of female attractiveness in Siberian moth. In: Entomologicheskiye issledovaniya v Sibiri (Entomological studies in Siberia), Volume 2. Krasnoyarsk Institute of Forest Press, 2000 p 156-164. (In Press; in Russian).

Baranchikov Yuri N. 2000. Alive-catch trap for big moths. In: Zaschita rasteniy (Plan protection) (In Russian).

Klun, Jerome A., Yuri N. Baranchikov, Victor C. Mastro, Yousef Hijji, Jesse Nicholson, Iral Ragenovich, and Tamara A. Vshivkova. 2000. A sex attractant for the Siberian moth, *Dendrolimus superans sibericus* (Lepidoptera:Lasiocampidae). J. Entomol. Sci. 35(2):158-166.

Sawyer, Alan. 2000. Analysis of 1999 Siberian Moth Pheromone Trap Data. Unpublished Report. USDA – APHIS-PPQ. 6 pg.

***Abstracts in Proceedings from Conferences*:**

Baranchikov Yuri N., V.M. Pet'ko, I.R. Ragenovich, J. Klun., V. Mastro V. 2000. Sex attractant and pheromone traps for Siberian moth monitoring. In: Biologicheskiye metody v zaschite lesa (Biological methods in forest protection). Proceedings all-Russia conference on forest protection, October 1999. Pushkino, Moscow Region. In press (in Russian).

Baranchikov Yuri N., Jerome A. Klun, Victor C. Mastro, Vladimir M. Pet'ko, Iral Ragenovich, and Vladislav G. Sukhovol'skiy. 1999. Siberian moth females' attractiveness to males: day time and moth age effects. In: Proceedings U.S. Department of Agriculture Interagency Research Forum on the Gypsy Moth and Other Invasive Species, January 19-22, 1999. ed. Sandra L.C. Fosbroke and Kurt W. Gottschalk. Gen. Tech. Report NE-266. USDA Forest Service, Northeastern Research Station. Pg. 19.

Baranchikov Yuri N., Jerome A. Klun, Daniel R. Kucera, Victor C. Mastro, Vladimir M. Pet'ko, and Iral Ragenovich. 1999. Pheromone monitoring of *Dendrolimus superans* Butler: Evaluation of three trap designs in Siberian Taiga.. In: Proceedings U.S. Department of Agriculture Interagency Research Forum on the Gypsy Moth and Other Invasive Species, January 19-22, 1999. ed. Sandra L.C. Fosbroke and Kurt W. Gottschalk. . Gen. Tech. Report NE-266. USDA Forest Service, Northeastern Research Station. Pg. 18.

Posters:

Klun, J.A., Y. Baranchikov, D. Kucera, V. Mastro, and I. Ragenovich. Serendipitous discovery of a potent sex attractant for the Siberian moth, *Dendrolimus superans sibericus* (Lepidoptera:Lasiocampidae). This poster was presented at:

- -Annual national meeting of the Entomological Society of America. Dec. 1999. Atlanta, GA.
- -Interagency Research Forum in Annapolis, MD. Jan 2000; and
- -Symposium on Invasive Species, May 2000; Beltsville, MD.

Work that will lead to PhD from Sukachev Institute of Forests, Russian Academy of Science:

Pheromone monitoring of Siberian moth – Vladimir Pet'ko

Titles of Diploma studies for students from the Faculty of Biology , Krasnoyarsk State University:

Evaluating the trap type for Siberian moth monitoring – Nina Yudina

Variation of male wing morphology and color in South Siberian populations of *Dendrolimus superans sibericus* – Irina Tshonovskaya

Female attractiveness to males in Siberian moth – Svetlana Ageyeva

Follow-up Activities:

Testing the most attractive synthetic blend across the range of the moth. This work was initiated in 2000 with funding provided by APHIS. Components used in Test 1were tested in the Urals, and The Russian Far East, and repeated in south Central Siberia. Analysis is not yet complete.

In 2000, fir race larvae were brought to the U.S. Rearing attempts to date have been successful with the successful rearing and mating of adults in laboratory conditions. Successful establishment of a colony will allow opportunities to continue to identify pheromone components and test for attractiveness in wind tunnels, etc.

Transferring results: –An agreement and purchase order have been developed with Morgan State University for preparing the synthetic attractant (Ald + OH), to be used in port trapping in the U.S. to detect introductions.