FINAL PROJECT REPORT
WTFRC Project Number: AE-04-429   (WSU Project 3643-7366)

Project Title: Mechanisms underlying mating disruption

PI:	Vincent P. Jones	**Co-PI(2):**	Jay Brunner
Organization:	WSU-TFREC	**Organization:**	WSU-TFREC
Telephone:	509-663-8181 x 273	**Telephone:**	509-663-8181 x 238
email:	vpjones@wsu.edu.	**email:**	jfb@wsu.edu
Address:	1100 N. Western Ave	**Address:**	1100 N. Western
City:	Wenatchee	**City:**	Wenatchee
State/Province/Zip	WA 98801	**State/Province/Zip:**	WA 98801

Budget History:

Item	Year 1: 2004	Year 2: 2005	Year 3: 2006
Salaries	22,724	23,634	24,377
Benefits	7,272	7,563	9,263
Wages	12,800	12,800	12,800
Benefits	2,048	2,048	1,408
Equipment			
Supplies	3,200	3,200	5,700
Travel	3,200	3,200	1,500
Miscellaneous			
Total	51,244	52,445	55,048

Objectives:
1. Examine the role that female and male mate choice plays in the mating success of CM and OBLR.
2. Examine the effect of male age on female reproduction in CM.
3. Determine if the delay in mating should be calculated on a calendar or a degree-day basis, and determine if we can predict severity of CM and OBLR problems based on the average delay in mating experienced during the spring related to weather patterns.
4. Investigate the importance of CM dispersal between MD and non-MD areas.

Significant findings:
- Our data show that CM females discriminate against males older than two days, resulting in a reduction in successful matings even in no-choice situations. CM males did not show significant age-based discrimination.
- OBLR males and females both showed age-based discrimination that resulted in a reduced percentage of successful matings.
- Female CM mated to older males showed significant reductions in reproductive output compared to females mated to young males. The effect was pronounced (20-45% reduction) but smaller than reductions found when female age was increased.
- We developed a method to partition the codling moth and OBLR models to allow us to determine male age distribution at any point in the season. Combined with other studies, we were able to show that male age effects result in a roughly 15% decrease in reproductive rate.
- Our previous studies showed conclusively that the delay in mating for both males and females should be calculated on a calendar date basis.
- We found in lab wind tunnels that CM could not successfully contact a lure when speeds were over 2.2 mph and OBLR were unable to successfully contact a lure over 3 mph.
- Studies in the field showed that under certain circumstances, the reduction in mating caused by wind velocity over 2.2 mph was significant compared to situations with no wind. The effects were highly location and orchard specific.
- Our studies to determine the importance of CM dispersal between MD and non-MD this year were unsuccessful because of weather and location-specific problems in the first generation. The hot spell in May and the rainy weather along with low trap catches at the test orchards resulted in our being unable to complete this objective.

Objective 1

Our data from last year showed that even in no-choice tests female CM discriminated against males that were >2 days old. The study also showed that 6-day-old males were able to successfully pass a spermatophore only 50% as often as males aged 0 or 2 days. In contrast, males showed little age-based discrimination against females, and even 6-day-old females were able to mate at roughly 87% of the rate of newly emerged females (Fig. 1).

In the no-choice trial with OBLR, mating success decreased with age in both sexes similarly compared to individuals paired on the day of emergence. By day 6, males were only 70% as efficient at mating as males paired on the day of emergence and females were about 66% as efficient (Fig. 2).

Fig. 1. Proportion of CM pairs where a spermatophore was successfully passed. Values corrected relative to the success for 0 day old individuals.
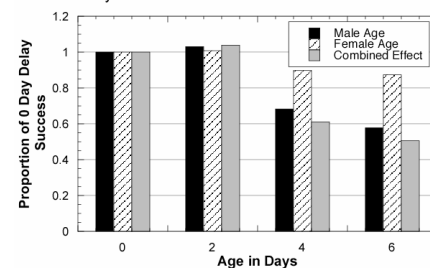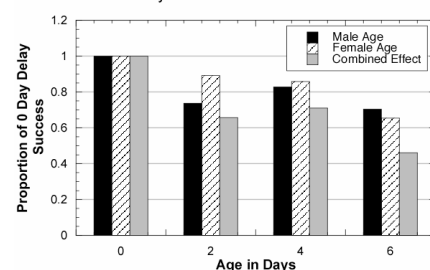

Fig. 2. Proportion of OBLR pairs where spermatophores were successfully passed. Values corrected relative to the success of 0 day old individuals.

Objective 2

Last year we finished our laboratory studies on the effect of CM females mating with males of different ages. We found that the net reproductive rate (average number of daughters produced over the average female life span) dropped as the male age increased past four days (Fig. 3). The reduction in population growth associated with older males being mated to females of a fixed age is similar (but of lower magnitude) to our previous studies where females of different ages were mated with fixed-age (1-day-old) males. In this study, there were two components affecting female reproductive output: (1) as male age increased, the incidence of no eggs produced also increased and (2) there was a change in reproductive rate not associated with number of eggs produced (*i.e.* a decrease in percent egg hatch).

Fig. 3. Effect of male age on female reproductive output. All females were mated at 4 days old.

These data, in conjunction with our previous data on female age at time of mating, clearly show that the ages of both sexes at time of mating are critical to population growth. Combined with our data in Objective 1, it appears that incidences of zero reproduction are likely to be caused at least in part by female rejection of older males. While young males may reject some older females, it appears to be relatively rare.

This year, we concentrated on integrating the effects of male age on female reproductive rate in our model of the effects of delayed mating. We accomplished this by taking the current CM phenology model and partitioning the output so that the number of moths emerging per day could be tracked. This allowed us to take field temperatures from TFREC and our information on field longevity of CM (reported below) and determine the male age distribution of the population at any point in time. This was then combined with our laboratory information on the effect of male age on female reproductive rate. When this was done, we were able to show that male effects were relatively constant (they caused roughly a 15% decrease in female reproductive rate) after the peak emergence of CM (Fig. 4). Before peak emergence, the population was increasing so rapidly that the male population was heavily skewed towards those just emerging so the effects on female reproductive output are relatively minor. After peak emergence, the effects on reproductive output fluctuate only mildly, even at the times between generations.

Objective 3

Last year we finished the original objective and showed with lab studies that the effects of delayed mating occur on a degree-day (DD) basis. In those studies we took newly emerged individuals and placed them for a constant number of DD in either 15 or 30°C chambers. For both OBLR and CM, the reproductive rates were very similar even though the moths placed in the 30°C chambers were aged for two calendar days vs. 10 days at 15°C for the other treatment.
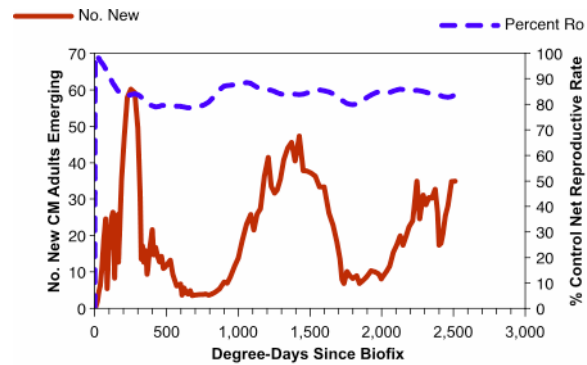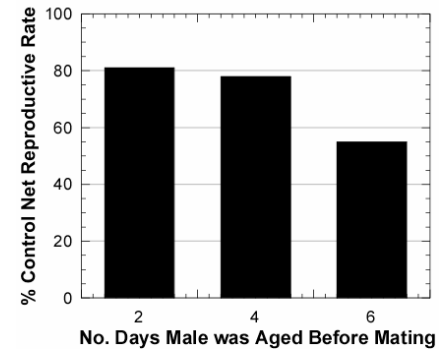
Fig. 4. Graph shows the effect of male age over the season in Wenatchee, WA based on phenology of emergence, temperature driven mortality, and male age on female reproductive rate. Solid line is the phenology of codling moth, dotted line the percentage of the reproductive rate of females mated to males on the day of emergence.
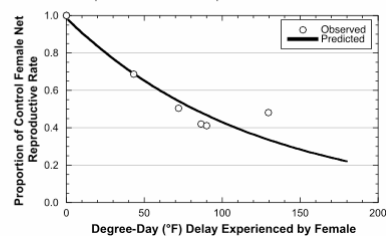
Fig. 5. Mortality rate of CM females in the field at TFREC in 2004-2005.
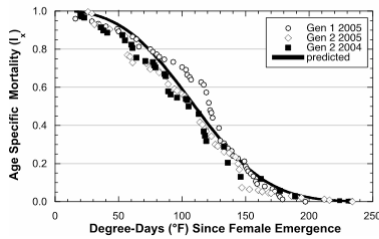
Fig. 6. Shape of the oviposition curve for CM females. Includes those with and without a delay in mating.
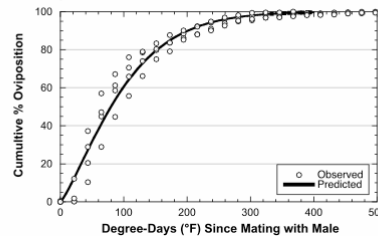
Fig. 7. Effect of delaying female CM mating on net reproductive rate. All rates scaled so undelayed females reproductive rate equals 1.

We also presented information that showed we were able to predict the longevity of adult OBLR and CM moths of both sexes based on DD (Fig. 5). In addition, we showed that the shape of the oviposition curve was easily predictable by the amount of time (measured in DD) since mating (Fig. 6). Finally, we were also able to show that the effect of a delay in mating could be predicted again using DD (Fig. 7).
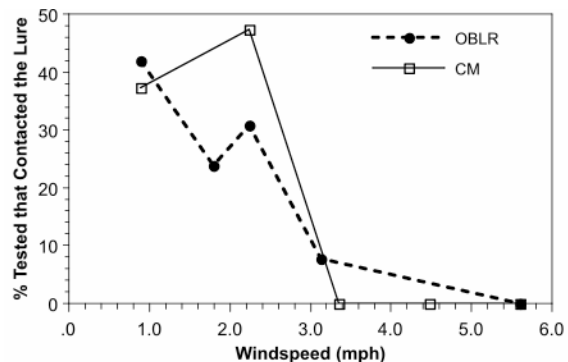
Last year, we expanded our original objective to look at the effect of wind velocity on mating. This objective has both a laboratory and a field component. In our laboratory wind tunnel, we were able to get information on the males' ability to fly upwind and locate a pheromone lure at different wind speeds. In the field, we placed anemometers in three apple orchards of varying configurations and at different times throughout the spring and summer.

In the lab wind tunnels, we found that male CM were unable to fly against a wind of 3.3 mph; they were all sucked backward into the rear of the tunnel where the fan was housed. If the wind speed was reduced, it was not uncommon for those moths to emerge from the back chamber and fly towards the lure. None of the moths was able to orient and locate the lure above 2.2 mph in our wind tunnel tests. Between 40 and 50% of the moths were able to locate the lure at 1.1-2.2 mph (Fig. 8).

In our laboratory OBLR tests, we did find that a relatively small percentage of male moths (7.7%) was able to orient and successfully contact the lure at 3 mph but none at higher wind speeds. There was a strong drop off in ability to locate the lures as wind speed increased (Fig. 8).

In the field, we tested three apple orchards: Block 16 at the Tree Fruit Research and Extension Center (TFREC), a commercial orchard in East Wenatchee, and a commercial orchard in Quincy. The tests were run at different times of the season so some of the differences could simply be a result of prevailing weather conditions. The biggest differences among the orchards were that the TFREC orchard is planted at 9x18 feet (≈270 trees/acre), the orchard in East Wenatchee is planted at 5x15 feet (≈580 trees/acre), and the Quincy orchard is planted at 5x13 feet (≈670 trees/acre). Additionally, the tree rows at the TFREC and East Wenatchee sites are positioned perpendicular to the prevailing wind direction, and the tree rows at the Quincy site are positioned parallel to the prevailing wind direction. In all situations, one anemometer was placed at the upwind edge of the orchard, and the others distributed throughout the orchard. We moved the anemometers at roughly 1-2 week intervals.

Fig. 8. Effect of wind speed on the ability of OBLR and CM to contact a lure in wind tunnel assays. All moths pre-conditioned to wind velocity for 15 min before flying.

Analysis. We analyzed the data by determining the daily time of sunset and examined the wind speed

for two hours before and two hours after sunset (this corresponds to most CM flight). We recorded the average wind speed at five-minute intervals (TFREC and East Wenatchee) and one-minute intervals at the Quincy orchard. Each day, we recorded the percentage of intervals in the four-hour flight period where the average wind speeds were >2.2 mph (speed above which CM were unable to locate the lure in our wind tunnel experiments).

Results. The TFREC orchard showed the highest wind velocities and the greatest percentage of times where moth flight would be unlikely to lead to mating. During the first period, the percentage of unsuitable times was between 75 and 95% of the entire flight period for anemometers on the edge or close to the edge of the orchard, with 55% being unsuitable for interior locations (Table 1). Over all sensor locations over the period of 14 June to 26 July, 48% of the time the average wind speed during the flight period was >2.2 mph (Table 1).

The orchard in East Wenatchee has a wind break about 150 feet in front of the block. At this orchard, we virtually never (0.3%) exceeded the 2.2 mph threshold for the average wind speed. However, the gust speed was over the threshold 38% of the time, with the greatest frequency in the interior of the orchard (Table 1).

The Quincy orchard is oriented so that the prevailing wind parallels the tree rows. In this configuration, the station on the edge of the orchard had the highest frequency of wind velocity exceeding the 2.2 mph threshold, as would be expected. The second highest total came from the station in the same row but 80 m towards the center of the block.

While it is tempting to assume that the differences in wind speed in the orchard are related to the different tree spacing, we cannot do so because the data were not taken concurrently. This means that some of the differences are likely driven by the seasonal and daily fluctuations occurring at the different times the stations were out, as well as the locations themselves. We expect that the tree density does affect weather readings and hence the percentage of times that the male moths can successfully locate calling females, but the exact contribution of tree density vs. location vs. time of the year cannot be determined from this study.
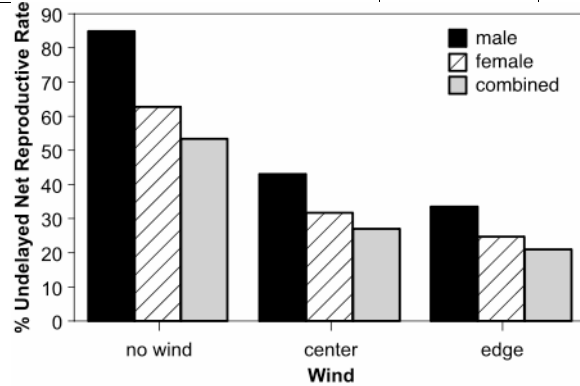
Of primary importance, our data do show that relatively modest wind velocities can dramatically affect the ability of male CM and OBLR to locate females. If wind velocities correspond to high temperatures over a key portion of the flight, the delay in mating may dramatically lower the reproductive capacity of the population, leading to lower pest pressure. Secondly, our data suggest that the edges of the orchard experience the greatest reductions in mating time because wind velocities are highest there. This is an interesting possibility because it would suggest that in windy

Table. 1. Percentage of recording intervals occurring 2 hrs before and after sunset that had wind speeds >2.2 mph.

Dates	% Intervals > 2.2 mph			Location	Tree Spacing
	Average[1]	Highest Sensor	Lowest Sensor		
14 June to 19 June	82.2	95.1	55.6	TFREC	9 x 18
20 June to 12 July	32.1	51.9	6.9	TFREC	
13 July to 18 July	74.4	84	56.9	TFREC	
19 July to 26 July	45	49.4	41.4	TFREC	
27 July to 9 Aug	0.11	0.4	0	E. Wenatchee	5 x 15
14 Aug to 17 Aug	1.04	3.1	0	E. Wenatchee	5 x 13

21 Aug to 9 Sept	5.7	25.3	0	Quincy	5 x 13

[1]Average of all 6 sensors scattered throughout the orchard

locations the upwind side of the orchard would have a sort of natural delay of mating and may not require heavy border treatments to reduce damage in those areas. Finally, in designing and planting orchards, maximizing wind flow may reduce the overall codling moth pressure by maximizing wind velocity.

Fig. 9. Comparison of male and female age on net reproductive rate from 14 June to 26 July at TFREC. No wind shows effects of age alone, center indicates effects using wind speed in the interior of the block, edge using wind speed at the edge of the block.

To determine how important the delay in mating caused by wind could be, we used daily wind speed in our TFREC orchard to determine the reduction in female reproduction caused by wind speeds >2.2 mph. This model combined the wind speed information with the survival and age distribution of both males and females to determine the overall impact of the natural delay in mating as a percentage of the net reproductive rate of females that were able to mate on the day of emergence with newly emerged males.

The results of the model were evaluated by first considering the effects by sex separately, then combined, using no wind effects. Secondly, the model was run for the same period in the same fashion but incorporating the proportion of times that flight was inhibited by wind velocity >2.2 mph. The black bars on Fig. 9 show the effects of male age on female reproductive rate, the striped bars the effect of female age at time of mating, and the grey bars the combined effect on a female's reproductive rate during the period of 14 June to 26 July (the period for which we had wind data). Examination of the black bars shows that male effects alone cause a 15% reduction in population growth rate, female effects yield a roughly 37% drop and the combined effects result in a drop of about 47% compared to individuals that experience no delay. The wind effects are rather dramatic for both sexes, and both result in a decrease of >70% in the net reproductive rate for individuals that experience no delay.

Objective 4

Our studies on dispersal over the past two years showed that the scales of our experiments were too small because we still caught multiple marked individuals in the traps that were the greatest distance from our protein-treated areas. Even so, our studies showed that moths flew an average of 390 feet and we still caught multiple marked moths at distances >800 feet away. Our studies did suggest that there might be differences in the average flight distance between plots where Assail or Guthion were used as cover sprays. Parts of the experiments planned this year were to investigate if these differences in flight distance were related to cover sprays applied.

We set up three experiments to examine codling moth dispersal between MD and non-MD areas during the spring. Unfortunately, the warm period in the spring accelerated the emergence and although we were able to apply our markers, rain began and lasted for roughly two weeks on and off and dramatically affected both the flight and the retention of our markers. In addition, in an orchard where we had caught hundreds of moths last year we caught only 23 moths during the first flight this year, with over 140 traps placed. As such, in the second generation, we needed to find new sites and

our planned experiments (looking at movement from MD to non-MD and the effects of cover sprays) were impossible to perform. Instead of the planned experiments, we concentrated on examining the

role of CM movement from abandoned or poorly managed areas to well managed areas, which was easily testable during a single generation. Those experiments are reported in Objective 3 of our grant on dispersal (AE-04-428).