Reproductive biology of *Dioryctria abietivorella*
PMTAC final report
March 31, 2009

Research in 2008 focussed on flight phenology, female calling behaviour, and adult longevity. The following report follows the outline of the research proposal for 2008.

Mating Behaviour

It is important to know when moths are reproductively active, during the adult lifespan, season and day, in order to develop pest management tactics that target reproductive behaviours such as pheromone-based monitoring, mating disruption or attract and kill technologies.

1. Light trap monitoring

   Objectives:

   - To determine the flight phenology and reproductive status of female *D. abietivorella* moths in seed orchards throughout the flight season.
   - **Management objective:** Knowledge of reproductive biology is essential for the eventual development of successful pheromone–based management of *Dioryctria*. With information on the timing of female flights, we can better target pheromone-based management tactics in the orchard. We will determine flight duration and likely moth origin inside or outside the orchard of interest. Mated moths originating outside the orchard and dispersing into a treated orchard would negatively impact mating disruption efforts. Further, our studies will assess mating status and frequency of female *Dioryctria* throughout the season which will delineate the times at which females are reproductively active throughout the season. In general, knowledge of reproductive behaviour of the target insect is essential in the development of pheromone-based pest management strategies.

   Progress to date:

   Female seasonal flight activity and mating status were monitored using an improved protocol modified from 2007. In June 2008, two black light traps were placed in Douglas-fir blocks at KRC, Vernon Seed Orchard (VSOC) and PRT. The traps, separated by at least 500 m, were hung on a line strung between two trees and positioned in the upper-mid canopy. Traps were run five days a week and monitored twice during that period. Females were captured from 20 June to 19 September, 2008. The collected females were dissected to assess mating status based on the presence and number of spermatorphores in the *bursa copulatrix* and to confirm species identification. Because of the difficulties in identification to species, taxonomic groups (Neunzig 2003) will be used in analyses. Females from the *abietella*, *schuetzeella*, *ponderosae*, *zimmermani* and *auranticella* species groups were captured. Multiple mating occurs in females of all species collected (Fig. 1). *Abietella* gr. and *schuetzeella* gr. females remated more frequently than females from other groups (Fig. 1). The distribution of mating frequency varied by species group (chi-square=188.130, p=<0.000). Seasonality appears to influence mating frequency for some, but not all, of the species groups (Fig. 2). There is a significant effect of season in *schuetzeella* gr. mating frequency (Mann-Kendall test p=0.024), while *abietella* gr. females do not show a seasonal trend (Mann-Kendall test p=0.454) when analyzed. The remaining groups were captured in numbers too low to conduct any analyses. We are in the process of finding a reliable way to measure spermatophore size to determine if male reproductive investment changes throughout season. In 2009, the light trapping experiment will be expanded to incorporate the beginning of the flight period in April and May and flight activity in other host blocks (pine and spruce). These data will indicate the importance of host association on moth capture and mating status throughout the season. Wing area measurements for female *D. abietivorella* have been completed and will be used as a covariate in analyses.

Male *D. abietivorella* adult flight was also monitored weekly between 4 May to 30 September, 2008 using pheromone traps located in KRC, VSOC and PRT Douglas-fir blocks. Trap capture of male moths was compared with female moths captured in light traps (Fig. 3). Females stopped appearing in the light traps after 17 August, 2008 while males were still captured in pheromone-baited traps after this time. It should be noted that 96 of the 97 *D. abietivorella* females caught had mated at least once. Females may not disperse before mating and therefore may not encounter the light traps. As this technique may not attract virgin females it would not be useful for tracking flight phenology of the entire population. It is interesting to note that males are present and flying in late summer and early fall when no females were captured in the light traps. Because only mated females were captured, it is difficult to know if females have died off or are not mated and not captured in traps late in the season.

2. Calling behaviour

Objectives:

- To determine when, within day and season, female moths release pheromone to attract males.
- To determine if female calling behaviour varies with female age.
- To determine if female attractiveness varies with female age.
- **Management Objective:** This information will help to develop appropriately timed and positioned pheromone-based management strategies. Knowing exactly when females call will enable us to time pheromone release to maximize disruption of pheromone-based mating behaviour, while minimizing the amount of expensive pheromone used. Furthermore, it may be possible to modify pheromone release through the season if we know that aging females call differently or become less attractive with age. This information can also be used to maximize the efficacy of other pest management strategies targeting active adults. In general, the design of integrated pest management strategies is dependent on basic biological knowledge of the target pest. Development of pheromone-based control strategies depends on understanding pheromone-mediated reproductive behaviour.

Progress to Date:

Originally this experiment was to be conducted at various times throughout the season to assess the effect of season on calling behaviour. However, we were only able to rear adults by August 2008 due to the unseasonably cold weather conditions. Therefore, this experiment was conducted only during the late season and addressed the periodicity of female calling behaviour over a 24-hour period and how this behaviour is modified by female age. We conducted five trials with the first occurring in mid-August. Three trials were conducted in the field and two in the laboratory at KRC. Females were from one of three ages groups: young (0-1 day old), middle (5-7 days) and old (10-12 days). In the field trials, insects were placed individually in a mesh-capped 207 ml plastic cup and provided with sugar water. The cup was suspended from a Douglas-fir branch at a height of approximately 1.5 m. Female behaviour was monitored every 30 minutes over a 24-hour period. Because of the lateness in the season and resulting cool temperatures further trials were conducted indoors. The same protocol was used for trials inside the research station with minor modifications. During the first indoor trial, we did not provide a host source but did so for the second trial. The presence of foliage seemed to stimulate oviposition behaviour rather than calling behaviour as females were observed ovipositing when foliage was present in the cups.

Preliminary results indicate that a greater percentage of 5-7 day old females will call, followed by 11-12 day and then 0-1 day old females (26%, 22% and 7% respectively). The relative percentage of females calling in each age group varied between trials (Table 1). Calling was initiated anywhere between 30 to 560 minutes after sunset/lights off and these extremes were demonstrated by females 0-1 day old. The mean onset of calling (minutes after sunset/lights off) by age groups varied between trial dates (Fig. 4) making it difficult to draw any conclusions at this point. Calling duration ranged between 30 to 540 minutes and, with the exception of these extremes, female calling duration did not appear to vary widely among age groups (see Fig. 5).

Using larger sample sizes and more trials, this experiment will be repeated in 2009 to accurately determine if differences in calling behaviour are demonstrated by females of different ages and if there is an effect of season. Additionally, because there appear to be differences in the production of chorionated eggs (discussed later in the Oviposition Behaviour section) I will separate young females into two age groups: 0 day and 1 day old. In fall 2008, some of the remaining field-collected cones contained mature larvae that had not exited. These cones were placed in large plastic containers and set outside until 7 December, 2008, then placed in a -2 C cooler to overwinter. The adults resulting from the overwintering larvae will be used to conduct calling trials in early May.

Trade-offs between reproduction and longevity

It is important to know the potential adult lifespan of moths so that pest management activities targeting mating and reproduction can be appropriately timed. It is important to assess longevity of mated and virgin moths as reproductive activity often results in a trade-off with adult longevity in moths thus resulting in a shortened adult lifespan. Knowledge of adult longevity will also allow us to pick appropriate age intervals for experimentation on calling and oviposition behaviours (below).

1. Adult Longevity

Objectives:

- To determine the lifespan of virgin and mated male and female *D. abietivorella* moths in the laboratory.
- To determine if lifespan is modified by mating status.
- **Management objectives:** This information will help guide insecticide control programs, determine extent of generation overlap, and help in making decisions about future damage potential based on current *D. abietivorella* densities. For example, if overwintered adults of *D. abietivorella* live only a short period, then perhaps a single well-timed spray will result in season-long control. Further the longevity of mated and virgin females is crucial knowledge for the development of pest management strategies targeting reproductive behaviour.

Progress to Date:

Experiments measuring the lifespan of mated male and female adult *D. abietivorella* in 2008 did not result in successful matings. Mating status was confirmed by dissection and none of the females contained spermatophores. The average adult lifespan does not appear to differ between sexes nor between moths held individually or with another moth in the same cup (Table 2). The lifespan of moths in this experiment is shorter than those initially observed in 2007, but this difference may be due to dissimilar rearing conditions (diet reared in 2007 versus cone-fed in 2008). Additionally, the lifespan of females used in the August and September 2008 calling trial was greater than 11 days. The longevity experiment was conducted in late September/early October and it could be that the shorter lifespan of moths observed in 2008 is due to a seasonal effect. Because of these apparently contradictory findings and only being able to assess virgin moths in 2008, this experiment will be repeated in 2009.

My results indicate that the presence of a second moth in the same rearing container does not reduce lifespan of either individual (Table 2). There was no significant reduction in lifespan as a result of pairing and therefore it can be assumed that any changes we observe in the longevity of mated pairs in 2009 are due to a mating and not a pairing effect.

Proposed research: April 2009 – March 2010

Our main objectives for this season will be to assess seasonal flight phenology, mating status throughout the flight period, female calling behaviour, adult longevity and egg production. All work, unless otherwise stated, will be conducted in the Douglas-fir breeding orchards located at the Kalamalka Research Centre (KRC). All insects for laboratory and field experiments will be reared from Douglas-fir cones collected at KRC. Voucher specimens of male and female *D. abietivorella*, harvested from light trap catches at several times through the season as well as sample individuals from each experiment, will be deposited in the Strickland Entomological Museum, University of Alberta.

Figures

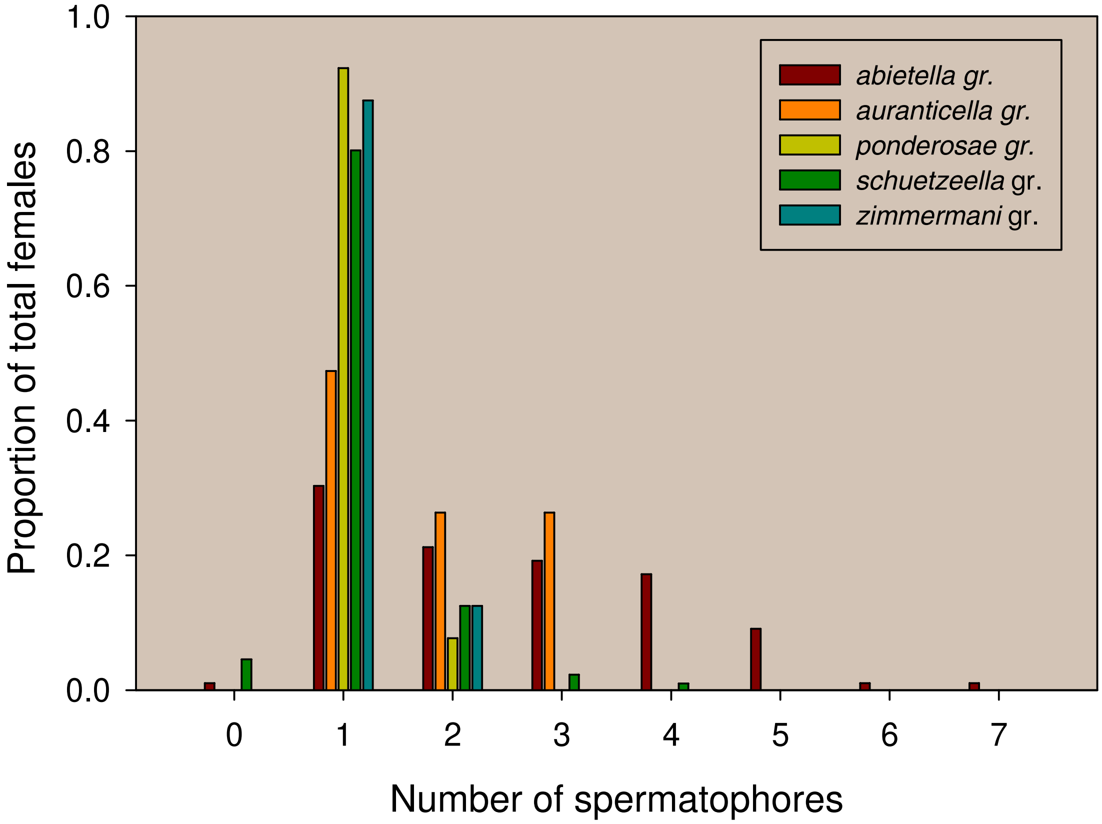

Fig. 1. Mating frequency of female *Dioryctria* spp. Bars represent the proportion of the total number of females from each species group. Data was pooled from six traps throughout the sixteen week trapping period.

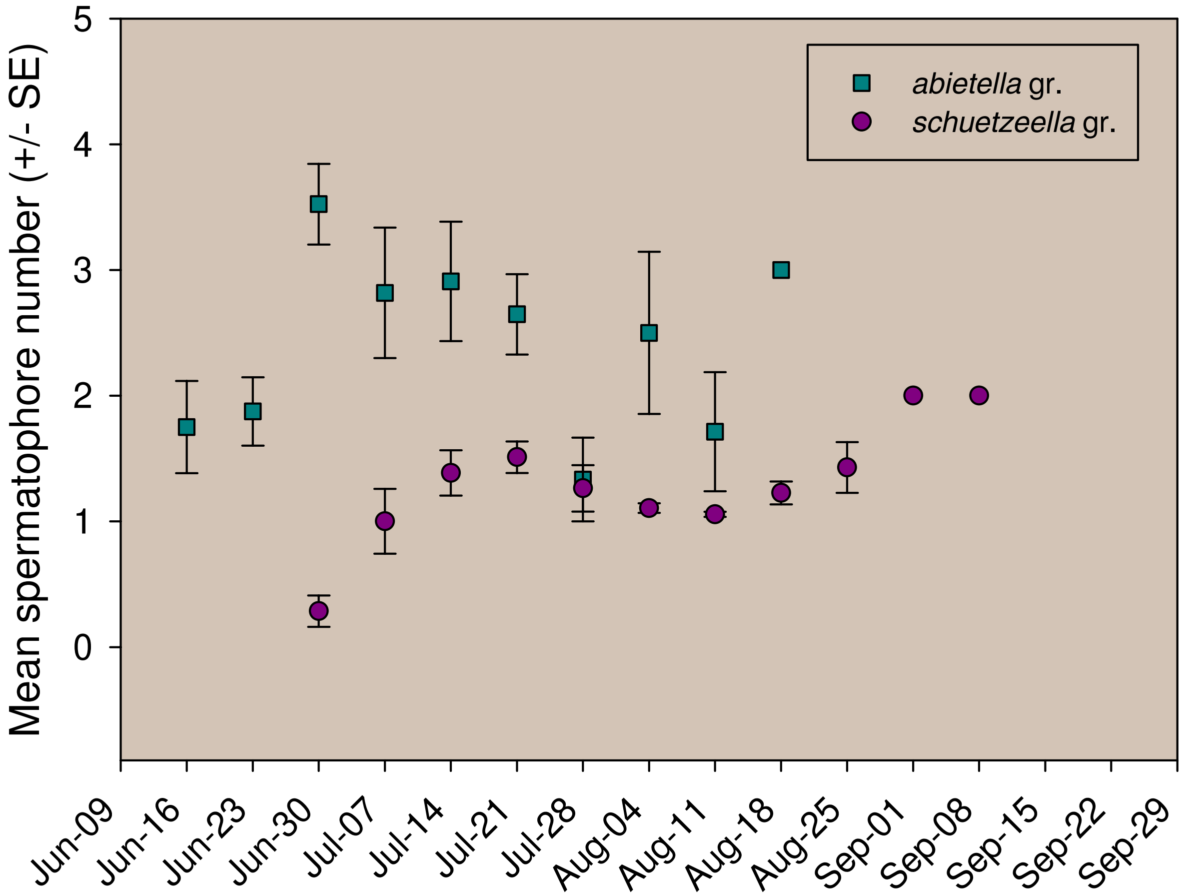

Fig. 2. Seasonal spermatophore number (mating frequency) of female *Dioryctria* spp. Points show mean spermatophore number by species group caught over a five day period. Data was pooled from six traps (two at each site) throughout the sixteen week trapping period.

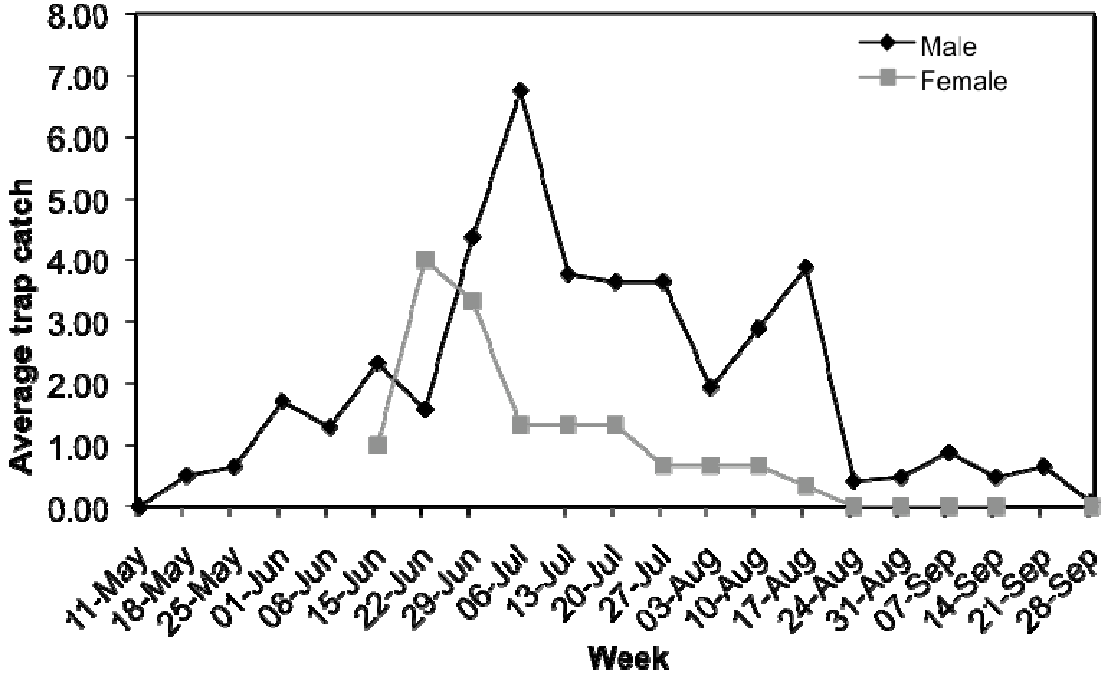

Fig. 3. 2008 seasonal flight of male and female *Dioryctria abietivorella* in British Columbia. Males were caught using pheromone-baited diamond traps and females were caught using UV light traps. Data is compiled trap catches at 3 sites conducted between May 4th to October 4th, 2008. Trapping was conducted in Douglas-fir blocks at the Kalamalka Research Centre, Vernon Seed Orchard and PRT in the Okanagan Valley, British Columbia.

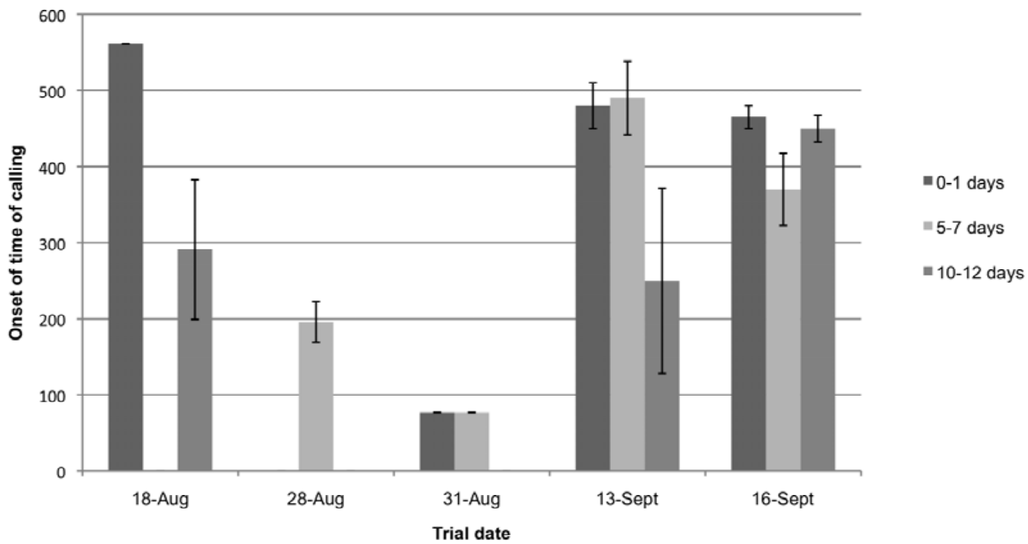

Fig. 4 Mean onset of calling (min. after sunset/lights-off, +/- SE) by virgin female *D. abietivorella* from three ages groups (days post-eclosion).

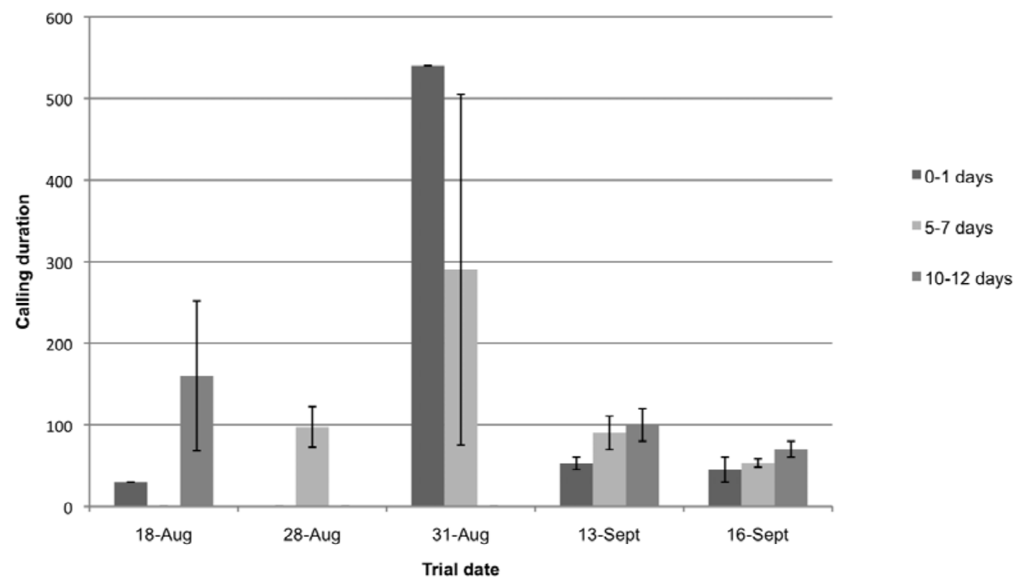

Fig. 5 Mean duration of calling (min., +/- SE) by virgin female *D. abietivorella* from three ages groups (days post-eclosion).

Table 1. Number of adult female *D. abietivorella* observed and calling and percent calling (in brackets) during 24-hour calling trials.

Location	Date	Age (in days)					
		0-1 days		5-7 days		10-12 days	
		# observed	# calling	# observed	# calling	# observed	# calling
Outdoor	Aug 18	3	1 (33)	2	0 (0)	3	3 (100)
	Aug 28	14	0 (0)	18	8 (44)	0	N/A
	Aug 31	22	0 (0)	11	3 (27)	9	1 (11)
Indoor	Sept 13	30	4 (13)	29	6 (21)	19	3 (16)
	Sept 16 *	27	2 (7)	28	6 (21)	14	3 (21)

* host material (Douglas-fir foliage) present in holding container

Table 2. Average lifespan and number of observed individual adult *D. abietivorella* (measured in days, ± SE).

Sex	Treatment		N	
	Alone	Paired	Alone	Paired
Male	10.6 (± 0.81)	9.2 (± 0.66)	19	10
Female	10.3 (± 0.86)	10.1 (± 0.79)	13	13