Is it possible to use mass-reared or field-collected diapaused codling moth larvae, *Cydia pomonella* (Lepidoptera: Tortricidae), to predict spring biofix?

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ABSTRACT

Codling moths, *Cydia pomonella* (L.), from a mass-reared colony induced into diapause and from locally collected overwintering populations were placed in the field inside mesh cages in the fall of 1997 to determine whether they would synchronize their spring emergence with the wild population and thus could be used as a tool to set biofix. Our results show that the laboratory-reared moths emerged at approximately the same time regardless of the location where they spent the winter. Locally collected (and caged) wild material always emerged later than the remaining wild population and thus was no better at predicting biofix than were laboratory-reared insects.

Key words: *Cydia pomonella*, diapause, biofix, emergence

INTRODUCTION

The codling moth, *Cydia pomonella* (L.) (Lepidoptera: Torticidae), is the key pest of apples and pears in the Pacific Northwest (Madsen and Procter 1982). It is a multivoltine species that possesses facultative diapause (Riedl 1983; Brown 1991). In the Okanagan and Similkameen Valleys of British Columbia, codling moth typically completes two generations per year (Madsen and Vakenti 1973), and in the Yakima Valley of Washington, USA, it completes two generations per year with a possible small third generation occurring in some seasons (Newcomer and Whitcomb 1924). Wild codling moths spend the winter as mature 5th-instar larvae in diapause, defined by Dickson (1949) as a physiological stage of arrested development that enables an organism to survive unfavorable conditions. Silken hibernacula are spun under loose bark scales, in litter at the base of trees, on tree props or fruit bins in the orchard or on farm buildings near cull piles (Beers *et al.* 1993). Overwintering larvae break diapause and pupate inside their cocoons in early spring about the time when the first apple blossoms show pink color (Beers *et al.*

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1993). In southern BC, peak flight activity of overwintered adults occurs in early May, and in late July-early August for the summer generation (Madsen and Procter 1982).

Biofix is a biological fix point used to synchronize phenology (or day-degree) models with insect development (Beers *et al.* 1993). Phenology models for *C. pomonella* were developed by Riedl *et al.* (1976), and are widely used to predict codling moth egg hatch and to time more precisely the application of pesticide cover sprays. To determine biofix for codling moth, growers typically place pheromone-baited traps in the orchard before apple flower-bud development to capture the emerging overwintered adults. The date male moths first begin to be consistently captured in pheromone traps (e.g., one or more moths captured on successive days) is used to set biofix. Codling moth pheromone-baited traps have been shown to be only about 10% efficient at capturing feral moths when deployed at the standard trap density of one per ha (Anonymous 1997). When feral populations are small or when trap densities are lower than recommended, it might be difficult to determine when to set biofix, which in turn will lower the predictive accuracy of the codling moth phenology model.

We have developed the technology to induce laboratory-reared *C. pomonella* into diapause in the open tray diet system used for mass-rearing these insects in BC, Canada (Bloem *et al.* 1997, 1999). Mass-rearing under conditions of diapause induction allows for the collection of large numbers of overwintering larvae into corrugated cardboard rolls. Some of the benefits of producing insects in this manner have been discussed elsewhere (Bloem *et al.* 1998, 1999). Here we examine the possible use of laboratory-reared larvae in diapause as a tool to help set biofix in the field. Specifically, we asked the questions: 1) Do codling moths induced into diapause in the laboratory emerge similarly to wild *C. pomonella* when placed in the field and exposed to the same winter field conditions, and 2) can field emergence of the laboratory-reared insects be used to accurately set biofix?

MATERIALS AND METHODS

Experiment 1: Effect of Induction Conditions on Spring Emergence

Sample Preparation and Collection. On 18 August 1997, laboratory-reared codling moth larvae were induced into diapause under mass-rearing conditions at the SIR rearing facility in Osoyoos, BC, by altering the photoperiod and temperature during rearing as outlined in Bloem *et al.* (1997). However, to improve the efficiency of induction into diapause the temperature during scotophase was lowered to 21°C. Mature diapaused larvae were collected into C-flute corrugated cardboard rolls (15.25 cm diam. x 2.50 cm) when exiting the diet. The infested rolls were stored at 15°C, 0L:24D and 60% RH inside black polyethylene bags until needed.

Samples of diapaused summer generation wild *C. pomonella* were collected from infested apple orchards in Kelowna and Creston, BC, by placing corrugated cardboard bands around the trunks of apple trees in mid-August, and removing the bands infested with overwintering larvae from the trees in early November 1997. Bands were stored as indicated above for laboratory-reared insects. Diapaused codling moth larvae from Osoyoos, BC, were collected from infested apples removed from local orchards in mid-September 1997, by SIR Program staff. Apples were placed into 12 large plastic bins (125 x 50 x 40 cm) that had been lined with corrugated cardboard strips. The bins were covered with thin muslin cloth and placed under shelter in an Osoyoos orchard for 6 weeks to allow the larvae to exit the fruit. At that time, bins were uncovered, apples were discarded, and the cardboard strips removed and stored in bags as described above.

On 15 November 1997, the cardboard rolls and bands containing diapaused laboratory-reared and wild codling moth larvae were prepared for field placement inside a walk-in cold room (0-2°C). Material from each of the four cohorts (laboratory, Kelowna, Creston

and Osoyoos) was divided into three equal groups, and each group was placed into an individual cylindrical fiberglass mesh and wire cage (23 cm diam. x 60 cm). Cages with laboratory-reared material contained no fewer than 100 larvae in diapause. The number of larvae in each wild sample varied, but was never less than 25 larvae per cage.

Field Placement and Cage Monitoring. On 20 November 1997, the cages containing wild-collected and laboratory-reared diapaused larvae were transported inside coolers to an apple orchard in each of three locations - Kelowna, Creston and Osoyoos, BC. The orchard at each location was an established traditional planting of 'Red Delicious' trees (avg. tree height ca. 4 m, row spacing 5.5 x 3.7 m). Because codling trap captures throughout the SIR treatment area (i.e., in Osoyoos and Creston) were generally low in 1997, the orchards were chosen for having known codling moth infestations, as well as for general similarities in orchard structure. Four cages, one from each cohort, were hung in each orchard at the three locations in apple trees at a height of 1.5-2.0 m above ground and within 10 m of one another. The cages remained in the orchards until the following spring. In mid-April 1998, pheromone-baited codling moth traps were hung (one trap/ha and five traps/orchard; traps were hung in the upper 1/3 of the tree canopy) in the same orchards where the cages were located, but no closer than 25 m to the cages, to capture wild codling moth males emerging from diapause. Adult moth emergence inside each cage, and trap captures at each location were checked daily beginning on 15 April 1998. Biofix at each location, both inside the cages and in the orchard, was set when one or more male moths were captured on successive days.

Experiment 2: Synchroneity of Emergence of Laboratory-Reared and Wild Moths

Sample Preparation. Laboratory codling moth larvae were induced into diapause, allowed to enter corrugated cardboard rolls and stored until needed as described above. On 30 November 1997, the cardboard rolls with diapaused larvae were prepared for field placement inside a cold room (0-2°C). Material was divided into six groups and placed into cylindrical fiberglass mesh and wire cages as described for Experiment 1. Each cage contained no fewer than 100 larvae and was kept in the cold room until needed.

Field Placement and Cage Monitoring. In early December 1997, the cages containing laboratory-reared diapaused larvae were transported inside coolers to pre-selected orchards located roughly along a north-south gradient from Creston, BC, Canada, to the north and Medford, Oregon, USA, to the south. Although Creston is actually 400 km east of Osoyoos in the Kootenay Mountains of BC, it was chosen as our "northern-most" site because of its cooler temperatures and historically late codling moth emergence in the spring compared to other locations at the same latitude. Locations in between the north and south endpoints were: Osoyoos, BC, Canada, Oroville, Washington, Wenatchee, WA, and Hood River, Oregon, USA. One cage with diapaused material was hung in an apple orchard at each of these locations where it remained until the following spring. In late March 1998, pheromone-baited codling moth traps were hung and used as indicated above. Adult moth emergence in each cage and trap captures at each location were checked daily beginning on 30 March 1998. Biofix for caged material and wild populations was established as previously indicated.

RESULTS AND DISCUSSION

The biofix dates for laboratory-reared and field-collected diapaused codling moth larvae caged at different locations in southern British Columbia, and the biofix dates for the wild populations captured in pheromone-baited traps at each location, are presented in Table 1. As expected, biofix for orchard populations of wild moths occurred first in Osoyoos, followed by Kelowna, and then Creston. At all locations, biofix for the caged laboratory-reared material occurred before biofix was set for the wild population using trap

Table 1

Biofix dates for caged laboratory-reared and field-collected diapaused *Cydia pomonella* larvae held at different locations in southern British Columbia, and biofix dates using adult male moth captures in pheromone-baited traps at these locations in the spring of 1998.

	Biofix date (d/mo.)		
Source of material	Osoyoos	Kelowna	Creston
Pheromone-baited trap	29/4	03/5	21/5
Caged diapausing larvae			
Laboratory-reared	27/4	30/4	04/5
Wild collected			
Osoyoos	04/5	10/5	06/5
Kelowna	06/5	09/5	12/5
Creston	01/6	- [a]	09/6

[a] No adults emerged from the Creston sample that was overwintered in Kelowna. The source of larval mortality could not be determined.

catch data. The number of days prior to orchard biofix that caged laboratory-reared material consistently began emerging was +2, +3, and +17 days for the Osoyoos, Kelowna, and Creston locations, respectively. Similarly, at all locations, the caged laboratory-reared moths began emerging before (+2 to +36 days) any of the caged field-collected material. Although these data are not presented, at each location once the laboratory-reared material began to emerge the pattern of emergence followed a typical bell-shaped curve as described by Bloem *et al.* (1997). Unlike the results for laboratory-reared codling moth larvae, caged field-collected larvae that overwintered at the same location where they were collected always emerged after the first trap captures occurred at that site. For example, biofix for the wild population in Osoyoos occurred on 29 April 1998, while biofix for the caged field-collected larvae from Osoyoos occurred on 4 May 1998 at the Osoyoos site.

Results from the experiment comparing biofix dates for diapaused laboratory-reared *C. pomonella* maintained in the field at different locations along a north-south gradient (from British Columbia, Canada, to Oregon, USA) with biofix dates for wild populations at the same locations are shown in Table 2. As in the first experiment, wild moth trap captures occurred earlier in the year at more southerly locations. Biofix occurred on 22 April 1998, in Medford, OR, USA, and on 21 May 1998, in Creston, BC, Canada. Unfortunately, biofix for the caged laboratory-reared material occurred between 27 April and 6 May 1998 at all sites, with no clear south-to-north trend.

Our results suggest that SIR colony codling moths induced into diapause in the laboratory and emerged under field conditions cannot be used to accurately set biofix at sites south of the SIR rearing facility. At these sites, biofix determined by emergence of caged laboratory-reared material occurred increasingly later than biofix for field populations determined using pheromone traps. However, as the experiment moved north of Osoyoos laboratory-reared moths emerged increasingly earlier than wild moths. As such, diapaused codling moths produced at the SIR facility would appear to have potential for use in fruit growing areas of southern BC to predict biofix, better time the initiation of spring sterile moth releases by the SIR Program and/or to establish times to set-out pheromone traps. Additional research would be needed to determine the consistency with which emergence of colony material precedes wild emergence at each location.

Table 2

Spring 1998 biofix dates for laboratory-reared diapaused *Cydia pomonella* larvae overwintered in the field at different locations along a north-south gradient, and biofix dates for orchard populations of *C. pomonella* captured in pheromone-baited traps at these same locations.

		Biofix date (d/mo.)		Time between emergence
Location		Caged lab-reared material [a]	Wild male moth trap captures	of lab-material and 1st trap captures (days)
N	Creston, BC	04/5	21/5	+17
↑				
	Osoyoos, BC	27/4	29/4	+2
	Oroville, WA	04/5	02/5	-2
	Wenatchee, WA	02/5	27/4	-5
	Hood River, OR	06/5	27/4	-9
↓				
S	Medford, OR	06/5	22/4	-14

[a] Laboratory material was reared and induced into diapause at the SIR Program codling moth mass-rearing facility in Osoyoos, BC.

It is interesting to note that field emergence of the caged laboratory-reared codling moths most closely approximated the timing of emergence of the Osoyoos wild population. This laboratory colony has been in continuous culture since 1988, initially at the Summerland Pacific Agriculture and Agri-Food Research Centre and then at the SIR mass-rearing facility in Osoyoos. The majority of the wild material used to "found" the colony came from orchards in the south Okanagan Valley and subsequent attempts to introduce wild genetic material into the colony have also come largely from orchards in and around Osoyoos.

Locally collected and caged wild material was no better at predicting biofix than were laboratory-reared insects. Wild diapaused larvae collected, caged and maintained at the same location emerged 1-2 weeks after moths were captured in biofix traps placed in that orchard (Table 1). Why locally collected, wild, diapaused material emerged consistently later than the non-collected population is not known. One possible explanation is that our field-collected samples were too small, and thus showed narrower emergence extremes relative to the wild population captured in the pheromone traps. Another possibility may be related to the fact that field samples were only collected in the fall (mid-August) and thus were taken from summer (or second) generation populations exclusively. According to Riedl (1983), a certain percentage of codling moth populations are composed of genetically univoltine larvae that enter diapause after one generation even under favorable long-day conditions. It is possible that larvae that enter diapause at the end of the spring (or first) generation are the first ones to emerge as adults the following spring, with second generation larvae (entering diapause in the summer and fall) emerging somewhat later. Cisneros (1971) and Phillips and Barnes (1975) also suggest that the first individuals to enter diapause are the first to emerge the following year; however, according to Riedl (1983) the evidence for this is still unclear. A third explanation for our results might be that there was a temperature difference between where the cages were placed and the location of overwintering sites chosen by feral populations. Larvae that spun hibernacula under tree bark and in other typical overwintering sites near the ground could have

accumulated heat units faster than did the larvae inside the cardboard rolls in our cages hung in apple trees and thus emerged sooner. Although, this argument would not explain the emergence pattern observed for the laboratory-reared material (no north-south variation in emergence date and Osoyoos laboratory material emerged similarly to Osoyoos orchard population).

Reactivation of larval development after diapause must occur at the right time to synchronize adult emergence in the spring with fruit development (Riedl 1983). The role of temperature in the regulation of physiological development is the basis upon which predictive day-degree models are constructed. Data collected by the SIR Program between 1993 and 1999 showed that site-specific biofix dates in the Okanagan and Creston Valleys, BC, varied by more than 4 weeks (e.g., 9 May in 1993 and 14 June in 1999 in Creston). However, in our experiments, artificially moving diapaused codling moths to different locations, with different winter-spring conditions, did not appear to have a notable impact on their emergence date. Wild material collected and caged at Osoyoos, BC, emerged on 4 May 1998 in Osoyoos and on 6 May 1998 in Creston, BC, compared with orchard biofix dates of 29 April 1998 and 21 May 1998 for Osoyoos and Creston, respectively (Table 1). Likewise, caged laboratory-reared insects placed at Medford, OR (the southern most location in our experiment) emerged on 6 May 1998 and 2 days earlier at the northern most location in Creston, BC, on 4 May 1998 (Table 2). In contrast, orchard biofix at these locations occurred on 22 April and 21 May 1998, respectively.

Although the primary factors regulating diapause induction are short-days and cool temperatures (Riedl 1983), Garlick (1938, 1948) as discussed in Putman (1963) suggested a partial genetic basis to voltinism in the codling moth. Brown *et al.* (1979) also proposed a genetic-nutritional mechanism for diapause induction in the first generation when the primary overwintering cues are not present. In our experiments, the fact that the geographical location where diapause induction occurred played a more important role in determining when the insects emerged from diapause than did the conditions under which they spent the winter and spring (this held for both for laboratory-reared and field-collected insects) suggests a possible genetic component to diapause termination. Codling moth larvae from a localized population may be genetically predisposed to emerge within a limited range of dates that are selected based on historical weather patterns for the area. However, additional work is still needed to fully understand the factors that regulate termination of diapause in the field.

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