

PREEMPTIVE MEASURES TO MANAGE THE RED CLOVER CASEBEARER MOTH IN OREGON CLOVER SEED CROPS

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Introduction

The red clover casebearer moth, *Coleophora deauratella* (Lepidoptera: Coleophoridae), is an invasive insect species predominantly occurring in the red clover (*Trifolium pratense* L.) seed-growing regions of North America. Since its first detection in Oregon in 2011, intermittent monitoring of this pest using pheromone traps in red clover seed fields from 2014 to 2019 has indicated high moth activity that can inflict economic damage to the crop by reducing seed yield (Walenta et al., 2020). Although high adult moth captures occurred, corresponding larval populations were not detected, potentially indicating a limiting biotic factor in Oregon. We speculated that the number of larvae might be regulated by the mechanical disruption of the insect life cycle due to silage cutting of the red clover crop prior to seed production, by presence of a natural enemy (egg or larva parasitoids), or by the presence of an additional favorable host plant. Since previous monitoring efforts were limited to commercial red clover seed fields, and the host range of this insect in Oregon is currently unknown, we employed a DNA-based method to determine whether other seed crops might be at risk of infestation by the red clover casebearer moth and to identify noncrop host plants of this insect.

Adult moth flights occur during May through August, and eggs are laid directly on newly set red clover heads. Highly concealed first- to third-instar larvae feed on developing seeds within florets. The fourth instar is easier to detect, as it constructs a portable case while feeding. During harvest in the late summer/early fall, the mature larvae crawl onto the soil surface, where they overwinter in sealed cases within crop residue until the following spring. The red clover casebearer moth has only one generation per year.

Larvae are capable of consuming two to three developing seeds per day and can cause up to 80% seed loss in red clover stands, especially in the second year of the crop (Walenta et al., 2020). Red clover seed crops in western Canada have been modified to single-year seed production systems because of the extensive damage caused by the pest by year 2 (Evensen et al., 2010). The establishment of this pest in Oregon's

red clover seed fields poses a risk to seed growers' profitability in the world's largest clover seed growing region. This pest may also impede grass seed growers from utilizing an important crop in their rotation. Therefore, it is imperative to formulate a management plan before this pest reaches its damaging potential in Oregon.

Foliar-applied insecticides with contact modes of action can be ineffective at killing the highly concealed larvae. The adult flight is protracted (i.e., moths fly for several weeks), so repeated insecticide applications may be required to effectively manage this pest and reduce its effects on seed yield. Pollinators are critical for maintaining high seed yield, making multiple insecticide applications a cause of concern for pollinator health. A phenology model to predict adult flights will be a valuable tool for growers to make informed management decisions. Targeting adults with a pheromone-based mating disruption technique has been demonstrated to be effective for adult moth suppression, significantly reducing larval numbers and increasing seed yield in an earlier study conducted in Canada (Mori and Evenden, 2015).

The objectives of this study were to test mating disruption methods, develop phenology models, examine whether additional hosts may exist, and identify the presence of natural enemies. Knowledge generated from this work will help formulate a management plan against the red clover casebearer moth in Oregon clover seed crops.

Materials and Methods

A sex-pheromone bait utilizing a pheromone blend of female *C. deauratella* (10:1 Z7-12:OAc to Z5-12:OAc, Evenden et al., 2010) was utilized to capture adult male moths in 15 commercial red clover seed production fields and 1 experimental red clover site at OSU's Hyslop Research Farm in western Oregon. A single green UniTrap was placed in each field at least 100 feet from the field edge and at crop canopy height. A septum baited with the pheromone lure was placed in the pheromone housing at the top of the trap, and the bucket on the bottom contained an insecticide vapor strip to euthanize captured moths. Pheromones were

replaced after the first 30 days. Traps were monitored weekly for 8–10 weeks. Monitoring efforts ended in late July to mid-August, depending on harvest schedules at each location. Weekly monitoring activities included: (1) collecting adult moth specimens from each trap for identification and quantification, and (2) evaluating red clover heads for larvae presence and/or feeding damage.

Phenology model

The number of moths per trap per sampling date was converted to a cumulative proportion of total trap catches over the season. Accumulation of growing degree days (GDD) began on January 1 and ended on August 4, 2020. The weather data to calculate degree-days, using daily maximum and minimum temperatures, were obtained from weather stations corresponding to the nearest field site (http://pnwpest.org/dd/model_app). An air temperature of 53°F was used as the base temperature to calculate GDD. Three-parameter nonlinear regression models (Weibull, Gompertz, and logistic) were fit using the cumulative proportion of trap catches as the dependent variable (y) and cumulative degree-days as the independent variable (x) in JMP 15 Pro (SAS Institute Inc.).

Mating disruption technique

In 2020, a mating disruption experiment was conducted on a commercial third-year red clover field site in Marion County, OR. In this study, X-mate disruption devices (Alpha Scents, West Linn, OR) were used at the recommended rate of 20 devices/acre to test whether pheromone-mediated mating disruption can suppress *C. deauratella* damage. Two green UniTraps were placed in each plot to evaluate communication disruption, at 13–14 inches above the soil surface and 41 feet from the center of the plot. Four treatment plots contained mating disruption devices that were placed 13–14 inches above the soil surface. The four control plots remained untreated. Each 164-foot x 164-foot plot was separated by 82-foot buffers.

Traps were checked weekly for adult captures, and data on larval densities were recorded from the third week onwards. Twenty-five flower heads were randomly collected weekly from each plot to determine larval infestation and feeding injury. Data were analyzed using generalized linear repeated-measures mixed-effects models (GLMM), where the pheromone treatment was specified as a fixed effect, and replicate (site) within the week was treated as a random effect (SAS Institute Inc.).

Gut content analyses

A DNA-based method allows detecting host-plant DNA signals to infer an insect pest's dietary history. Representative insect samples (very first captures of the season) collected from seven different western Oregon sites were subjected to gut content analysis to examine the crop or weed hosts on which the insect may have fed or developed. DNA was extracted from insects using DNAeasy Blood & Tissue Kit (Qiagen). Polymerase chain reaction (PCR), using universal primers for the chloroplast gene *trnL* and the ribosomal gene ITS, were used to amplify regions of plant DNA from the guts of insects according to methods described in Cooper et al. (2019). PCR products were direct sequenced using a PacBio platform. The NCBI website's BLAST search function was used to identify plant sequences based on similarity to archived sequences.

Natural enemy survey

Several species of parasitoid wasps are known to be associated with *Coleophora* species in Europe and other countries of their origin. Discrepancy in the numbers of male moths collected using pheromone-baited traps and the number of larvae found upon dissection of individual florets have led us to speculate that natural enemies may exist in our red clover seed cropping systems. While sampling red clover heads for larval presence and/or damage at the mating disruption experiment site (Marion County), larvae-infested florets were brought to the laboratory and reared until the later instars successfully pupated, to determine whether any parasitism occurred.

Results and Discussion

Phenology model

A three-parameter logistic model described the relationship between cumulative proportion of trap catches and cumulative GDDs in western Oregon (Figure 1). According to the model, the median flight of red clover casebearer moth occurs after 323 GDDs have been accumulated after January 1. In 2020, these GDDs were accumulated between June 17 and June 23.

Mating disruption

Overall, male captures in assessment traps positioned with mating disruption pheromone-treated plots were reduced by $38 \pm 17.8\%$ compared to captures in traps in the control plots ($\chi^2 = 10.15$, $df=1$, $P = 0.0014$)

over the 5-week trapping period (Figure 2). The subsequent decline of damage ($72.6 \pm 8.3\%$) ($t = 4$, $df = 3$, $P = 0.028$) in terms of the feeding holes and larval density in treated plots compared with untreated plots indicated promising potential for using this mating disruption technique for pest suppression (Figure 3).

Gut content analyses

The data in both ITS and trnF sequences corresponded to plants in the Fabaceae family, with 47% of the sequence data corresponding to *Trifolium* species (*T. repens*, *T. pratense*, *T. occidentale*), 7% to *Medicago sativa*, and 1% to *Vicia* species. The rest of the sequences corresponded to plants in the Poaceae family, including weed species that commonly occur within red clover fields or field borders, suggesting that adult moths seek out additional food resources while foraging. In the future, sampling of additional plant species (identified in this study) in field stands, along with laboratory testing, can elucidate whether these plant species are suitable hosts for this insect by allowing its successful development.

Natural enemy survey

Two parasitoid wasps, belonging to the Pteromalidae and Ichneumonidae families, that are known to attack lepidopteran larvae were found during sweep net sampling and while dissecting the collection of seed heads from the western Oregon fields. At least 2% of field-collected larvae were associated with a parasitoid wasp in the family Pteromalidae (*Catolaccus aeneoviridis*), and identification was further confirmed by the Insect Pest Prevention and Management Program, Oregon Department of Agriculture. Further confirmation of the biocontrol potential of this parasitoid wasp species is needed.

In summary, the first-year data using a mating disruption technique are promising and warrant additional testing to provide growers with a viable control option to help mitigate damage caused by *C. deauratella*. The phenology model will be further validated for adult flight activity during the 2021 growing season.

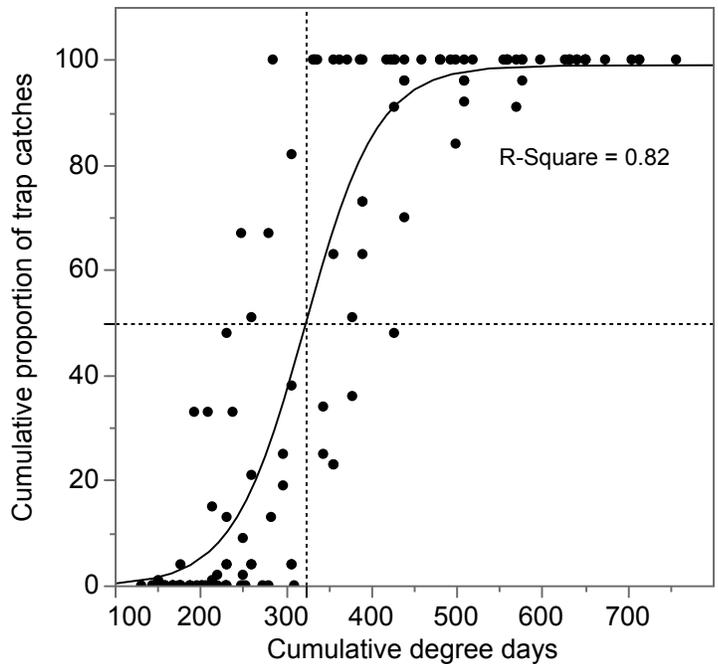


Figure 1. Cumulative proportion of trap catches of male *C. deauratella* in sex-pheromone-baited traps during 2020 plotted against cumulative degree-days (base 53°F from January 1). The vertical dotted line indicates the degree days accumulated corresponding to median moth flight.

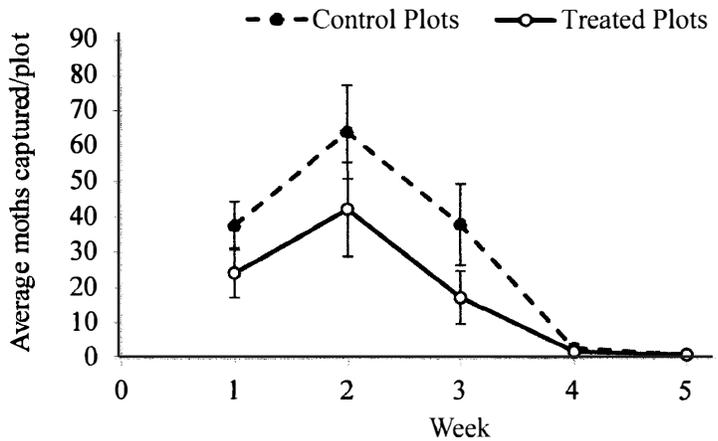


Figure 2. Number of *C. deauratella* adult male moths captured per plot per week in assessment traps using mating disruption pheromones.

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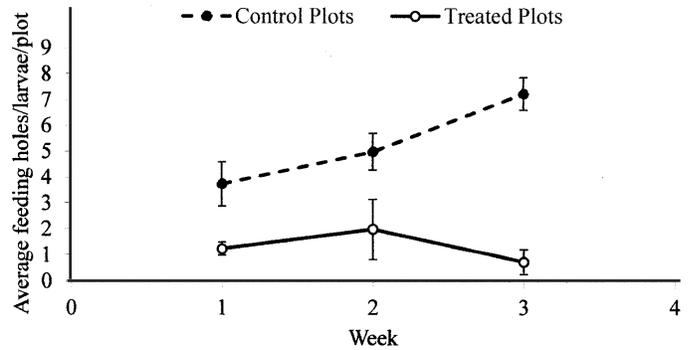


Figure 3. Number of *C. deauratella* feeding holes and/or larva detected per plot per week in seed head samples randomly collected in the mating disruption pheromone experiment.