AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2013 – 2015

TITLE: Developing methods to improve efficiency of *Tyta luctuosa*, a biological control agent of field bindweed (*Convolvulus arvensis*)

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SUMMARY: *Tyta luctuosa* (TL) s a known defoliator of field bindweed and readily consumes foliage in laboratory settings. Larvae are highly host specific and will complete development only on field bindweed, *Convolvulus arvensis*. The moth has been field released since 1980 in western states including Washington and Oregon. However, the lack of empirical data regarding efficacy and establishment success continues to hinder adoption of this biological weed control program. Traditional sampling techniques (visual scouting, light traps) are time consuming and ineffective due to the cryptic, diurnal nature of TL larvae and adults. We are in need of a tool to detect the establishment of previous releases. Pheromone research provides a safe, effective, and economical way to monitor emergence and flight patterns of this weed biocontrol agent.

Techniques have been developed to disrupt mating, or attract and kill adult Lepidoptera for many pest species including: corn earworm (*H.zea*), cotton bollworm (*H. armigera*), tobacco budworm (*H.virescens*), fall armyworm (*S. frugiperda*), and diamondback moth (*P. xylostella*). We propose that the same techniques can be used to monitor, attract, and enhance mating of beneficial weed control agents such as TL. Only very recently has the concept of pheromone-monitoring for beneficial insects been explored. The female-produced sex pheromone for the *Tyta luctuosa,* the bindweed moth, was identified about 10 years ago but had never been field-tested. Moreover, the authors admitted that the synthesized blend is likely missing one or more components, and that further research is needed.

One of the biggest drawbacks to biological weed control is the apparent lack of post-release studies that quantify success. There is a similar dearth of knowledge regarding efficacy of TL; although the bindweed moth has been released for more than 2 decades, surprisingly little empirical data have been collected regarding larval feeding capacity and effects on field bindweed growth. From 2013-15, our research team investigated these issues with the following objectives:

Objective 1: Quantifying herbivory and response to pesticides in a controlled environment. We proposed an assay experiment to evaluate the impact of herbicides on *T. luctuosa* feeding capacity and field bindweed growth.

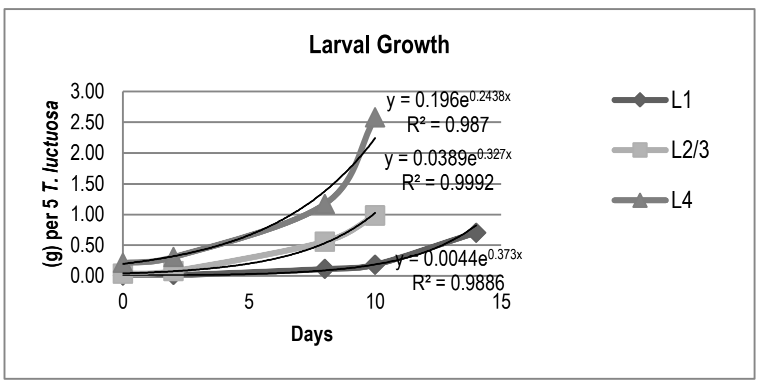
Objective 2: Releasing moths and monitoring dispersal into perennial field crops. At this point, it is unknown how far the bindweed moth can travel from a release point. We proposed a field-trapping study to determine if TL can be sampled effectively using pheromone traps.

PROCEDURES:

(OBJ. 1): Supplies for this objective were purchased in spring 2013. However, an unexpected issue arose at the commercial insect rearing facility and we were unable to obtain larvae in time for the experiment to be conducted. Officials at the rearing facility have since resolved the problem, and will be able to supply for future research. The reason for the failure is unknown, but hypotheses include: extended cold storage of eggs, poor host plant quality, and lack of genetic variation in the population. This issue brings up an important constraint of this research that may need to be addressed; at this time, there is only one professional facility that rears the bio-control agent. In the future, it may become necessary to collaborate with Oregon Dept. of Ag. or other public or private sector companies to rear the bindweed moth on a

large scale. We did, however, conduct a feeding-assay experiment in the lab to estimate growth habits and determine which larval stage (L1 thru L4) were most effective at consuming field bindweed. 5 TL larvae were placed within an experimental unit (mesh bag), and fed fresh bouquets of field bindweed until pupation. Larval weight and fresh plant biomass were recorded.
(OBJ. 2): In 2013 and 2014, we set pheromone traps at locations where larvae had been released in years prior. A few additional sites were monitored as control points, where no intentional release had been made. With the exception of the wildlife refuge, most releases were made in or near cropland. Specifically, we focused efforts on perennial crops such as small fruits, orchards, vineyards, and mint. Trapping began in late May and continued throughout September. Number of moths trapped were recorded and reported as 'moths per day' for each site. For one site (BSR), population dynamics were estimated and analyzed statistically.

SIGNIFICANT ACCOMPLISHMENTS:
The larval assay feeding study revealed some important baseline data that will be crucial to know as we move forward with this research. Never before had the feeding capacity of TL been tested in the Pacific Northwest. Therefore, it was unclear which larval stage would be the most effective to release against field bindweed. With the trial that we conducted, it became apparent that the bindweed moth exhibits exponential growth, typical of Lepidopteran larvae, but that the rates of increase are not equivalent between the life stages (FIG. 1a). While growth of larvae follow exponential growth estimates for early (LI; R^2=0.988) and mid-stage ((L2-3; R^2=0.999) larvae, late stage larvae (L4) reach a period of enhanced growth in the days just prior to pupation. Larger instars also consume bindweed at a greater rate than earlier instars, as evidenced by the steeper slope in FIG 1b.



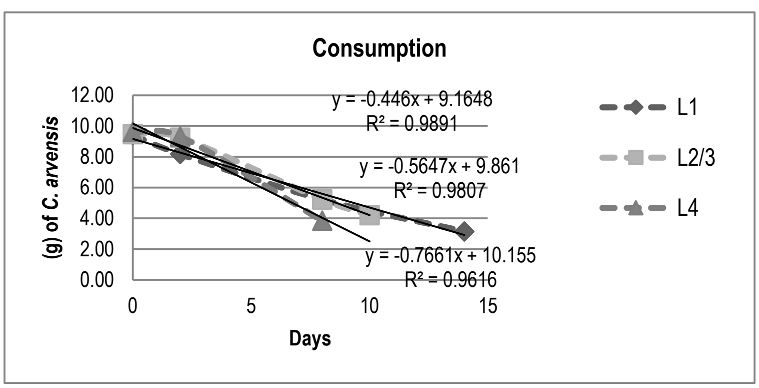


FIGURE 1 – Growth (a) and consumption (b) patterns for OBJ 1. Different age larvae (L1, L2-3, L4) were exposed to ambient temp and light (lab bench). Measurements were taken at 0,2,8,10, and 14 days. n=6 cups, 2 reps per treatment.

Pheromone trapping was effective. There were less than 20 non-target moths detected in pheromone traps, indicating high specificity of the formulated semiochemical blend. Adult moths were detected at many of the sites where larvae had been previously released (TBL. 1). All positive trap locations in 2013 were located within the Willamette Valley, OR., indicating that TL is able to overwinter in this region. Adult male moths were detected from 9 previous release points within the Willamette Valley but were not detected in Eastern Oregon (TBL.1). Unexpectedly, a total of 40 moths were detected at 2 of the 8 check locations. In each case, the control sites were located ≤5 miles directly east of a release point, suggesting that the BCA is able to disperse at least a few miles. To date, there are only 3 other locations in the U.S. with a documented, established population of this BCA.

Flight activity measured over 2.5 years near Rickreall, OR, revealed patterns consistent with bi-voltinism, which has been reported in the agent's native range but never documented in the U.S.. Trap catch data were averaged across years and plotted against cumulative growing degree days using a fifth-order polynomial multiple regression (FIG. 2). Peak emergence occurred on or before April 21st each year and TL activity extended throughout September.

TABLE 1 - Current totals of male TL moths detected (OBJ. 2). Experimental field sites range from the Willamette Valley (JCT to DYT) and Eastern OR (TYV to LGR) to Washington (WSU.1-6). Year of the first intentional release of larvae is specified. Control traps (X.A – X.G) indicate areas that are at least 5 miles away from a known release point.

site	YEAR *of initial release*	SYSTEM	JUNE	JULY	AUG *moths per day*	SEPT	TOTAL *total no. moths*
SPR	2011	strawberry	0	0	0	0	0
JCT	2011	OG vegetable	0.36	0	0.06	0.06	11
PCH	2012	home landscape	0	0	0	0	0
MNR	2014	mint	TBD 2015				
HRN	2012	hazelnut	0	0	0	0	0
LKS	2014	mint	TBD 2015				
BRN	2012	home landscape	0	0	0	0	0
FWR	2011	wildlife refuge	0	0	0	0	0
PMH	2011	home landscape	0.43	0	0.20	0.14	26
CVS	2012	research farm	0.43	1.21	0.20	0.07	35
DVR	2014	mint	TBD 2015				
CHR	2012	blueberry	0.14	0.36	0.11	0.07	17
KEN	2012	riparian	0.14	0.29	0.11	0.09	19
JFR	2011	blueberry	0.21	0.07	0.17	0.09	20
AMR	2012	caneberry	1.57	1.14	0.51	0.30	96
BSR	1998	wildlife refuge	1.79	4.07	4.30	3.57	220
DYT	2011	caneberry	0	0	0.01	0.01	3
TYV	2011	rangeland	0	0	0	0	0
CVE	2010	roadside	0	0	0	0	0
HPN	2010	roadside	0	0	0	0	0
LGR	2010	roadside	0	0	0	0	0
WSU.1	1999	mint	0	0	0	0	0
WSU.2	2013	mint	0	0	0	0	0
WSU.3	2013	grapes	0	0.21	0.01	0	4
WSU.4	2013	research farm	0	0	0	0	0
WSU.5	2014	OG mint	TBD 2015				
WSU.6	2014	OG asparagus	TBD 2015				
X.A – Unionvale	—		0	0	0.01	0.03	3
X.B – Alpine	—		0	0	0	0	0
X.C – Halsey	—		0	0	0	0	0
X.D – Creswell	—		0	0	0	0	0
X.E – Scio	—		0	0.50	0.37	0.22	37
X.F – Monmouth	—		0	0	0	0	0
X.G – Garden City, KS	—		0	0	0	0	0

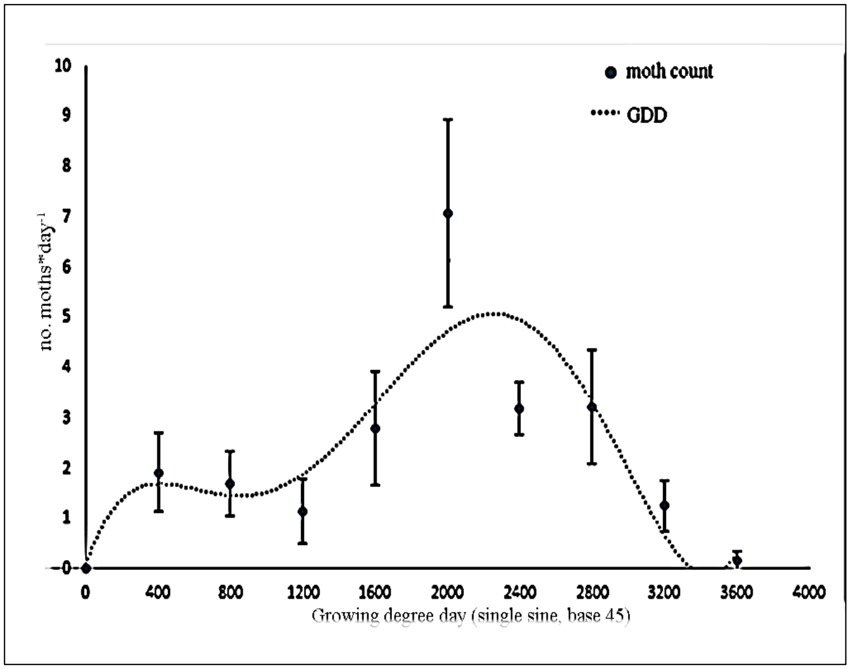


FIGURE 2 – Seasonal activity of male TL moths (±SE) vs. cumulative growing degree-days at a known establishment site. Data were collected using pheromone traps and were averaged across years. Two peaks of activity are evident: spring (~May 15th) and summer (~August 10th).

BENEFITS & IMPACT:

- Lab assay provided the first documented estimate of feeding capacity within this ecoregion, and revealed that *T.luctuosa* displays exponential growth, the rate of which varies per life stage.
- Pheromone trapping conducted throughout the Willamette Valley indicated that TL is able to overwinter in this region. Although the agent has established at one site in Oregon, the fate of other releases was previously undetermined. To date, there has been only 1 moth recovered from Washington releases, and 0 from Eastern Oregon.
- Using pheromones to sample the bindweed moth is an effective means of estimating presence/absence of the agent, and the ease of application will likely appeal to other integrated weed control professionals throughout the country.
- To date, there are only 3 other locations in the U.S. with a documented, established population of *T.luctuosa.* Detection and seasonal activity data collected by our team will be shared with national evaluators of biocontrol success.

ADDITIONAL FUNDING RECEIVED: The Oregon Mint Commission has provided some money to determine if the bindweed moth could be effective for integrated weed control in peppermint.

FUTURE FUNDING:

In order to mitigate crop loss from field bindweed, we need to be able to attract moths to feed on bindweed within the crop. Many studies have shown that in order to enhance reception by female moths, plant volatiles can be added to the pheromone lure. Plant volatiles are detected by female Lepidoptera and indicate host plant quality and suitable oviposition sites. If we can identify the volatiles or other semiochemical cues produced by field bindweed, it may be possible to lure females onto isolated patches of bindweed within a production field, thus enhancing herbivory where it is needed most within the landscape. We have applied for a grant from ARF (2015-17) to pursue this research question.