FINAL PROJECT REPORT
WTFRC Project Number: AE-06-604 (WSU Project 13C-3643-5367)

Project Title: Evaluation of tachinid parasitoids for OBLR in apples

PI:	Vincent P. Jones	**Co-PI(2):**	Tom Unruh
Organization:	WSU-TFREC	**Organization:**	USDA-ARS, Wapato
Telephone:	509-663-8181 x 273	**Telephone:**	509-454-6563
email:	vpjones@wsu.edu.	**email:**	unruh@yarl.ars.usda.gov
Address:	1100 N. Western Ave	**Address:**	5230 Konnowac Pass Rd.
City:	Wenatchee	**City:**	Wapato
State/Province/Zip	WA 98801	**State/Province/Zip:**	WA 95951

Co-PI(3):	Dave Horton
Organization:	USDA-ARS, Wapato
Telephone:	509-454-5639
email:	horton@yarl.ars.usda.gov
Address:	5230 Konnowac Pass Rd.
City:	Wapato
State/Province/Zip	WA 95951

Budget History:

Item	Year 1: 2006
Salaries[1]	10,046
Benefits	1,707
Wages	6,240
Benefits	686
Equipment	0
Supplies[2]	1,000
Travel[3]	1,000
Miscellaneous	
Total	20,679

[1] Half-time Ag Project Assistant - Nik Wiman.
[2] Supplies include rearing supplies, routine lab and field supplies.
[3] Travel to in-state plots and vehicle costs.

Objectives:

1. Develop rearing methods for *Nemorilla pyste* and *Nilea erecta.*
2. Test the means by which the two tachinid flies locate leafroller hosts.
3. Examine the effect of Esteem® and Intrepid® on the tachinid parasitoids within the host larvae.

Significant findings:

- Both tachinid species can be reared in the lab using colony OBLR larvae as hosts. Colony-reared CM larvae are also suitable hosts for the flies, although tachinid parasitism of CM is unlikely to occur naturally in the field.

- Over the course of this study it was determined that *Nilea erecta* has a different mode of attack than previously reported in the literature; eggs are injected subcutaneously into the caterpillar and are not deposited externally on the cuticle. This suggests that its impact has been underestimated because there are no obvious indications of parasitism on the host caterpillar.

- With *Nemorilla pyste*, the time of attack has a strong influence on parasitism success. Eggs are deposited externally on leafroller larvae. If the eggs have not hatched by the time the molt takes place, they are shed along with the exoskeleton during the molting process and parasitism is unsuccessful. This may not be a problem with *Nilea erecta* because the eggs are inserted beneath the surface of the exoskeleton.

- *Nemorilla pyste* were long lived in the laboratory, and egg production occurred over most of adult female life.

- Flies of both species reared from OBLR treated with sublethal doses of Esteem® and Intrepid® developed more rapidly than flies reared from control larvae and did not demonstrate increased mortality in any lifestage. However, it appears that adult reproductive potential of *Nemorilla pyste* was reduced by exposure to Esteem®.

Objective 1. *Develop rearing methods for Nemorilla pyste and Nilea erecta.*

In 2006, 308 tachinid-parasitized leafrollers were recovered from eight commercial orchards, of which six were conventionally managed and two were organically managed. Parasitoids were collected from field populations of leafroller larvae and from sentinel OBLR larvae placed in the field. Parasitized larvae were distinguished by the presence of tachinid eggs on the larval body or on the cast cuticle or headcapsule of larvae and pupae. Field exposure to insecticides in both conventional and organic orchards may have been the cause of high mortality (62%) among the collected parasitized larvae; just 50 male and 45 female *N. pyste* and 14 male and 6 female *N. erecta* were successfully reared to the adult stage. Field-parasitized larvae were placed individually on pinto-bean diet in small cups. After leafroller pupation and tachinid emergence, tachinid pupae were removed from diet cups and placed in small Petri dishes where flies were reared to the adult stage. Species identifications were made using characters described in a recent taxonomic description and by comparison with voucher specimens identified by Dr. James O'Hara (Diptera Systematics Unit, Agriculture Canada).

Adult flies were sexed and were placed in cages with water and honey-water solutions. Each cage contained up to 20 adult flies at a 1:1 sex ratio. Colony-reared OBLR larvae were placed on shoots of apple foliage inserted into 100 ml water-filled tubes. These small artificial "trees" were placed into the cages and were removed daily so that all leafroller larvae could be checked for

parasitism. We have found that the tachinids will also directly parasitize hosts on artificial diet, without the presence of leaves, although rates of parasitism are somewhat lower. The artificial diet method will be used to keep tachinid colonies going through winter when apple foliage is in short supply. Initially, cages were exposed to indirect sunlight for part of each day to encourage mating, but when the season changed they were placed in an incubator (22°C, 70% RH, 16L:8D). Magnetic fluorescent lighting in the incubator was supplemented with electronic fluorescent lighting, which flickers at a speed that exceeds flicker fusion rate of higher Diptera (≈250 Hz). More recently, flies are successfully being reared in growth rooms under the same photoperiod and humidity but with the addition of a halogen light source for several hours a day to simulate natural light. Mating of flies in the cages is typically observed during the period when the halogen light is on.

Parasitized OBLR larvae collected from the cages were placed on artificial pinto-bean diet in small cups, and their head capsule width was measured to determine larval instar. Host development was monitored daily until tachinids emerged or, in cases where parasitism was not successful, the adult leafroller emerged.

Results - Nemorilla pyste: Mating in cages was frequently observed two days after adult emergence, and parasitized larvae began to appear roughly three days after mating. The sex of emerging *N. pyste* adults was not related to the number of eggs per host larva or the size of larvae attacked. The maximum number of eggs per leafroller was 12, although the average was 2.1 (± 1.7) eggs among all larvae attacked (n = 567). Notably, the mean number of eggs from wild and sentinel *N. pyste* collections was 1.1 (± 1.01) eggs per larva, and the maximum number was eight. Eggs were typically oviposited dorsally on or near the pronotum and head, but eggs also occurred on other locations. The success of *N. pyste* parasitism increased as a function of the number of eggs oviposited on hosts (Fig. 1). As higher numbers of eggs per host imply greater competition between parasitoid larvae, this result was unexpected. This relationship may actually reflect host suitability, where the most vulnerable hosts are more highly targeted by female *N. pyste.* It is not known whether the eggs on superparasitzed larvae originated from the same or multiple females, but future observations will determine this. Other tachinid species have shown an aversion to attacking previously parasitized hosts, but the higher density of eggs may also be related to the relatively high number of parasitoids and the few OBLR larvae within the small cage.

Adult *N. pyste* were remarkably long lived under laboratory conditions (Fig. 2), and egg production occurred over most of adult female life. Future experiments will address survival of the tachinids in the field, as lab-derived longevity estimates are typically not realistic assessments of field survival due to the provision of nutritional supplements, constant temperatures, and lack of mortality factors that occur in

Fig. 1. Parasitism success of *N. pyste* as related to the number of eggs per host larva.

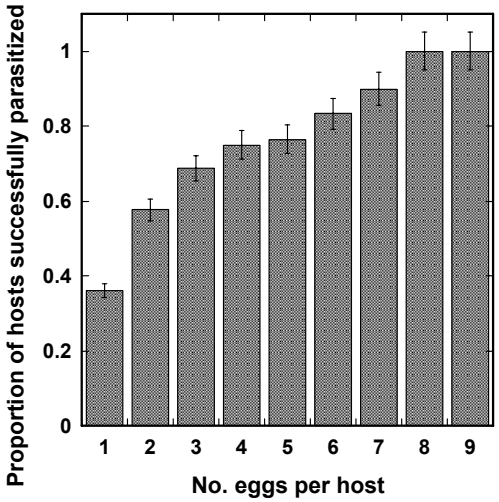
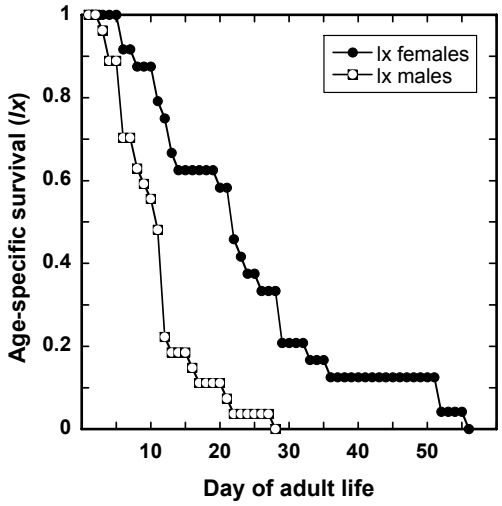


Fig. 2. Proportion of *N. pyste* surviving at different times after adult emergence in the lab.



the field. Parasitism success, which entails death of the host and subsequent emergence of at least one parasitoid, was surprisingly low (51%) in relation to the number of larvae attacked. From a population dynamics perspective, this low rate of success is compensated to some degree by the intermittent emergence of up to three parasitoids per host larva. However, those flies are often smaller and may have lower fitness and reproductive rates than singly emerging flies.

Explanations for the low level of parasitism success may be attributed to laboratory effects or rearing methods, *i.e.*, cage size, adult density, or functional dependence on the number of larvae available to the flies. However, under our hypothesis, the molting schedule of host larvae and the time required for *N. pyste* eggs to hatch determine the window of opportunity for successful parasitism. To determine the day of egg eclosion, parasitized larvae were dissected at different intervals from the time of parasitism. Preliminary results indicate that *N. pyste* eggs may require as many as six days of incubation on the host before hatching. At 22° C, OBLR larvae take four days as fourth instar larvae and nine days as fifth instar larvae (unpublished data). Although more dissection data are needed, our results to date suggest that the success of parasitism in *N. pyste* is highly dependent on the timing of oviposition as it relates to the host molting schedule; if the host caterpillar molts before egg hatch, the egg remains on the cast exoskeleton and parasitism does not occur. Given that leafroller larvae molt at increasingly longer time intervals as they approach pupation, this may partially explain why *N. pyste* females target later instar larvae.

Results – Nilea erecta: Although mating of *N. erecta* was observed in rearing cages, OBLR larvae were not visibly parasitized and therefore most larvae were not reared. This later proved to be a mistake when we found *N. erecta* emerging from OBLR that had been exposed to the adult flies in cages but had no eggs deposited on them. We found this contrary to literature predictions that *N. erecta* would oviposit externally on the host; it appears that *N. erecta* injects its eggs beneath the cuticle of the host. This finding has major implications for collecting and identifying leafroller larvae parasitized by *N. erecta* and explains why so few of this species were collected in 2006. Because larvae with external tachinid eggs were the only wild and sentinel larvae that were reared, larvae that yielded *N. erecta* had also been parasitized by an externally ovipositing tachinid species (*i.e.*, multiparasitism). With no clear external physical indication of *N. erecta* parasitism, experiments with this species will entail rearing of all leafroller larvae exposed to gravid adult females. Although internal incubation of eggs by female *N. erecta* requires greater female investment than externally deposited ones by other species, inserting the eggs may be a more effective mode of attack because eggs cannot be shed by hosts during molts, which appears to be a limitation for *N. pyste*. However, this strategy may also incur certain risks, such as longer exposure of the parasitoid larvae to the host immune system and the risk of pathogen-induced host death via the oviposition puncture. Nonetheless, we hypothesize that our rearing experiments will demonstrate higher rates of parasitism success with *N. erecta* compared to *N. pyste*, but we also expect lower rates of egg production due to higher maternal investment in eggs. These experiments are in progress.

Objective 2. *Test the means by which the two tachinid flies locate leafroller hosts.*

No progress has been made on this objective, but this work will proceed in the spring.

Objective 3. *Examine the effect of Esteem® and Intrepid® on the tachinid parasitoids within the host larvae.*

Insect growth regulator (IGR)-induced pest mortality is often delayed until specific phenological events such as pupation occur, and parasitoids may be exposed to the compounds through utilization of intoxicated hosts during this period. Field observations this year (reported above) suggest that tachinid parasitism of IGR-intoxicated hosts does occur in treated orchards. Exposure

of tachinid flies to Esteem® and Intrepid® is of concern because the larvae of tachinids are internal parasitoids which are exposed and respond not only to the hormones of the host but also potentially to these IGR compounds widely used for leafroller control. The mode of action of the IGR insecticides is to manipulate the level of two of the key hormones involved in the molting process, Juvenile hormone (JH) and molting hormone (MH); Esteem® mimics JH and Intrepid® mimics MH.

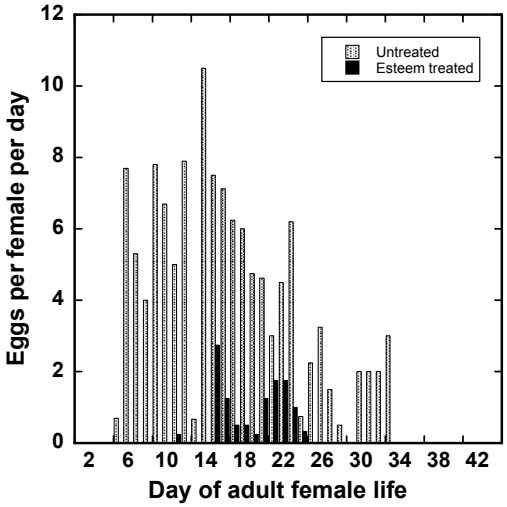
While studies have shown that JH and MH analogs generally have low acute toxicity to fish, birds, and mammals, some JH analogs are known to affect a range of non-target insects, including predators and parasitoids from disparate taxonomic groups. MH analogs such as Intrepid® are purportedly specific to Lepidoptera but may affect adult egg production and mortality in non-target insects. However, the effect of these compounds on internal parasitoids has rarely been considered.

We tested the effects of Esteem® and Intrepid® on the development of *N. pyste* by exposing host OBLR larvae to sublethal doses of the compounds and then allowed treated larvae to be parasitized by the flies. Leaf discs were punched from apple leaves using a 7 mm cork borer, and each was treated by application of 10 µl of solution. Solutions were either water (control) or sublethal rates of Esteem® or Intrepid®. Sublethal doses were defined as those that cause less than 10 to 12% larval mortality. All solutions included 80 ppm of the surfactant Sylgard® 309 to facilitate even dispersal over the disc surface. After solutions on the leaf discs had dried, discs were presented to leafroller larvae that had been starved for 24 hours. Larvae that fully consumed the disc within 24 hours were placed in tachinid rearing cages for parasitism. Parasitized larvae were then placed individually into cups provided with pinto-bean diet, and were reared in growth chambers (22°C, 70% RH, 16L:8D).

Results - Esteem treatment: While OBLR larvae that were fed leaf discs treated with Esteem® took an average of ≈2 days longer to pupate than control larvae, the immature development time of *N. pyste* was significantly shorter. This result suggests that the parasitoids were directly affected by the treatment of their hosts with Esteem®. The direct effects of the JH analog on *N. pyste* were more important than the indirect effects from hormone-induced physiological changes causing delayed pupation of the host because, although the host responded with increased time as a larva, the parasitoid's development accelerated.

Mortality of *N. pyste* reared from treated larvae was not elevated in any development stage. The effects of Esteem® exposure were most pronounced during the pupal period of the parasitoid, which was significantly shorter (9.2 days) compared to parasitoids reared from untreated larvae (16.9 days). Unfortunately, adult reproductive potential was reduced by exposure to Esteem®-treated hosts (Fig. 3), perhaps because of premature development of reproductive structures or because of the importance of JH in egg maturation. Nine pairs of adult *N. pyste* males and females reared from Esteem®-treated hosts produced just 48 eggs on 30 larvae, of which only six larvae were successfully parasitized, or 0.66 fertile eggs per female over the course of adult life. Apparently both fecundity and fertility were affected by exposure to Esteem®, although more data will be needed to statistically compare reproductive data from tachinids reared from treated and untreated hosts.

Fig. 3. Egg production of adult female *N. pyste* reared from untreated and Esteem-treated OBLR larvae.



Results - Intrepid treatment: Results from early testing of sublethal doses of Intrepid® were remarkably similar to those obtained in the Esteem® tests, suggesting that the compound

affected parasitoids directly. It took significantly longer for intoxicated hosts to pupate and significantly less time for *N. pyste* to emerge from the host body than on non-intoxicated hosts. As with the Esteem® treatments, we also saw a significantly more rapid pupal development period of *N. pyste* (11.42 d) in the Intrepid® treatments compared to controls (16.91 d). Results from adult fertility and fecundity tests are in progress.