FINAL REPORT
Project Title: Effects of new insecticides on natural enemies

CO-PIS Tom Unruh, Dave Horton, USDA-ARS Yakima
Richard Hilton, Helmut Riedl, OSU, Medford & Hood River
Elizabeth Beers, WSU, Wenatchee
Nick Mills, U.C., Berkeley

OBJECTIVES (2002-2004):

1. Test acute toxicity of new insecticides to 7 arthropods using standard bioassay
2. Develop bioassay methods to measure sub-lethal effects on beneficial insects
3. Test sub-lethal effects in those cases where acute effects are modest.
4. Model acute and sub-lethal toxicity data to provide field testable predictions of pesticide effects and to better represent combined effects

SIGNIFICANT FINDINGS:

- Acute and sublethal bioassay methods were developed and used for 7 species including: the European earwig, the common green lacewing, a predatory mite, *Deraeocoris brevis, Anthocorus nemoralis, Colpoclypeus florus, Mastrus ridibundus*.
- Acute toxicity were tested for 7 to 13 insecticides and responses were often different among species; generally the neonicotinyl insecticides, Actara, Assail, Provado and Success were acutely toxic with the exceptions of earwig and predatory mite.
- The sublethal assay results also varied by species: negative effects were seen for Esteem, Intrepid and especially Novaluron, compounds which were not acutely toxic.

FUNDING: WTFRC funding by lab

Lab – scientist <arthropod tested> /year	2002	2003	2004
Wenatchee – Beers <predatory mite>	10,000	10,000	0
Hood River – Riedl <predatory mirid bug>	10,000	10,000	10,000
Medford – Hilton <European earwig>	10,000	10,000	10,000
Wapato – Unruh & Horton <leafroller parasitoid & predatory anthocorid bug	10,000	10,000	10,000
Berkeley – Mills <green lacewing, codling moth parasitoid>	10,000	10,000	20,000
TOTAL	50,000	50,000	50,000

USDA-IFAFS matched $220,000 over 3 years ($86,000, $86,000, $60,000)

INTRODUCTION

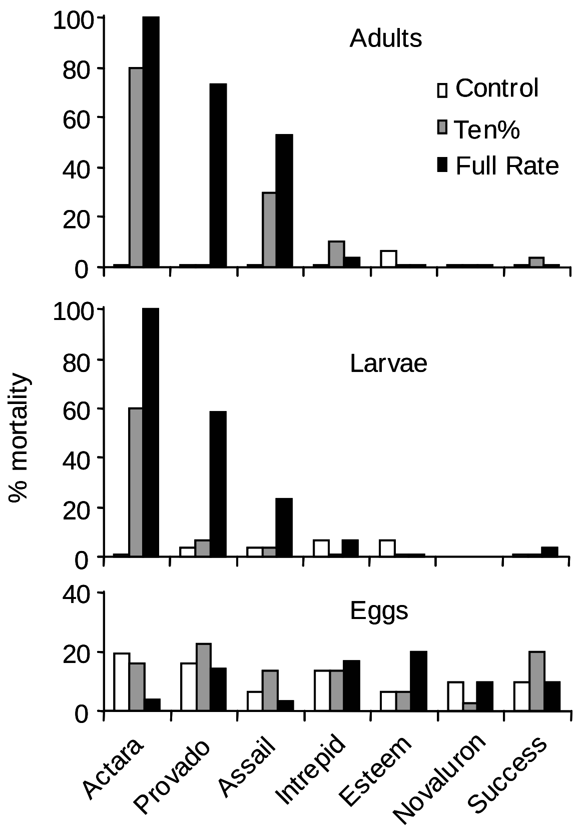
The purpose of this work was to discover the negative effects, if any, of the new, nominally more selective, insecticides being used for pest control in pome fruits in the Pacific Northwest. These studies represent 3-4 years of effort in 5 laboratories by six scientists working with 7 natural enemy species and testing effects of 7 or more new insecticides. Because each arthropod natural enemy has its own requirements, new methods were required. The uniqueness of the bioassay procedures used for each test organism lead to differences in responses and differences in the best ways to report this data for each species as is evident in pages that follow. The last page of the report provides a tubular summary of the effects and the page that precedes it outlines where we hope to go in the future to better summarize and understand sublethal effects..

Green lacewings, *Chrysoperla carnea*, predator of pear psylla, mealybugs, and moth eggs

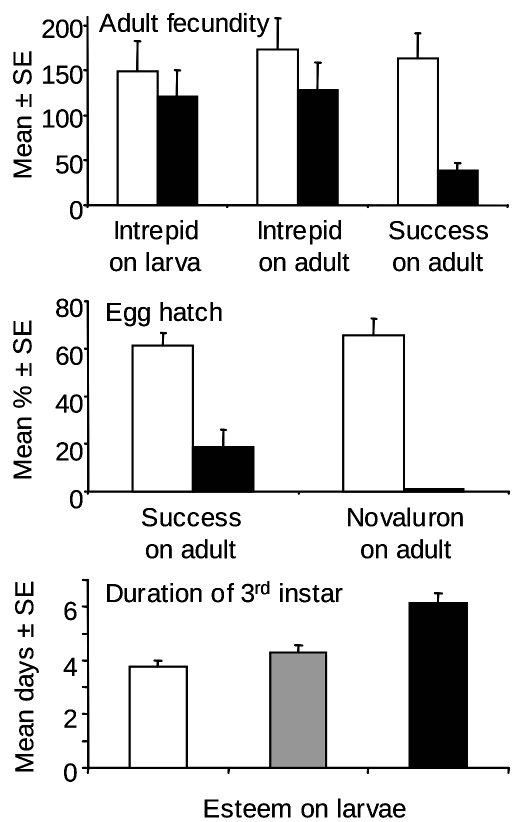
- Acute toxicity of neonycotinyls to lacewing adults and larvae, but not eggs
- Sublethal effect of larval or adult exposure to Intrepid, reducing adult fecundity
- Sublethal effect of adult exposure to Novaluron and Success, reducing egg hatch
- Sublethal effect of larval exposure to Esteem, increasing duration of the 3rd larval instar

We developed a 'worst case scenario' lab assay to determine both the acute (48 h) toxicity of a topical exposure, and the lethal and sublethal toxicities of a combined route of exposure (topical, residual, and oral) of each insecticide on eggs, larvae (2 day old) and adults (2 day old) of the lacewings. Following treatment, all lacewing stages were kept at 23°C, 70% R.H. and 16 hours of light. Measurements from assays included development time, survivorship, adult size (hind tibia length), fecundity (first 14 days) and success of egg hatch. Only those measurements that showed notable acute or sublethal effects are shown below for the influence of each insecticide on either larval or adult lacewings. Sublethal effects were followed into the F1 generation to check on the production of viable offspring. All acute topical assays used 100% and 10% field rates and distilled water controls. The sublethal bioassays were restricted to control plus the highest concentration that permitted greater than 25% survival from the acute assay.

Acute toxicity of neonicotinyls



Sublethal effects of IGRs and Success on adult fecundity, egg hatch and development time of 3rd instar larvae



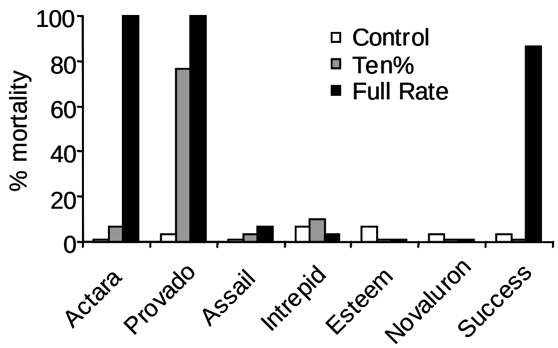
***Mastrus ridibundus*, introduced parasitoid of codling moth cocoons**

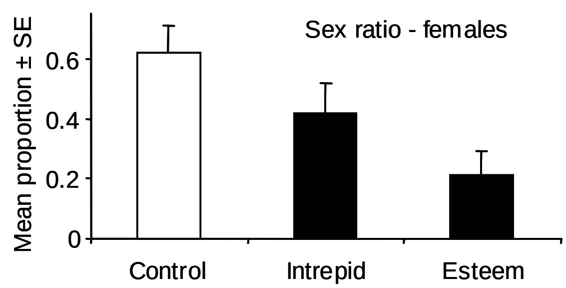
- Acute adult toxicity of Provado at 10% rate, and Actara and Success at full rate
- Sublethal effect of exposure to neonicotinyls, Novaluron and Success, reducing adult fecundity
- Sublethal effect of exposure to IGRs, biasing progeny sex ratio toward males
- Sublethal effect of exposure to neonicotinyls and Success, reducing adult survivorship

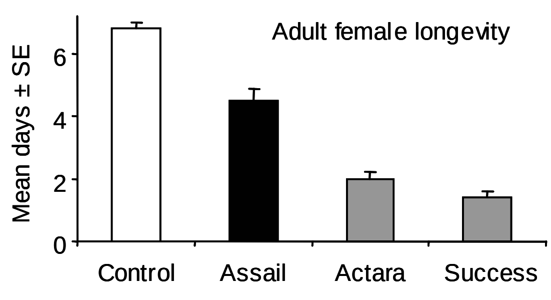
We developed a ‘worst case scenario’ lab assay to determine both the acute (48 h) toxicity of a topical exposure, and the lethal and sublethal toxicities of a combined route of exposure (topical, residual, and oral) of each product on adults (2-d old). Following treatment, all female parasitoids were kept at 23°C, 70% R.H. and 16 hours of light, and given 8 codling moth cocoons to parasitize each day. Measurements from assays included development time, survivorship, adult size (hind tibia length), and fecundity (first 6 days) and sublethal effects were followed through to F1 progeny to check on the production of viable offspring. Adult females (1.5-d old) were the only stage assayed in both sets of assays since the other stages are never exposed to insecticides in the field.

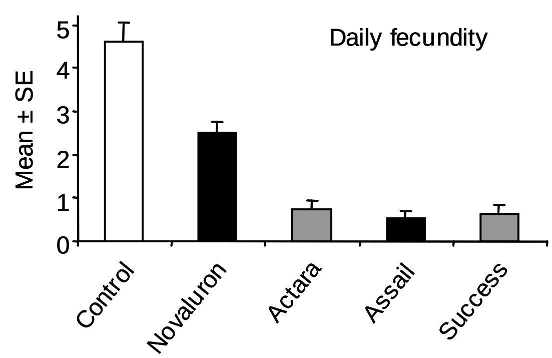
Acute toxicity and reduced survivorship of neonicotinyls and Success

Sublethal effects of neonicotinyls, IGRs, and Success on sex ratio and daily fecundity









***Anthocoris nemoralis*, introduced predator of pear psylla and other soft-bodied insects**

Table 1. Acute toxicity (48 hour) in topically treated females.

Product	High rate	Low rate
Provado	100	100
Agrimek	100*	30**
Assail	100	100
Pyramite	100	78
Actara	100	0
Success	0	0
Novaluron	0	0
Esteem	0	0
Intrepid	0	0

*Also killed untreated males added to Petri.
** 100% of females died before ovipositing.

Provado, Agri-Mek, Assail, and Pyramite caused high rates of mortality within 48 hours of treatment in female *A. nemoralis* (Table 1). Untreated males added to clean petri dishes containing females treated with a high rate of Agri-Mek also died within 48 hours, presumably due to contact with females during mating attempts. Females that survived 48 hours following treatment with a low rate of Agri-Mek nonetheless died before depositing eggs. No acute toxicity effects were noted for Success, Novaluron, Esteem, Intrepid, and low rate of Actara.

Newly eclosed adult females were sprayed as described, moved to a clean Petri dish and provide with untreated, psylla-infested pear leaves, and an untreated male was added to each dish; snow peas were provided as oviposition substrates. Acute toxicity was defined as death of the female within 48 hours of treatment. For females surviving longer than 48 hours, 15-day fecundity, 15-day female survival, hatch rates of eggs, development time of hatched offspring (hatch to adult eclosion), and survival of offspring (F1) was recorded. Offspring were reared on a diet of pear psylla eggs and nymphs.

Sublethal effects were monitored for Success, Novaluron, Esteem, and Intrepid, all compounds not causing acute toxicity (Table 2). For each effect, I express the treatment result as a percentage of the control result. Thus (for example), fecundity in the "Success - high" treatment was only 24% of fecundity shown by untreated controls. Values above 100% for development time indicate that development rate was slowed in offspring of treated females (relative to development in offspring of untreated females). The assays showed substantial effects of Success on fecundity and (for High rates) female longevity, with a suggestion also that offspring from Success-treated females showed reduced survival. Intrepid may have had modest negative effects on female longevity and fecundity. Novaluron had especially strong effects on hatch of eggs deposited by the treated females. All eggs deposited by Novaluron-treated females (high rate) failed to hatch; the low rate of Novaluron also resulted in strongly reduced egg hatch. Esteem did not have any detrimental effects on *A. nemoralis* for the life-history characteristics that were monitored.

Table 2. Effects of Success, Intrepid, Esteem, and Novaluron on fecundity, female longevity, egg hatch, and offspring development times. All values expressed as percentage of control.

	Success		Intrepid		Esteem		Novaluron	
EFFECT MONITORED	High	Low	High	Low	High	Low	High	Low
15-day fecundity*	24	71	83	75	107	100	74	80
15-day female survival	67	100	89	78	100	107	100	90
Egg hatch	99	98	90	99	107	100	0	32
Development time	105	109	100	100	106	104	--	--
Offspring survival	76	95	106	109	116	116	--	--

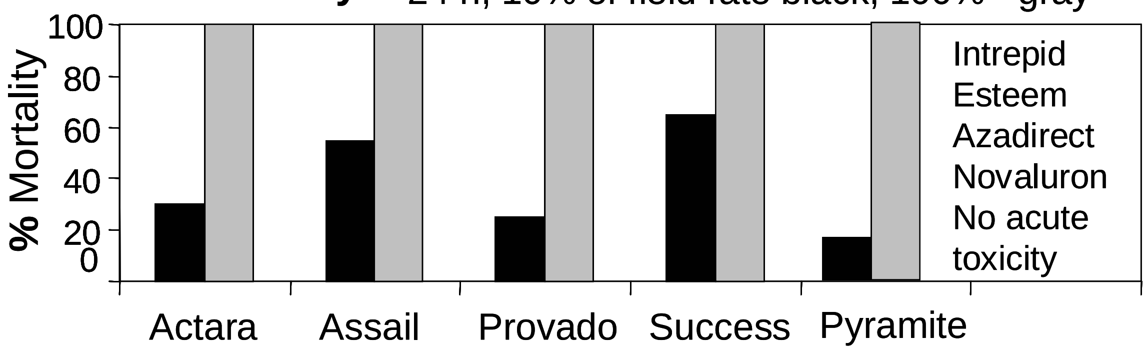
* Calculated including eggs of females that died before reaching 15-day age

***Colpoclypeus florus*, imported parasitoid of leafrollers**

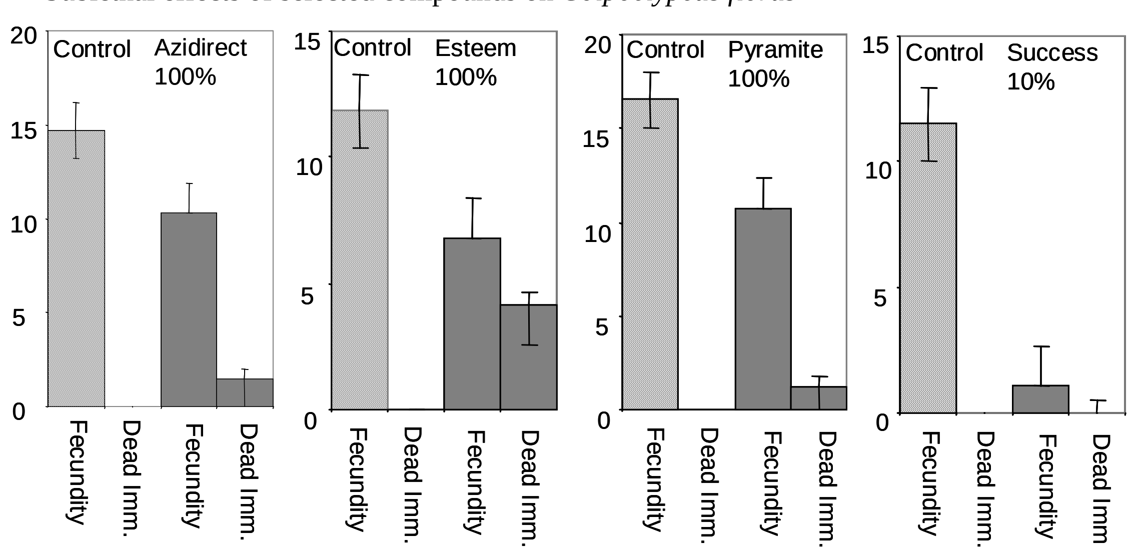
We evaluated acute toxicity for both adult female and males and recorded mortality after 24 h. The sublethal bioassays used the 3 routes of exposure combined. We used control plus concentrations that permitted greater than 25% survival from the acute assay for sublethal studies. Three- to five-d-old mated females were used and they were provided 2 hosts sequentially for 3 days each. With the first host the wasp, the host-leaf roll and honey droplets in the Petri dish were sprayed. The second host, the leaf and Petri substrate and the food were untreated.

- Acute adult toxicity to all the neonicotinyls and Pyramite at field rate and substantial mortality at 10% rates.
- Sublethal effects from field rates of Aza-Direct, Esteem, and Pyramite could be at least partially explained as death of eggs and larval wasps, mostly in the first clutch of eggs.
- Sublethal effects of 10% rate of Success are similar to that seen for the neonicotinyls (not shown) and are due to poor long-term survival and no reproduction of the adult.
- There were no sublethal effects of exposure to Intrepid at both rates(not shown)
- 100% mortality/sterility was cause by exposure to Novaluron (not shown)
- No significant effects on sex ratio were observed in any sublethal assay (not shown)
- Offspring of females exposed to Esteem also showed 46% reduction in fecundity; no other significant F-1 sublethal effects were observed.

Acute Toxicity – 24 h; 10% of field rate black; 100% - gray



Sublethal effects of selected compounds on *Colpoclypeus florus*

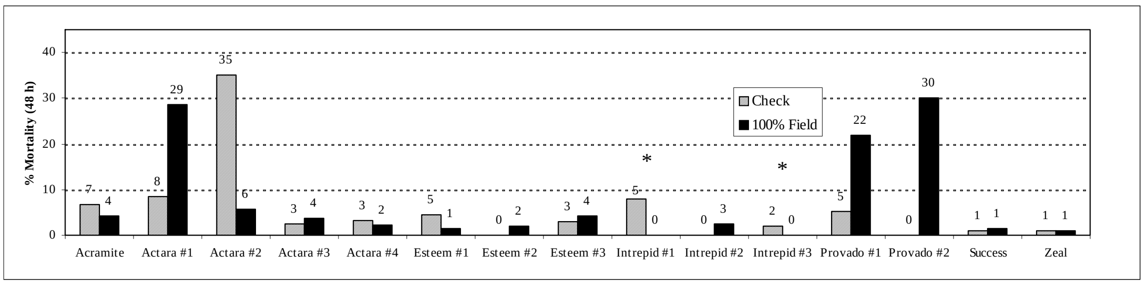


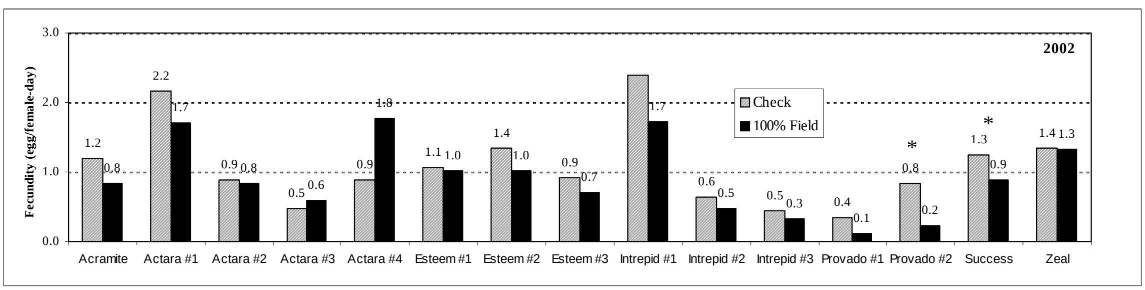
Galendromus occidentalis (western predatory mite), spider mite predator

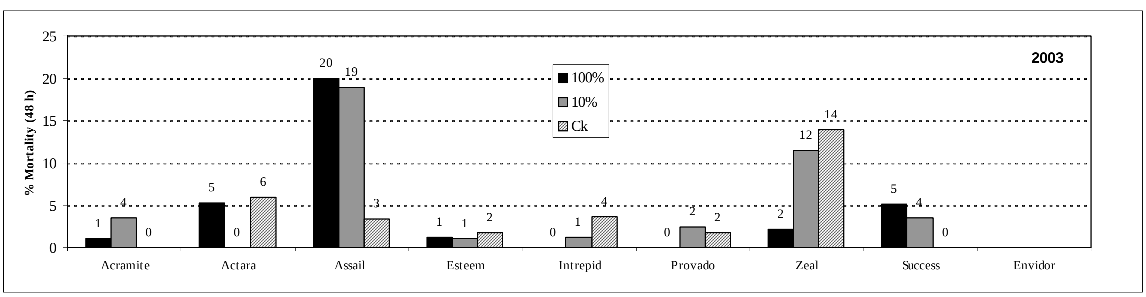
Acute mortality, fecundity and longevity of adult female *G. occidentalis* were tested in a topical-residual leaf disk bioassay. Twenty females/disk were treated in a Potter Spray Tower, and evaluated over a 7-d period. Acute toxicity was evaluated after 48 h. Tests done in 2002 used *G. occidentalis* females taken directly from commercial orchards; those in 2003 were from a cohort of eggs, and <24 h old.

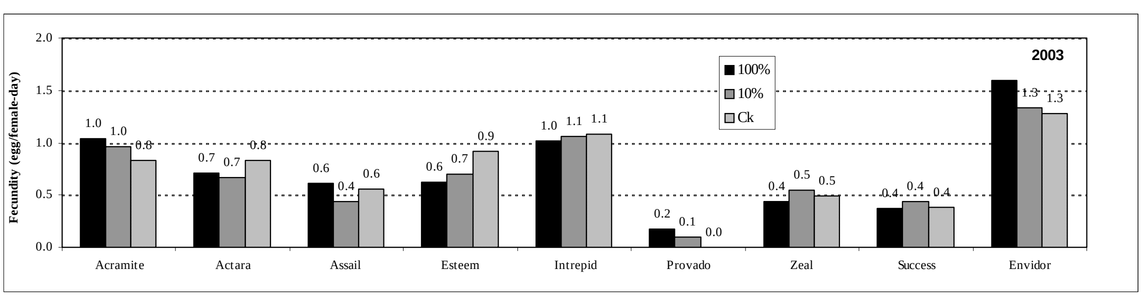
2002. Significant mortality caused by the test pesticide did not exceed 30% in 2002. Two neonicotinyls, Actara and Provado, caused the highest mortality, however, there was a signficant increase in mortality in two Intrepid assays. Significant reductions in fecundity occurred in only two assays, Provado and Success.

2003. None of the increases in mortality caused by pesticides were significant; however, the 10% and 100% field rate of Assail caused ≈20% mortality of adult females, and both rates of Zeal caused 12-14% mortality. Provado caused a slight increase in fecundity, and Esteem a slight decrease in fecundity.







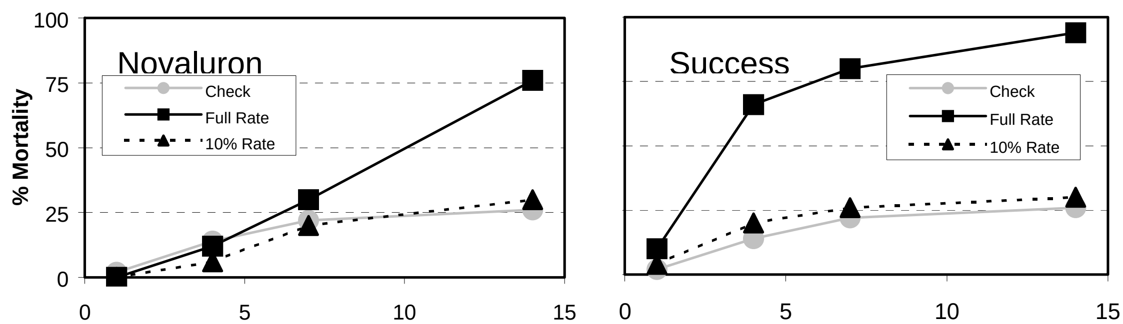


European earwig, *Forficula auricularia*, predator of aphids, pear psylla, and Lep. eggs

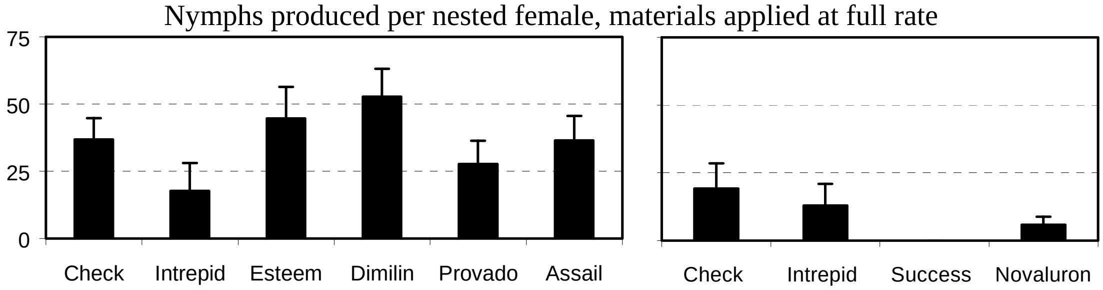
- High level of acute toxicity from Success to adults and nymphs
- Major sublethal effects from exposure to Success and Novaluron
- Moderate level of acute toxicity from neonicotinyls to adults and nymphs
- Moderate reduction in overall fecundity from exposure to Intrepid

Due to the European earwig's protracted life cycle, bioassays for sublethal effects required a minimum of seven months. Mortality often occurred in a delayed manner, this was particularly evident with the effect on nymphs from exposure to Success and novaluron where mortality continued to increase for up to two weeks following treatment. In the initial test on adults in 2002-3, the only significant sublethal effect observed was a 52% reduction in overall fecundity with Intrepid. This test was repeated unsuccessfully in 2003-4 and again in 2004-5 with novaluron and Success as additional treatments. In the 2004-5 test, overall fecundity was reduced by 32% with Intrepid, 68% with novaluron and 100% with Success. Low survivorship in the long term bioassays was a consistent problem and complicated measurement of possible carryover effects in the F1 generation. A total of thirteen materials were tested over the course of this study.

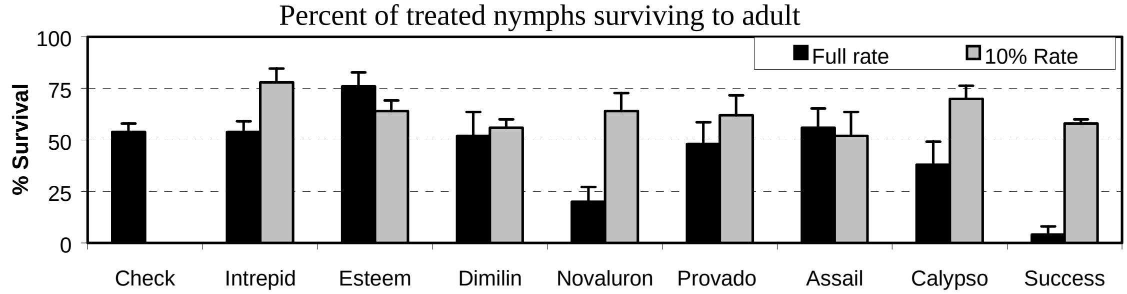
Percent mortality of nymphs up to 14 days after treatment



Nymphs produced per nested female, materials applied at full rate



Percent of treated nymphs surviving to adult

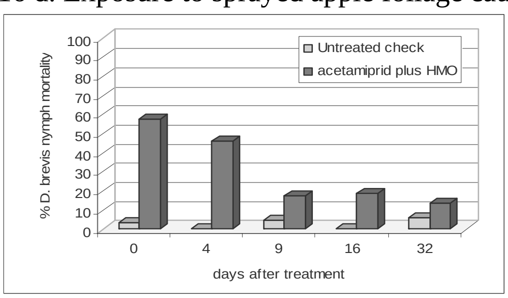


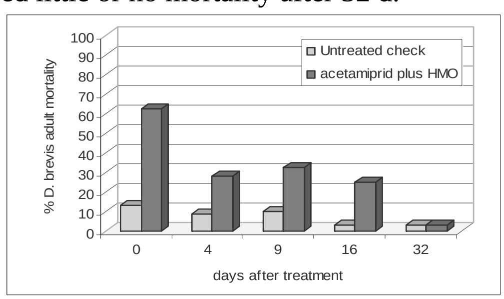
***Deraeocoris brevis*, predator of pear psylla and other insects and mites**

- Acute toxicity of Actara, Assail, Calypso, Clutch, and Provado; AgriMek; Danitol; and Imidan to *D. brevis* nymphs and adults
- Acute toxicity of Actara, Calypso, and Clutch, Provado; Danitol; Esteem, novaluron; and Imidan on *D. brevis* eggs
- Sublethal effect of adult exposure to novaluron; Success; AgriMek, reducing egg numbers and hatch
- Sublethal effect of adult exposure to AgriMek, increasing nymph development time in subsequent generation
- Sublethal effect of nymph exposure to Intrepid and AgriMek, increasing development time
- Moderate residual toxicity of Assail on apple foliage, causing mortality for 3-4 weeks
- High impact of neonicotinyls Assail, Actara, Calypso, Provado and AgriMek on *D. brevis* on pear in the field, with no or little recovery for at least 5 weeks

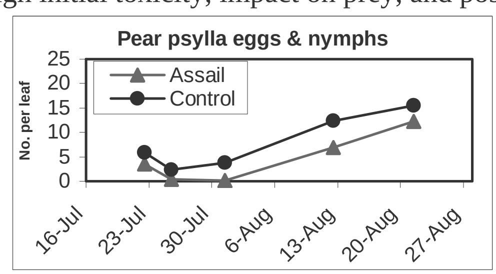
Laboratory tests: The acute toxicity of the following insecticides to *D. brevis* eggs, nymphs and adults was assessed: Actara, Assail, Calypso, Clutch and Provado (neonicotinyls); Esteem, Intrepid, and novaluron (IGRs); AgriMek and Success (fungal metabolites); Danitol (pyrethroid); Nexter (pyridazinone); and Imidan (organophosphate). Insecticides were tested at the full and 10% field rate with distilled water serving as untreated check. Insecticides with more than 25% survival in the acute bioassays were further evaluated to determine sublethal effects on development and reproduction. These included AgriMek, Assail, Intrepid, novaluron, and Success. Results have been published (see Kim et al. {2004} in Arthropod Management Tests, Vol. 29; and Kim et. al. {2006; in press} in BioControl, Vol. 51).

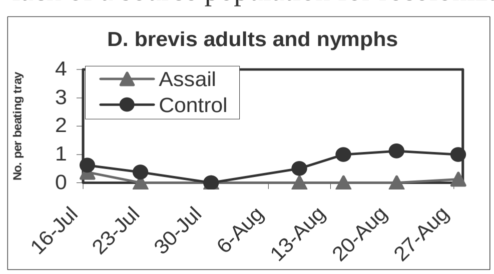
Semi-field test: The residual toxicity of Assail (acetamiprid) was evaluated by exposing *D. brevis* nymphs and adults to apple foliage treated in the field by air blast sprayer (3.4 oz/acre). Fresh deposits caused about 60% mortality in both stages. Impact declined to 15 to 20% mortality after 16 d. Exposure to sprayed apple foliage caused little or no mortality after 32 d.





Field tests: Spray trials using the full field rate were conducted to assess the impact of Actara, Assail, Calypso and Provado; AgriMek; and Mitac on *D. brevis* populations on pear. As an example, the impact of Assail on prey and predator are shown below. AgriMek and all neonicotinyl insecticides tested eliminated *D. brevis* from the orchard for at least 5 wks due to high initial toxicity, impact on prey, and possibly lack of a source population for recolonization.



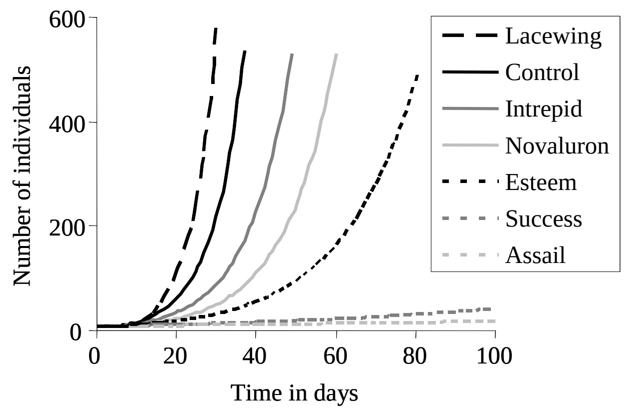


Demographic Approach to Risk of Pesticides to Natural Enemies

Traditionally the impact of pesticides on natural enemies has been based on acute mortality from topical exposure or accumulation from residues on the foliage, and expressed as an LC_{50} referring to the concentration of the product that causes 50% mortality of the natural enemy species. For the newer generation insecticides that have been developed to replace the organophosphates, impacts on natural enemies include more subtle effects such as reduced fecundity and male biased sex ratios, and thus LC_{50}s are no longer capture the overall effect on natural enemy populations. Sublethal effects are also more complex and thus it is less intuitive whether a 60% reduction in fecundity has a greater impact than 50% mortality. To resolve this problem, we have developed age-structured population models to incorporate the influence of any changes in life history parameters brought about by the impact of pesticides on the growth rate of natural enemy populations.

This demographic approach is based on an age-structured Leslie matrix model of life-history elements (survivorship, development rate and fecundity), allowing the incorporation of both acute and sublethal effects of exposure to pesticides. The model examines the impact of pesticides on natural enemies in terms of population growth or recovery time after exposure, providing a holistic population-level endpoint for the toxicology assays that can be used for comparisons among natural enemy species and products. The endpoints that have been considered are the intrinsic rate of population increase (*r*), the net reproductive rate (R_0), and the difference in time that it takes an exposed population to grow from 10 to 1000 individuals in comparison to a control population (*D*).

As an illustration, we present the results of this approach applied to the assay results from a predator, *Chrysoperla carnea*, and a parasitoid, *Mastrus ridibundus*. The graph compares the population growth of *M. ridibundus* populations in the absence (control) and presence of various insecticides, and in relation to the population growth of *C. carnea* (control). Note that *C. carnea* is in general a more rapid rate of population growth than *M. ridibundus* and so could recover more quickly after pesticide disturbance. Note also that the acute toxicity of Success to *M. ridibundus*, is matched by the reduced adult survivorship and fecundity caused by Assail, with the male bias in offspring sex ratio caused by Esteem not far behind.



The table shows the consistency of the three population endpoints and provides a comparison between natural enemy species and pesticide products.

	Chrysoperla carnea			*Mastrus ridibundus*		
	r	R_0	*D*	*r*	R_0	*D*
Control	0.175	60.52	0	0.128	12.40	0
Provado	0.011	1.32	393	-0.020	0.63	
Actara	-0.011	0.75		-0.020	0.63	
Assail	-0.004	0.91		0.007	1.18	630
Success	-0.006	0.85		0.016	1.41	251
Intrepid	0.158	44.76	4	0.094	7.20	14
Esteem	0.175	60.52	0	0.054	3.42	52
Novaluron	-0.124	0.00		0.079	5.37	27

Table 1. Acute toxicity summaries for 7 natural enemy species (2002-2004)
0=no effect, 1=up to 25% mortality, 2 = 25-50% mortality; 3= 50-75% mortality;
4 =75-100% mortality. Acute toxicities in the 3-4 range predict that few insects will survive a spray of this product in the field.

<u>Acute Toxicity</u> **0 = none** **1 = trivial** **2 = modest** **3 = strong** **4 = severe** **n = no data**	Earwig Nymph, adult	Lace-wing egg, larva, adul	Predatory mite (Adult)	*D. brevis;*	*A. nemoralis;* High, low rates	*C. florus* adult	*M. ridibundus* adult
Provado 1.6F	3,2	0, 3, 3	1	3, 4, 4	4,4	3	4
Actara 25WDG	n	0, 4,4	0	3, 4, 4	4,0	3	4
Assail 70WP	2,2	0, 1,3	1	0, 3, 4	4,4	3	0
Intrepid 2F	0,0	0, 0,0	1	0, 0, 0	0,0	0	0
Esteem 0.86EC	0,0	0, 0,0	0	2, 1, 0	0,0	0	0
Success 2SC	4,4	0, 0,0	1	1, 0, 0	0,0	4	3
Novaluron	3,0	0, 0,0	n	3, 1, 0	0,0	0	0
Pyramite 60W	n	n	n	0, 4, 3	4,4	3	n
Agri-Mek 0.15EC	3,1	n	n	0, 4, 4	4,2	n	n

Table 2. Sublethal effects of various insecticides on 7 insects tested. A value of 2 suggest that an insect exposed to this product will dye off in the orchard after this spray, more or less rapidly depending on the species tested.

<u>Sublethal effects</u> **0 = trivial** **1 = modest** **2 = strong** **n = no data**	Earwig Nymph, SR, fecundity	Lace-wing larva, adult, SR, fecundity	Predatory mite (fecundity)	*D. brevis*	*A. nemoralis; Fecundity, longevity, egg hatch*	*C. ;florus Female fecundity*	*M. ridibundus* Adult, sex ratio, fecundity
Provado 1.6F	0,0,0	2,1,0,1	1	n	n	n	n
Actara 25WDG	n	2,n,0,n	0	n	n	n	2,0,2
Assail 70WP	0,0,0	1,1,0,1	0	0,0,0	n	n	1,0,2
Intrepid 2F	0,01	0,1,0,1	0	0,1,0	1,0,0	1	0,1,0
Esteem 0.86EC	0,0,0	1,0,0,0	1	n	0,0,0	0	0,2,0
Success 2SC	2,0,2	0,2,0,2	1	0,0,2	2,1,0	2	2,0,2
Novaluron	2,0,2	0,2,0,2	n	0,0,2	1,0,2	2	0,1,2
Pyramite 60W	n	2,1,0,1	n	n	n	1	n
Agri-Mek 0.15EC	1,0,0	2,n,0,n	n	0,0,2	n	n	n