FINAL REPORT

Title: Identification, biology and insecticide resistance of green apple aphid and spirea aphid.

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Objectives:

1) *Species Identification.*

a) Morphological Comparisons. Extensive samples of green apple aphid (GAA), *Aphis pomi* De Geer, and the morphologically similar spirea aphid (SA), *A. spiraecola* Patch, collected from apple orchards and ornamental hosts in B.C. and Washington State were examined and measured to determine if the species can be reliably separated based on morphological features. Inspection of slide-mounted specimens from eastern Canada, Utah, and New York were included for comparative purposes.

b) Molecular Diagnostics. A Visiting Research Fellow in Dr. Foottit's laboratory developed molecular markers based on DNA microsatellites for the separation of GAA and SA. Results from these tests were compared with traditional morphometric examinations.

2) *Comparative Biology*.

a) Sampling Field Populations. Extensive and intensive sampling of aphids collected from commercial apple orchards and from ornamental hosts throughout the year to determine species distribution and abundance. Samples collected from apple early in the season inspected for the presence of SA forms from overwintering eggs.

b) Development and Fecundity. Aphids of both species reared in the laboratory under a range of temperatures to determine developmental thresholds and differences in fecundity. SA and GAA reared on potted apple seedlings to determine population growth rates.

3) *Insecticide Resistance*.

a) Baseline Susceptibilities. Clones of SA and GAA from B.C. and WA tested for susceptibility to common aphicides, including imidacloprid (Admire□, Provado□), pirimicarb (Pirimor□) and dimethoate (Cygon□).

b) Quantification of Degradation Enzyme Activities. Comparison of detoxification enzyme activities and synergism of aphicides for resistant clones of SA or GAA.

Most of the proposed objectives have been met or are nearing completion. Additional early season samples from commercial apple orchards in WA will be collected in 2002. The large number of aphid samples are still being prepared and examined for the presence of SA from overwintering eggs (fundatriginae). Gugs Lushai, a Postdoctoral Fellow working in Dr. Foottit's laboratory, has used one of the four microsatellite markers in an attempt to separate SA and GAA and is completing studies with the remaining markers. They hope to have this component of the research completed during the coming calendar year.

Determination of baseline susceptibilities for a resistant and a susceptible clone of SA from commercial apple orchards are continuing for several additional insecticides. These bioassays will be completed by the end of the three year study period. Attempts will be made to synergize the toxicity of imidacloprid and pirimicarb to a clone of SA having low levels of resistance to these materials.

Upon completion of the above studies, results of this three year study will be provided in the

form of scientific papers, articles in industry newsletters, and an additional report to the Research Commission. No additional funding is required for the completion of this research.

Significant Findings:

- SA and GAA cannot be reliably separated based on morphological characteristics. SA in particular is highly variable in color, size, and morphological features.
- Differences in one microsatellite gene sequence can be used to separate SA from GAA for aphids from most regions, but it was not diagnostic for aphids from southern B.C. and WA where the species occur together on apple. These preliminary results suggest that these two closely related species might interbreed on apple. Additional loci are being examined for their diagnostic ability.
- SA is widespread on apple, particularly in southern Washington. In late summer GAA is rarely found on apple in southern WA, it becomes increasingly common to the north, and was the only 'green apple aphid' found in commercial orchards north of Vernon, B.C. This north-south gradient in species abundance mirrors the increase in GAA populations recorded from orchards at higher elevations.
- SA are found on apple early in the season, indicating that they overwinter on this host, but we have not yet found the forms that develop from overwintering eggs. Large colonies consisting of all growth stages have been found on apple by the end of April, suggesting that they overwinter asexually on this host during mild winters.
- Laboratory studies have shown that GAA has a lower developmental threshold compared with SA. The latter species is more tolerant of high temperatures, likely explaining why SA predominates on apple in central and southern WA during summer.
- Based on probit analyses and LC_{50} values, susceptibility of SA to insecticides is more variable compared with GAA. SA are 4 to 5-fold less susceptible, on average, to imidacloprid and pirimicarb, but approximately 1.5 times more susceptible to dimethoate.
- Levels of detoxifying enzymes are higher for SA compared with GAA, which likely accounts for the lower susceptibility of SA to insecticides.
- Aphids appear to be an increasing problem on apple. The rosy apple aphid, *Dysaphis plantaginea* (Passerini), for example, can regularly be found damaging apple throughout the summer rather than migrating to alternate hosts.

Methods:

Species Identification. For traditional morphometric analyses, a large number of samples of 'green apple aphids' from commercial orchards and alternative hosts in southern B.C. and central and north central Washington were collected and preserved in ethanol and sent to Dr. Foottit for species identification. Morphological features were counted and measured for both species, and the results compared with specimens from other areas of North America.

Separation of species based on molecular diagnostic techniques required the preparation of suitable molecular markers from microsatellite flanking regions for clones of SA and GAA maintained in colony. A phage library was used to design primers that could be used in polymerase chain reaction (PCR) to amplify gene sequences that would allow for the rapid and accurate determination of species. DNA from individual aphids could then be extracted and the appropriate gene sequence amplified. The resulting fragment can then be compared with known gene sequences from SA and GAA

Comparative Biology. Aphid populations on apple were sampled throughout the growing season in organic and conventional orchards to provide information on the distribution and relative abundance of the two species. Sampling from a sufficient number of organic and conventional orchards will help determine the effect of insecticide sprays on populations of the two aphid species and determine if SA

is better able to develop on apple during summer when the terminals are beginning to harden off. Material collected in spring was examined for the presence of specialized aphid morphs that develop from overwintering eggs (fundatriginae) to determine if SA has adapted to use apple as a primary host.

Both species of aphid were also reared in the laboratory on excised disks of apple leaves under several temperature regimes to provide information on aphid development, survival, and reproduction; factors that can influence aphid population growth and the degree of damage to the host trees.

Insecticide Resistance. Clones of SA and GAA from organic and conventional orchards in B.C. and WA were established in the laboratory on potted apple. Baseline susceptibilities to a number of insecticides were determined for these clones based on LC_{50} values derived from probit analyses. For the insecticide bioassays, 3rd instar nymphs were reared on leaf disks of apple dipped in one of several concentrations of the test material. Levels of detoxifying enzymes were determined for the clones used in the insecticide bioassays.

Results and Discussion:

Species Identification. Morphological examination of a large number of slide-mounted aphids from commercial orchards and alternate hosts over a period of two years did not allow for unequivocal separation of the two species. The morphometric study conducted by Dr. Foottit showed that a significant proportion of aphids of both species overlapped in length measurements or numbers and location of physical structures such as abdominal tubercles. Winged adults of GAA and SA can be distinguished by the veins in the forewings, the former species being distinctly pigmented. Reliably differentiating wingless aphids of these two species is important, as winged forms are often not produced until later in the season or when colonies become crowded.

In a previous study, physical characteristics used in Europe to distinguish the two species in the laboratory, such as the relative length of the last rostral segment and the number of hairs on the cauda (Blackman & Eastop, 1984), did not prove to be diagnostic for specimens collected in North America (Halbert & Voegtlin, 1992). Our results also demonstrate that apterae of the two species from western North America cannot be distinguished by morphological characteristics.

A molecular diagnostic technique based on microsatellite flanking region sequences revealed a number of important traits in the genotype diversity. Four broad clusters (Figure 1) indicated the presence of several genotypes across the continent. Eastern samples of GAA from Nova Scotia and Quebec are closely related to European types, while the similarity of samples within B.C. suggest a single source with subsequent genetic drift. The genetic similarity and grouping of SA and GAA from WA and southern B.C. into the prevalent genotype (cluster 4) suggests that the two species are very closely related and that they possibly interbreed on apple where their ranges overlap. Fertile hybrids could introduce genes for insecticide resistance from SA to populations of GAA. Examination of additional gene sequences and inclusion of more samples should help determine if these species hybridize.

Samples of GAA collected early and late in the growing season from Kelowna, Summerland and Cawston, B.C., indicate low genetic variability in the local population. As the season progresses, however, the dominant genotype observed early in the season declines; suggesting immigration from outside sources or selection for rare genotypes present early in the season. The significance of this genetic change is not known. Based on our studies, there is little variation between clones of GAA with respect to morphology or susceptibility to insecticides.

Comparative Biology. This study has provided important information required for the management of 'green apple aphids' on apple in B.C. and Washington. Extensive sampling has shown that SA is the predominant species in southern Washington. This distribution is consistent with laboratory results

showing that SA is more tolerant of hot temperatures than GAA. Conversely, GAA predominates in cooler coastal areas, northern regions, and at higher elevations. Management programs should be designed to take these regional population differences into consideration. Additional samples from south central WA early in the growing season will determine seasonal differences in species abundance for that region.

The widespread distribution and abundance of SA in commercial orchards in WA is similar to findings in other regions. It has recently been shown, for example, that most pest apple aphids in the eastern U.S. are in fact SA (Pfeiffer *et al.* 1989, Mayer & Lunden, 1996). In greenhouse studies with potted Red Delicious apple, Kaakeh *et al.* (1993) found that GAA and SA reached similar densities and caused similar levels of damage. They concluded that the economic injury level would be about the same for these species. Their study was not designed to determine if SA is better able to remain on apple during the summer months. In addition to other factors such as insecticide applications and greater tolerance to heat, higher populations of SA might occur later in the summer on apple if this species can better survive on terminals that have largely ceased growing.

Large colonies of aphids of various ages were discovered on young apple trees as early as April in some locations, suggesting that GAA and SA can overwinter asexually on apple in some years. Samples collected early in the season from B.C. are still being examined for fundatriginae of SA.. The addition of samples that we plan to collect early in 2002 from WA where SA is the dominant species will help to determine if this species overwinters as eggs on apple.

The two species of 'green apple aphid' differ in biology. The GAA overwinters as eggs on apple and feeds throughout the summer on a restricted range of summer hosts, mainly apple, pear and hawthorn. The SA utilizes spirea as a primary host and migrates to a wide range of secondary hosts, including apple. In 1979, however, SA was found to overwinter successfully on citrus, and it has been suggested that this species has made another recent host shift to utilize apple as a primary host (Pfeiffer, 1991). Eggs of SA have not yet been found on apple, however, and it is possible that significant numbers overwinter as parthenogenetic forms. Additional migrants would be expected to arrive during summer from outside orchards.

Insecticide Resistance. Laboratory bioassays involving 12 aphid clones collected from commercial orchards in WA in the fall of 2000 showed that SA was approximately 5 to 10 times less susceptible to imidacloprid than GAA (Table 1). These results are comparable to those for aphids collected from B.C. orchards in 1999, where the average LC_{50} values were 0.17 and 0.87 ppm AI for GAA and SA, respectively (Table 2); which is almost identical to the overall averages obtained between 1997 and 2001 for 19 clones of GAA and 17 of SA. Although GAA from unsprayed or organic apple were more susceptible to imidacloprid in 1997, there were no consistent differences for aphids from conventionally managed orchards compared with those from organic orchards or unsprayed apple.

The decrease in susceptibility to imidacloprid recorded after 1997 (Table 2) could have resulted from increasing use of this product, but the small change in LC_{50} values does not indicate the development of significant levels of resistance. Within any particular year, LC_{50} values did not differ greatly between clones of each species and the relative difference between species remained more or less constant. The small year to year differences likely reflect changes in test conditions and materials.

Although high levels of resistance to imidacloprid have not yet been demonstrated for any species of aphid, Devine *et al.* (1996) reported that clones of green peach aphid, *Myzus persicae* (Sulzer), and tobacco aphid, *M. nicotianae* Blackman, resistant to nicotine also showed low levels of resistance to this aphicide. Sprays of this material might be ineffective against populations of SA that develop even low levels of resistance, as the margin of overkill is not particularly high for this species.

Pirimicarb was generally less toxic to SA than GAA and there were no consistent differences in susceptibility between clones from unsprayed or organic apple and those from conventional

orchards (Tables 3 and 4). LC_{50} values for clones from B.C. were approximately half those of WA clones, however, which possibly correlates with the higher rates of detoxification enzyme activity recorded for GAA and SA clones from WA (Table 7). Higher esterase activity for aphids from WA was particularly evident when α-naphthyl-butyrate was used as the degradation substrate.

Previous studies have shown that SA is significantly more resistant to a wide range of carbamate and organophosphate insecticides, including azinphosmethyl (Hogmire *et al.*, 1990, 1992). Contrary to these reports, we found that dimethoate is generally more toxic to SA than GAA (Tables 5 and 6). LC_{50} values for various clones of the two species did not always differ significantly, but these results suggest that sprays of dimethoate might be advisable in late summer where SA predominates. Higher levels of degradation enzyme activity for SA (Table 7) might increase the toxicity of dimethoate, or GAA might be less susceptible due to an unknown resistance mechanism.

Conclusions:

Spirea aphid (SA) is the predominant 'green apple aphid' species occurring in orchards in Washington State. To the north and in cooler coastal areas it is largely replaced by the morphologically similar green apple aphid (GAA). The distributions of these two species is best explained by differing climatic requirements; GAA has a lower developmental threshold, but SA is better able to tolerate warm conditions.

Winged GAA can be separated from winged SA based on the presence of darkened veins in the forewings of the former species, but apterae cannot be reliably distinguished based on morphological characteristics. Preliminary analyses of microsatellite gene sequences suggests that the two species might interbreed, but inclusion of aphids from additional sampling sites and examination of other gene loci might provide a diagnostic method that will accurately separate the two species.

In certain years, large colonies of asexually reproducing SA of various ages can be found on apple early in the spring, particularly in newly-planted orchards. We have not yet been able to state with certainty that SA does not overwinter on apple in the egg stage, and additional sampling is planned for early in 2002 in areas where this aphid is the predominant species on apple.

SA is significantly less susceptible than GAA to imidacloprid (Admire□, Provado□) and pirimicarb (Pirimior□), but not dimethoate (Cygon□). These differences possibly relate to higher rates of detoxification enzyme activity, specifically esterases, for SA. Insecticide bioassays are nearing completion for a number of additional insecticides. Differences in susceptibilities to insecticides between the two species is an important consideration for the management of aphids on apple. Efficacy trials should be conducted against the more resistant species, and aphid management programs should take into account the relative abundance of the two species throughout the season.

Budget:

Identification, biology and insecticide resistance of green apple aphid and spirea aphid.
Tom Lowery

Project Duration: 1 April, 1999 to 31 March 2002 (3 years).

	1999/2000	2000/2001	2001/2002
Salaries[1]	$20,250	$21,042	$19,792
Benefits (20%)	$4,050	$4,208	$3,958
Materials and Supplies	$6,200	$3,750	$1,750
Travel	$1,000	$1,000	$1,000
Publication costs	--	--	$1,500
Total[2]	$31,500	$30,500	$28,000
WTFRC Funds	**$15,750**	**$15,250**	**$14,000**

AAFC-MII Funds	$15,750	$15,250	$14,000

[1]Includes ~$10,000/yr to Dr. Foottit for contract slide mounting and technical salary; and technical assistance at PARC (D.T. Lowery and summer students).
[2]Includes $1,000/yr to Dr. Peryea for collection of aphid samples.

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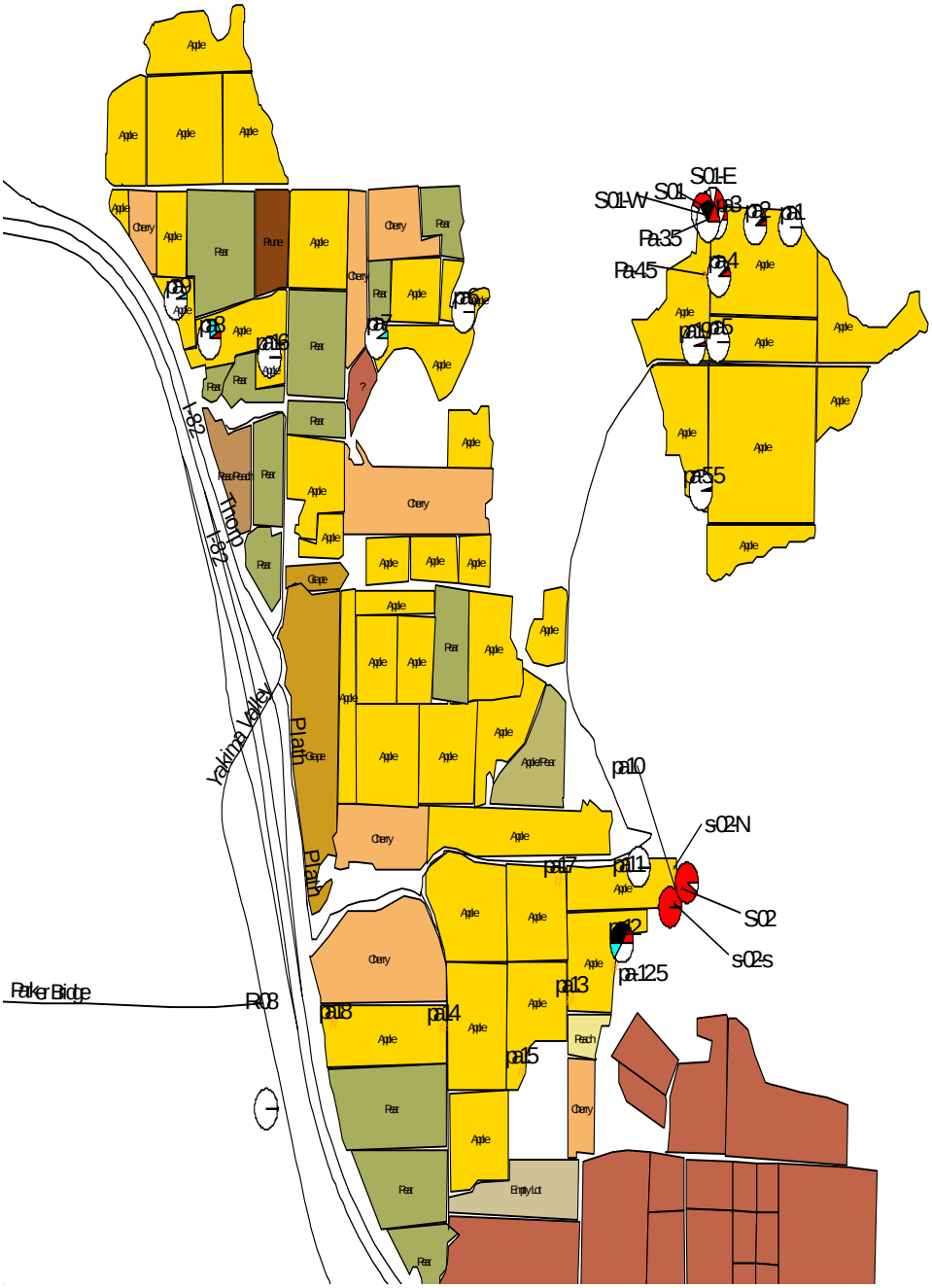

Figure 1. Genetic relationship of green apple aphid and spirea aphid (SA) from B.C., Washington and eastern North America based on distance-neighbour joining analysis of sequenced microsatellite loci (~1600 base pairs) rooted by Nova Scotia samples being used as out groups. Branch length is proportional to genetic change.

Table 1. Susceptibilities (LC_{50} ppm AI from probit analyses) of green apple aphid (GAA), *Aphis pomi*, and spirea aphid (SA), *A. spiraecola*, to imidacloprid (Admire , Provado) for aphids collected in 2000 from orchards and from ornamental crab apple in Washington State.

Species	Location	Culture	LC_{50}	95% CI	Chi^2
GAA	Quincy	Conventional	0.15	0.12-0.19	4.40
"	"	"	0.16	0.13-0.20	1.63
"	"	"	0.16	0.13-0.21	1.00
SA	"	"	0.74	0.57-0.94	3.29
"	Wenatchee	Crab Apple	0.74	0.57-0.93	1.36
"	Donald	Conventional	0.76	0.60-0.94	0.50
"	Columbia View	"	0.78	0.62-0.96	2.60
"	Wenatchee	Crab Apple	0.84	0.71-0.98	1.87
"	Grandview	Conventional	0.84	0.69-1.02	1.98
"	Wenatchee	"	0.89	0.71-1.11	1.06
"	Quincy	"	0.90	0.72-1.11	0.60
"	Chelan	"	1.12	0.99-1.39	1.77

Table 2. Summary of average susceptibilities (LC_{50} ppm AI from probit analyses) of green apple aphid (GAA), *Aphis pomi*, and spirea aphid (SA), *A. spiraecola*, to imidacloprid for aphids established in culture from 1997 to 2000. Numbers of aphid clones indicated in parentheses.

Year	Species	Location	Practice	Avg. LC_{50}	Species Avg. by Year
2000	GAA	WA	conventional	0.16 (3)	0.16 (3)
"	SA	"	"	0.88 (7)	0.85 (9)
"	"	"	unsprayed/organic	0.74 (2)	
1999	GAA	B.C.	conventional	0.13 (2)	0.17 (7)
"	"	"	unsprayed/organic	0.19 (5)	
"	SA	"	conventional	0.79 (5)	0.87 (6)
"	"	"	unsprayed/organic	1.31 (1)	
1998	GAA	WA	conventional	0.30 (4)	0.30 (4)
"	SA	"	"	1.25 (1)	1.25 (1)
1997	GAA	B.C.	conventional	0.12 (2)	0.09 (5)
"	"	"	unsprayed/organic	0.07 (3)	
"	SA	"	"	0.55 (1)	0.55 (1)

GAA: overall average LC_{50} = 0.17 ppm AI; range 0.06-0.54 ppm AI; number clones tested = 19SA: overall average LC_{50} = 0.86 ppm AI; range 0.51-1.53 ppm AI; number clones tested = 17

Table 3. Susceptibilities (LC_{50} ppm AI from probit analyses) of green apple aphid (GAA), *Aphis pomi*, and spirea aphid (SA), *A. spiraecola*, to pirimicarb (Pirimor) for aphids collected in fall 2000 from orchards and from ornamental crab apple in Washington State.

Species	Location	Culture	LC_{50}	95% CI	Chi^2
GAA	Quincy	Conventional	0.57	0.48-0.66	2.95
"	"	"	1.18	0.99-1.43	4.33
"	"	"	1.66	1.40-1.95	2.11
Spirea	Wenatchee	Crab Apple	1.40	0.85-2.29	5.22
"	"	"	2.17	1.82-2.51	0.40
"	Quincy	Conventional	2.48	2.08-2.93	3.06
"	Columbia View	"	3.58	2.96-4.21	0.44
"	Grandview	"	4.07	3.38-4.87	1.21
"	Chelan	"	4.95	1.31-13.02	7.09
"	Donald	"	5.26	4.33-6.22	4.55
"	Quincy	"	6.20	5.26-7.14	2.06
"	Wenatchee	"	6.30	5.42-7.22	0.01

Table 4. Summary of average susceptibilities (LC_{50} ppm AI from probit analyses) of green apple aphid (GAA), *Aphis pomi*, and spirea aphid (SA), *A. spiraecola*, to pirimicarb (Pirimor™) for aphids established in culture in 1999 and 2000. Numbers of aphid clones indicated in parentheses.

Year	Species	Location	Practice	Avg. LC_{50}	Species Avg. by Year
2000	GAA	WA	conventional	1.14 (3)	1.14 (3)
"	SA	"	"	4.69 (7)	4.05 (9)
"	"	"	unsprayed/organic	1.79 (2)	
1999	GAA	B.C.	conventional	0.56 (2)	0.59 (7)
"	"	"	unsprayed/organic	0.60 (5)	
"	SA	"	conventional	1.92 (5)	2.04 (6)
"	"	"	unsprayed/organic	2.64 (1)	

GAA: overall average LC_{50} = 0.75 ppm AI; range 0.36-1.66 ppm AI; number clones tested = 10SA: overall average LC_{50} = 3.24 ppm AI; range 0.50-6.30 ppm AI; number clones tested = 15

Table 5. Susceptibilities (LC_{50} ppm AI from probit analyses) of green apple aphid (GAA), *Aphis pomi*, and spirea aphid (SA), *A. spiraecola*, to dimethoate (Cygon) for aphids collected in fall 2000 from orchards and from ornamental crab apple in Washington State.

Species	Location	Culture	LC_{50}	95% CI	Chi^2
GAA	Quincy	Conventional	16.2	12.9-20.5	1.10
"	"	"	27.9	23.2-33.7	4.15
"	"	"	55.4	48.3-62.5	0.18
SA	Wenatchee	Crab Apple	8.7	7.4-10.3	1.42
"	Quincy	Conventional	12.3	10.1-14.8	4.07
"	Grandview		15.6	13.9-17.5	2.08
"	Quincy	"	19.9	16.4-24.2	2.75

"	Wenatchee	Crab Apple	20.0	17.1-23.2	1.77
"	Columbia View	Conventional	21.5	18.4-24.8	1.70
"	Chelan	"	25.4	22.2-28.9	2.17
"	Wenatchee	"	25.4	20.9-31.0	1.98
"	Donald	"	30.1	25.5-35.1	3.70

Table 6. Summary of average susceptibilities (LC_{50} ppm AI from probit analyses) of green apple aphid (GAA), *Aphis pomi*, and spirea aphid (SA), *A. spiraecola*, to dimethoate (Cygon™) for aphids established in culture in 1999 and 2000. Numbers of aphid clones indicated in parentheses.

Year	Species	Location	Practice	Avg. LC_{50}	Species Avg. by Year
2000	GAA	WA	conventional	33.2 (3)	33.2 (3)
"	SA	"	"	21.5 (7)	19.9 (9)
"	"	"	unsprayed/organic	14.4 (2)	
1999	GAA	B.C.	conventional	10.0 (2)	10.2 (7)
"	"	"	unsprayed/organic	10.2 (5)	
"	SA	"	conventional	8.8 (5)	8.5 (6)
"	"	"	unsprayed/organic	7.5 (1)	

GAA: overall average LC_{50} = 17.1 ppm AI; range 4.8-55.4 ppm AI; number clones tested = 10SA: overall average LC_{50} = 15.3 ppm AI; range 6.3-30.1 ppm AI; number clones tested = 15

Table 7. Detoxification enzyme activity, esterase activity/minute/mg protein, for green apple aphid (GAA), *Aphis pomi*, and spirea aphid (SA), *A. spiraecola*, collected in fall 2000 from orchards and from ornamental crab apple in Washington State.

Species	Location	Culture	Acetate	Butyrate
GAA	Quincy	Conventional	1.74	0.47
"	"	"	2.19	0.57
"	"	"	2.71	0.67
SA	Wenatchee	Crab Apple	1.37	0.55
"	"	"	2.01	0.69
"	"	Conventional	2.29	0.89
"	Quincy	"	2.77	0.88
"	"	"	3.18	1.02
"	Columbia View	"	2.21	0.66
"	Grandview	"	2.06	0.67
"	Donald	"	2.39	0.83
"	Chelan	"	2.52	0.87

GAA: 2000 acetate average = 2.21; butyrate average = 0.57; number clones tested = 31999 acetate average = 1.23; butyrate average = 0.29; number clones tested = 4SA: 2000 acetate average = 2.31; butyrate average = 0.78; number clones tested = 91999 acetate average = 1.27; butyrate average = 0.49; number clones tested = 6