Baseline Susceptibilities to Imidacloprid for Green Apple Aphid and Spirea Aphid (Homoptera: Aphididae) Collected from Apple in the Pacific Northwest

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ABSTRACT Susceptibilities to the neonicotinyl insecticide imidacloprid were determined for clones of apple aphid, *Aphis pomi* De Geer, and spirea aphid, *Aphis spiraecola* Patch, collected from conventional and organic apple orchards and from crab apple and wild apple in Washington state and British Columbia over a period of 6 yr. For aphids collected during 1996–1998, adults were dipped in test solutions by using the Food and Agriculture Organization protocol, and third instars and adults were reared on treated apple leaf disks. During the final 3 yr of study, bioassays involved only third instars on treated leaf material. Tests showed that *A. spiraecola* was significantly more tolerant to imidacloprid compared with *A. pomi.* Depending on the bioassay method and aphid developmental stage, average LC_{50} values for *A. spiraecola* were 4.4–5.7 times higher than those for *A. pomi* established under the same test conditions. Clones of both species from Washington were marginally more tolerant to imidacloprid than clones from British Columbia, but the differences were generally not significant. Average measures of susceptibility for clones from organic orchards or unsprayed trees also did not differ from those for clones from conventional orchards, and there was no evidence for increasing LC_{50} values over the 6 yr of study. Differences in susceptibility to insecticides between these two anatomically similar species should be considered during the testing of new products for use on apple.

KEY WORDS aphids, imidacloprid, *Aphis pomi*, *Aphis spiraecola*, toxicity, tolerance

THE SPIREA APHID, *Aphis spiraecola* Patch, is a highly polyphagous species found on plants from >20 families, including economically important crops such as citrus (Blackman and Eastop 1984). It was first recorded in the Okanagan Valley of British Columbia on apple in 1981 (Forbes and Chan 1989) and in south central Washington state a few years later (Halbert and Voegtlin 1992). In the field, it is virtually indistinguishable from the apple aphid, *Aphis pomi* De Geer, (Blackman and Eastop 1984, Halbert and Voegtlin 1992), which was previously thought to be the predominant species of apple aphid on apple in North America. Recently, however, it has been demonstrated that *A. spiraecola* is the more common species on apple in Virginia, West Virginia, Maryland (Pfeiffer et al. 1989), and south central Washington (Mayer and Lunden 1996). In the latter area, *A. spiraecola* was the only species in 44% of samples and the primary species in an additional 44% of samples collected from 75 south central Washington orchards over a 2-yr period. It is likely that *A. spiraecola* was often misidentified in the past, but these recent records might reflect a host shift for this aphid. Spirea was known to be the primary host, but according to Pfeiffer (1991) it seems that *A. spiraecola* now successfully uses both citrus and apple as primary hosts.

Beginning in the mid 1990s neonicotinyl insecticides, such as imidacloprid, were approved in various countries to control aphids and other pests of apple. These materials are particularly effective against homopteran pests such as aphids (Mullins 1993) due to their good systemic activity and high oral toxicity (Elbert et al. 1998). Concurrent with the introduction of the neonicotinyl insecticides, increasing public concern about pesticide safety and possible damage to the environment has resulted in government initiatives, such as the U.S. Food Quality Protection Act, to restrict or eliminate the use of many organophosphate and carbamate insecticides. Faced with the impending loss of many insecticides currently registered for use on apple, there will be a greater reliance on imidacloprid and related materials for the management of aphids.

Studies by Kerns et al. (1998) and Elbert et al. (1998) showed that clones of green peach aphid, *Myzus persicae* (Sulzer), differed in susceptibility to imidacloprid, whereas Devine et al. (1996) recorded pos-

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Table 1. Collection sites, host records, production practices, collection year, and orchard or site code for *A. pomi* and *A. spiraecola* from British Columbia and Washington

Collection location	Host, production practice	Yr	Orchard or site code(s)	Map no.[a]
BC, Salmon Arm	Apple, conventional	1997	BC1-97	1
BC, Armstrong	Apple, wild unsprayed tree	1999	BC2-99	2
BC, Vernon	Apple, nursery	1996	BC3-96	3
BC, Summerland	Apple, research orchard	1997	BC4-97	4
BC, Summerland	Apple, research orchard	1999	BC4-99a, BC4-99b	5
BC, Summerland	Apple, conventional	1999	BC5-99	6
BC , Okanagan Falls	Apple, conventional	1999	BC6-99	7
BC , Oliver	Apple, conventional	1999	BC7-99a, BC7-99b, BC7-99c	8
BC, Osoyoos	Apple, conventional	1997	BC8-97	9
BC, Cawston	Apple, organic	1997	BC9-97	10
BC, Cawston	Apple, organic	1999	BC10-99, BC11-99	11
BC, Cawston	Apple, organic	2001	BC12-01	12
WA, Oroville	Apple, conventional	1998	WA13-98	13
WA, Columbia View	Apple, research orchard	2001	WA14-98a, WA14-98b	14
WA, Columbia View	Apple, research orchard	2000	WA14-00	15
WA, Wenatchee	Apple, research orchard	1998	WA15-98	16
WA, Wenatchee	Apple, research orchard	2000	WA15-00	17
WA, Wenatchee	Crab apple, unsprayed	2000	WA16-00a, WA16-00b	18
WA, Quincy	Apple, conventional	2000	WA17-00, WA18-00, WA19-00	19
WA, Moxee	Apple, conventional	1998	WA20-00	20
WA, Wapato	Apple, conventional	1998	WA21-00	21
WA, Grandview	Apple, conventional	1998	WA22-00	22

[a] Collection sites (BC, British Columbia; WA, Washington) shown on Fig. 1 indicated by these numbers.

sible cross-resistance for lines of tobacco aphid, *Myzus nicotianae* Blackman, and *M. persicae* tolerant to nicotine. In the laboratory, exposure of a clone of cotton aphid, *Aphis gossypii* Glover, to imidacloprid over 13 generations resulted in an approximately eight-fold increase in resistance (Wang et al. 2002). Of particular interest, the resistant line also was highly cross-resistant to the pyrethroid insecticide fenvalerate, up to 108.9-fold on cotton. These studies suggest that the development of resistance to imidacloprid is a possibility and it is important to implement resistance management strategies that can prolong the effectiveness of these new materials. As a first step, it is important for future resistance monitoring to accurately determine baseline susceptibilities to the neonicotinyl insecticides for economically important aphid species (Elbert et al. 1996).

Hogmire et al. (1990, 1992) demonstrated that significant differences in susceptibility to a number of insecticides existed between *A. pomi* and *A. spiraecola* and that these differences should be considered in the development of aphid management programs and testing of new products for use on apple. The purpose of our study was to evaluate several clones of *A. pomi* and *A. spiraecola* from insecticide-treated and untreated hosts in south central British Columbia and central Washington for their susceptibility to imidacloprid. Neonicotinyl insecticides were not used on apple in British Columbia before the onset of this study, whereas imidacloprid (Provado) was registered in Washington one year previously. Studies were conducted over several years (1996–2001) to allow for detection of low levels of resistance development immediately after the introduction of imidacloprid for the management of aphids and other pests of apple in the two countries.

Materials and Methods

Aphid Cultures. During the 1996–2001 growing seasons, aphids were collected from conventional and organic apple orchards, wild apple, and ornamental crab apple in south central British Columbia and central Washington (Table 1; Fig. 1). Neonate aphids from this collection were reared on excised leaf disks in small, ventilated self-sealing petri dishes until maturity to eliminate parasites and diseases. Individual adult aphids were then transferred to caged apple seedlings held in a growth chamber at 20°C and a photoperiod of 16:8 (L:D) h. Aphids from a single clone were then used to establish the laboratory culture. Specimens were identified to species (by R.G.F.) at Agriculture and Agri-Food Canada-Eastern Cereals and Oilseeds Research Centre (AAFC-ECORC), Ottawa, which houses the Canadian National Insect Collection.

Apple seedlings ('McIntosh') used for culturing aphids were grown in a growth chamber at 22°C in 4.5-liter plastic pots containing a soil mixture consisting of two parts Pro-Mix 'BX' (Premier Horticulture, Steinbach, Manitoba, Canada), one part sterilized potting soil (Greenleaf Products, Abbotsford, British Columbia, Canada), and one-half part perlite (Supreme Perlite, Portland, OR). A photoperiod of 16:8 (L:D) h was supplied by a mixture of incandescent and fluorescent lighting, and a dilute solution of 20–20-20 fertilizer was applied weekly to maintain vigorous growth suitable for aphid development.

Insecticide Bioassays: Topical Treatment. For clones collected during the first two seasons, apterous adult aphids were exposed to insecticide following the recommended Food and Agriculture Organization (FAO) protocol for the detection of insecticide resistance (FAO 1979). Aphids were grasped lightly

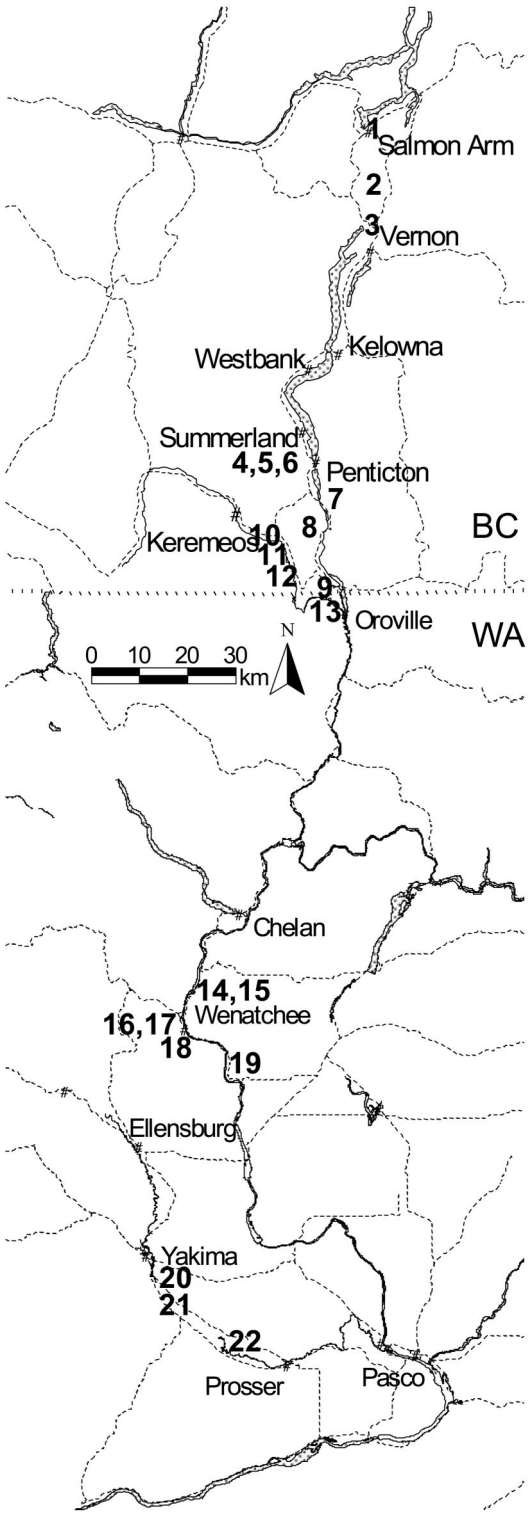

Fig. 1. Location of sites in south central British Columbia and central Washington where clones of apple aphid and spirea aphid tested for susceptibility to imidacloprid were collected. Refer to Table 1 for collection information.

with fine forceps by a rear leg and submerged for ≈2 s in test solutions of imidacloprid (Admire, 21.4% [AI], Bayer Canada Ltd., Calgary, Alberta, Canada). Mortality to imidacloprid is not fully expressed until several days after exposure (Knaust and Poehling 1992), so after treatment, aphids were reared, 10 per dish, for 3 d on apple leaf disks in small petri dishes as outlined by Lowery and Smirle (2003). Based on preliminary results involving 10-fold dilutions, aphids were exposed to five to seven test concentrations of imidacloprid, including appropriate controls. The surfactant Agral 90 (Zeneca Agro, Calgary, Alberta, Canada) was added at a uniform concentration (0.25 ml/liter) to all treatments; controls consisted of the surfactant solution only. Aphid survival was assessed after 3 d, and probit analysis (SAS Institute 2000) was used to establish the lethal concentration required to kill 50% of the test population (LC_{50}).

Insecticide Bioassays: Leaf Disk Residual. Apple leaf disks dipped in solutions of imidacloprid (Admire, 21.4% [AI], Bayer Canada Ltd.) were used to rear aphids,10 per dish, on treated leaf disks in small petri dishes as outlined by Lowery and Smirle (2003). Agral 90 (0.25 ml/liter) was again added to all solutions, including the control. Mortalities were assessed after 3-d feeding. During the first 2 yr, separate trials were conducted with third instar and adult *A. pomi* and *A. spiraecola;* bioassays for the remaining 3 yr used only third instars. For both species, trials with adults or third instars were replicated at least twice; from five to seven concentrations of imidacloprid were used for each instar of both species. LC_{50} values were determined by probit analysis (SAS Institute 2000).

Results and Discussion

Topical Treatment. For adult *A. pomi* dipped in solutions of imidacloprid, lethal concentrations required to kill 50% of the test population (LC_{50}) ranged from 0.38 to 1.46 ppm for clones from conventional orchards in Osoyoos, British Columbia, and Oroville, Washington, respectively (Table 2). The range in values represents a nearly four-fold difference in susceptibility. The LC_{50} value for a clone from an organic orchard in Cawston, British Columbia, was intermediate and only differed significantly, based on nonoverlapping 95% confidence intervals, from the lowest and highest values. Clones of *A. pomi* from British Columbia were only marginally more susceptible to imidacloprid compared with those from Washington; average LC_{50} values were 0.73 versus 1.06 ppm, respectively.

Due to differences between slopes and intercepts, the range in LC_{90} values was even greater. The largest value of 41.54 ppm was >23 times larger than the smallest (Table 2). It should be noted that for adults of clone WA15-98 dipped in test solutions of imidacloprid the results fit the probit line poorly, resulting in a broad confidence interval for the resulting LC_{90} value. However, this value is only about twice that of the next highest LC_{90} value. In agreement with these results, the largest LC_{50} and LC_{90} values for third

Table 2. Lethal concentrations (ppm [AI]) of imidacloprid (Admire) for adult *A. pomi* and *A. spiraecola* dipped in test solutions based on probit analyses of mortality rates after 72 h

Province/state	Yr	Code[a]	*n*	Slope (SE)	Intercept (SE)	LC_{50} (95% CL)	LC_{90} (95% CI)	χ^2 (*P*)
A. pomi								
BC	1997	BC8-97	820	1.84 (0.23)	0.78 (0.12)	0.38 (0.22–0.56)	1.88 (1.19–3.97)	9.29 (0.05)
BC	1997	BC4-97	420	2.54 (0.38)	0.63 (0.13)	0.57 (0.42–0.71)	1.80 (1.37–2.78)	0.83 (0.66)
BC	1997	BC1-97	510	1.88 (0.34)	0.24 (0.14)	0.75 (0.56–1.05)	3.60 (2.13–10.20)	1.64 (0.44)
BC	1997	BC9-97	760	2.03 (0.24)	0.24 (0.08)	0.76 (0.61–0.91)	3.25 (2.53–4.69)	0.47 (0.79)
BC	1996	BC3-96	800	2.42 (0.29)	−0.18 (0.14)	1.19 (0.90–1.46)	4.02 (3.28–5.29)	0.76 (0.68)
WA	1998	WA14-98a	410	0.74 (0.12)	0.35 (0.12)	0.33 (0.20–0.61)	18.04 (5.75–143.39)	0.93 (0.63)
WA	1998	WA14-98b	400	1.66 (0.19)	−0.11 (0.11)	1.16 (0.83–1.54)	6.85 (4.79–11.35)	1.31 (0.52)
WA	1998	WA15-98	380	0.85 (0.19)	−0.09 (0.14)	1.28 (0.67–4.32)	41.54 (9.17–1784)	0.06 (0.97)
WA	1998	WA13-98	400	1.54 (0.21)	−0.25 (0.11)	1.46 (1.06–2.00)	9.96 (6.15–21.45)	2.24 (0.33)
A. spiraecola								
BC	1997	BC4-97	420	2.13 (0.27)	−1.79 (0.28)	6.90 (5.34–8.57)	27.51 (20.55–42.54)	5.98 (0.11)
WA	1998	BC15-98	490	1.29 (0.14)	−0.63 (0.12)	3.08 (2.21–4.18)	30.41 (19.41–57.73)	0.34 (0.84)

[a] Clone collection site (BC, British Columbia; WA, Washington) and orchard production practice indicated in Table 1.

instars of *A. pomi* reared on treated leaf disks of apple also were established for clone WA15-98 (Table 4). Although LC_{90} values are intrinsically more variable, they are useful in the determination of diagnostic doses for studies of potential resistance development. A diagnostic dose of ≈40 ppm, which would result in nearly complete mortality for all the aphids we tested, is ≈10 times higher than that suggested previously based on tests with a single clone of *A. pomi* (Lowery and Smirle 2003). For a susceptible strain of *M. persicae*, Elbert et al. (1996) established a diagnostic dose of 60 ppm based on the FAO protocol, whereas for the cotton aphid, it was 100 ppm.

Only two clones of *A. spiraecola* were tested in this manner, but the higher LC_{50} values demonstrated that adults of this species are less susceptible to imidacloprid compared with *A. pomi* (Table 2). On average, *A. spiraecola* is ≈5.7-fold less susceptible to imidacloprid, average LC_{50} value 4.99 ppm, than *A. pomi*, average LC_{50} value 0.88 ppm. The LC_{50} values of the two clones of *A. spiraecola* differed significantly based on nonoverlapping 95% confidence intervals, but there was no difference between LC_{90} values.

Elbert et al. (1996), using a modified FAO dip test, established LC_{50} values for *M. persicae* exposed to imidacloprid of 2.6 and 2.2 ppm 2 and 3 d after treatment, respectively. Tests with several clones of *M. persicae* and *M. nicotianae* produced values ranging from 1.6 to 9.0 ppm 2 d after treatment. For insecticide-resistant strains of these aphids from Japan, France, and the United States, Nauen et al. (1998b) determined LC_{50} values based on a modified FAO adult dip test ranging from 1.0 to 4.8 ppm, compared with 0.47 ppm for a susceptible laboratory strain. Adult dip bioassays with several clones of *M. persicae* and *M. nicotianae* produced LC_{50} values from 1.5 to 7.2 ppm (Devine et al. 1996).

Compared with *M. persicae* and *M. nicotianae*, adult *A. pomi* seem to be marginally more susceptible to imidacloprid. Many of the LC_{50} values overlapped, however, and the slightly lower values could simply reflect the smaller size of *A. pomi* relative to *M. persicae* and *M. nicotianae*. Adult *A. pomi* dipped in insecticide solutions would have received a higher relative dose compared with the other two species due to a proportionately higher surface area-to-volume ratio. Our results show that the contact toxicity of imidacloprid to adult *A. spiraecola* is comparable with that previously published for *M. persicae* and *M. nicotianae*. LC_{50} values for the two clones we tested fell within the ranges reported for the other species.

Leaf Disk Residual. LC_{50} values for adult *A. pomi* reared for 3 d on treated leaf disks of apple ranged from 0.11 ppm for a clone from an organic orchard in Cawston, British Columbia, to 0.83 ppm for aphids from a research orchard in Wenatchee, WA (Table 3). As for dipped aphids, clones from Washington (average LC_{50} value 0.52 ppm) were slightly more tolerant to imidacloprid compared with clones from British Columbia (average LC_{50} value = 0.17), with two of the four Washington clones differing significantly from all British Columbia clones based on nonoverlapping 95% confidence intervals. For all clones combined, the average LC_{50} value for adult *A. pomi* dipped in test solutions of 0.88 ppm (Table 2) was substantially higher than the average value of 0.32 ppm for adult aphids reared for 3 d on treated leaf disks of apple (Table 3). According to Mullins (1993), toxicity of imidacloprid to aphids occurs primarily by ingestion, which would explain the lower values for aphids reared on treated leaf disks. Applications to leaf material would, however, involve oral and contact toxicity because aphids would make physical contact with residues on leaf surfaces.

The largest LC_{90} value for adult *A. pomi* reared on treated leaf disks (2.56 ppm) was only 5.3 times larger than the smallest (0.48 ppm) (Table 3), compared with a difference of >23-fold when adult aphids were treated as per the FAO protocol (Table 2). An approximate diagnostic dose of 3.0 ppm for adult *A. pomi* reared on treated disks might, therefore, provide a more accurate tool for resistance monitoring. Dipping adult aphids in test solutions is a simpler method and according to Nauen and Elbert (1997), it is preferable to combine the FAO protocol with bioassays using treated leaf disks.

For *A. spiraecola*, the clone from Washington was significantly ($P < 0.05$) more tolerant to imidacloprid

Table 3. Determination of lethal concentrations (ppm [AI]) of imidacloprid (Admire) applied to leaf disks of apple to adult *A. pomi* and *A. spiraecola* from south central British Columbia and central Washington based on probit analyses of mortality rates after 72 h

Province/State	Yr	Code[a]	*n*	Slope (SE)	Intercept (SE)	LC_{50} (95% CI)	LC_{90} (95% CI)	χ^2 (*P*)
A. pomi								
BC	1997	BC9-97	420	1.96 (0.21)	1.91 (0.23)	0.11 (0.09–0.13)	0.48 (0.34–0.78)	2.14 (0.54)
BC	1997	BC4-97	420	1.75 (0.20)	1.47 (0.21)	0.14 (0.11–0.19)	0.78 (0.52–1.43)	0.94 (0.82)
BC	1996	BC3-96	810	2.39 (0.57)	1.95 (0.45)	0.15 (0.05–0.26)	0.52 (0.29–16.45)	12.08 (0.01)
BC	1997	BC8-97	450	1.70 (0.25)	1.24 (0.19)	0.19 (0.13–0.25)	1.05 (0.69–2.10)	3.42 (0.33)
BC	1997	BC1-97	420	2.68 (0.34)	1.63 (0.24)	0.25 (0.20–0.31)	0.74 (0.55–1.15)	1.30 (0.73)
WA	1998	WA13-98	530	2.02 (0.25)	1.17 (0.16)	0.26 (0.20–0.33)	1.13 (0.82–1.81)	2.35 (0.31)
WA	1998	WA15-98	600	2.29 (0.31)	0.94 (0.14)	0.39 (0.30–0.49)	1.42 (1.07–2.17)	2.36 (0.31)
WA	1998	WA14-98b	630	2.13 (0.22)	0.50 (0.10)	0.58 (0.45–0.72)	2.33 (1.81–3.27)	0.35 (0.95)
WA	1998	WA14-98a	380	2.61 (0.43)	0.22 (0.14)	0.83 (0.63–1.05)	2.56 (1.85–4.47)	0.73 (0.70)
A. spiraecola								
BC	1997	BC4-97	420	1.25 (0.17)	0.49 (0.17)	0.40 (0.28–0.69)	4.25 (1.96–15.76)	1.41 (0.70)
WA	1998	WA15-98	620	1.51 (0.15)	−0.59 (0.12)	2.44 (1.86–3.11)	17.20 (12.31–26.99)	2.62 (0.27)

[a] Clone collection site (BC, British Columbia; WA, Washington) and orchard production practice indicated in Table 1.

compared with the clone from BC based on nonoverlapping LC_{50} values (Table 3), but their LC_{90} values did not differ ($P > 0.05$). Based only on this limited sample, the diagnostic dose for adult *A. spiraecola* reared on treated leaf disks would likely exceed 20 ppm, compared with only 3.0 ppm for *A. pomi.*

For third instars of *A. pomi* reared for 3 d on treated leaf disks of apple, LC_{50} values determined from probit analyses ranged from 0.05 to 0.50 ppm, for a 10-fold difference in susceptibility (Table 4). Compared with rearing of adult *A. pomi* on treated leaf disks, third instars were on average about twice as susceptible to imidacloprid. For *A. pomi* from British Columbia, the average LC_{50} value for clones from organic orchards of 0.15 ppm differed little from that for clones from conventional orchards, average value 0.12 ppm (Table 4), suggesting that previous exposure to synthetic insecticides did not alter the response of this species to imidacloprid. Average LC_{50} values for the 13 British Columbia clones and seven Washington clones of *A. pomi* were 0.13 and 0.20 ppm, respectively. However, if the clone from the research orchard in Wenatchee that is significantly more tolerant to imidacloprid compared with all other clones is removed from the calculation, the average LC_{50} value for *A. pomi* from Washington would be 0.15 ppm. Imidacloprid (Provado) was registered for use on apple in Washington 2 yr before it was approved in British Columbia, but except for the single Washington clone (WA15-98), there was little difference in susceptibility between aphids from the two locations. Additionally, there was no strong trend to suggest that tolerance to imidacloprid increased over the 6 yr of study.

LC_{90} values for the 20 clones of *A. pomi* varied by <12-fold (Table 4), suggesting that bioassays involving third instars might provide greater accuracy than those with adults. In addition, the highest and lowest LC_{50} values corresponded with the highest and lowest LC_{90} values. A diagnostic dose for third instars of *A. pomi* of ≈3.0 ppm should help discriminate between susceptible and resistant populations. This value is twice the spray concentration that eliminated all *A.*

Table 4 Determination of lethal concentrations (ppm [AI]) of imidacloprid (Admire) applied to leaf disks of apple to third instars of *A. pomi* from south central British Columbia and central Washington based on probit analyses of mortality rates after 72 h

Province/state	Yr	Code[a]	*n*	Slope (SE)	Intercept (SE)	LC_{50} (95% CI)	LC_{90} (95% CI)	χ^2 (*P*)
BC	1997	BC4-97	420	1.96 (0.21)	2.50 (0.27)	0.05 (0.04–0.06)	0.24(0.17–0.37)	3.07 (0.38)
BC	1996	BC3-96	630	1.87 (0.17)	2.21 (0.19)	0.07 (0.05–0.08)	0.32(0.25–0.44)	2.20 (0.33)
BC	1997	BC9-97	550	1.86 (0.19)	2.14 (0.22)	0.07 (0.06–0.09)	0.34(0.25–0.52)	1.29 (0.73)
BC	1997	BC8-97	420	1.77 (0.20)	1.97 (0.24)	0.08 (0.06–0.10)	0.41(0.28–0.70)	3.97 (0.27)
BC	2001	BC12-01	480	2.03 (0.22)	2.09 (0.22)	0.09 (0.07–0.12)	0.40(0.30–0.60)	0.25 (0.88)
BC	1999	BC5-99	400	0.72 (0.09)	1.61 (0.20)	0.11 (0.07–0.15)	0.63(0.43–1.09)	4.11 (0.13)
BC	1999	BC4-99b	420	0.82 (0.09)	1.71 (0.19)	0.13 (0.10–0.16)	0.60(0.44–0.91)	1.53 (0.47)
BC	1997	BC1-97	420	1.87 (0.19)	1.60 (0.20)	0.14 (0.11–0.18)	0.68(0.47–1.13)	0.73 (0.87)
BC	1999	BC6-99	420	0.64 (0.06)	1.22 (0.13)	0.15 (0.11–0.19)	1.10(0.76–1.83)	3.88 (0.14)
BC	1999	BC2-99	420	0.74 (0.08)	1.34 (0.15)	0.16 (0.12–0.21)	0.93(0.66–1.49)	1.87 (0.39)
BC	1999	BC4-99a	420	0.87 (0.10)	1.42 (0.17)	0.20 (0.15–0.25)	0.85(0.62–1.31)	1.62 (0.44)
BC	1999	BC11-99	520	0.93 (0.09)	1.41 (0.15)	0.22 (0.18–0.26)	0.87(0.67–1.24)	5.61 (0.13)
BC	1999	BC10-99	420	0.90 (0.08)	1.33 (0.15)	0.23 (0.18–0.28)	0.95(0.71–1.39)	2.15 (0.34)
WA	1998	WA14-98b	420	1.18 (0.20)	1.16 (0.15)	0.10 (0.04–0.18)	1.26(0.75–2.87)	3.83 (0.43)
WA	1998	WA14-98a	630	1.17 (0.12)	0.95 (0.11)	0.15 (0.11–0.21)	1.92(1.21–3.69)	1.04 (0.60)
WA	2000	WA19-00	830	1.56 (0.11)	1.27 (0.10)	0.15 (0.12–0.19)	1.01(0.77–1.41)	4.40 (0.11)
WA	2000	WA17-00	680	1.90 (0.15)	1.50 (0.13)	0.16 (0.13–0.20)	0.77(0.60–1.06)	1.63 (0.44)
WA	2000	WA18-00	460	1.54 (0.15)	1.21 (0.13)	0.16 (0.13–0.21)	1.11(0.78–1.79)	1.00 (0.61)
WA	1998	WA13-98	420	1.21 (0.17)	0.87 (0.14)	0.19 (0.12–0.28)	2.17(1.23–5.41)	0.62 (0.73)
WA	1998	WA15-98	630	1.69 (0.25)	0.51 (0.11)	0.50 (0.38–0.64)	2.84(1.88–5.66)	2.72 (0.26)

[a] Clone collection site (BC, British Columbia; WA, Washington) and orchard production practice indicated in Table 1.

Table 5. Determination of lethal concentrations (ppm [AI]) of imidacloprid (Admire) applied to leaf disks of apple to third instars of *A. spiraecola* from south central British Columbia and central Washington based on probit analyses of mortality rates after 72 h

Province/State	Yr	Code[a]	*n*	Slope (SE)	Intercept (SE)	LC_{50} (95% CI)	LC_{90} (95% CI)	χ^2 (*P*)
BC	1999	BC4-99b	440	0.56 (0.06)	0.38 (0.10)	0.51 (0.34–0.72)	5.06 (3.35–8.69)	1.25 (0.54)
BC	1999	BC7-99c	420	0.83 (0.13)	0.52 (0.17)	0.53 (0.13–1.41)	2.50 (1.04–62.31)	5.42 (0.07)
BC	1997	BC4-97	590	1.89 (0.19)	0.50 (0.10)	0.54 (0.42–0.68)	2.57 (1.90–3.90)	4.06 (0.40)
BC	1999	BC7-99a	420	0.85 (0.08)	0.33 (0.10)	0.68 (0.54–0.84)	3.06 (2.24–4.67)	2.31 (0.32)
BC	1999	BC7-99b	410	0.77 (0.07)	0.30 (0.10)	0.68 (0.53–0.86)	3.55 (2.56–5.50)	0.27 (0.87)
BC	1999	BC4-99a	380	0.92 (0.12)	−0.25 (0.13)	1.31 (0.99–1.66)	5.26 (3.81–8.58)	3.33 (0.19)
BC	1999	BC6-99	420	0.88 (0.09)	−0.38 (0.12)	1.53 (1.20–1.93)	6.55 (4.82–9.97)	0.28 (0.87)
WA	2000	WA16-00b	450	2.02 (0.21)	0.27 (0.10)	0.74 (0.57–0.93)	3.17 (2.35–4.77)	1.36 (0.51)
WA	2000	WA21-00	520	2.10 (0.23)	0.25 (0.10)	0.76 (0.60–0.94)	3.10 (2.34–4.59)	0.50 (0.78)
WA	2000	WA14-00	800	1.39 (0.10)	0.15 (0.07)	0.78 (0.62–0.96)	6.45 (4.80–9.36)	2.60 (0.27)
WA	2000	WA22-00	690	2.15 (0.20)	0.16 (0.09)	0.84 (0.69–1.02)	3.32 (2.58–4.62)	1.97 (0.58)
WA	2000	WA16-00a	790	2.03 (0.15)	0.15 (0.07)	0.84 (0.71–0.98)	3.60 (2.88–4.75)	1.87 (0.39)
WA	2000	WA15-00	480	1.85 (0.16)	0.09 (0.09)	0.89 (0.71–1.11)	4.38 (3.26–6.40)	1.06 (0.59)
WA	2000	WA19-00	540	1.79 (0.16)	0.09 (0.08)	0.90 (0.72–1.11)	4.65 (3.46–6.83)	0.60 (0.74)
WA	2000	WA20-00	830	1.80 (0.13)	−0.13 (0.07)	1.18 (0.99–1.39)	6.05 (4.72–8.26)	1.77 (0.41)
WA	1998	WA15-98	420	1.75 (0.20)	−0.17 (0.11)	1.25 (0.92–1.62)	6.73 (4.85–10.60)	0.63 (0.73)

[a] Clone collection site (BC, British Columbia; WA, Washington) and orchard production practice indicated in Table 1.

pomi from caged apple seedlings in a previous study (Lowery and Smirle 2003). LC_{50} values determined for third instars of *A. spiraecola* clearly show that imidacloprid is less toxic to this species compared with *A. pomi.* For the 16 clones of *A. spiraecola* tested in this manner, all of the values (Table 5) were higher than any of those for third instars of *A. pomi* (Table 4). The range in LC_{50} values for *A. spiraecola* of 0.51–1.25 ppm represents a difference of only 2.5-fold. The two most tolerant clones from Washington differed significantly in susceptibility to imidacloprid from three of the British Columbia clones based on nonoverlapping 95% confidence intervals. The susceptibility of the seven British Columbia clones was similar, however, to that for the nine Washington clones; average LC_{50} values were 0.82 and 0.91 ppm, respectively. The earlier registration of imidacloprid in Washington in relation to BC had not influenced susceptibility of this species to any great extent. Although a clone from crab apple in Wenatchee was the most susceptible Washington clone, it was not significantly different from five clones from conventional Washington orchards based on overlapping 95% confidence intervals. The LC_{50} value for a second clone from crab apple at the same site fell approximately midway between the upper and lower values.

Considering that *A. spiraecola* is a widely distributed polyphagous species that occurs on citrus as well as apple, it is perhaps surprising that LC_{50} values for third instars reared on treated leaf disks of apple were less variable than those for *A. pomi. A. spiraecola* clones tested in this study varied greatly in size and appearance compared with *A. pomi* (D.T.L., unpublished data). Individuals of one clone were bright yellow and a little more than half the size of individuals from the largest clone, which had an apple green background color similar to that of *A. pomi.* Physical characteristics could evolve independently and perhaps more rapidly in comparison with physiological properties. A review of the literature suggests that *A. spiraecola* has only recently become common in the Pacific Northwest (Forbes and Chan 1989, Mayer and Lunden 1996), perhaps as a result of an adaptation allowing use of apple as a primary host (Pfeiffer1991). A narrow genetic base could explain the similar response to imidacloprid for a number of clones from British Columbia and Washington.

In previous studies, systemic treatment of lettuce seedlings with imidacloprid through a hydroponic rearing system resulted in LC_{50} values for a number of insecticide resistant and susceptible populations of *M. persicae* of 9.3–45.8 ppm based on adult mortality after 24 h (Kerns et al. 1998). Using a similar systemic application technique, Cahill et al. (1996) established LC_{50} values for several susceptible and resistant strains of tobacco whitefly ranging from 0.5 to 4.4 ppm. These methods are substantially different than treatment of leaf disks, making it difficult to compare the susceptibility of these species with that of *A. pomi* and *A. spiraecola.* However, Nauen et al. (1998a) showed that LC_{50} values for tobacco whitefly reared on leaf disks treated with imidacloprid did not differ significantly from those for leaves treated systemically.

For third instars of *A. spiraecola,* LC_{90} values differed by <2.7-fold (Table 5). As for third instars of *A. pomi,* almost all of the LC_{90} values for *A. spiraecola* were proportional to their respective LC_{50} values, demonstrating that there was little variation in response to imidacloprid among the tested clones; slopes and intercepts did not vary greatly. A diagnostic dose of ≈7.0 ppm would suggest clones or populations that should be tested further for possible resistance to imidacloprid. This diagnostic dose is more than twice that determined for *A. pomi* under the same test conditions.

In conclusion, bioassays involving dipping of adult aphids in test solutions as per the FAO protocol are relatively simple to conduct, but rearing third instars on treated leaf material produced more reliable LC_{50} and LC_{90} values. Depending on the bioassay method, *A. spiraecola* was shown to be from 4.5-fold to 5.8-fold more tolerant to imidacloprid compared with *A. pomi.* An insufficient number of clones were tested to accurately establish diagnostic doses for adult *A. spirae-*

cola dipped in test solutions or reared on treated leaf disks. For third instars reared on treated leaf material, the diagnostic dose is ≈7.0 ppm. For *A. pomi*, an approximate diagnostic dose of 40.0 ppm was established for dipped adults, whereas for both adults and third instars reared on treated leaf disks the value is around 3.0 ppm. Our results support earlier findings that *A. spiraecola* and *A. pomi* differ in susceptibility to a number of insecticides (Hogmire et al. 1990, 1992).

Clones from organic orchards were not more susceptible than those from conventional orchards, suggesting that prior exposure to synthetic insecticides had not influenced susceptibility levels. As well, testing of numerous clones over a period of 6 yr did not reveal the development of resistance in either species.

These findings should be considered during testing of new products for the control of aphids on apple. Spray rates established using the more susceptible species might not provide a sufficiently high rate of overkill for the other aphid species, possibly leading to control failure when sprays are applied improperly to large trees. Ideally, novel materials should be tested against susceptible and resistant clones of both species. Considering the differing levels of susceptibility to imidacloprid for these two aphid species, additional information relating to their distribution, relative abundance, and biology would assist in the development of an effective pest management strategy.

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References Cited

Blackman, R. L., and V. F. Eastop. 1984. Aphids on the world's crops: an identification and information guide. Wiley, Chichester, United Kingdom.

Cahill, M., K. Gorman, S. Day, I. Denholm, A. Elbert, and R. Nauen. 1996. Baseline determination and detection of resistance to imidacloprid in *Bemisia tabaci* (Homoptera: Aleyrodidae). Bull. Entomol. Res. 86: 343–349.

Devine, G. J., Z. K. Harling, A. W. Scarr, and A. L. Devonshire. 1996. Lethal and sublethal effects of imidacloprid on nicotine-tolerant *Myzus nicotianae* and *Myzus persicae.* Pestic. Sci. 48: 57–62.

Elbert, A., R. Nauen, M. Cahill, A. L. Devonshire, A. W. Scarr, S. Sone, and R. Steffens. 1996. Resistance management with chloronicotinyl insecticides using imidacloprid as an example. Pflanzenschutz-Nachrichten Bayer 49: 5–54.

Elbert, A., R. Nauen, and W. Leicht. 1998. Imidacloprid, a novel chloronicotinyl insecticide: biological activity and agricultural importance, pp. 50–73. *In* I. Ishaaya and D. Degheele [eds.], Insecticides with novel modes of action; mechanisms and application. Springer, Berlin, Germany.

[FAO] Food and Agriculture Organization. 1979. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides: method for adult aphids - FAO method no. 17. FAO Plant Protection Bull. 27: 29–32.

Forbes, A. R., and C. K. Chan. 1989. Aphids of British Columbia. Agric. Canada Tech Bull. 1989-E.

Halbert, S. E., and D. J. Voegtlin. 1992. Morphological differentiation between *Aphis spiraecola* and *Aphis pomi* (Homoptera: Aphidiae). Great Lakes Entomol. 25: 1–8.

Hogmire, H. W., M. W. Brown, and V. L. Crim. 1990. Toxicity of slide dip application of five insecticides to apple aphid and spirea aphid (Homoptera: Aphididae). J. Entomol. Sci. 25: 10–15.

Hogmire, H. W., H. W. Brown, J. J. Schmidt, and T. M. Winfield. 1992. Population development and insecticide susceptibility of apple aphid and spirea aphid (Homoptera: Aphididae) on apple. J. Entomol. Sci. 27: 113–119.

Kerns, D. L., J. C. Palumbo, and D. N. Byrne. 1998. Relative susceptibility of red and green color forms of green peach aphid to insecticides. Southwest. Entomol. 23: 17–24.

Knaust, H.-J., and H.-M. Poehling. 1992. Effect of imidacloprid on cereal aphids and their efficiency as vectors of BYD virus. Pflanzenschutz-Nachrichten 45: 381–408.

Lowery, D. T., and M. J. Smirle. 2003. Comparison of bioassay techniques for determining baseline susceptibilities to imidacloprid for green apple aphid (Homoptera: Aphididae). J. Econ. Entomol. 96: 1864–1871.

Mayer, D. F., and J. D. Lunden. 1996. Apple and spirea aphids (Homoptera: Aphididae) on apples in south central Washington. J. Entomol. Soc. Br. Columbia 93: 35–37.

Mullins, J. W. 1993. Imidacloprid: a new nitroguanidine insecticide, pp. 183–198. Am. Chem. Soc. Symp. Ser. No. 524.

Nauen, R., and A. Elbert. 1997. Apparent tolerance of a field-collected strain of *Myzus nicotianae* to imidcaloprid due to strong antifeedant responses. Pestic. Sci. 49: 252–258.

Nauen, R., B. Koob, and A. Elbert. 1998a. Antifeedant effects of sublethal dosages of imidacloprid on *Bemisia tabaci.* Entomol. Exp. Appl. 88: 287–293.

Nauen, R., H. Hungenberg, B. Tollo, K. Tietjen, and A. Elbert. 1998b. Antifeedant effect, biological efficacy and high affinity binding of imidacloprid to acetylcholine receptors in *Myzus persicae* and *Myzus nicotianae.* Pestic. Sci. 53: 133–140.

Pfeiffer, D. G. 1991. Biology and management of aphids on apple, pp. 169–183. *In* K. Williams [ed.], New directions in tree fruit pest management. Good Fruit Grower, Yakima, WA.

Pfeiffer, D. G., M. W. Brown, and W. W. Varn. 1989. Incidence of spirea aphid (Homoptera: Aphididae) in apple orchards in Virginia, West Virginia and Maryland. J. Entomol. Sci. 24: 145–149.

SAS Institute 2000. SAS version 8.1. Cary, NC.

Wang, K.-Y., T.-X. Liu, C.-H. Yu, X.-Y. Jiang, and M.-Q. Yi. 2002. Resistance of *Aphis gossypii* (Homoptera: Aphididae) to fenvalerate and imidacloprid and activities of detoxification enzymes on cotton and cucumber. J. Econ. Entomol. 95: 407–413.

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