

Use of Shaking Treatments and Preharvest Sprays of Pyrethroid Insecticides to Reduce Risk of Yellowjackets and Other Insects on Christmas Trees Imported Into Hawaii

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ABSTRACT Insects are commonly found by Hawaii's quarantine inspectors on Christmas trees imported from the Pacific Northwest. To reduce the risk of importing yellowjacket (*Vespula* spp.) queens and other insects, an inspection and tree shaking certification program was begun in 1990. From 1993 to 2006, the annual percentage of shipped containers rated by Hawaii quarantine inspectors as moderately or highly infested with insects was significantly higher for manually shaken trees than for mechanically shaken trees. Between 1993 and 2001, 343 insect species in total were recovered from Christmas trees. Live western yellowjacket [*Vespula pensylvanica* (Saussure)] queens were intercepted both from containers certified as manually shaken and from containers certified as mechanically shaken. The standard manual shaking protocol removed about one-half of the queens from Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] trees that were naturally infested with western yellowjacket queens. We investigated the use of preharvest sprays of permethrin as a complement to shaking procedures used to control yellowjackets and other insects. Western yellowjacket queens and honey bees (surrogates for wasp pests) were exposed to Noble fir foliage that had been sprayed in the field with permethrin >6 wk before harvest. Pesticide residues provided complete control (moribundity or mortality) in both species. The sprays did not affect needle retention or quality of Noble fir foliage. We conclude that preharvest sprays of pyrethroid insecticides could be used in combination with mechanical shaking to greatly reduce the quarantine risk of yellowjacket queens and other insects in exported Christmas trees.

KEY WORDS yellowjackets, Christmas trees, pyrethroid insecticides, permethrin, tree shaking

Christmas trees are a major seasonal import to the state of Hawaii. Each year, thousands of trees arrive in Honolulu from the Pacific Northwest in refrigerated sea-freight containers. Insects are commonly found on these trees by Hawaii Department of Agriculture (HDOA) quarantine inspectors. In a 1988 study, HDOA found that 90.2% of the 448 containers of Christmas trees imported from the Pacific Northwest contained live insects. HDOA officials were most concerned about the presence of queens of several different species of yellowjackets (*Vespula* spp.) occasionally intercepted in Christmas tree shipments.

Although most yellowjackets nest in the ground, mated queens sometimes choose Christmas trees as an overwintering site. *Vespula pensylvanica* (Saussure), the western yellowjacket, has been established in Hawaii since 1919 (Williams 1927) and is categorized as both a public nuisance and a threat to populations of native insects (Stone and Pratt 1995). It has been hypothesized that a new, more aggressive race of this species was introduced into the islands in 1977, probably on Christmas trees (Stone and Pratt 1995, Howarth and Mull 1992), and this event was associated with the infestation of the islands of Maui and Hawaii (N.J.R., unpublished data). The ecological impact of yellowjackets in Hawaii is magnified because Hawaii has no native generalist predator wasp species.

The 1988 study led the HDOA to impose a zero tolerance the following year for all live yellowjacket and hornet queens in Christmas trees. In response, state departments of agriculture in Washington and Oregon in cooperation with HDOA initiated an inspection and tree shaking certification program in 1990. Current program rules require exporters to shake trees after harvest to remove insects via either of two protocols: 1) manual shaking of 10% of the trees

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in a consignment performed by striking the butt of each tree on a hard surface in the presence of an agricultural inspector (with a requirement for manual shaking of all trees if one or more live yellowjackets is observed); and 2) mechanical shaking (using a motor- or PTO-driven shaking unit) of 100% of the trees in the consignment, with no inspection requirement (at origin) for yellowjackets (Oregon Department of Agriculture, 2004).

There have been no published assessments of the efficacy and adequacy of the shaking program. Our objectives were to 1) summarize quarantine interceptions occurring during the period 1993–2006; 2) compare insect infestation levels associated with the two shaking treatments; and 3) investigate the use of pre-harvest sprays of pyrethroid insecticides as a potential supplement to shaking treatments to reduce the risk of yellowjacket queens and other insects on imported Christmas trees.

Materials and Methods

Quarantine Interception Data. Sea-freight containers holding Christmas trees from the Pacific Northwest were inspected by HDOA upon arrival in the port of Honolulu. Before being released to the importer, each container had to be cleared by an HDOA quarantine inspector. Phytosanitary certificates issued by the Washington or Oregon departments of agriculture indicated whether the trees were manually or mechanically shaken at origin. After opening container doors, the HDOA inspector inspected the area around the door area at the front of the container for insects. If the phytosanitary certificate indicated the trees were 10% manually shaken, the inspector removed a subsample of trees from the container and shook them to dislodge arthropods, which were collected for subsequent identification. This inspection method was also used occasionally for containers listed as 100% mechanically shaken. Inspectors subjectively classified containers as uninfested, lightly infested, or moderately/heavily infested. When live yellowjackets were found, the yellowjackets were collected for identification, and the container was sealed and transported to a screened-in area at the HDOA Plant Quarantine Station. There the trees were individually unwrapped and shaken (butt rapped three times on a hard surface) in the presence of a quarantine officer. All arthropods dislodged during these 100% inspections were collected and identified to species or morpho-species by HDOA entomologists using keys and/or comparison with identified specimens. The identification dataset presented herein is derived from the quarantine inspections of imported Christmas trees between 1993 and 2001 (data for specimens collected more recently has not been collated). Species names were compared with the Bishop Museum Arthropod Checklist (2008) and other published sources to determine whether the species was exotic (not established in Hawaii). If a determination could not be made (due to incomplete identification), the species in question was assumed not to be exotic.

Manual Shaking to Remove Yellowjackets Queens. A trial on the efficacy of manual shaking was carried out in Puyallup, WA, in 1990. A Quonset screen cage was placed over a planting of eight lightly sheared Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] trees that were 1.5–2.0 m high and 0.9 m apart. A colony of *V. pensylvanica* was placed inside the cage on 17 October underneath a polyethylene rain shelter and provided honey and water. Yellowjacket queens were allowed to mate and settle naturally into hibernating locations. The cage was removed on 26 November. Each tree was closely examined, and the locations of queens within trees were noted. Trees were cut 10–15 cm from the base and the butts rapped forcefully against a sheet of 1.27-cm (0.5-in.)-thick plywood placed on ground. After each set of three raps, dislodged queens were collected and counted. Sets of raps were continued until sets produced no additional queens. Trees were then dissected, and individual branches removed and closely inspected for additional queens.

Application of Pyrethroid Insecticides. In 2005 and 2006, pyrethroid insecticides were applied to Noble fir Christmas trees (≈ 1.8 m [6 ft] in height, spaced 1.7 m [5.5 feet] apart) growing in a commercial planting in Orting, WA. Trees at least two rows apart showing good growth form were selected and tagged for spray treatments by using a randomized complete block design with each tree considered as a replicate. There were four blocks of trees in 2005 and five blocks in 2006. The exposed foliage on the trees were sprayed until wet on all sides with a mist blower (Solo Port 423 gas-powered mist blower, Solo, Newport News, VA) from a distance of ≈ 2 –3 m. Insecticide spray treatments used in 2005 were 0.0049% esfenvalerate (Asana XL, 8.4% [AI], DuPont, Wilmington, DE), 0.184% permethrin (Astro, 36.8% [AI], FMC, Philadelphia, PA), and 0.010% bifenthrin (TalstarOne, 7.9% [AI], FMC). The concentrations used corresponded to the maximum allowable rates for Christmas trees or for ornamental trees on the product labels. The treatment variable in 2005 was a combination of insecticide type and application date. Trees were sprayed one time, either on 11 November or 30 November (≈ 1 or 3 wk before harvest). Harvest date was 6 December. Two sets of experimental controls were used (one set for each spray date). These trees were sprayed only with water. The treatment variable in 2006 was application date. All trees other than the experimental control were sprayed one time only with 0.184% permethrin either on 26 September, 17 October, or 31 October ($\approx 1, 3, \text{ or } 6$ wk before harvest). Harvest date was 11 November. Trees assigned to the experimental control were sprayed with water on 31 October. On the day of harvest, trees were cut as close to ground level as possible using a chain saw, and transported by truck to the research facilities at Washington State University, Puyallup, located ≈ 10.5 km from the field site. Data in 2005 on rainfall accumulation were obtained from a weather station at Washington State University (Puyallup) (data provided courtesy of Washington State University AgWeatherNet). Data in 2006 are from the

Chinook Estates weather station in Orting. These two stations were located ≈ 10 and 2 km from the field site, respectively.

Shaking Arthropods from Permethrin-Treated Trees. In 2006, the Noble fir Christmas trees described above were harvested in the rain and brought to a research building kept at $\approx 18^\circ\text{C}$. Trees were stored along the walls and sorted by treatment to minimize insect movement among treatments. Insects were shaken from trees the following day after the foliage had dried. Trees were shaken for 10 s in a vertical position on a Lit'l Shakee electric-powered mechanical shaker with a seven inch-diameter cone vibrating at 700 rpm (McCanse Engineering, Inc, Oregon, IL). Living arthropods were collected from the floor beneath the trees during a 2-min search period and placed into 70% ethanol for later identification. Insects were identified to family level using keys in Borror et al. (1976). Although insects also were shaken from trees harvested in 2005, the data are not shown because trees were lightly infested (typically only a few arthropods per tree).

Bioassay Assessment of Pyrethroid Residues on Harvested Trees. Insects used to assess efficacy of insecticide residues included worker honey bees (used as surrogates for pest species of wasps), western yellowjacket (*V. pensylvanica*) queens, and queens of the European paper wasp, *Polistes dominulus* (Christ). Honey bees (*Apis mellifera* L.) used in 2005 and 2006 were supplied by a local bee keeper in Puyallup, WA. Western yellowjacket queens were obtained from an excavated nest near Moxee, Yakima County, WA, on 18 November 2006. These were kept chilled until needed for bioassays. European paper wasp queens were collected under the eaves of a house near Tenino, WA, on 26 October 2006 and also kept refrigerated until needed. Insects were confined for 24 h to branchlets 6.5–7.0 cm in length clipped from either the inner or outer portion of the canopy (as indicated in Tables 4 and 5). Insects and branchlets were enclosed in small, clear plastic cylinders (4.0 cm in diameter by 6.2 cm in length) with snap top lids. Branchlets considered to be "outer" foliage were from the current year's (season's) growth located within 10 cm of the exterior cone shape of the tree. "Inner" foliage was collected at least 30-cm-horizontal distance from the outer cone and from the middle portion of the tree. Each cage held three or five honey bees, or one or two queens of *V. pensylvanica* or *P. dominulus*. We used fewer wasps than honey bees because the supply of wasp queens was limited. At the end of the bioassay period (4 or 24 h), insects were scored as living, moribund, or dead. Those that were unable to right themselves when prodded were considered moribund, whereas those that showed no movement of any body part were considered dead. Holding temperatures during bioassays were $\approx 13^\circ\text{C}$ in 2005 and $\approx 18^\circ\text{C}$ in 2006. A warmer building was chosen for bioassays the second year because some of the worker bees died from cold exposure during the holding period the first year.

Exposure of Insects to Foliage Dipped in Permethrin Solutions (2006). To study variation in permethrin tolerance among insect species used in bioassays, we confined insects to foliage of Noble fir that had been dipped in various concentrations of permethrin. One branch was dipped into each concentration for 3 s and hung indoors to dry. The next day, 15 current season shoots (6.5–7.0 cm in length) were clipped from each branch and set up individually in bioassay arenas (five shoots per insect species per concentration) as described above.

Foliage Quality Test (2005). To determine whether field treatments of pyrethroid insecticides had an effect on postharvest quality of trees, three branches ≈ 20 cm in length composed of the current and previous-season's growth were clipped from the outer portion of the canopy of each tree 12 d after trees were harvested from the field. Samples were displayed in racks on a table in a windowless room at a constant temperature of $\approx 13^\circ\text{C}$. Branches were weighed on the same day they were clipped from trees, and again after 2, 5, 8, and 10 d. At the end of this holding period, dry weights were measured after drying at 60°C for 48 h. Moisture content was calculated as $100 \times (\text{wet weight} - \text{dry weight}) / \text{dry weight}$.

Analysis. Data on the percentage of sea-freight containers classified as moderately or heavily infested with insects was arcsine square-root transformed (Steel and Torrie 1980) before analysis using a paired *t*-test (SAS Institute 2001). Pairs were comprised of data associated with mechanical and manual shaking within each calendar year. Percentage mortality data for honey bees exposed 4-h to residues of different pyrethroid insecticides was transformed as described above and then analyzed by analysis of variance (ANOVA) by using independent variables of spray type, spray date, and an interaction term (control data excluded). Tukey's multiple comparison tests were then used to separate main effects means (the interaction term was not significant). Mortality of honey bees exposed 4-h to pesticide residues on branches clipped from inner and outer canopies of harvested trees that had been treated at various preharvest treatment intervals was analyzed using Kruskal–Wallis tests to determine effect of preharvest treatment interval. Separate analyses were carried out for inner and outer branch samples, and control data were excluded. Using the same data set, a direct statistical comparison of inner versus outer branches was accomplished with a Friedman rank sum test with each tree considered as a separate block (Hollander and Wolfe 1973). Parametric tests were avoided because of low sample numbers upon which percentages were based.

For 24-h mortality data involving western yellowjacket queens and honey bees, pairwise Fisher exact tests (Kirkman 1996) (carried out separately for each insect species) were used to determine the effect of preharvest treatment interval on the efficacy of permethrin residues. Each test included 2 by 2 contingency cells holding mortality/survival data associated with exposure to foliage treated 6 wk before harvest and foliage treated 1 or 3 wk before harvest. Mori-

Table 1. Number of sea-freight shipping containers with Christmas trees imported into Hawaii, percentage of these listed as having received the 100% mechanical shaking treatment versus 10% manual shaking treatment, and the percentages of containers within these shaking treatment categories classified by Hawaii Department of Agriculture inspectors as uninfested (0), lightly infested (L), or medium-to-heavily infested (M/H) with insects

Yr	Containers	% each type, ^a 100%/10%	% containers infested with insects					
			100% trees mechanically shaken			10% trees manually shaken		
			0	L	M/H	0	L	M/H
1993	495	50.5/49.5	81.6	17.2	1.2	57.2	40.8	2.0
1994	515	68.0/32.0	74.0	25.4	0.6	55.8	41.2	3.0
1995	420	71.0/29.0	18.5	74.5	7.0	18.9	45.9	35.2
1996	452	68.0/32.0	53.1	41.7	5.2	27.9	58.6	13.5
1997	407	64.9/35.1	65.2	31.8	3.0	28.7	62.2	9.1
1998	353	73.1/26.9	68.7	31.2	0.1	37.5	50.0	12.5
1999	407	76.2/23.8	33.2	63.9	2.9	18.5	58.8	22.7
2000	325	74.5/25.5	32.2	66.1	1.7	7.2	71.1	19.3
2001	337	78.0/22.0	48.7	43.7	7.6	4.1	79.7	16.2
2002	290	75.9/24.1	83.6	16.4	0	31.4	62.9	5.7
2003	299	92.3/7.7	55.8	44.7	1.7	52.1	8.7	39.2
2004	227	78.0/22.0	53.7	46.3	0.0	2.0	78.0	20.0
2005	207	75.6/24.4	78.6	23.9	1.9	9.6	71.3	19.2
2006	170	82.4/17.6	5.7	91.4	2.9	0.0	80.0	20.0
Avg	350.3	73.5/26.5	53.8	44.2	2.6	25.1	57.8	17.0

^a Percentages of containers holding trees that were either mechanically shaken (100% of trees) or manually shaken (10% of trees in consignment) at destination to remove insect pests, according to information on the phytosanitary certificate.

bundity/mortality response for insects exposed to foliage dipped in permethrin was analyzed by probit analysis using a \log_{10} transformation of pesticide concentration (SAS Institute 2001). Significant differences in response were defined by nonoverlap of 95% confidence intervals for LC_{50} values.

Data on postharvest moisture retention was subjected to a repeated measures analysis of variance (ANOVA) (SAS Institute 2001). The model used moisture content (averaged over three branch samples per tree for each repeated measure) as the dependent variable, and block, spray treatment, application date and treatment \times application date as independent variables. A polynomial transformation was used to implement the repeated measures analysis, and probabilities were calculated using Wilks' Lambda (SAS Institute 2001). The preceding analysis led to a significant interaction involving spray treatment and application date, which was explored by reclassifying each spray treatment-date combination as an independent treatment, and carrying out an ANOVA and Tukey's honestly significant difference (HSD) multiple comparison test using day 5 data only (SAS Institute 2001). Day 5 data were selected because the range of treatment differences was maximal at this time.

Results

Quarantine Interception Data. From 1993 to 2006, an average of 350.3 sea-freight containers (6.1–12.2 m in length) containing Christmas trees were imported each year from the Pacific northwest through Hawaii's main port of Honolulu (Table 1). The percentage of Christmas tree containers classified as 100% mechanically shaken ranged from 50.5 to 92.3%. The remainder of the imported containers held trees that were manually shaken (10% of trees in consignment man-

ually shaken, 90% not shaken). The yearly average percentage of shipping containers rated by quarantine inspectors as moderately or highly infested with insects after mechanical and manual shaking was 2.6 and 17.0%, respectively (Table 1). This difference was statistically significant ($t = 6.88$, $P < 0.0001$, paired t -test; $N = 14$). From 1993 through 2001, 343 species of arthropods were intercepted on Christmas trees, representing 14 insect orders, 113 families, and 270 genera (Fig. 1A; data on families and genera not shown). Two-hundred and seventy-eight (81.0%) of the species intercepted are not known to occur in the state of Hawaii (Fig. 1B). The families of insects represented by the greatest number of species were Curculionidae (weevils, 43 species), Carabidae (ground beetles, 24 species), Coccinellidae (lady beetles, 21 species), and Pentatomidae (stink bugs, 30 species) (Fig. 2A and B). Of these 118 species, at least 99 are not established in Hawaii. Interceptions representing the greatest ecological threat were in the families Formicidae (ants; eight species) and Vespidae (yellowjackets, hornets, and paper wasps, seven species) (Fig. 2C). None of the ant species intercepted is known to be established in Hawaii, and only one (*V. pennsylvanica*) of the seven intercepted vespid species is established. Vespid species intercepted in Christmas trees since 1993 are *Vespula germanica* (F.), *Vespula vulgaris* (L.), *Polistes* sp., *Dolichovespula arenaria* (F.), *D. norvegicoides* (Sladen), and *Vespula atropilosa* (Sladen), although only a single dead specimen of the latter species was collected.

Annual interceptions of *Vespula* queens from 1994 through 2006 ranged from 19 to zero, depending on the year (Table 2). *V. pennsylvanica* was by far the most common species collected. Queen interceptions were highest in 2001, with a total of 19 queens collected from six containers. Four of these containers held trees

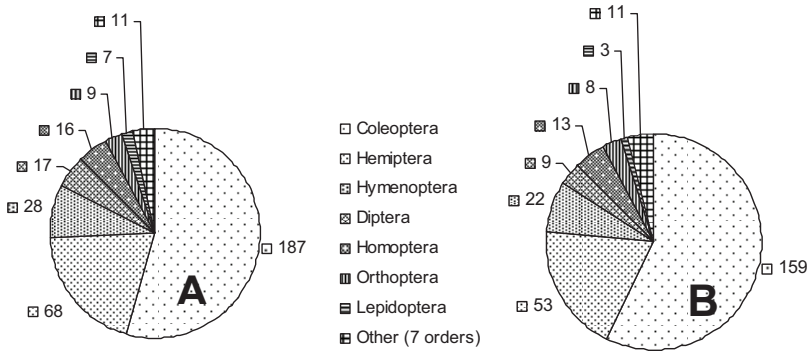


Fig. 1. Number of insect species within insect orders intercepted on Christmas trees 1993–2001. (A) Total species. (B) Species not known to be established in Hawaii, based on Bishop Museum Arthropod Checklist (2008) and other published sources.

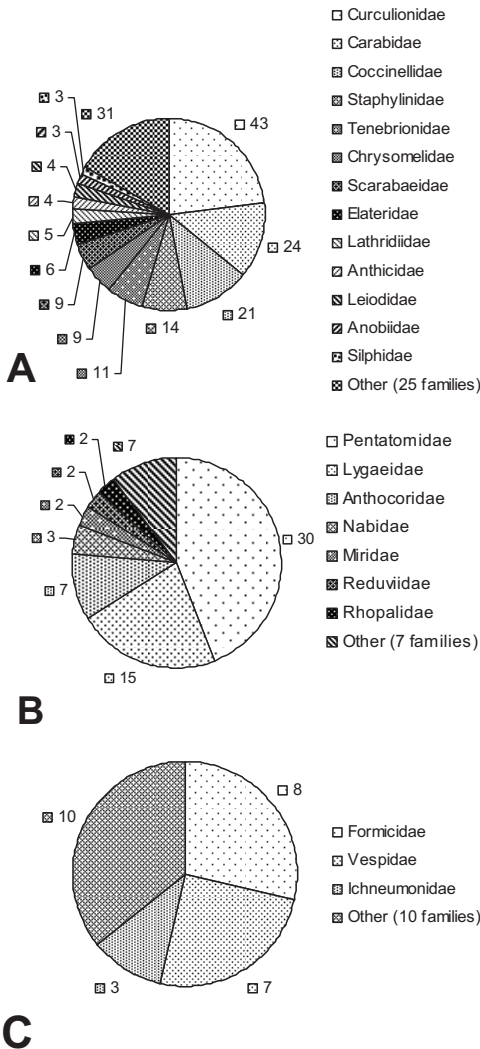


Fig. 2. Number of species within insect families intercepted in Christmas trees 1993–2001. (A) Coleoptera. (B) Hemiptera. (C) Hymenoptera.

certified as 100% mechanically shaken. These four containers held a total of ten queens.

Manual Shaking to Remove Yellowjackets Queens.

The eight trees from the *V. pensylvanica* enclosure held a total of 58 living overwintering queens, with a range of three to 16 queens per tree (Table 3). In total, 31 wasps (53%) were dislodged by the first series of three raps (the industry standard for phytosanitary certification by using the 10% shake treatment). This treatment completely removed queens from only two of the eight trees. In total, 10 wasps (17%) on two trees were not dislodged by six hard raps, and four wasps (all on the same tree) were not dislodged even by 12 raps.

Rainfall Accumulations between the Time of Pyrethroid Sprays and Harvest (2005–2006). Total rainfall experienced by Christmas trees from the time of spraying to harvest was low in 2005; 83 and 19 mm, respectively, fell during the 3- and 1-wk intervals between spraying and harvest. In 2006, rainfall was exceptionally high; 262, 241, and 224 mm, respectively, fell during the ≈6-, 3-, or 1-wk intervals between spraying and harvest.

Shaking Arthropods from Permethrin-Treated Trees (2006). The five trees in the experiment control (sprayed with water) yielded 13 specimens in the orders Hemiptera and Coleoptera when shaken. In contrast, only six specimens in these two orders were

Table 2. Numbers of yellowjacket (*Vespula* spp.) queens intercepted by Hawaii Department of Agriculture inspectors between 1994 and 2006

Yr	<i>V. pensylvanica</i>	<i>V. vulgaris</i>	<i>V. germanica</i>
1994	12	1	1
1995	7	1	0
1996	1	1	0
1997	0	0	0
1998	2	0	0
1999	1	0	0
2000	11	1	0
2001	18	1	0
2002	0	0	0
2003	0	0	0
2004	0	0	0
2005	1	0	6
2006	0	0	0

Table 3. Number of *V. pensylvanica* queens dislodged from unshaded Christmas trees by mechanical trunk impacts

Tree no.	Tree ht (m)	No. queens dislodged per 3-rap ^a series				No. queens found upon dissection	Total no. queens in tree
		1	2	3	4		
1	1.5	5	1	0	— ^b	0	6
2	2.0	5	4	0	—	4	13
3	0.9	3	0	0	—	0	3
4	2.0	3	1	0	—	0	4
5	2.0	4	5	0	0	0	9
6	0.9	5	5	5	1	0	16
7	2.0	3	0	0	0	0	3
8	1.9	3	1	0	0	0	4

^a Each "rap" was a forceful strike of the bottom end of the tree bole against plywood that was 1.27 cm (0.5 in.) thick.

^b The fourth series of raps was omitted for trees numbers 1–4.

shaken from the 15 trees treated with permethrin at 1, 3, or 6 wk preharvest (Fig. 3). Statistics indicated that the total number of insects in these two orders in the experimental control was significantly greater than total counts obtained from trees treated with permethrin 3 or 6 wk before harvest, but not greater than those treated 1 wk before harvest ($P < 0.05$, Wilcoxon two-sample tests, using one-sided normal approximation; SAS Institute 2001). Other living organisms shaken from trees included centipedes (class Chilopoda), and arthropods in the orders Opiliones (harvestmen), Collembola (springtails), Dermaptera (earwigs), and Diptera (flies) (data not shown).

Bioassay Assessment of Pyrethroid Residues on Harvested Trees. Percentages of honey bee workers moribund or dead 4 h after confinement to treated Noble fir foliage were significantly ($F = 11.7$; $df = 2, 18$; $P < 0.001$) different depending on the type of pesticide sprayed (Table 4). No significant difference was associated with either spray date (i.e., 1 or 3 wk before harvest) ($F = 0$; $df = 1, 18$; $P = 1.0$) or the interaction of pesticide type and spray date ($F = 0.3$; $df = 2, 18$; $P = 0.76$) (2005 data). Permethrin was significantly more effective than either esfenvalerate or bifenthrin, and

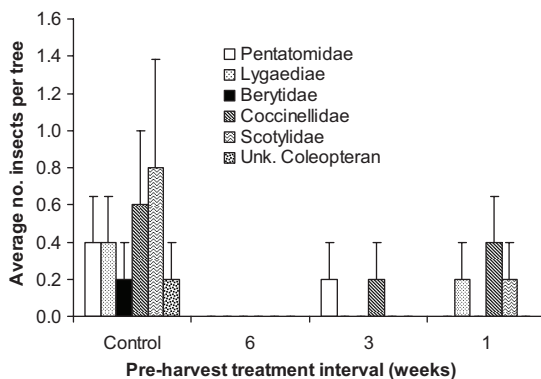


Fig. 3. Number of insects in the orders Hemiptera and Coleoptera shaken from Noble fir Christmas trees treated with permethrin in 2006. Mean \pm SE, $N = 5$. Unk, unknown family.

the latter two did not statistically differ from one another (Tukey's HSD test, $\alpha = 0.05$, 18 error df). The 2006 data for "outer" branch samples had a significant effect of preharvest treatment interval on mortality of honey bees ($P = 0.03$, $\chi^2 = 6.9$, $df = 2$, Kruskal-Wallis test; SAS Institute 2001). Average percentage of mortality on foliage treated 1, 3, and 6 wk before harvest was 76, 56, and 24%, respectively. The same analysis carried out using data from inner branch samples indicated no significant differences ($\chi^2 = 0$, $df = 2$, $P = 1.0$). A statistical comparison of honey bee mortality on outer or inner canopy showed that outer foliage was associated with significantly greater mortality ($\chi^2 = 9.6$, $df = 1$, $P = 0.002$; $N = 15$, Friedman rank sum test).

One-hundred percent of honey bee workers and western yellowjacket queens exposed to foliage treated with permethrin 1, 3, and 6 wk before harvest (2006) were moribund or dead after a 24-h exposure to inner or outer foliage, regardless of preharvest treatment interval (Table 5). Considering dead insects only (excluding those that were moribund), there was a consistent trend for higher mortality as preharvest treatment interval was reduced. Contingency table analyses indicated significantly ($P = 0.03$) higher mortality of yellowjacket queens exposed to outer foliage treated 1 wk before harvest compared with outer foliage treated 6 wk before harvest (Fisher exact test; Kirkman 1996). The same comparison involving honey bees indicated no significant mortality differences at the 5% probability level, although results approached the level of significance ($P = 0.06$; Fisher exact test). Comparisons involving preharvest treatment intervals of 3 wk versus 6 wk also indicated no significant differences at the 5% probability level for either yellowjacket queens ($P = 0.08$) or honey bee workers ($P = 0.06$), although results were marginally significant. Comparisons involving inner foliage were not made because of low sample sizes (Table 5, footnotes).

Exposure of Insects to Foliage Dipped in Permethrin Solutions (2006). Susceptibility to permethrin residues on Noble fir foliage as measured by percentage of insects moribund or dead after 24 h of continuous exposure was highest in yellowjacket queens, intermediate in worker honey bees, and lowest in queens of the European paper wasp (Table 6). LC_{50} values (95% confidence intervals) were 0.0002 g/liter (AI) (confidence intervals not estimable), 0.0012 g/liter (AI) (0.0007–0.0020), and 0.0079 g/liter (AI) (0.0030–0.0214) for yellowjacket queens, honey bees, and paper wasps, respectively (probit procedure, SAS Institute 2001). Paper wasps were significantly more tolerant (0.0079 versus 0.0012 g/liter) to permethrin than honey bees (based on nonoverlap of confidence intervals). Yellowjacket queens (0.0002 g/liter) were even more susceptible than honey bees, but statistical comparisons based on LC_{50} values for yellowjacket queens were not possible, because 95% confidence intervals could not be estimated. Concentrations of permethrin associated with 100% control (moribundity plus mortality) were 0.002, 0.02, and 0.2

Table 4. Percentage of honey bees moribund or dead after 4-h exposure to branch samples from Noble fir trees treated preharvest with pyrethroid insecticides

Yr	Canopy sampled	Treatment	Preharvest treatment interval (wk)	% moribund (SE ^a)	% dead (SE)	% control ^b	N ^c
2005	Outer	Asana	1	85 (5.3)	6 (3.2)	91	36 ^d
			3	56 (10.1)	11 (7.9)	67	36
		Astro	1	44 (12.0)	56 (12.0)	100	36
			3	53 (16.6)	44 (17.6)	97	36
		TalstarOne	1	92 (5.3)	8 (5.3)	100	36
			3	81 (5.3)	8 (2.8)	89	36
2006	Outer	Check	1	0 (0.0)	0 (0.0)	0	36
			3	6 (5.6)	0 (0.0)	6	36
			6	24 (7.5)	76 (7.5)	100	25 ^e
		Astro	3	44 (17.2)	56 (17.2)	100	25
			6	76 (9.8)	24 (9.8)	100	25
			6	4 (4)	0 (0)	4	25
Inner	Astro	1	76 (11.7)	12 (8.0)	88	25	
		3	88 (8.0)	12 (8.0)	100	25	
		6	68 (10.2)	12 (8.0)	80	25	
	Check	6	0 (0)	0 (0)	0	25	

^a Results associated with each tree were considered as replicates.

^b Moribund plus dead bees.

^c Number of worker honey bees.

^d Bees exposed in groups of three to three branch samples from each of four trees.

^e Bees exposed in groups of five to one branch sample from each of five trees.

g/liter (AI) for yellowjacket queens, honey bees, and paper wasps, respectively.

Foliage Quality Test (2005). Moisture content (calculated as $100 \times (\text{wet weight} - \text{dry weight}) / \text{dry weight}$) in branch samples collected after harvest from trees sprayed with pyrethroid insecticides ranged from 127 to 138% among treatments at day 0, but gradually declined to a moisture content of 18–38% over the 10-d display period (Fig. 4). Ranks of treatment averages at various time intervals changed little over the sampling period, as indicated in the figure by lines that were nearly parallel. Repeated measures ANOVA results indicated a significant ($P > 0.0001$) effect of time (time interval over which samples were weighed), but no significant effect of tree block ($P = 0.22$), or spray treatment ($P = 0.51$) (repeated measures ANOVA using Wilks' Lambda, SAS Institute 2001). However, there was a significant interaction involving spray treatment (the type of chemical sprayed) and application date (11 versus 30

November). This interaction was explored by considering each spray treatment-date combination as an independent treatment, and analyzing treatment differences associated with day 5 moisture content data only. Results using this one-way ANOVA indicated a significant difference among treatments ($F = 5.9$; $df = 10, 21$; $P = 0.0003$). Tukey's multiple comparison test indicated that branch samples from trees in the "check" treatment (treated with water only) associated with the first application date (11 November) and trees treated with permethrin on the second application date (30 November) yielded branch samples with significantly ($P < 0.05$) greater moisture content than trees treated with permethrin or bifenthrin on the first treatment date (SAS Institute 2001; Table 7). No needle loss was observed from any of the branches.

Table 6. Percentage of insects moribund^a or dead^b (dead only in parentheses) after 24-h exposure to Noble fir foliage dipped in permethrin insecticide in 2006

Concn (grams/liter [AI])	<i>V. pensylvanica</i> ^c	<i>A. mellifera</i> ^d	<i>P. dominulus</i> ^e
0	0 (0)	0 (0)	0 (0)
0.0002	50 (40)	4 (4)	0 (0)
0.002	100 (30)	68 (40)	20 (0)
0.02	100 (20)	100 (84)	70 (0)
0.2	100 (70)	100 (100)	100 (10)
2.0 ^e	100 (90)	100 (100)	100 (90)

^a Insects were counted as moribund if they could not right themselves when prodded.

^b Insects were counted as dead if they did not move any body part when prodded.

^c Two insects per replicate, five replications.

^d Five bees per replicate, five replications.

^e Approximately equal to maximal concentration permitted by Astro insecticide product label.

Table 5. Percentage of insects moribund^a or dead^b (dead only in parentheses) after 24-h exposure to Noble fir foliage field treated with 0.184% permethrin in 2006

Preharvest treatment interval	<i>V. pensylvanica</i> ^c		<i>A. mellifera</i> ^d	
	Outer	Inner	Outer	Inner
Control	0 (0)	0 (0)	4 (0)	0 (0)
Six weeks	100 (20)	100 (0)	100 (88)	100 (72)
Three weeks	100 (60)	100 (20)	100 (96)	100 (92)
One week	100 (70)	100 (20)	100 (100)	100 (92)

^a Insects were counted as moribund if they could not right themselves when prodded.

^b Insects were counted as dead if they did not move any body part when prodded.

^c Two queens were used for each outer branch sample, and one wasp for each inner sample (five replications).

^d Five bees were used for each branch sample (five replications).

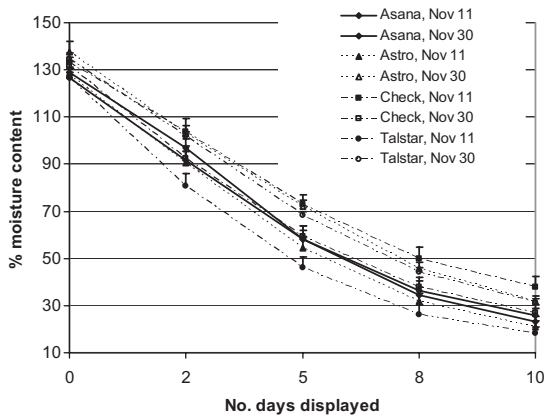


Fig. 4. Effect of preharvest pyrethroid spray type on postharvest moisture loss in branch samples (2005). Mean \pm SE, $N = 4$.

Discussion

Preharvest mist applications of permethrin to Christmas trees at label rates >6 wk before harvest resulted in complete control of two hymenopterous species exposed to treated foliage at the time of harvest. Residues were apparently higher on foliage from the outer canopy; yet, exposure to inner canopy foliage also provided 100% control of yellowjacket queens, and honey bee workers used as surrogates for wasp pests. Treatments were effective despite the occurrence of significant rainfall after spray applications. In addition to affecting hymenopterous species, permethrin sprays reduced the numbers of Hemiptera and Coleoptera found on trees that had been sprayed 3 or 6 wk earlier. Permethrin has relatively low toxicity to mammals (an LD_{50} value of 998 mg/kg oral toxicity and $>2,000$ mg/kg dermal toxicity) (FMC 2001), and products containing this active ingredient are already approved by the U.S. Environmental Protection Agency for use on Christmas trees and other ornamental trees. Preharvest permethrin sprays, in addition to tree shaking at the time of harvest, seemed to be a promising means for reducing pest risk on exported trees and the associated risk of quarantine rejections.

Astro, the permethrin product used in our study, is registered for use at the rate we tested (0.184% per-

methrin) on "ornamental trees," but Christmas trees are not listed on the label and would not be considered a valid site use (Rose Kachadoorian, personal communication). Ambush, which is another emulsifiable concentrate formulation of permethrin, is registered for use on Christmas trees. However, maximum label rates for this product (expressed as amounts per acre) would ordinarily require the applicator to use concentrations of AI much lower than those we used in our study. For example, based on the amount of spray we applied to each Christmas tree (≈ 275 ml) by using a mist blower, the maximum allowable concentration of permethrin using this product would be 13% of the concentration used in our study. In our 2005 bioassay results using honey bees, TalstarOne, sprayed 1 and 3 wk before harvest at a concentration of 0.010% bifenthrin, was almost as effective as the Astro treatment (applied at a concentration of 0.184% permethrin). Like Astro, TalstarOne is labeled for "ornamental trees" but not "Christmas trees." OnyxPro, which is also an emulsifiable concentrate form of bifenthrin, is registered for Christmas trees and can be used at a concentration as high as 0.026% active ingredient. Unlike Astro and TalstarOne, OnyxPro is a restricted use insecticide. We plan to include emulsifiable concentrate formulations of bifenthrin in future tests of preharvest sprays for control of insects in Christmas trees.

In total, 343 insect species have been intercepted on Christmas trees, including various species of ants and yellowjackets, which are a serious threat to Hawaiian ecosystems. Currently, the only treatment protocol required for Christmas trees exported to Hawaii involves shaking and inspection of trees. Although mechanical shaking was significantly more effective than manual shaking of trees, both techniques, as currently practiced, were inadequate for removal of yellowjacket queens. Experimental results presented herein demonstrated that almost half of yellowjacket queens in Christmas trees were not removed by the manual shaking protocol. Evidence for the ineffectiveness of mechanical shaking procedures includes the fact that interceptions of yellowjacket queens occurred in multiple shipments of Christmas trees accompanied by phytosanitary certificates that stated that trees had been 100% mechanically shaken. However, these interceptions do not prove the inadequacy of the mechanical shaking procedure itself. Growers store shaken trees in open shipping yards for various periods before container loading. Possibly trees were infested after shaking, or else trees might not have been shaken sufficiently. The effectiveness of the shaking will be influenced by the design of the shaker, the weight of the tree, how the tree is held on the shaker and how long it is shaken. Trees should be shaken just before loading containers destined for export. Although improved shaking practices are an important first step to reducing quarantine risk, they are unlikely to solve the problem completely due to logistical and practical considerations. Our research shows that preharvest permethrin sprays could be used as an additional measure to significantly reduce pest risk.

Table 7. Effect of different pyrethroid spray treatments on the moisture content of branch samples 5-d after initial display

Spray treatment	Date applied	Moisture content (%)
Check (water only)	11 Nov.	73.0a ^c
Permethrin	30 Nov.	72.0a
Bifenthrin	30 Nov.	68.4ab
Check (water only)	30 Nov.	59.3abc
Esfenvalerate	11 Nov.	58.3abc
Esfenvalerate	30 Nov.	58.0abc
Permethrin	11 Nov.	54.5bc
Bifenthrin	11 Nov.	46.1c

^a Means followed by the same letter are not significantly different ($P = 0.05$; Tukey's multiple comparison test).

Another potential approach to preventing yellowjacket queens in Christmas trees imported into Hawaii is to use traps with chemical attractants to trap out pest or invasive species of yellowjackets, or to assess the risk of queens establishing within Christmas trees on-farm. Workers of the western yellowjacket are strongly attracted to heptyl butyrate (Davis et al. 1969), whereas workers and spring queens of the western yellowjacket, as well as *V. germanica*, *V. vulgaris*, and other yellowjackets, hornets, and paper wasps are attracted to the combination of acetic acid and 3-methyl-1-butanol (Landolt 1998; Landolt et al. 1999, 2007). Trapping programs on Christmas tree farms in Washington and Oregon may reduce yellowjacket populations, as was shown in fruit orchards by Davis et al. (1973), or may indicate risk by relating numbers of yellowjackets trapped to the potential to recruit fall queens into Christmas trees. Unfortunately, none of the lures is attractive to fall queens.

Before testing preharvest sprays of pyrethroid insecticides, we made several attempts to control insects postharvest using aerosol formulations of pyrethrum released from pressurized canisters placed near the doors of sea freight containers packed with Christmas trees. In one of these tests, honey bees were placed within window-screen cages that were either buried within the pile of trees or left on top of the trees in various areas of the container. The aerosols killed 100% of bees in the cages placed on tops of the trees, but bees in the buried cages were unaffected, apparently due to poor circulation of the aerosol.

The postharvest moisture retention study indicated that branches that received applications of the Astro product on 11 November had statistically significant lower moisture levels than one set of the check branches. However, the moisture loss profiles from branch tips in all treatments and the lack of any needle loss were indicative of trees that would be expected to display well in the homes of consumers.

Yellowjacket wasps were particularly sensitive to pyrethrum residues, and complete control was achieved in a foliage dip exposure bioassay using a concentration one-thousandth the concentration permitted on the Astro product label. Permethrin, as a type I pyrethroid, has a negative temperature coefficient, with the result that it is generally more effective at lower temperatures (<15.5°C) (Ware and Whitacre 2004). Sea-freight containers are typically maintained at ≈4°C. Permethrin has good UV tolerance, and residues are normally efficacious on crop foliage for 4–7 d (Ware and Whitacre 2004). In our case, effective residues persisted in the field at least 46 d. Exceptional persistence was probably due to cool temperatures and low solar radiation typical of the Pacific Northwest during autumn.

In Hawaii, western yellowjacket colonies are much larger than reported on the U.S. mainland. Nests of this yellowjacket in Hawaii can be 130 cm wide and 124 cm high (Nakahara and Lai 1981, cited by Chang 1988), with cell numbers often >20,000 and up to a half million (Gambino 1992). The islands of Maui and Hawaii, in particular, support large populations of yellow-

lowjackets in certain areas of each island (Gambino 1992). A large yellowjacket colony in Volcanoes National Park (Hawaii Island) was estimated to consist of at least 1 million wasps (Stone and Pratt 1995). Extensive studies in Washington State (MacDonald et al. 1974) showed nest sizes at the end of the season with 600–12,300 cells.

Although not intercepted as often as *V. pensylvanica*, *V. vulgaris* is another yellowjacket species intercepted in Christmas tree shipments. Between 1994 and 2003, five queens in total were intercepted in Christmas trees, never more than one per year. This species was briefly established at higher elevations on the island of Maui in the 1970s but has not been collected there since and is presumed to have died out (N.J.R., unpublished data). *Vespula germanica* has been intercepted in Christmas trees even less frequently. A single queen was intercepted by HDOA in 1994. No queens of this species were intercepted again until 2007, when multiple *V. germanica* queens were found. Interceptions may be more common in the future, because this species is still expanding its range in the western United States (Akre et al. 1989).

Yellowjackets are both a public health hazard and a public nuisance. They readily attack any animal that disturbs their nest and are attracted to food consumed out-of-doors. In Hawaii, *V. pensylvanica* was found to consume large numbers of prey insects, potentially reducing populations of native insect species. In total, 22,000 prey items were taken in a single day by yellowjacket workers of a large nest (Howarth and Mull 1992, Stone and Pratt 1995). Native biota in New Zealand was seriously affected by the introductions of *V. vulgaris* and *V. germanica* (Toft and Rees 1998, Beggs and Rees 1999, Beggs 2001). Absence of native generalist predator wasp species renders the fauna and flora of the Hawaiian Islands particularly sensitive to the introduction of generalist predators such as yellowjackets.

In conclusion, the use of an integrated approach that includes the application of preharvest permethrin sprays and thorough mechanical shaking just before loading Christmas trees into containers would significantly reduce the risk that yellowjackets and other generalist predators will be inadvertently introduced into the Hawaiian Islands. Future research should focus on the factors which affect the efficiency of manual and mechanical shaking, including current industry shaking practices.

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