The Dissipation of Tebuconazole and Propiconazole in Boronia (*Boronia megastigma* Nees)

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The broad spectrum, systemic fungicides tebuconazole and propiconazole are used to control rust in boronia (*Boronia megastigma* Nees). Gas chromatography combined with either a benchtop quadrupole mass spectrometer or a high-resolution mass spectrometer allowed for the monitoring of both pesticides in boronia leaves, flowers, and concrete. Field trials were established at two sites to determine the rate of dissipation of tebuconazole and propiconazole in boronia. At site 1, two application rates of 125 and 250 g active ingredient/hectare (ai/ha) tebuconazole were employed. Treatments were repeated 17 days later. At harvest, 286 days after the final application, tebuconazole was detected at levels of 0.06 ± 0.05 and 0.5 ± 0.1 [mg/kg $\pm$ standard error, on a dry matter basis (DMB)] in the leaves collected from plots treated with 125 and 250 g ai/ha of tebuconazole, respectively. The oil produced from the flowers collected at the final harvest had residues of tebuconazole at levels of 0.06 ± 0.03 and 0.10 ± 0.08 mg/kg for the 125 and 250 g ai/ha application rates, respectively. Two repeat applications of 125 g ai/ha propiconazole were also used at site 1. Residues of propiconazole were detected at 0.09 ± 0.03 mg/kg (DMB) 286 days after the final application. At site 2, treatments of 125 g ai/ha of tebuconazole were applied twice. At harvest, 279 days after the final application of tebuconazole, residues were recorded at 0.30 ± 0.09 mg/kg in the leaves (DMB) while the oil produced had 0.20 ± 0.07 mg/kg.

INTRODUCTION

Boronia megastigma Nees (Brown Boronia) is a native shrub of the Rutaceae family, endemic to Western Australia. The yellow to reddish brown flowers of boronia are extracted with organic solvents to produce a waxy extract (concrete) used in the food and fragrance industry. Despite the development of rust tolerant clones, fungal infection is a recurrent problem in the production of boronia. The broad spectrum, systemic fungicides Folicur (tebuconazole) and Tilt (propiconazole) are applied in the spring before the dispersal and multiplication of the basidiospores. Both fungicides inhibit the biosynthesis of ergosterol (*2*) and are used in a wide range of crops to control fungi such as powdery mildew, leaf spot, rust, and root rot.

Many papers have been published dealing with the detection and degradation rates of pesticides in water, soil, and vegetative material. Pesticides in essential oils derived from citrus and mint by steam distillation have also been widely studied. However, studies on the dissipation and carryover of pesticide residues in solvent-extracted products such as boronia concretes and absolutes are limited. A study monitoring propiconazole levels in boronia attributed much of the decrease in propiconazole in

leaf material to dilution effects as a result of growth (*4*). In this study using similar analytical methodology, a benchtop quadrupole mass spectrometer in the selected ion monitoring (SIM) mode and a high-resolution mass spectrometer (HRMS) in the SIM mode were coupled with gas chromatographs to quantify both tebuconazole and propiconazole in boronia leaf material and oil. Observations were made on field trials to determine the rates of dissipation of tebuconazole and propiconazole during the growth of boronia through to flowering. The levels of residues of both systemic fungicides in treated leaves and in the flowers produced several months after pesticide application are studied. This study provides data, not only to determine residue levels in boronia concrete produced with optimal field use of pesticides but also to provide a basis from which to assess residues levels that may occur when nonroutine fungicide applications are used to control outbreaks of rust late in the growing season.

EXPERIMENTAL PROCEDURES

Field Trial Plot Layout and Treatment Schedule. Two trial sites were established within commercial boronia crops located in Southern Tasmania. Boronia is grown in double rows with diagonal spacing of 0.5 m between each plant in the row, with foliage extending to a height of 1 m. At site 1, six randomized blocks were delineated. Each block

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consisted of a 10 m section of boronia row with buffer zones of 4 m. On three of the 10 m sections, 125 g active ingredient (ai)/hectare (ha) of tebuconazole (commercial product Folicur 250 EW containing 250 g/L tebuconazole) was applied while another three were treated with 250 g ai/ha. Propiconazole (125 g ai/ha) (commercial product Tilt EC 250 containing 250 g/L propiconazole) was applied to the remaining crop. At the second site, one application of 125 g ai/ha tebuconazole was applied on each of three replicates. The high potential for fungal infection at site 1 necessitated the application of systemic pesticides to all areas of the boronia. Control plots for tebuconazole were sprayed with 125 g ai/ha of propiconazole while plots treated with 125 g ai/ha of tebuconazole provided controls for the propiconazole experiments. At site 2, rust presented few problems to the crop health and controls were taken from the untreated section of the crops. Pesticide applications were repeated at site 1 after 14 days while at site 2 a second application of 250 g ai/ha of tebuconazole occurred 10 days after the first. The equivalent of 92 mL/ha of Topwet (commercial product of Schering), a nonionic surfactant, was added to each formulation. At site 1, an automated tractor-driven spraying unit, calibrated to deliver the units required, was used. At site 2, pesticides were applied with a Buchmester backpack attached to a Matabi 4 m boom with four cone nozzles. Samples were collected immediately before, and 2 h after, each application and then at 1, 4, 16, 33, 73, and 95 days after the last application. Dry weights were determined from subsamples dried for 72 h at 35 °C, and the results were used to correct for moisture content for all samples analyzed. At the final harvest, in addition to the collection of leaf samples, boronia flowers were combed from the stems and extracted with petroleum ether to produce boronia concrete.

Measurement of Plant Growth. Growth measurements followed the methods previously employed by Reddy et al. and Roberts et al. in *B. megastima* Nees (*5, 6*). Ten boronia plants were selected at random for each site. One lateral on each plant was marked. Plant heights, the lengths of selected laterals, and the number of nodes on each lateral were recorded at each sampling date. At the end of the experiment, the plants were pruned to the initial height and the prunings, which represented the entire seasonal growth, were collected, dried at 35 °C, and weighed.

Chemicals. Analytical grade propiconazole (99%) was supplied by Ciba-Geigy, Victoria (Australia). Tebuconazole (99.6%) was obtained from Bayer. Endosulfan (99.8%) was purchased from the Australian Government Analytical Laboratories (AGAL), Pymble, NSW. Methanol and chloroform were from Mallinckrodt and of ChromAR high-performance liquid chromatography (HPLC) grade.

Standard Solution Preparation. Tebuconazole (5.2 mg) and propiconazole (5.9 mg) were dissolved in chloroform and made up to 50 mL. This resulted in a 100 μg/mL stock solution from which 10, 1, and 0.1 μg/mL working standard solutions were prepared by volumetric serial dilution.

Extraction Procedure. Boronia leaves were stripped from the woody stems and ground under liquid nitrogen with a stainless steel mortar and pestle. Ground leaves (2-3 g) were weighed into 30 mL vials and extracted in 5 mL of methanol with sonication for 10 min at room temperature. Aliquots (1 mL) of the extracts were transferred quantitatively to gas chromatography (GC) vials and spiked with 5 μL of a 192 μg/mL solution of the internal standard endosulfan. (Endosulfan was found to be a satisfactory internal standard as the retention time was close to but did not interfere with the target analytes, the peak shape was excellent and reproducible, and diagnostic ions were available for SIM. The insecticide, endosulfan, is not commonly used in the essential oil industry and is not registered for use on boronia.) One replicate was subsampled five times to obtain a measure of variability within samples. The % coefficient of variation for the repeat sample analyses was less than 4% for both tebuconazole and propiconazole.

At harvest, the flowers were combed from the plants and extracted at 20 °C in 4 × w/v of petroleum ether on a shaker for 2 h. The solvent was decanted, and the extraction was repeated. The washes were combined, and the solvent was removed using a rotary evaporator at reduced pressure and at 40 °C. At the end of the initial dry down period, the temperature was increased to 60 °C for 1 min to remove solvent residues. The yield of oil from flowers from sites 1 and 2 were 0.39 [standard error (SE) ± 0.01] and 0.38% (SE ± 0.02), respectively.

Table 1. Recoveries of Fungicides in Boronia Leaves

fungicide	fortification level (mg/kg)	% recovery ± SE
tebuconazole	25	72 ± 2 (*n* = 4)
	2.5	68 ± 3 (*n* = 6)
	0.1	75 ± 6 (*n* = 4)
propiconazole	25	58 ± 1 (*n* = 4)
	2.5	57 ± 2 (*n* = 6)
	0.1	59. 1 ± 0.8 (*n* = 4)

Subsamples (10-20 mg) were dissolved in 1 mL of methanol, and 5 μL of a 192 μg/mL solution of endosulfan was added.

Calibration Curves. Standard curves were established by spiking methanol extracts of leaves and oils produced from boronia that had not been treated with the target pesticides. In the analysis of field samples with residues expected to be above 2 mg/kg, a series of boronia extract solutions ranging from 1.04 to 20.8 μg/mL was prepared and 5 μL of a 192 μg/mL endosulfan solution was added to each vial. The samples were analyzed by a GC mass selective detector (MSD) in the SIM mode. Samples that had levels of residues above the upper range were diluted with methanol and reanalyzed. For samples with residue expected to be below 2 mg/kg, calibration curves were established in the range of 0.2 ng/mL to 1.04 μg/mL or 0.3 μg/mL to 2 mg/kg relative to boronia leaf (dry matter basis, DMB). Residues in oil samples were related to calibration curves covering the range of 0.05-1 mg/kg. Samples expected to have residue levels within these low concentration ranges were analyzed using GC HRMS.

Recoveries in Fortified Samples. Samples of boronia leaves, known to be free of the target analyte, were fortified with tebuconazole and propiconazole at levels equivalent to 0.1, 2.5, and 25 mg/kg. Recoveries are listed in Table 1. Fortification levels of the fungicides did not substantially affect their corresponding recoveries in the assayed concentration range. Causon (*1*) states, "Although it is desirable to attain recovery as close to 100% as possible in order to maximize the sensitivity of the method, it is unlikely that recoveries of 50% or more will compromise the integrity of the method." The low recoveries are most likely due to the dispersal of the target analytes through the vegetative material. The responses recorded for fortified samples are compared to the responses of extracted blank matrix fortified at the same concentration but which are no longer in contact with the vegetative material. Correction factors are determined on each day of analyses to account for this effect.

Apparatus and Chromatography. Samples were analyzed either on a Hewlett-Packard (HP) 5890 GC directly coupled via an open split interface to a HP 5970B MSD or a HP 5890 GC coupled to a Kratos Concept ISQ mass spectrometer. Within each system, a BPX5 fused silica capillary column (25 m, 0.22 mm id, 0.25 μm film thickness) was used.

Quadrupole GC-MS. Automatic injections of 1 μL (splitless) were applied to the BPX5 column, which was supplied with helium at a head pressure of 27 pounds per square inch (psi). The GC oven was held at 50 °C for 1 min followed by a temperature gradient of 10 °C/min to 290 °C, which was held for 5 min. The injector and transfer line temperatures were 250 and 290 °C, respectively. Ions monitored were *m/z* 195 for the internal standard, *m/z* 250 for tebuconazole, and *m/z* 259 for propiconazole.

Analysis by GC HRMS. Splitless injections (1 μL) of samples were analyzed using a helium carrier gas flow program of 30 psi/min from 25 to 40 psi, held for 0.1 min, then at 30 psi/min to 25 psi, then at 1 psi/min to 35 psi. The GC injection temperature was 260 °C, and the oven temperature was programmed from 60 to 290 °C at 20 °C/min. The ions of highest abundance were not necessarily selected for monitoring as matrix components with interfering *m/z* values sometimes had the same retention time as potential target ions. The ions monitored by SIM were *m/z* 259.0291 ($C_{12}H_{13}Cl_2O_2$) and 261.0262 for propiconazole, 250.0743 ($C_{12}H_{13}ClN_3O$) and 252.0714 for tebuconazole, and *m/z* 194.9534 for endosulfan, the internal standard. A dwell time of 300 ms/ion and a 50 ppm voltage sweep were employed for all ions. A resolution of 10000 (10% valley definition) was used, and the ion at

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Table 2. Levels of Residues of Tebuconazole (mg/kg) Detected in Boronia at Sites 1 and 2[a]

events	n	days	residues of tebuconazole detected (mg/kg DMB)			
			site 1			site 2
			125 g ai/ha	250 g ai/ha		125 g ai/ha
		days	mean ± SE	mean ± SE	days	mean ± SE
application 1	3	0	54 ± 1	131 ± 3	0	23 ± 3
	3	17	10.9 ± 0.1	31 ± 13	10	4.7 ± 0.5
application 2	3	0	153 ± 6	172 ± 18	0	51 ± 8
	3	1	130 ± 23	168 ± 22	1	58 ± 9
	3	4	92 ± 19	155 ± 16	5	43 ± 3
	3	16	26 ± 1	53 ± 6	14	9.6 ± 0.5
	3	33	9 ± 2	24 ± 3	32	4.4 ± 0.1
	3	53	3.1 ± 0.5	10 ± 1	58	0.90 ± 0.05
	3	95	3 ± 1	5 ± 1	93	1.0 ± 0.2
	3	169	0.4 ± 0.4	5 ± 2	172	bdl
	3	229	0.5 ± 0.3	0.9 ± 0.3	222	0.15 ± 0.07
	3	286	0.06 ± 0.05	0.5 ± 0.1	279	0.30 ± 0.09

[a] Detetion limit, 0.04 mg/kg.

Table 3. Levels of Residues of Propiconazole (mg/kg) Detected in Boronia at Site 1[a]

| events	n	days	residues of propiconazole detected (mg/kg DMB) 125 g ai/ha
			mean ± SE
application 1		0	sample not taken
		17	sample not taken
application 2	3	0	66 ± 5
	3	1	71 ± 9
	3	4	43 ± 4
	3	16	26 ± 3
	3	33	5.3 ± 0.2
	3	53	0.75 ± 0.06
	3	95	0.32 ± 0.02
	3	159	0.3 ± 0.2
	2	229	0.03 ± 0.01
	3	286	0.09 ± 0.03

[a] Detection limit, 0.004 mg/kg.

m/z 242.9856 from perfluorokerosene was used as the lock mass for both the analytes and the internal standard. Electron ionization was undertaken at a source temperature of 210 °C and an electron energy of 70 eV, with an accelerating voltage of 5.3 kV. The two diastereomers of propiconazole, the mass spectra of which are essentially indistinguishable, were both monitored and their areas were summed for quantification calculations. The mass spectra of propiconazole (*4*) and tebuconazole (*3*) have been previously reported.

RESULTS AND DISCUSSION

The standard curves for tebuconazole related the ratio of the peak area of tebuconazole (m/z 250)/the peak area of endosulfan (m/z 194) to the concentration of tebuconazole (μg/mL). Similarly, the standard curve for propiconazole related the ratio of the peak area of propiconazole (m/z 259)/the peak area of endosulfan (m/z 194) to the concentration of propiconazole (μg/mL). Linear regressions were fitted to both relationships with correlation coefficients ranging from 0.983 to 1.000. The range of concentrations under which calibration curves were obtained did not affect the corresponding correlation coefficient.

Table 2 lists the levels of tebuconazole residue detected in the boronia leaves at specified days after the first treatment for sites 1 and 2. Table 3 shows the levels of propiconazole residues detected in boronia at site 1. All results have been corrected for recoveries as detailed in the Experimental Procedures.
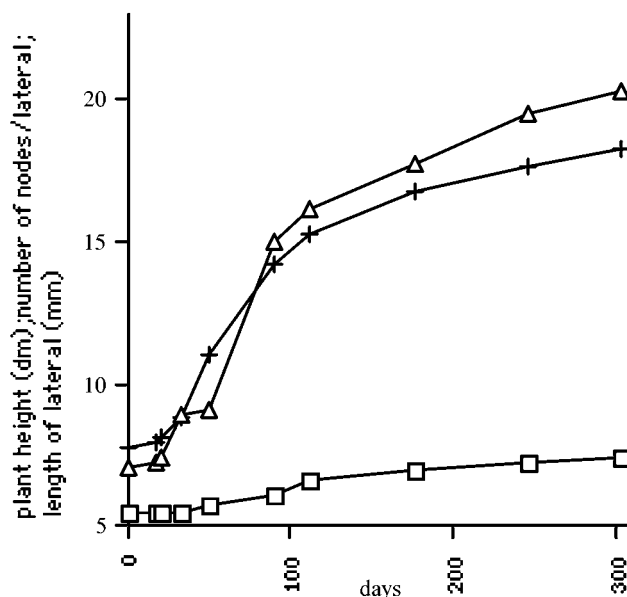
Figure 1. Increases in mean heights (□), numbers of nodes/lateral (△), and lateral lengths (+) of 10 selected boronia bushes throughout the growing season.

Tebuconazole and propiconazole are systemic pesticides. Decreases in the levels of pesticide residues are affected by growth dilution as well as by the breakdown of the ai. To account for the dilution of pesticides by plant growth, the percentage increase in plant heights, lateral lengths, and the number of nodes on each lateral for the 10 plants selected at each site were measured at each sampling date. At the end of the season, the total amount of increase in dry matter was measured by cutting the selected bushes back to the original height measured at the beginning of the trial (see Measurement of Plant Growth in the Experimental Procedures). The total vegetation was then dried and weighed. Figure 1 shows the increase in plant height, lateral length, and the numbers of nodes/lateral recorded over the period of the field trial at site 1. At site 2, the growth parameters measured showed a similar pattern. The percentage increases in lateral lengths and the numbers of nodes per lateral were calculated, and the mean increases of these two parameters were determined. The increase in vegetative bulk (DMB) (as weighed at the end of the season) was then apportioned through the period of the field trial based on the mean increase of lateral lengths and numbers of nodes. The increases in bulk between sampling dates were then used to calculate correction factors, which were applied to adjust the residue concentrations listed in Tables 2 and 3, thereby eliminating the effects of growth dilution. The decreases in the adjusted pesticide residue concentrations reflect the dissipation rate of tebuconazole and propiconazole in boronia leaves. The residues determined at each sampling time can then be expressed as a percentage of the amount detected at the time of the most recent application.

$$\% \text{ pesticide remaining} = \frac{\text{mg/kg (DMB) of ai at specific time}}{\text{mg/kg (DMB) at time of most recent application}} \times 100$$

The percentages of tebuconazole and propiconazole remaining at various sampling times at sites 1 and 2 are plotted in Figure 2. The concentrations of tebuconazole and propiconazole decrease most rapidly over the first 2 weeks following the final
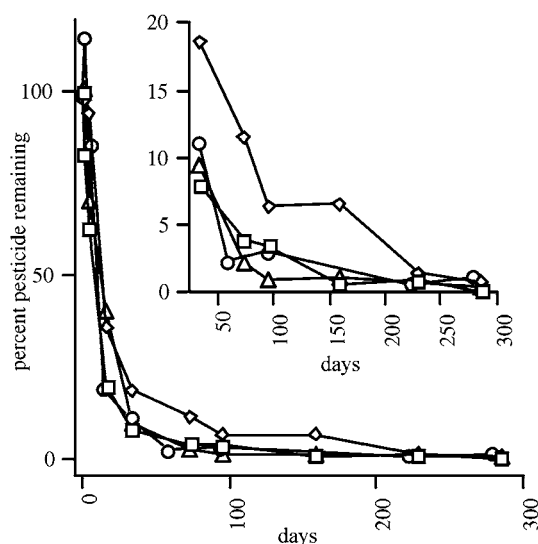
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Figure 2. Residues of tebuconazole remaining in boronia (adjusted for growth dilution and expressed as percentages of the levels detected immediately after pesticide application) in boronia applied at rates of 125 (□) and 250 (◇) g ai/ha at site 1 and 125 g ai/ha (○) at site 2. Percentage decreases in propiconazole at site 1 applied at 125 g ai/ha (△). Inset-enlarged graph showing detail of dissipation between 33 and 286 days.

Table 4. Levels of Tebuconazole Residues Detected in Vegetative Material (Wet Weight) Related to Levels Detected in Boronia Concrete

	residue levels of tebuconazole (mg/kg)		
	site 1		site 2
application rate ($n = 3$)	0.125 kg/ha ai	0.250 kg/ha ai	0.125 kg/ha ai
boronia leaf (mg/kg × 10^{-2})	2 ± 2	16 ± 4	9 ± 3
boronia flowers (mg/kg × 10^{-4})	3 ± 2	5 ± 3	7 ± 2
boronia oil (mg/kg × 10^{-2})	6 ± 5	10 ± 10	20 ± 10

application. After 1 month, less than 10% of both tebuconazole and propiconazole remain. The rate of dissipation for both pesticides is, however, significantly lower in boronia than that reported in peppermint (*3*) where 6 ± 1% ($n = 8$) and 2.1 ± 0.4% ($n = 9$) of tebuconazole and propiconazole, respectively, remained after approximately 2 weeks.

Also of note are the differences in the relative rates of dissipation of tebuconazole residues observed for the two dosage rates applied at site 1. On the day of application at site 1, where 250 g ai/ha had been applied to boronia, the levels of residues detected were only 1.1-fold of those to which the standard 125 g ai/ha had been applied. Throughout the days and weeks that followed, however, that relationship changed. At 73 days after the final applications, the residue levels detected in boronia leaves to which 250 g ai/ha of tebuconazole had been applied were three times the level detected in boronia treated with 125 g ai/ha tebuconazole. At harvest, the levels in the leaves treated with the higher application rate were eight times the levels detected in the leaves that were subject to the standard rate. Assuming that the degradation of tebuconazole by exposure to the physical effects of weathering (rain, wind, abrasion, and sunlight) is consistent irrespective of the concentration of residual ai, then it may be proposed that boronia has a limited capacity to metabolize tebuconazole. Any residues in excess of that limit may dissipate at a slower rate. This has implications for the horticultural industry in that the application of pesticide in excess of the recommended rate may result in a disproportionate increase in residues detected in the final product.

At harvest, the boronia flowers are combed from the stems and extracted in petroleum ether to produce boronia concrete. The yields of oil produced at sites 1 and 2 were 0.38 ± 0.01% and 0.39 ± 0.02%. Table 4 lists the mg/kg tebuconazole detected in the leaves and flowers (wet weight) and the oils subsequently extracted from the flowers. In addition, the levels of tebuconazole concentration detected in the oils were compared to the weight of flowers used to produce the boronia concrete. It was found that there was no relationship between the level of the pesticide residues in the leaves of boronia bushes and the amount of contamination subsequently detected in the flowers harvested

from them. At site 1, flowers harvested from bushes whose leaves showed residue levels of 0.02 mg/kg tebuconazole had only 1.5% that level of pesticides while plants with leaves containing 0.16 mg/kg tebuconazole produced flowers with only 0.3% that level. This indicates that the systemic translocation of tebuconazole from leaves to flowers is not proportional to the concentration in the leaves. The residue found in flowers may instead occur by the incidental harvesting of contaminated leaves.

This study has shown that after 1 month, 5.9 and 7.6% of tebuconazole residues at sites 1 and 2, respectively, were still detectable in boronia treated with the standard application rate of 125 g ai/ha. However, when the application rate is doubled, the relative rate of dissipation is significantly reduced. This disparity was not as marked in the levels of tebuconazole subsequently detected in the flowers. It may be proposed that limited systemic translocation of tebuconazole from leaves to flowers occurs. In steam-distilled oils, residual pesticides still present in harvested material are concentrated in the oil. Unlike solvent-extracted oil, however, this is offset by the lower volatility of many pesticides, their thermal lability, and the extensive contact of residues with water and steam inherent in the process of oil production. In one study, only 0.09% (SE ± 0.02) of tebuconazole detected in peppermint leaves was found to codistill with the peppermint oil (*3*). The production process of solvent-extracted concretes has no such advantage. Potentially all of the residues that are present in the flowers at harvest and that are soluble in petroleum ether may coextract. With boronia flowers yielding 0.38% of concrete, pesticide residues that coextract can therefore be up to 250 times more concentrated in the extract than in the flowers from which they were extracted.

The Australian Food Standards Code (standard A14 schedule 1) specifies a maximum residue limit (MRL) for tebuconazole in barley and wheat of 0.05 mg/kg. Boronia is used, among other applications, in beverages and perfumes. Most MRLs are established in relation to whole food products, and to date, acceptable levels of tebuconazole and propiconazole contamination in essential oils have not been established. Although tebuconazole residues at concentrations of 0.2 mg/kg were detected in boronia concrete in this study, the level would be significantly diluted when used to enhance the flavor of whole foods or cosmetics.

The use of tebuconazole is not as common in the Australian boronia industry as that of propiconazole; yet, tebuconazole has been proposed as an alternative to limit the development of pesticide resistance fungi over prolonged periods of propiconazole use. This study has highlighted that both pesticides should only be applied at the recommended levels and that annual monitoring of essential oil harvests should be undertaken in the production of solvent-extracted oils.

ABBREVIATIONS USED

ai, active ingredient; bdl, below detection limit; DMB, dry matter basis; GC, gas chromatography; ha, hectare; HRMS, high-resolution mass spectrometry; MRL, maximum residue limit; MSD, mass selective detector; *m/z*, mass-to-charge ratio; psi, pounds/square inch; SE, standard error; SIM, selected ion monitoring.

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