# Acaricide susceptibility in Ontario and B.C. populations of two spotted spider mite Tetranychus urticae

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**2015 PMR REPORT # 04** **SECTION F: ORNAMENTALS AND GREENHOUSE - Insect Pests**

**CROP:** Sweet pepper (*Capsicum annuum* L.), cucumber (*Cucumis sativus* L.), eggplant (*Solanum melongena* L.)

**PEST:** Two spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae)

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**TITLE:** **ACARICIDE SUSCEPTIBILITY IN ONTARIO AND B.C. POPULATIONS OF TWO SPOTTED SPIDER MITE *TETRANYCHUS URTICAE***

**MATERIALS:** AGRI-MEK SC (Abamectin), NEXTER (Pyridaben), ACRAMITE 50 WS (Bifenazate), VYDATE (Oxamyl), KANEMITE 15 SC (Acequinocyl), CARZOL SP (Formetanate HCl), OBERON 4 SC (Spiromesifen) and ENVIDOR 240 SC (Spirodiclofen) were provided by the respective chemical companies.

**METHODS:** Populations of two spotted spider mites (TSSM) *Tetranychus urticae* were collected in 2011, 2012 and 2013 from Bleneim, Harrow and Leamington Ontario research and commercial production greenhouses (GH1, GH2, GH3, GH5 and GH6). TSSM were collected from cucumber, eggplant and pepper plants. In 2012, a population was also collected from a greenhouse in Agassiz B.C. (GH4). The TSSM strains were maintained afterward at AAFC London on young bean (*Phaseolus vulgaris* L. ) plants and held in an insectarium at 25±2 °C, 50±5 RH% and a photoperiod of 16:8 h (L:D). An acaricide-susceptible laboratory strain has been cultured at AAFC for many years and has had no exposure to pesticides within the past 10 years. The lab strain has previously been determined not to be susceptible to mitochondrial complex election transport inhibitor (METIs) acaricides, for example pyridaben and acequinocyl, or carbamate acaricides, for example oxamyl and formetanate HCl.
All acaricides were prepared in RO water at stock concentrations of 1000 ppm (or 5000 ppm for VYDATE). Working solutions were prepared in a range of 5 to 6 concentrations and stored for 2 weeks at 4°C.
Acaracide toxicity bioassays were conducted with single adult females using the leaf disc method. *P. vulgaris* leaf discs (40 mm dia) had been placed ad axial surface down on 5 ml of 0.7% solidifying agar medium (pH 5.8) in 50 mm Gelman plates. Leaf discs were dipped in solutions of each formulated chemical for 10 s, and dried on a wire mesh for 20 min or until dry. Ten adult TSSM were transferred on to each leaf disc with a fine paint brush, with the exception of trials using spirodiclofen, formetanate HCl and spiromesifen, which used 10 TSSM larvae and nymphs per disc.
At least 3 separate series of bioassays were run with the laboratory strain at each of 5-6 concentrations for each acaricide giving a minimum of 120 TSSM (3 bioassays x 4 replicates/bioassay x 10 adults/nymphs/replicate) for each concentration tested. Tested concentrations were selected based on preliminary trials to provide a range of 0%-100% mortality. Probit analysis (SAS Institute, 2008) of the data generated was then completed to develop regression lines and determine the $LC_{50}$, $LC_{90}$ and fiducial limits (FL) for the lab strain.
For each greenhouse-collected population and each acaricide, a minimum of at least 3-4 replicates of 10 TSSM was exposed to the $LC_{90}$, a discriminating concentration (DC), on 3 separate days. Those

populations where average mortality at the DC fell below 30% were selected for $LC_{50}$ and $LC_{90}$ determination as described previously.
The resistance ratio (RR) was determined for populations tested with acaricides to which the lab strain is still considered susceptible (excludes the METIs and carbamates). The RR is calculated as the $LC_{50}$ GH strain/$LC_{50}$ lab strain.

**RESULTS:** As outlined in Tables 1 and 2.

**CONCLUSIONS:** *T. urticae* populations collected from Ontario and B.C. greenhouses and tested with the 8 acaricides showed a wide range of susceptibility (Table 1). In general, the populations collected from one research greenhouse (GH1), where minimal or no chemical treatments were used, indicated no resistance. In contrast, the populations collected from commercial vegetable production greenhouses following more conventional chemical control practices were determined to have higher levels of resistance to a greater number of the acaricides tested. For example, TSSM from GH2 had recently been exposed to several acaricides, and there was a > 600-fold RR to bifenazate, and >100-fold RR for abamectin for the $LC_{90}$ compared to the laboratory strain. Based on the criteria that resistance can be defined as a response to the DC ($LC_{90}$) where less than 30% mortality is determined, GH3 was resistant to 4 of the 6 acaricides tested. Unfortunately $LC_{50}$ and $LC_{90}$ values for abamectin, acequinocyl, oxamyl and pyridaben could not be calculated as this strain was lost due to contamination of the colony by predatory mites. Tests with a TSSM collection from an Agassiz BC greenhouse (GH4) detected resistance to 3 of 8, including formetanate HCl, spirodiclofen and spiromesifen. The RR for spirodiclofen was determined to be >50 at the $LC_{50}$ and >100 at the $LC_{90}$ relative to the lab strain (Table 2). Of the two remaining populations, GH5 was more susceptible to the acaricides tested. Only resistance to pyridaben was observed using the discriminating concentration ($LC_{90}$) (Table 1) and the RR was determined to be >10 at the $LC_{50}$ and >30 at the $LC_{90}$. In contrast, GH6 was determined to be resistant to acequinocyl, pyridaben, formetanate HCl and spiromesifen (Table 1). A minimum of 20000 ppm was estimated for the pyridaben $LC_{50}$, indicating a RR of >100, while the RRs for acequinocyl, formetanate HCl and spiromesifen in GH6 TSSM were between 10 and 40.
Acaricide-resistance and cross-resistance is a growing concern for Canadian greenhouse vegetable growers. For many years growers have relied heavily on foliar treatments of chemical acaricides and it appears that this reliance has led to resistance in an increasing number of *T. urticae* populations. These findings indicate that caution should be taken by growers in selecting acaricides and a resistance management plan implemented.

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**Table 1.** Mean percent mortality of six *T. urticae* greenhouse populations collected from Ontario and B.C. greenhouses between 2011 and 2013 exposed to a discriminating concentrations (DC) or lab strain $LC_{90}$ values for 8 registered acaricides.

| Acaricide (a.i.) | Lab strain $LC_{90}$ (FL) | Slope | Mean percent mortality at $LC_{90}$ for each acaricide | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | GH 1 | GH 2 | GH 3 | GH 4 | GH 5 | GH 6 |
| ACRAMITE (Bifenazate) | 12 ppm (8.1, 22) | 1.6 | 80.0 | 0 | 93 | 100 | 67.1 | 64.6 |
| AGRI-MEK (Abamectin) | 4.4 ppm (2.6, 10.7) | 1.2 | 95.0 | 40.0 | 24.0 | 46.4 | 64.5 | 96.3 |
| CARZOL (Formetanate HCl) | 23.5 ppm (17.3, 39.4) | 2.1 | ND | ND | ND | 57.4 | 50.9 | 27.5 |
| ENVIDOR (Spirodiclofen) | 394 ppm (177, 1533) | 1.1 | 75.0 | 34.0 | 51.4 | 3.3 | 64.9 | 65.7 |
| KANEMITE (Acequinocyl) | 53.5 ppm (41, 78.8) | 2.5 | 100 | 43.3 | 22.6 | 100 | 91.8 | 28.1 |
| NEXTER (Pyridaben) | 820 ppm (605, 1268) | 1.9 | 65.0 | 80.0 | 26.7 | 82.6 | 24.5 | 7.1 |
| OBERON (Spiromesifen) | 274 ppm (31.1, 12315) | 0.3 | ND | ND | ND | 48.5 | 69.4 | 27.5 |
| VYDATE (Oxamyl) | 4640 ppm (2690, 12240) | 1.5 | 100 | 100 | 24 | 82.3 | 100 | 100 |

**Table 2.** The acaricide $LC_{50}$ and $LC_{90}$ values (± FL) and resistance ratios determined for the lab *T. urticae* strain and two greenhouse strains collected from one Ontario and one B.C. greenhouse.

| Acaricide (a.i.) | Lab strain $LC_{50}$ / $LC_{90}$ (FL) | Slope | GH4 strain $LC_{50}$ / $LC_{90}$ (FL) | RR $LC_{50}$ / RR $LC_{90}$ | GH6 strain $LC_{50}$ / $LC_{90}$ (FL) | RR $LC_{50}$ / RR $LC_{90}$ |
|---|---|---|---|---|---|---|
| ENVIDOR (Spirodiclofen) | 24.7 ppm (16.2, 40.9) / 394 ppm (177, 1533) | 1.1 | 1396 ppm[1] (788, 5244) / 421062 ppm (NA) | 56.5 / 1069 | ND | |
| OBERON (Spiromesifen) | 0.35 ppm (0.13, 1.5) / 274 ppm (31.1, 12315) | 0.3 | ND | | 8.2 ppm[2] (<0.1, 32.5)/ 1102 ppm (N.A.) | 23.4 / 4 |

[1] slope = 0.5 for GH4 strain with spirodiclofen; [2] slope = 0.4 for GH6 strain with spiromesifen; ND = not determined; NA = not available.