ACARICIDE RESISTANCE OF THE TWO-SPOTTED SPIDER MITE (*TETRANYCHUS URTICAE*) IN PACIFIC NORTHWEST HOPS

By

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ACARICIDE RESISTANCE OF THE TWO-SPOTTED SPIDER MITE

(*TETRANYCHUS URTICAE*) IN PACIFIC NORTHWEST HOPS

Abstract

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The two-spotted spider mite (*Tetranychus urticae*) is one of the greatest economic plant-feeding pests of agriculture in the world, attacking food crops, trees, and ornamentals, resulting in serious economic injury and occasionally plant death. In the Pacific Northwest, *T*. *urticae* is a common and severe pest in hops (*Humulus lupulus*), a perennial plant grown for its economically important strobile (or cone) that is used as a flavoring and stability agent in beer. Hops are a valuable commodity in Washington, Oregon, and Idaho, the most important hop growing regions in the U.S., accounting for nearly all of U.S. hop production. Severe infestations of *T*. *urticae*, however, can cause defoliation and dry, brittle cones, thereby reducing quantity of hop cones, as well as quantity and quality of alpha and beta acid constituents. Production of high quality hop plants and cones requires a rigorous integrated pest management program for *T*. *urticae*, and current control relies almost exclusively on the application of acaricidal pesticides. Unfortunately, *T*. *urticae* has a well-documented history of rapidly developing tolerance and/or resistance to most of the acaricides registered for their control. A greater understanding of the

mechanisms involved in developing resistant populations is expected to improve mite management strategies in hops.

My current study investigated the response of *T*. *urticae* to acaricide exposure in three distinct experiments. First, I determined baseline toxicity of a naïve *T*. *urticae* laboratory population to the acaricides, abamectin, bifenazate, and bifenthrin. Second, I established mite colonies resistant to acaricides through artificial selection. Third, I tested field populations for their susceptibility to the three candidate acaricides.

Baseline lethal concentrations (LC_{50}) values were identified after direct exposure using a Potter spray tower. In the second test, I determined that *T*. *urticae* are capable of developing increased tolerance to abamectin, bifenazate, and bifenthrin. Following ten acaricide applications, the LC_{50} of the abamectin-resistant strain increased 26-fold, the bifenazate-resistant strain increased 14-fold, and the bifenthrin-resistant strain increased 5-fold. In a comprehensive survey of field populations I detected a wide range of responses to the acaricides. During the 2012 and 2013 hop seasons, *T*. *urticae* response to abamectin was calculated at 1.29-107-fold, while bifenazate was calculated at 2.29-96.3-fold greater than the naïve laboratory population.

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INTRODUCTION

Spider Mite Biology

Of the more than 1,200 species of spider mites described (Bolland et al 1998; Milegeon et al. 2010), *Tetranychus urticae* Koch (Acari: Tetranychidae) (TSSM; two-spotted spider mite) is the most economically important plant-feeding pest mite in the world (Van Leeuwen et al. 2012). *T. urticae* is a generalist feeder and is among the most polyphagous arthropod herbivores (Agrawal 2000), feeding on more than 1,100 plant species belonging to more than 140 different plant families, including those that are known to produce toxic compounds (Grbic 2011; Van Leeuwen et al. 2012). *T. urticae* threatens greenhouse production and field, vine, and orchard crops, destroying economically important annual and perennial crops worldwide such as tomatoes, peppers, cucumbers, strawberries, corn, apples, grapes, hops, almonds, peppermint, and citrus (Jeppson et al. 1975).

All mites are classified as Acari, the most diverse taxon within the subphylum Chelicerata, with over 40,000 described species representing a wide range of life histories, from human and veterinary impact to agricultural damage (Grbic 2011). Of concern, the two main mite families injurious to agriculture are the Tetranychidae and the Eriophyidae (Hoy 2011). The two-spotted spider mite, *Tetranychus urticae,* belongs to the Tetranychidae family, an assemblage of web spinning mites collectively called spider mites. Spider mites (larvae, nymphs, and adults) produce webs from silk glands located at each palp (Alberti & Crooker 1985), hence the root of the 'spider' in the common name. Webbing may be used to protect against factors including wind and rain, natural enemies, and exposure to chemicals; for instance, spray droplets may become trapped in a barrier of webbing and fail to contact the mites (Davis 1952). Silk

webbing is used for a variety of different functions including dispersal, colony establishment, pheromone communication, adhesion to leaf substrate during quiescence (Gerson 1985), and it can play a role in mating (Penman & Cone 1972). Despite their very small size (about 1mm for adult females), the various species of spider mites can be devastating pests causing substantial economic injury. Given optimal conditions of high temperatures and low humidity, *T. urticae* populations can outbreak to high densities and cause serious damage to host plants.

T. urticae is generally known to be active on the underside of leaves, except under high population density. Crops with symptoms of spider mite infestations include a specking appearance and discoloration. Spider mites damage their host plants while feeding, using specialized piercing-sucking, stylet-like mouthparts to penetrate through the outer epidermal cells and into parenchyma cells (Park & Lee 2002), and thus removing chlorophyll and other cell contents (Tomczyk & Kropczyńska 1985). The loss of chlorophyll results in a visibly patchy discoloration of leaf tissue, as well as a reduced photosynthetic rate and production of nutrients (Park & Lee 2002). Economic injury occurs as high populations accumulate and feeding increases, leading to sufficient damage over a period of days. Extreme levels of damage can eventually cause leaf and fruit loss, complete defoliation, and death of the host plant (Van Leeuwen et al. 2012).

The life cycle of *T. urticae* progresses through a series of five stages, egg, larva, protonymph, deutonymph, before finally molting into an adult male or female (Crooker 1985). Sexes are dimorphic: males are smaller with a tapered posterior end to their body, while females are larger and more round in shape. Eggs appear as translucent pearl-like spheres, 0.1mm in diameter, and are deposited singly (Van Leeuwan 2012). The developmental period of the eggs varies from 3 days at 24ºC to 21 days at 11ºC (Cagle 1949). Eggs become reddish as they

develop, until hatching into a six-legged larva. The larvae, along with the next two eight-legged nymphal stages (protonymph and deutonymph) are all active immature stages that feed on the host plant, that are followed by a period of quiescence. During quiescence a mite is inactive and attaches itself to the leaf substrate (Crooker 1985). Time spent developing in each stage depends on temperature and humidity during the specific life stage (Herbert 1981; Cagle 1949). At optimal temperatures of about 30-32ºC, TSSM can develop from egg to adult in as little as 7-8 days (Zhang 2003). Outbreaks usually occur during the summer months when TSSM populations can increase rapidly, and there are numerous overlapping generations per year (Helle & Sabelis 1985). Males reach maturity first, then search and wait besides a female deutonymph in the resting state (Penman & Cone 1972). Copulation occurs almost immediately after an adult female emerges (Crooker 1985).

A fertilized female will produce offspring of both sexes, although with a skewed sex ratio of 3:1 females:male (Overmeer & Harrison 1969). If eggs are not fertilized, arrhenotokous parthenogenesis occurs, resulting in the production of haploid males (Helle 1985). The haplodiploidy genetic system enables a single female to initiate a new colony and cause a potential outbreak. Oviposition begins with an average of 5 or 6 eggs laid per day, with total egg production up to 100-150 in a lifetime (Sabelis 1985). Spider mite females of the genus *Tetranychus* lay their eggs within or under webbing (Gerson 1985).

T. urticae overwinter in a state of reproductive diapause (Tauber et al. 1986). Diapause is cued by a decreased photoperiod, temperature, and the decline in the quality of the host plant (Veerman 1985). In controlled greenhouse conditions, populations of *T. urticae* can continue unimpeded (Zhang 2003). Once *T. urticae* enter a diapause state they move from the host to hibernation sites, such as soil, tree bark, ground cover, and dried leaves (Veerman 1985; Kim &

Lee 2003). Only adult female *T. urticae* enter diapause, becoming brightly red due to the build-up of carotenoid compounds in the body that impede freezing and desiccation. Diapausing *T. urticae* females also terminate feeding and are negatively photokinetic (Veerman 1985). With the onset of improved environmental conditions and temperature in spring, *T. urticae* break diapause and emergence from their overwintering site seeking host plants (Kim & Lee 2003; Margolies & Kennedy 1985) for sustenance and oviposition sites.

Control and Acaricide Resistance

Historically in nature, natural enemies, disease, and poor plant nutrition kept *T. urticae* populations at low to unobservable densities. Modern agricultural systems, fertilizers, and an overuse of synthetic organic pesticides during and post-World War II created conditions favorable for *T. urticae* to increase to extremely high densities and experience outbreaks (van de Vrie et al. 1972). When crop production is not limited by water, nutrients, competition from weeds, or pest pressure, the plants in production become an excellent food source for mites. Additionally, monocultures of host plants along with synthetic organic pesticides contributed to high mite densities indirectly through the elimination of natural enemies (Roush & Hoy 1978), and from increased reproductive rates (Luckey 1968).

Despite the biological characteristics contributing to the potential pest status of *T. urticae* (i.e. fast generation time, high fecundity), and the changes in agroecosystems in the latter half of the 20th century, the rapid growth of spider mite outbreaks is especially due to their ability to develop rapid acaricide resistance (Cranham & Helle 1985). Acaricides are pesticides that are specifically targeted to suppress pest populations of mites (Walsh 2002). Pest populations can be

susceptible or resistant to a pesticide, and resistance occurs when a formerly susceptible population becomes less susceptible to acaricide applications (Price & Nagle, 2012).

Spider mite resistance to acaricides is a well-documented event (Grbic et al. 2011) and *T. urticae* in particular has been documented to have evolved resistance to over 95 acaricidal/insecticidal active ingredients (Van Leeuwan et al. 2010). Acaricide resistance in *T. urticae* has been reported from over 60 countries (DARP 2013), easily earning it the title of the world's top resistant animal pest (Van Leeuwan et al. 2010). Tolerance documented to acaricides can result after a few applications (Sato 2005). Furthermore, *T. urticae* can become fully resistant to new acaricides within two to four years, meaning that control of multi-acaricide resistant *T. urticae* has become increasingly difficult (Grbic 2011).

Resistance and control failures against *T. urticae* have been reported for pesticides such as organophosphates (Sato et al. 1994), organotins (Edge & James 1986; Flexner et al. 1988), hexythiazox (Herron & Rophail 1993), bifenthrin (Farnham et al. 1992), fenpyroximate (Stumpf & Nauen 2001; Sato et al. 2004), abamectin (Beers et al. 1998), and bifenazate (Van Leeuwen et al. 2006). While the number of effective active ingredients registered for control of *T. urticae* and other pests declines, fewer new acaricides are being produced in the market as they have a high cost associated with their use and application restrictions (Dekeyser 2005; Metcalf 1980).

More than 550 species of insects and mites have developed resistance to at least one class of insecticides/acaricides (Van Leeuwan et al. 2012). Studies and research of the genetic, biochemical, and molecular mechanisms involved are expected to contribute to better resistance management programs. Organisms can become resistant to pesticides by reducing the effective dose at the target site, which can be attributed to mechanisms such as behavioral resistance, reduced penetration or absorption at the cuticle level, sequestration, and metabolic detoxification

(Van Leeuwen et al. 2010). During detoxification, pests are able to shuttle out xenobiotic compounds, so that it never enters the cell of the target site. Another method is decreased target sensitivity; this includes structural modifications at the target site itself (mainly due to point mutations). The physiological target of the pesticide is less sensitive, and does not react as normally as in susceptible populations (Van Leeuwen et al. 2010). In addition to these mechanisms, resistance development in *T. urticae* can be accelerated by certain factors including arrhenotoky, high mutation rate, and inbreeding (Croft & Van De Baan 1988). These factors lead to faster fixation of resistance alleles in acaricide-selected populations of *T. urticae* because of females with acaricide resistant haploid males (Cranham & Helle 1985).

Efforts to manage *T. urticae* population have relied mostly on mites in the family Phytoseiidae. In some areas, *Phytoseilus persimilis* is widely used in biological control programs (Cho et al. 1995) as they specialize on spider mites in the genus *Tetranychus*. *P. persimillis* is an aggressive obligate feeder that multiplies and spreads rapidly, which can decrease mite populations quickly (Van den Boom et al. 2002). These predators, however, may not be able to suppress spider mite populations for an extended period of time (Kim et al. 1997) or, once established in a classical biological approach, cannot provide economic level of control (Trumble & Morse 1993). For example, inundative release programs in strawberries have effectively controlled two-spotted spider mite; however, the cost at sustaining such a program is not economically feasible due to the cost of mass production (Wyman et al. 1977). Additionally, this control has mainly been effective and useful in greenhouse crops (Zhang 2003), but failures are common in field crops and ornamentals. There is pressure to integrate biological control or develop integrated pest management strategies due to problems associated with chemical control (Hoy 2011), such as the harmful impacts on natural enemies, soil, water supply, agricultural

workers and consumers (van de Vrie 1985). Because biological control of *T. urticae* is difficult to reach at optimal efficiency and is time consuming due to the need to collect detailed information about crop systems, growers are more inclined to use a readily accessible chemical form of control.

Spider Mites in Pacific Northwest Hops

The two-spotted spider mite and several closely related sibling species within the family Tetranychidae are chronic pests in a number of crops in the U.S. Pacific Northwest. These include hops, peppermint, tree fruits, vegetables, legumes, and grass forage crops (Hollingsworth 2013). Historically, mite pests have been controlled by the introduction of a new acaricidal compounds with a novel mode of action. These acaricides were often used until resistance developed and field failures occurred. Some perennial crops have a greater opportunity for biological control, such as apple (Beers et al. 1998), however when these programs fail, acaricides are the main tools to control mite pests in the Northwest (Walsh 2009). In the Pacific Northwest, *T. urticae* is known to be one of the major arthropod pests in hops (Cannabaceae; *Humulus lupulus*), and has been difficult to control despite numerous acaricide applications within a single season.

Hops are one of the main ingredients used in the brewing process to add bitterness and keep freshness in production of beer (Neve 1991). The hop plant is a dioecious perennial plant that is planted in female monoculture and develops a basal woody crown. The woody crown in turn produces annual bines (flexible climbing stems), which will naturally climb and wrap around anything it can find and may reach heights of 7.6 m (Neve 1991). Under commercial production, hops are typically trellised in rows at heights of 5.5 m. Bloom is initiated by long

days, and unpollinated flowers develop into economically important cones that ripen between mid-August to mid-September. The bines are all harvested by late September and the cones are mechanically stripped from the bines. Hops are hardy cold-tolerant plants and the woody crowns can survive indefinitely, but market demands typically require growers to replant new cultivars every 3 to 7 years (Neve 1991). The commercial product in hops is the resin (which consists of alpha and beta acids) that is produced, along with hop oils, in glands known as the lupulin glands in the hop cone. The U.S. plays a large role in domestic and international trade of hops. By the mid-twentieth century, the Pacific Northwest (PNW) was one of the world's leading hop exporters. Ideal conditions, an established agricultural infrastructure, and improved capabilities all contributed to the success of PNW hops, which until the early 1900's were primarily grown in the eastern United States (Burgess 1964).

According to the Barth report (Barth-Haas Group 2011) the U.S. is the second largest producer of hops with 29.7% share of the world market in 2010. Currently in the U.S., the three main hops producing states are Washington, Oregon, and Idaho. The Yakima Valley of Washington State is one of the most important hop growing regions in the world. Approximately two-thirds of the hops produced in the Yakima Valley are exported internationally (USAhops). In 2012, 79% of U.S. hops were produced in Washington State, but it has been as high as 90% as recorded in 2010 (USDA-NASS). In 2010, there were 24,800 acres bearing hop yards and the crop was valued as 162.5 million dollars (USDA-NASS).

Hop plants are attacked by a suite of pests and diseases, including powdery mildew (*Podosphaera macularis*) and downy mildew (*Pseudoperospora humuli*) (Gent & Nelson 2009; Gent & Johnson 2009). The most common arthropod pests in hopyards are the hop aphid (*Phorodon humuli*), and *T. urticae* (Barbour 2009). The hop aphid is the more severe pest in

cooler conditions, while the two-spotted spider mite is more active in warmer, dry climates (Cranham 1985). *T. urticae* damage hops by feeding on leaves and cones, causing bronzing of leaves and giving them a stiff, parchment-like feel following *T. urticae* outbreak conditions. Severe *T. urticae* infestations can result in defoliation, and dry, brittle cones, thus reducing hop oil quality, alpha and beta acid quality, and yield (Barbour 2009). Acaricide and insecticide attempts usually fail due to pesticide resistance, difficulty of proper spray coverage from large canopies (James & Price 2000), and webbing. Despite substantial biological control efforts in hops (Pruzinski & Cone 1972; Campbell & Lilly 1999), it has largely been unsuccessful. Natural enemies of *T. urticae* in hopyards include the phytoseiids *Galendromus occidentalis* (James & Dreves 2009) and *Neoseilus fallacis* (James & Dreves 2009), as well as insect predators including the mite-eating lady beetles (*Stethorus sp*.) and minute pirate bug (*Orius tristicolor*). However, the abundance of these natural enemies are greatly influenced by the applications of insecticides/acaricides in the hopyard (James & Dreves 2009).

T. urticae control is essential for maximum yield of hops, as brewer rejection may occur if cones are damaged or discolored from mite feeding (Barbour 2009). In the hop industry, acaricides are the primary source of action when *T. urticae* populations exceed economic thresholds. Due to the combination of acaricide use and the rapid development of resistance documented in *T. urticae*, efforts should be made to evaluate and recommend the most effective acaricides for *T. urticae* control. Resistance of *T. urticae* to acaricides on hops is an urgent problem and better understanding of resistance is needed to advance management strategies and slow resistance development (Croft et al. 1987).

Objectives

A primary purpose of my project was to survey hopyard and lab populations of *T. urticae* for resistance to three selected commercially important registered acaricides. These are abamectin, bifenthrin, and bifenazate. These acaricides/insecticides were chosen because they are currently (or once were) widely favored for application in hopyards. Abamectin has been used for control of *T. urticae* in the Yakima Valley since becoming commercially available in 1988. Abamectin has a very short residual (Campbell 1989) and has become a predominant acaricide applied to control spider mite outbreaks on hops. Abamectin belongs to a class of avermectin and ivermectins, which are natural fermentation products of the soil bacterium *Streptomyces avermitilis* (Campbell 1989). Avermectins act on the GABA and glutamate-gated channels (IRAC). Bifenazate is a selective carbazate acaricide that was registered in Washington in 2002. Bifenazate provides quick knockdown of pests from direct contact and exhibits long residual control (Chemtura AgroSolutions, Lawrenceville, GA). Preliminary studies on the mode of action suggested that bifenazate might be a neurotoxin and act on GABA-gated chloride channels (Dekeyser 2004). However, recent studies have suggested a possible alternative target site in the mitochondrial encoded cytochrome b in Complex III of the electron transport chain (Van Leeuwen et al. 2006). Bifenthrin is a pyrethroid, which have become a favored class of insecticides in the world insecticide market, accounting for approximately 20% of worldwide insecticide use (Van Leeuwen et al. 2009). Bifenthrin was used initially for control of *T. urticae* on hops following registration in 1993. However, it quickly became unreliable due to its negative effects on the natural enemies of *T. urticae,* therefore, its use in hops has declined substantially. On the rare occasions bifenthrin is applied to hops, the target pests are hop aphids or caterpillar pests.

Overall, there are few recent detailed susceptibility studies of *T. urticae* to acaricides applied in hopyards in Washington State. Baseline levels of susceptibility and discriminating concentrations for detection of resistance or tolerance of *T. urticae* to three candidate acaricides were established in this study. This is a key step in examining the effectiveness of acaricides against *T. urticae*, as resistance monitoring is essential to all resistance management programs (Roush & Miller 1986). Toxicity information gained through phenotypic acaricide bioassays allows comparison of a susceptible strain to both field populations and selected lab colonies. Baseline reference values can be used to diagnose shifts in a *T. urticae* population's susceptibility to acaricides, and thus promote alternative chemistry or control practices if this tolerance or resistance increases. The bioassay data obtained in the laboratory can offer immediate practical guidance to individual growers and potentially replace the practice of conducting expensive field trials. Controlled laboratory data would allow for identification of effective products, and could help avoid unnecessary selection pressure.

In this thesis, I detail three experiments to fulfill the objectives of my project: (1) determine baseline concentration response curves of *T. urticae* populations susceptible to the acaricides abamectin, bifenazate, and bifenthrin (2) establish mite colonies resistant to acaricides through artificial selection, and (3) test selected field populations of spider mites and compare concentration-response curves to susceptible mites.

MATERIALS AND METHODS

Susceptible Strain

To establish baseline toxicity and concentration-response curves, an acaricide naïve susceptible strain of *T. urticae* was tested for response to specific acaricides applied to leaf discs at controlled concentrations. The susceptible acaricide naïve mites (henceforth to be identified as "susceptible") were obtained from our laboratory colony, and were kept in a walk-in growth chamber isolated from possible contaminants (i.e. pesticides and other arthropods). The founding *T. urticae* for this susceptible population were originally collected from weeds in Montana, and have been reared on beans in isolated conditions at the Irrigated Agricultural Research and Extension Center (IAREC) in Prosser, WA since 1995. The lack of exposure to pesticides and other environmental pressures warrants this *T. urticae* population as an ideal susceptible baseline strain.

Rearing

T. urticae were maintained and reared continuously on a food supply consisting of baby lima bean plants (also called Henderson baby lima beans or bush beans; *Phaseolus lunatus* L.), provided by Buckeye Seed Supply, Canton, OH, under conditions of 28 ± 1°C, 70±5 RH, and a 16 h light: 8 h dark photoperiod (Figure 1). New, uninfested baby lima bean plants were rotated into the mite colony every seven days. About 12 bags of plants were grown in the greenhouse every week, each bag with 60 ml of seeds sowed shallowly (1-2 cm deep) in medium grade vermiculite (Therm-o-rock West Inc.) (Figure 2). Cotyledons emerged within 3-5 days at 25±2°C

and were fully expanded within one week. Beans were kept in the greenhouse for a total of two weeks, until they were mature enough to be transferred to a *T*. *urticae* colony.

Cut leaf samples from the susceptible colony were first examined for predators (i.e. Western flower thrips or predator mites), to confirm an absence of contamination. The leaves were then laid on top of the clean replacement bean plants. As the cut leaves dry, the active stages of spider mites disperse onto the replacement plants. A handful of leaves (about 20 leaves, estimated to be around 300-500 mites total), were transferred to each new tray of plants (each tray consists of four vermiculite bags of planted beans), *T*. *urticae* usually colonize most parts of the replacement plant within three days, and within one week plants reach exhaustion and are no longer viable hosts for the spider mites. At this point, the colony requires new replacement plants. Each respective *T*. *urticae* colony maintained for my studies were kept isolated from uninfested beans and from other mite colonies. Furthermore, in attempt to prevent mite migration, the *T*. *urticae* colonies were maintained in Huffacker moats, in which the colonies were isolated in 27-L plastic tubs that were filled with soapy water.

Baseline Toxicity

Leaf disc bioassays were used to estimate the LC_{50} and LC_{90}, (the lethal concentrations that kill 50% and 90% of the population, respectively) of a particular acaricide. My bioassay method closely mimics methods described by Knight et al. (1990) to determine the direct acaricide efficacy of selected chemicals against *T*. *urticae*. Using a fine brush (10/0 Taklon), ten adult *T*. *urticae* females of the same age were placed on a bean leaf disc (2 cm diameter) on water-saturated cotton (4 cm x 4 cm) in a petri dish (6 cm diameter). Leaf discs were placed adaxial side down, and two leaf discs were placed in a single petri dish. Water saturated cotton

was pushed up against the perimeter of the leaf disc, in order to create a barrier and prevent mites from walking off the disk, since mite walk-off is sometimes observed in these tests (Knight et al. 1990).

I focused on three commercially formulated acaricides. The first was Bifenture ® EC, a pyrethroid provided by United Phosphorus (25.1% a.i. Bifenthrin). Second, Epi-mek® 0.15 EC an avermectin provided by Syngenta Crop Protection (2% a.i. Abamectin). The third miticide I used was Acramite® 4L SC, a carbazate provided by Chemtura Agro Solutions (43.3% a.i. Bifenazate). The recommended field concentrations of Bifenture, Epi-mek, and Acramite when controlling spider mites are 463 ml/ha, 464 ml/ha, and 1802 ml/ha, respectively. These dilutions correspond to U.S. values of 6.4 fl oz/acre, 6.4 fl oz/acre, and 1.5 pt/acre. Field rate solutions were prepared in the lab using a commercially relevant dilution rate of 100 gallons/acre using distilled water. The commercial formulated acaricides were then serially diluted with distilled water to prepare a series of concentrations.

Each bioassay consisted of 4-7 acaricide concentrations with 4-6 replicate discs per tested concentration. Mites exposed to distilled water in the Potter tower spray were used as the non-treated control treatment for each series of bioassays. Solutions of abamectin (Epi-mek®) doses ranged from 0.1-5.6 mg a.i. /L. Solutions of bifenazate (Acramite®) doses ranged from 0.44-44.9 mg a.i. /L. Lastly, solutions of bifenthrin (Bifenture®) doses ranged from 6-120 mg a.i./L. Leaf discs were treated topically with 2 ml of acaricide dilutions at various concentrations with a Potter spray tower (Burkard Manufacturing, Rickmansworth, Herts, UK; Potter 1952) (Figure 3), to ensure uniformity in the distribution of the spray liquid. The tower is calibrated to deliver 1.1 kg/cm2.

Thereafter, the treated leaf discs were kept at 25 ± 1°C and a 16 h light: 8 h dark photoperiod for 24-h after treatment. Individual *T*. *urticae* survival was determined by probing each mite with a fine brush (10/0 Taklon) under a dissecting stereomicroscope. Mites unable to move were classified as dead. Mites were also classified as dead if they were twitching, or were unable to walk at least a distance equivalent to their body length (usually about 3 steps). In the case of mites missing from the leaf disc due to mite walk-off (when a mite left the leaf disc and became entangled in the cotton), these individuals were eliminated from further analysis.

The leaf-disc bioassay method described in the previous paragraphs was used only for abamectin and bifenzate, and not for the pyrethroid bifenthrin. Because pyrethroids are known to cause mites to abandon treated leaves (Mochizuki 1994), as was observed in my preliminary attempts of the leaf-disc bioassay, the method was modified for bifenthrin. In this case, adult females were placed dorsal side down on a strip (3cm x 1cm) of double-sided sticky Scotch® tape, which was attached to a glass slide (7.5cm x 2.5 cm). My method is a modification of the slide dip method as described by Overmeer (1985), but rather than dipping the *T*. *urticae* in acaricidal solution, the *T*. *urticae* in my study were sprayed as described above in the Potter spray tower with the appropriate solution. Mortality was assessed 24-h after treatment. Mites unable to move their legs in a coordinated movement were considered dead.

T. *urticae* mortality data were corrected using Abbott's formula (Abbott 1925). Concentration-mortality regressions, LC_{50}, and LC_{90} within 95% confidence intervals, were estimated by probit analysis as described by Finney (1971). Probit regressions were estimated with Polo Plus (LeOra Software 2002). The results represent baseline toxicity data (Table 1) used in the following experiments of selection and field collections.

Selection for Resistance

Mites of the original susceptible population were selected for resistance to abamectin, bifenazate, and bifenthrin under laboratory conditions from July 2012 to November 2012. I started three new colonies of the susceptible strain in three separate walk-in growth chambers. These colonies were maintained as detailed above. After these new colonies were established, I waited two weeks until they each had a sufficient number of mites, and then sprayed one with the LC_{50} of the Epi-mek® formulation (0.224 mg a.i. abamectin/L), one with the LC_{50} of the Acramite® formulation (0.820 mg a.i. bifenazate/L), and one with the LC_{50} of the Bifenture formulation (17 mg a.i. bifenthrin/L). The LC_{50}'s were first determined by probit analysis from the susceptible population, and were used so that approximately 50% of mites survived for the succeeding generation.

A spray bottle was used to apply the concentration of acaricide from a standing distance of about 0.3 m. Colonies were sprayed eight times per selection treatment. After each individual spray, the colony was rotated 45° clockwise to ensure complete 360° coverage. If necessary, the plant was sprayed an additional 1-2 times when sufficient treatment was not obtained. Each spray released 1.25 ml of the acaricide solution; therefore, a total of about 10 ml of acaricide was directly sprayed onto the plant. Survivors after 24-h were used to initiate the next generation by cutting a handful of leaves from the plant, and placing them on a new replacement bean plant. The intervals between sprays for the first 10 selection treatments varied between 7 and 10 days. The 11^{th} and 12^{th} selection treatments were sprayed 14-18 days apart.

Bioassays were performed every two weeks, to observe any shifts in tolerance to an acaricide. After the 12^{th} selection treatment, acaricide applications were discontinued and the colonies were maintained without any further selection pressure. Bioassays were reinitiated in

July 2013 (36 weeks after the 12th selection treatment), in order to detect maintenance of tolerance, or reversion back to the population's pre-selection susceptible state. In the bioassays, solutions of abamectin (Epi-mek) concentrations ranged from 0.22-22.49 mg a.i./L, bifenazate (Acramite) concentrations ranged from 0.44-224.7 mg a.i./L, and bifenthrin (Bifenture) concentrations ranged from 1.2-120 mg a.i./L. Topical spray applications with the Potter spray tower consisted of 4-5 acaricide concentrations with 6 replicate discs per concentration. Mortality was assessed after 24-h in an identical manner as previously described for the baseline concentration-response curves.

Again, probit regressions were estimated with Polo Plus (LeOra Software 2002). From this analysis, LC_{50} and LC_{90} values were also computed for both acaricides. Resistance ratios (RR) were calculated for each acaricide by dividing the LC_{50} values of each selected colony by those from the susceptible strain (RR= LC_{50} of selection strains/ LC_{50} of susceptible strain).

Field Populations

Populations of mites from commercial hopyards located within the Yakima Valley of Washington State (Figure 4) were collected and bioassayed with abamectin, and bifenazate when mites were found in sufficient numbers. In these surveys of field *T*. *urticae* populations, I focused on abamectin, due to its commercial importance for the U.S. hop industry. Using a 10X hand lens and unaided visual observations, I identified hop leaves infested by spider mites and collected these leaves in a plastic bag. Bags of mite-infested leaves were transported to the lab in a cooling box within a few hours following collection. Mites were collected in August 2012, and again in June-September 2013. Discriminating doses were established for each acaricide. The discriminating dose for bifenazate was 224 mg a.i./L (this is 1/4 the concentration of the field

rate), and for abamectin was 22.5 mg a.i./L (this is the full field rate). A full field rate dose was not used for bifenazate, because throughout the duration of my project survival was never detected at this level. Therefore, a lower concentration was set for a more descriptive comparison of field collected populations to the baseline lab population.

In 2012, four populations were sampled from the Yakima Valley in hopyards near the towns of Prosser, Mabton, and Toppenish, Washington. Mite sampling and collection started later in 2012 than in 2013 (mid-August), and I was limited with a lack of collection for *T*. *urticae*. The weather in the Yakima Valley usually becomes cooler in the beginning of September; this triggers spider mites to initiate diapause. A diapausing spider mite is insufficient to use in a bioassay due to the change in metabolism and general resistance to acaricides (Van Leewan et al. 2009).

In 2013, mite collection started in June and continued throughout mid-September. Above-average temperatures and seasonably dry conditions fostered an especially favorable environment for spider mite populations to flourish in 2013. A warm September kept *T*. *urticae* from initiating diapause and *T*. *urticae* populations remained active through September 2013 without any indication of diapause. In total, 13 *T*. *urticae* populations were collected near Moxee, Prosser, Granger, and Mabton, WA. Beginning in June 2013, hopyards were surveyed and sampled every two weeks for spider mite presence. When mites were found in abundant numbers in the hopyard, a bioassay was performed to test for toxicity to either abamectin or bifenazate. In this case, mites were transferred off of the hop leaf, directly to the bean leaf disc. Some populations were reared on bean plants in the laboratory for 1-2 weeks until sufficient numbers of mites were obtained in a subsequent generation, and could then perform a bioassay.

These colonies were initiated with at least 50 mites. Mites were also slide-mounted and identified to confirm *T*. *urticae* species.

In 2013, 13 *T*. *urticae* populations were tested for abamectin resistance, and 12 of these 13 populations were tested for bifenazate resistance. Solutions of abamectin doses ranged from 0.225-39.3 mg a.i./L, and solutions of bifenazate ranged from 8.99-899 mg a.i./L. All *T*. *urticae* populations were tested within 24-72 h of collection from the hopyard.

Probit regressions were estimated with Polo Plus (LeOra Software 2002). From this analysis, LC_{50} and LC_{90} values were also computed. Resistance ratios (RR) were estimated at the LC_{50} level as RR= LC_{50} of field strains/ LC_{50} of susceptible strains. Hopyard treatment histories were compiled from grower records.

Figure 1. Susceptible *T. urticae* colony reared on baby lima bean plants and isolated in a walk-in growth chamber

Figure 2. Baby lima bean seeds sowed in vermiculite-filled plastic bags

Figure 3. Potter-Precision Laboratory Spray Tower

Figure 4. Hopyard in the Yakima Valley

RESULTS

Baseline Toxicity and Concentration-Response Curves

Results for baseline toxicity of the three acaricides tested against susceptible *T. urticae* are shown in Table 1. All acaricides tested produced 100% mortality of *T. urticae* at concentrations equivalent to the field rate, along with the half field rate and quarter field rate (data not shown). Subsequently, serial dilutions were conducted until individuals from the test population were able to survive the spray. After treatment with appropriate doses and mortality evaluation, initial susceptibility of *T. urticae* 24 hours after treatment was estimated for abamectin and bifenazate using the leaf disc bioassay method, and bifenthrin using the sticky-tape bioassay technique. Probit analysis estimated the LC_{50}'s of abamectin, bifenazate, and bifenture to be 0.228, 0.820 and 17.97 mg a.i./L, respectively, and the LC_{90} values to be 1.10, 1.37, and 99.3 mg a.i./L, respectively.

Performing this objective allowed me to identify a suitable bioassay technique for each acaricide. More importantly, the susceptible toxicity data provided critical reference information for my two subsequent objectives.

Selections for Resistance

The LC_{50} values of the susceptible population (Table 1) were used when selecting for resistance. Acaricide-selected colonies demonstrated a considerable decrease in the level of susceptibility over consecutive weeks and selection treatments. In the bifenazate-selected strain, the LC_{50} shifted from 0.820 to 11.99 mg a.i./L, a 14.6-fold increase (Table 2). After ten selections for resistance, abamectin-selected strain LC_{50} shifted from 0.228 to 6.096 a.i./L, a 26-

fold increase (Table 3). The bifenthrin-selected strain shifted from 17.97 to 87.44 mg a.i./L, a 4.8-fold increase (Table 4). Bioassays were performed on each colony with only the acaricide they were selected against. Two weeks after the tenth treatment, the abamectin-selected colony LC_{50} value declined to 4.56 mg a.i./L, and in some acaricide-selected colonies there were fluctuations over consecutive weeks (abamectin and bifenthrin colonies). Two more selection treatments were performed after the tenth spray, but bioassay data (not shown) were disregarded due to control mortality that exceeded 20%. Forty weeks after the round of 12 selection experiments (or 56 weeks after the first selection treatment) there was a significant increase in susceptibility, but LC_{50}'s did not return to pre-exposure level (Abamectin-selected colony= 0.88; bifenazate-selected colony= 3.04). Both the abamectin and bifenazate colonies returned to similar RR values at the LC_{50} level (abamectin=3.88; bifenazate= 3.71) (Tables 2 and 3).

2012 *T. urticae* Hopyard Populations

T. urticae from a hopyard in Toppenish, WA, appeared to have responses similar to the susceptible strain, this was also a baby hopyard (abamectin LC_{50}=0.295; RR-LC_{50}=1.29). Baby hops are those that are in their first year of planting. Other sites, however, required substantially greater concentrations of acaricides to provide mortality. For example, Prosser 1's LC_{50} to abamectin was 19.76 mg a.i./L (RR-LC_{50}= 86) and bifenazate LC_{50} was 3.84 mg a.i./L (RR=LC_{50}: 4.7). Of the five hopyards sampled for spider mites in 2012, bioassays tested with abamectin and bifenazate were performed against four *T. urticae* hopyard populations. In all, LC_{50}'s to abamectin ranged from 0.295 to 19.76 mg a.i./L, with RR values from 1.29-86 (Table 5). LC_{50}'s to bifenazate ranged from 1.88-3.84 mg a.i./L, with RR values from 2.42-3.39 (Table

6). Bifenthrin was not used against any of the *T. urticae* field populations collected in 2012 or 2013.

2013 *T. urticae* Hopyard Populations

T. urticae from 13 hopyards were bioassayed with abamectin (Table 8). Bifenazate bioassays were performed on *T. urticae* from 12 different hopyards (Table 7). The abamectin LC_{50}'s ranged from 1.35- 24.5 mg a.i./L, with low to high resistance ratios at the LC_{50} level to abamectin (5.96-107). The bifenazate LC_{50}'s ranged from 3.929- 96.30 mg a.i./L. In 2013, there was one organic hopyard sampled for spider mites (Granger 2, LC_{50} to abamectin=2.56; LC_{50} to bifenazate=3.93), and there were three baby hopyards (Prosser 3, Prosser 4, and Prosser 5). These 2013 *T. urticae* baby hopyards populations still exhibited a moderate degree of resistance (with LC_{50}'s ranging from 4.97-16.43 mg a.i./L). In fact, *T. urticae* population from Prosser 4 baby hopyard showed the second highest LC_{50} to abamectin.

In some cases, the quantity of collected spider mites were not sufficient to complete a bioassay; thus some of the field collected populations were reared on bean plants for two weeks until a population adequate for a bioassay had built up (Granger 1 and Mabton 1). Other times, only designated discriminating doses were used against field collected populations. For example, only one dose of 224 mg a.i/L of bifenazate was used, or 22.5 mg.a.i./L of abamectin was used. In all cases of abamectin treatments, complete bioassays were performed with a range of doses to obtain concentration-response data (Table 8), but mortality percentages at the discriminating dose are shown for additional information.

Two of the hopyard populations in 2013 were also sampled in 2012 (Prosser 1 and Prosser 3). Prosser 3, however, were planted with new baby hops in 2013. Between 2012 and

2013 Prosser 1 population showed a significant difference in the LC_{50} values, decreasing from 19.67 in 2012 to 3.42 in 2013. Prosser 3 population's resistance to abamectin remained consistent between samples from August 2012 and August 2013, with an LC_{50} of 7.23 in 2012, and LC_{50} of 7.85 in 2013.

The highest LC_{50} value to abamectin was recorded near Moxee at the Moxee 1 population (LC_{50}=24.54; RR=107). The highest bifenazate LC_{50} value was recorded in Granger, at the Granger 3 population (LC_{50}=78.9; RR=96.30). The lowest mortality to a bifenazate discriminating dose was recorded in the Granger 4 population (73% mortality).

Spray records were obtained from some growers of the sampled hopyards. Total number of sprays prior to sampling, along with the number of abamectin and bifenazatae applications are listed (Table 9). Table 10 displays the total acaricides/insecticides sprayed during the 2013 season. The spray date and information from Table 9 and Table 10 are then graphed along with *T. urticae* LC_{50} (Table 8) from hopyards that were sampled more than once during the season (Figures 5, 6, and 7).

Earlier attempts at sampling and above-average temperatures during the 2013 summer, allowed me to record the progression of resistance to abamectin in four hopyards (Prosser 2, Prosser 3, Prosser 4, and Granger 3), while other hopyards only had a single sample. Prosser 2 population's LC_{50} to abamectin increased 7-fold in ten weeks (Figure 5), Prosser 3 increased 1.5-fold in 4 weeks (Figure 6), and Prosser 4 increased 1.44 in five weeks (Figure 7). There were five bioassays performed from the *T. urticae* Prosser 2 population throughout the summer. Despite the fact that there were six application treatments throughout the season (Table 9), there were consistently high numbers of mite counts every week (20-30 mites per leaf), whereas mite populations in other hopyards fluctuated on a week-to-week basis.

DISCUSSION

Baseline Toxicity

My study identified baseline responses of a susceptible colony to the acaricides bifenazate, abamectin, and bifenthrin. The greatest concentration tested on all samples was equivalent to the field rate, in which no spider mites survived and there was 100% mortality. Given this information, the recommended rate would ensure efficacy against spider mites in commercial hopyards without any previous exposure to these acaricides. The lethal concentration estimates in these studies provided the valuable reference data required for my subsequent laboratory and field studies.

Bioassay Techniques

I used three different commercially formulated acaricides consisting of various ingredients added to the formulations, and different percentages of active ingredient present in the formulated product. It is important to take this into consideration when comparing active ingredients and different products from other studies. Moreover, detection of resistance can be affected depending on the type of bioassay performed (Dennehy et al. 1983). A range of techniques has been used including leaf and leafless bioassays (Welty et al. 1988). Some researchers have used lack of any sustained mite movement (Pree & Wagner 1987; Flexner et al. 1988) as criterion for death, while others have required that mites can be scored alive if they are able to walk at least the full body length (Welty et al. 1987; Beers et al. 1998). Some researchers have developed closed cell arenas to prevent mite walk-off (Keena & Granett 1987). Using different techniques to evaluate mite toxicity to acaricides can affect the outcome of results. In

all, there are numerous ways to interpret bioassays, but one important criterion is that they be kept consistent within a study (Hoy 2011). In my study, bifenthrin bioassays were prepared with sticky tape, which is considerably different from the leaf disc bioassay. For this reason, the three bioassays in my study should not be strongly compared to each other. Spider mites repelled by pyrethroids usually display reactions such as avoidance and run-off. Therefore, the bioassays with bifenthrin were modified for these reasons.

Selection Experiments

When using the LC_{50} concentration to establish resistant *T. urticae* strains, I found that spider mites are capable of developing resistance at different rates. Conducting studies on susceptible mites in laboratory settings, in this case, can reveal important clues in the ability of a pest to develop resistance. Mite colonies were never exposed an acaricide prior to selection, yet *T. urticae* developed a much higher resistance ratio to abamectin, than to bifenazate and bifenthrin following 10 selection exposures at the susceptible LC_{50} concentration. This suggests that *T. urticae* develops resistance faster to abamectin than to the bifenazate and bifenthrin. This could be due to the fact that abamectin exhibits a translaminar activity within leaves, and mites may have been exposed to a continual residual dose by feeding on the plant tissue (Campbell 1989; Walsh et al. 1996). Mites were transferred from their treated plant onto new beans within 24 hours, however, so this may not be a sufficient cause and further investigation is required. Because of the ability of *T. urticae* to develop resistance to abamectin quickly, this suggests there should be additional precautions when applying this in the field.

Bifenthrin displayed the lowest increase in tolerance in response to 10 selection sprays. Reasons for the low 5-fold increase in response to bifenthrin could be attributed to the type of

bioassay performed, but could also be due to the *T*. *urticae* behavioral reaction following exposure to pyrethroids. *T*. *urticae* in this colony could have sought refuge in non-exposed areas of the plant where the sprayed pesticide did not contact. Although I have no direct evidence from my selection experiments, avoidance behaviors have been recorded in both lab and field settings in other studies. It is known that synthetic pyrethroids are repellent to spider mites (Gerson & Cohen, 1987), affecting their inter- and intra-plant distribution (Penman & Chapman 1983; Walsh & Grove 2005). Mites are repelled when sprayed directly, as well as when coming in touch with pyrethroid deposits on leaves. Spider mites will sometimes display spin-down, in addition to their run-off behavior. Spin-down means that mites use their webs to drop off of pyrethroid treated plants or leaves. Penman and Chapman (1983) demonstrated that spin-down is an important component of dispersal from bean plants heavily sprayed with pyrethroids. Caution must be used when spraying in a field with spider mites, as resurgence of spider mites become evident one week to several months post treatment (Gerson & Cohen 1987). In addition to having a repellent and resurgent effect on spider mites, they are also repellent to their natural enemies in field settings. Therefore, bifenthrin is not widely used in the field to control spider mites. This fact also explains my reasons for withdrawing from bifenthrin bioassays in the field experiments.

In the artificial selections experiments, colonies were consistently sprayed with the original LC_{50} of the susceptible colony, and this dose remained consistent throughout the duration of my resistance selection objective. My method contrasts with other studies in which the researchers continually increased the dose from each selection treatment (Sato et al. 2005), or used LC_{90} concentrations (Van Leeuwen et al. 2008). These studies reported LC_{50}'s and RR values much higher than those in my experiment. Van Leeuwen et al. (2006) first studied

resistance to bifenazate in a laboratory selected *T. urticae* strain. The researchers artificially selected for bifenazate resistance using the LC_{90}, increasing the concentration after each treatment. Their selection method generated a 100,000-fold resistance after 22 selection treatments. Additionally, Sato et al. (2005) reported selection experiments where only adult survivors were used to inoculate a new plant. They applied doses ranging from 4.36 -58.10 mg a.i abamectin/L and increasing concentrations over the course of treatment; while I only exposed my colony to 0.228 mg a.i. each week. By the end of five selections, resistance in the Sato et al. (2005) *T. urticae* strain increased 342-fold. Although my study does not present results of susceptibility shifting to same magnitude of the previous two experiments, it still lends valuable information to the field of acaricide resistance. My results suggest that resistance can develop even in the presence of a low selection pressure. It may also suggest that the number of applications plays a factor influencing the degree of resistance.

After selection treatments ended and colonies were maintained, I detected a reversion to susceptibility in the acaricide-selected colonies. This suggests that without any pressure, spider mites will revert back to their original susceptible state. Reversion of resistant strains has been documented in the two-spotted spider mite by Flexner et al. (1989) and Sato et al. (2005). Flexner et al. (1989) isolated field resistant colonies in a laboratory setting, and documented that a resistant colony had reverted to susceptibility in six generations. Another resistant colony underwent an immigration dilution in which susceptible mites were added to the colony. During this process, susceptibility was achieved in 3 generations. In an applied sense, this has two important implications. First, constant selection pressure is necessary to maintain resistance in a population. And second, that immigration and emigration in the field can affect the resistance level of populations.

Field Populations

An LC_{50} to abamectin higher than the field rate (22.5 mg a.i./L) was recorded in the Moxee 1 population (24.5 mg a.i./L), the second highest resistance was recorded in Prosser 4 (16.4 mg a.i./L). The two highest records to abamectin were unexpected for different reasons. First, Prosser 4 *T. urticae* populations were sampled from a baby hopyard. Explanations to the relatively high level of infestations and high resistance ratios could be attributed to *T. urticae* that emerged in the spring and sought refuge in the new baby hop plants. Interestingly, however, the crop planted before hop production in Prosser 4 was Concord grapes. Spider mites are generally known to be pests of wine grapes but are not pests on Concord grapes (Walsh 2013). The two-spotted spider mites could also have dispersed into the hopyard from neighboring fields. Dispersal can occur over short distances by crawling, which is an important means for movement into crop fields as well as throughout the plant (Margolies & Kennedy 1985). Aerial dispersal is another important form of locomotion and has been shown to be responsible in other crop systems. Bradenburg and Kennedy (1982) reported that wind dispersal was a key factor for the movement of *T. urticae* in field corn to infestations in other surrounding crops.

The Moxee 1 population was interesting because this hopyard had one of the fewest number of acaricide applications. Moxee 1 along with Moxee 2 only had two applications of acaricides applied before I sampled from these fields. Both these populations were situated in a distinct growing region called the Moxee Valley. The other two regions of the Yakima Valley are the lower Yakima Valley and the Yakama Nation. The Moxee Valley is situated at the northern end of the Yakima Valley, and is distinct for its high density of hopyards as well as its slightly cooler climate (USAhops). Aroma varieties of hops are more prevalently grown here,

rather than alpha hops (USAhops). Because of the differences in location, varieties, and climate between all populations, resistance could be affected by any, or all of these factors.

Results from field populations were variable, ranging from low to high RR values both between populations and years. In 2012, resistance ratios to abamectin exceeded those to bifenazate. In 2013, resistance ratios seemed to be within a similar range of each other, with the highest resistance ratio recorded for abamectin in the Moxee 1 population. A main difference between the two years was weather. Spider mite outbreaks are affected by hot and dry weather, along with water stress, wind, and dust (Walsh 2002). Overall, the summer of 2012 started off cooler but then finished hot and dry (WSU AgWeatherNet). While the weather conditions in 2013 started off warm, followed by a heat wave that was the earliest heat wave of that degree since 1992. It was also the second warmest summer on record, dating back to 1991 (WSU AgWeatherNet). This may have attributed to higher resistance ratios and higher *T*. *urticae* abundance observed in hopyards in 2013.

In addition to my study, there have been previous reports on the level of resistance to both abamectin and bifenazate. Vostrel (2010) reported that bifenazate use in Czech hops gave good control of spider mites according to bioassays. The highest rate tested, which was the recommended concentration in the field, resulted in 100% mortality. Similarly, I also reported 100% mortality to the field rate of bifenazate in all bioassays and strains (artificial and field). Van Leeuwen et al. (2005) documented that bifenazate also had a very high efficiency in controlling a field-collected resistance strain of *T*. *urticae*, while demonstrating no signs of cross resistance.

Campos et al. (1995) first reported a decreased activity of abamectin while monitoring ornamental nurseries in California. Resistance ratios at the LC_{95} level ranged from 1 to 658 and it

was documented that increased resistance ratios were correlated with the number of abamectin applications per year, along with the total number of applications of abamectin. Perhaps the Moxee 1 population that reported the highest resistance ratio of abamectin (despite only 2 acaricide applications) could have endured a longer overall history of abamectin use. Campos et al. (1996) reported abamectin resistance from strains originating from Florida, the Netherlands, and the Canary Islands. They further supported the claim that abamectin resistance had a correlation with the frequency of treatments in the field. They also proposed that resistance development differed among locations (Campos et al. 1996). Beers et al (1998) reported on decreased *T. urticae* susceptibility to abamectin from populations collected from pear orchards in Washington. Vassiliou and Kitsis (2013) report LC_{50} values to abamectin in greenhouses from Cyprus; their highest LC_{50} value recorded to abamectin indicated a 1356-fold increase over the susceptible population. Out of the four acaricides bioassayed in their study, abamectin resistance ratios displayed the greatest levels.

Concluding Remarks

Overall, many studies have screened for the resistance of field populations of spider mites and have monitored their resistance over multiple years. None, to my knowledge, have documented the progression of resistance in a field population in a single crop season, as I have presented in my study.

For management of *T. urticae*, previous work demonstrated that abamectin, bifenazate, and bifenthrin give poor field control due to resistance development. My work supports varying levels of resistance of *T. urticae* in hopyards, and therefore caution should be taken while applying these acaricides. The highest resistances were found with abamectin; therefore use of

this acaricide is an added risk. Although high levels of resistance were not widespread in my sampled hopyards, values of a few populations suggest that *T*. *urticae* has the capability to reach higher resistance levels. Registered acaricides in hopyards should be used rationally and in rotation to delay resistance development. The use of novel acaricides with distinct modes of action may help in controlling resistant *T*. *urticae* populations. Supplementary acaricides and cross-resistances should be investigated in addition to the three in my study, as there are many others registered in this crop and applied in hopyards. This will aid in a more comprehensive study.

The development of resistance is of concern in the agricultural community. In my study, I have presented evaluations of artificially selected strains and field populations that are useful for implementing management programs. There is much work to be done on the *T*. *urticae*-hop system in the Pacific Northwest, not to mention other crops, and I hope my data contributes to information lending to improved programs and serves as a framework for future studies, including resistance monitoring and molecular mechanisms of resistance.

Table 1. Baseline toxicity and probit analysis of acaricides to susceptible *T. urticae*.

Acaricide	Field rate (mg a.i./L)	% Mortality at Field Rate	LC_{50} (mg a.i./L)	LC_{90} (mg a.i./L)	Slope±SEM	N	Bioassay method
Abamectin	22.5	100	0.228 (0.122–0.325)	1.107 (0.737–2.51)	1.867±0.071	4100	Leaf disc
Bifenazate	899	100	0.820 (0.788–0.851)	1.377 (1.27–1.524)	5.687±0.461	2195	Leaf disc
Bifenthrin	120	100	17.97 (8.42–44.60)	99.30 (41.49–5497)	1.726±0.075	2300	Sticky tape

Table 2. Selection for resistance to bifenazate, in a colony originally consisting of only susceptible mites before selection treatments. Estimated LC_{50} and LC_{90} values are recorded in weeks succeeding the first selection treatment.

Selection Number	Weeks	N	% Mortality at 224 mg a.i./L	LC_{50} (mg a.i./L) (95% CI)	LC_{90} (mg a.i./L) (95% CI)	RR [(1)] LC_{50}	Slope±SEM
0[(2)]	0	2950	100	0.820 (0.788–0.851)	1.377 (1.276–1.524)	–	5.687±0.461
2	2	139	100	0.817 (0.550–1.131)	4.072 (2.524–10.67)	1	1.837±0.361
4	4	208	100	1.760 (1.324–2.257)	6.580 (4.807–10.44)	2.15	2.237±0.292
6	6	264	100	2.748 (1.067–6.213)	15.343 (6.608–630.1)	3.35	1.716±0.246
8	8	136	100	4.058 (2.862–5.371)	15.358 (10.67–28.79)	4.94	2.217±0.378
10	10	159	100	6.581 (1.580–19.80)	37.685 (13.83–928.5)	8.03	1.691±0.224
10	12	157	100	11.995 (8.424–16.23)	41.238 (28.52–75.96)	14.63	2.390±0.397
12	56	148	100	3.045 (1.693–4.895)	20.923 (12.19–47.73)	3.71	1.531±0.239

Concentration of acaricide treated on selected colonies each week was 0.89 mg a.i./L

(1) Resistance Ratio (RR)= LC_{50} selected colony/ LC_{50} susceptible colony

(2) Before Selection

Table 3. Selection for resistance to abamectin, in a colony originally consisting of only susceptible mites before selection treatments. Estimated LC_{50} and LC_{90} values are recorded in weeks succeeding the first selection treatment.

Selection Number	Weeks	N	% Mortality at 22.5 mg a.i./L	LC_{50} (mg a.i. /L) (95% CI)	LC_{90} (mg a.i./L) (95% CI)	RR [(1)]- LC_{50}	Slope±SEM
0[(2)]	0	4100	100	0.228 (0.122–0.325)	1.107 (0.737–2.514)	–	1.867±0.071
2	2	240	100	1.94 (1.31–2.58)	6.60 (4.90-7.87)	8.51	1.204±0.161
4	4	406	100	1.357 (0.273–2.691)	8.083 (4.39–20.55)	5.95	1.654±0.231
6	6	200	100	1.737 (0.855–3.044)	9.457 (5.07–29.20)	7.62	1.741±0.196
8	8	200	92.5	3.037 (2.128–4.207)	22.296 (14.30–42.42)	13.32	1.480±0.176
10	10	200	72.5	6.096 (3.970–9.83)	103.304 (46.09–429.2)	26.73	1.043±0.161
10	12	200	75	4.56 (2.250–9.30)	54.522 (23.3–271.7)	20	0.095±0.194
12	52	160	100	0.800 (0.289–1.351)	4.723 (3.06–8.86)	3.5	1.662±0.343
12	56	180	100	0.885 (0.633–1.204)	3.870 (2.655–6.619)	3.88	1.999±0.248

Concentration of acaricide treated on selected colonies each week was 0.224 mg a.i./L

(1) Resistance Ratio (RR)= LC_{50} selected colony/ LC_{50} susceptible colony

(2) Before Selection

Table 4. Selection for resistance to bifenthrin, in a colony originally consisting of only susceptible mites before selection treatments. Estimated LC_{50} and LC_{90} values are recorded in weeks succeeding the first selection treatment.

Selection Number	Weeks	N	%Morality at 120 mg a.i./L	LC_{50} (mg a.i./L) (95% CI)	LC_{90} (m.g./L) (95% CI)	RR[(1)]-LC_{50}	Slope±SEM
0[(2)]	0	2300	100	17.974 (8.423–44.609)	99.307 (41.5–5497)	–	1.726±0.075
2	2	240	100	14.545 (11.49–17.34)	37.606 (30.85–50.3)	0.809	3.106±0.453
6	6	240	100	25.593 (14.66–33.98)	61.003 (48.1– 84.1)	1.42	3.397±0.717
10	10	160	75	87.437 (57.18– 112.6)	190.589 (136.8– 855)	4.86	3.787±1.314

Concentration of acaricide treated on selected colonies, each week was 17 mg a.i. /L

(1) Resistance Ratio (RR)= LC_{50} selected colony/ LC_{50} susceptible colony

(2) Before Selection

Table 5. Toxicity of abamectin to *T. urticae* hopyard populations collected in 2012.

Population	N	% Mortality at 22.5 mg a.i./L [(1)]	LC_{50} (mg a.i./L) (95% CI)	LC_{90} (mg a.i./L) (95%CI)	$RR^{(2)}$-LC_{50}	Slope±SEM
Prosser 1	120	57.5	19.675 (18.91–180.6)	159.489 (61.38–2791)	86.29	1.249±0.384
Prosser 3	240	80	7.234 (5.407–9.971)	45.232 (28.05–93.1)	31.72	1.610±0.193
Toppenish	160	100	0.295 (0.163–0.442)	1.787 (1.14–3.653)	1.29	1.637±0.281
Mabton 1	200	87.5	5.844 (2.53–14.52)	46.018 (17.3–901.9)	25.63	1.430±0.189

[(1)] Abamectin discriminating dose. 22.5 mg a.i.(field rate)
[(2)] Resistance Ratio (RR)= LC_{50} field population/ LC_{50} susceptible colony

Table 6. Toxicity of bifenazate to *T. urticae* hopyard populations collected in 2012.

Population	N	% Mortality at 224 mg a.i./L[(1)]	LC_{50} (mg a.i./L) (95% CI)	LC_{90} (mg a.i./L) (95% CI)	RR[(2)]-LC_{50}	Slope±SEM
Prosser 1	160	100	2.787 (13.09–43.79)	104.412 (35.22–1181)	3.39	0.814±0.170
Prosser 3	240	-	1.888 (5.40–9.97)	55.168 (22.19–408.3)	2.29	0.874±0.183
Toppenish	119	100	-	-	-	-
Mabton 1	120	-	3.841 (2.209–6.44)	49.400 (23.10–202.3)	4.68	1.155±0.203

(1) Bifenazate discriminating dose: 224 mg a.i. /L (¼ of the field rate.) Where multiple concentrations could not be tested, or perform a probit analysis, the discriminating dose was identified for comparison.

(2) Resistance Ratio (RR)= LC_{50} field population/ LC_{50} susceptible colony

Table 7. Toxicity of bifenazate to *T. urticae* hopyard populations collected in 2013.

Population	Month Sampled	N	% Mortality at 224 mg.a.i/L[(1)]	LC_{50} (mg a.i./L) (95% CI)	LC90 (mg a.i./L) (95%CI)	$RR^{(2)}$-LC_{50}	Slope±SEM
Prosser 1	July	60	100	-	-	-	-
Prosser 2	August	160	85	55.80 (30.4-85.40)	216 (65–280)	68.04	1.583±0.220
Prosser 3	August	160	88	25.486 (3.87–66.98)	181.56 (68.715–5358)	31.08	1.503±0.211
Prosser 4	August	160	90	6.867 (1.36–13.66)	44.39 (23.84–130.2)	8.37	1.581±0.420
Prosser 5	August	157	95	9.314 (3.913–15.69)	90.39 (54.08–209.5)	11.39	1.298±0.238
Mabton 1	July	60	96	-	-	-	-
Granger 1	August	157	82	47.859 (11.39 –138.08)	265.3 (101.2–8486)	58.29	1.723±0.219
Granger 2^{Org}	August	120	100	3.929 (0.343–7.113)	18.71 (11.81–55.79)	4.79	1.891±0.642
Granger 3	August	197	76	78.967 (55.99 –107.5)	444.7 (304.3–747.2)	96.30	1.707±0.191
Granger 4	July	60	73	-	-	-	-
Granger 5	July	160	92.5	-	-	-	-
Moxee 1	August	160	90	18.882 (9.714 – 30.14)	160.420 (96.36– 354.9)	23.02	1.379±0.224

(1) Bifenazate discriminating dose: 224 mg a.i./L (¼ of the field rate). Where multiple concentrations could not be tested, the discriminating dose was identified for comparison.

(2) Resistance Ratio (RR)= LC_{50} field population/ LC_{50} susceptible colony

Table 8. Toxicity of abamectin to *T. urticae* hopyard populations collected in 2013.

Population	Month Sampled	N	% Mortality at 22.5 mg a.i./L[1]	LC_{50} (mg a.i./L) (95% CI)	LC_{90} (mg a.i./L) (95% CI)	RR[2]-LC_{50}	Slope±SEM
Prosser 1	June	116	95	3.42 (1.416–8.055)	133.96 (35.05–4625)	15	0.804±0.198
Prosser 2	Mid June	100	95	1.89 (0.216–3.972)	40.85 (15.37–1780)	8.30	0.916±0.313
Prosser 2	Mid July	200	87.5	2.85 (1.756–4.040)	20.86 (14.109–37.44)	12.5	1.482±0.209
Prosser 2	Late July	198	89	7.12 (2.123–15.36)	30.81 (14.527–580.12)	31.2	2.014±0.249
Prosser 2	Mid August	198	73	11.65 (6.862–19.41)	50.95 (27.52–252.46)	51.09	2.00±0.270
Prosser 2	August	397	72	13.99 (3.668–23.99)	46.76 (26.86–330.7)	61.35	2.447±0.233
Prosser 3	Late July	180	85	4.97 (1.278–9.965)	28.86 (13.61–304.6)	21.79	1.278±9.965
Prosser 3	Late August	220	75	7.85 (4.15–15.64)	60.96 (26.25–469.0)	34.42	1.440±0.186
Prosser 4	Mid July	200	62.5	11.37 (6.01–35.94)	192 (76.20–2159)	49.86	1.042±0.256
Prosser 4	September	339	51	16.43 (10.28–33.91)	119 (48.41–1795)	72.06	1.469±0.211
Prosser 5	July	220	92.5	8.471 (5.98–10.50)	22.80 (17.03–44.35)	37.15	2.980±0.686
Mabton 1[C]	July	199	92	4.24 (2.54–6.0)	24.25 (15.62–51.60)	18.59	1.693±0.299
Granger 1[C]	July	180	80	8.24 (6.22–10.73)	40.85 (27.21–79.67)	36.11	1.842±0.265
Granger 1	August	238	92.5	7.471 (5.77–8.98)	16.851 (13.750–23.23)	32.76	3.628±0.602
Granger 2[Org]	June	180	100	2.56 (1.82–3.36)	9.79 (7.19–15.19)	11.22	2.200±0.295
Granger 3	July	178	74	9.715 (7.48–12.79)	40.30 (26.49–84.8)	42.61	2.074±0.332
Granger 4	July	240	92.5	1.359 (0.145–3.909)	28.14 (8.51–1365)	5.96	0.974±0.127
Granger 5	July	200	95	8.799 (0.122–0.325)	17.83 (13.20– 34.84)	38.59	4.179±0.660
Moxee 1	July	160	50	24.54 (11.31–187.82)	635 (113–5939)	107.64	0.907±0.323
Moxee 2	August	200	77.5	13.36 (10.95–16.53)	32.43 (24.02–58.71)	58.622	3.329±0.628

[1] Abamectin discriminating dose: 22.5 mg a.i. (field rate)
[2] Resistance Ratio (RR)= LC_{50} field population/ LC_{50} susceptible colony
[C] Reared on bean plants for 2 weeks
[Org] Organic hopyard

Table 9. Spray records of hopyards sampled for *T. urticae* populations.

Population	# of acaricide/ insecticides applied in 2013 hop season	# of acaricides/ insecticides applied prior to sampling	# times abamectin applied	# times bifenazate applied	Date of First and Last Application Sprays
Prosser 1	-	-	-	-	-
Prosser 2	7	Mid June: 0 Mid July: 2 Late July: 5 Mid August: 7 Late August: 7	2	1	June 5-July 31
Prosser 3	7	Late July: 5 Late August: 7	1	1	June 20-August 20
Prosser 3(1)	3	3	1	0	June 15-July 20
Prosser 4	7	Mid July: 4 Early September: 7	1	1	June 20-August 20
Prosser 5	-	-	-	-	-
Mabton	-	-	-	-	-
Granger 1	5	July: 2 August: 5	1	1	June 30-August 13
Granger 2(Org)	0	0	0	0	-
Granger 3	5	3	1	1	June 30-August 13
Granger 4	4	3	1	0	June 22-August 14
Granger 5	9	6	2	1	June 16- August 6
Moxee 1	2	2	1	0	June 25-July 13
Moxee 2	2	2	1	0	June 25-July 13

Empty fields indicate spray records that could not be obtained from the grower.
(1) 2012 spray record
(Org) Organic hopyard

Table 10. List of acaricides/insecticides applied in sampled hopyards during 2012 and 2013 hop season. Grouped based on IRAC Mode of Action (MoA) Classification.

Mode of Action (Group)	Active ingredient	Registered name	Company
Sodium channel modulators (Group 3)	bifenthrin (Chemical family: pyrethroids)	Bifenture EC	United Phosphorus, Inc.
Nicotinic acetylcholine receptor agonists/ antagonist	imidacloprid (Chemical family: neonicitinoids)	Admire Pro Couraze	Bayer CropScience Chemicova Inc.
Chloride channel activators (Group 6)	abamectin	Abba Ultra Agri-mek	MANA Crop Protection Syngenta Crop Protection
Mite growth inhibitors (Group 10)	hexythiazox	Savey	Gowan Company
	etoxazole	Zeal	Valent U.S.A. Corporation
Mitochondrial complex III electron transport inhibitors (Group 20)	acequinocyl	Kanemite	Arysta LifeScience North America, LLC
Mitochondrial complex I electron transport inhibitors (Group 21)	fenpyroximate	Fujimite	Nichino America, Inc.
Inhibitors of acetyl Coenzyme A carboxylase (Group 23)	spirodiclofen	Envidor	Bayer CropScience
Neuronal inhibitors (Unknown MOA)	bifenazate	Acramite	Chemtura Corporation

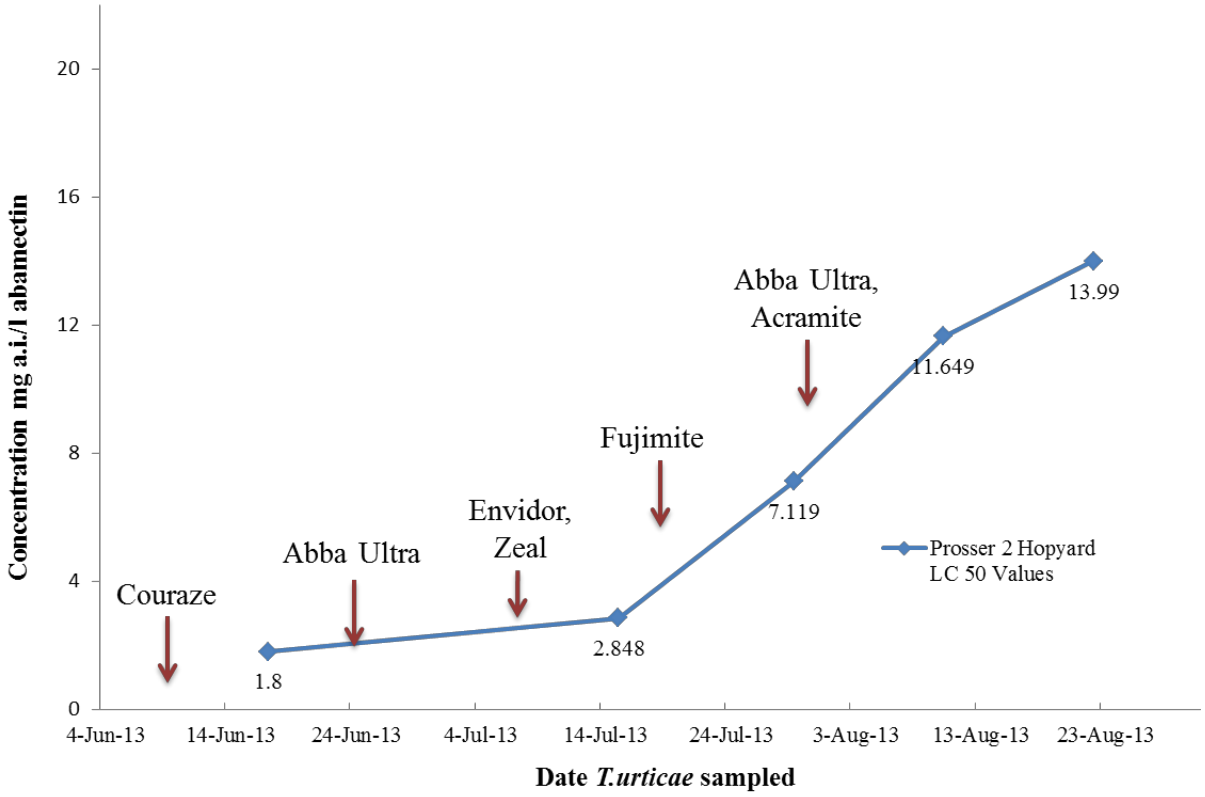

Figure 5. LC_{50} values of Prosser 2 *T. urticae* population in response to abamectin, and in relation to the acaricide applied, as depicted by the arrows above.

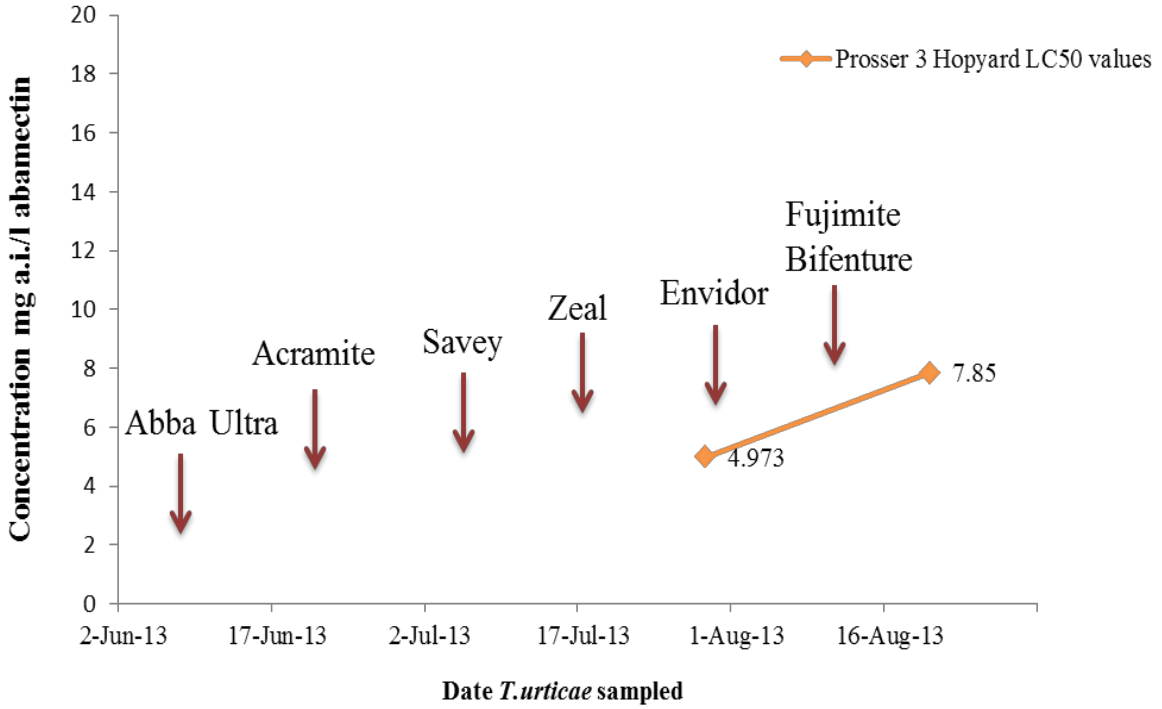

Figure 6. LC_{50} values of Prosser 3 *T. urticae* population in response to abamectin, and in relation to the acaricide applied, as depicted by the arrows above.

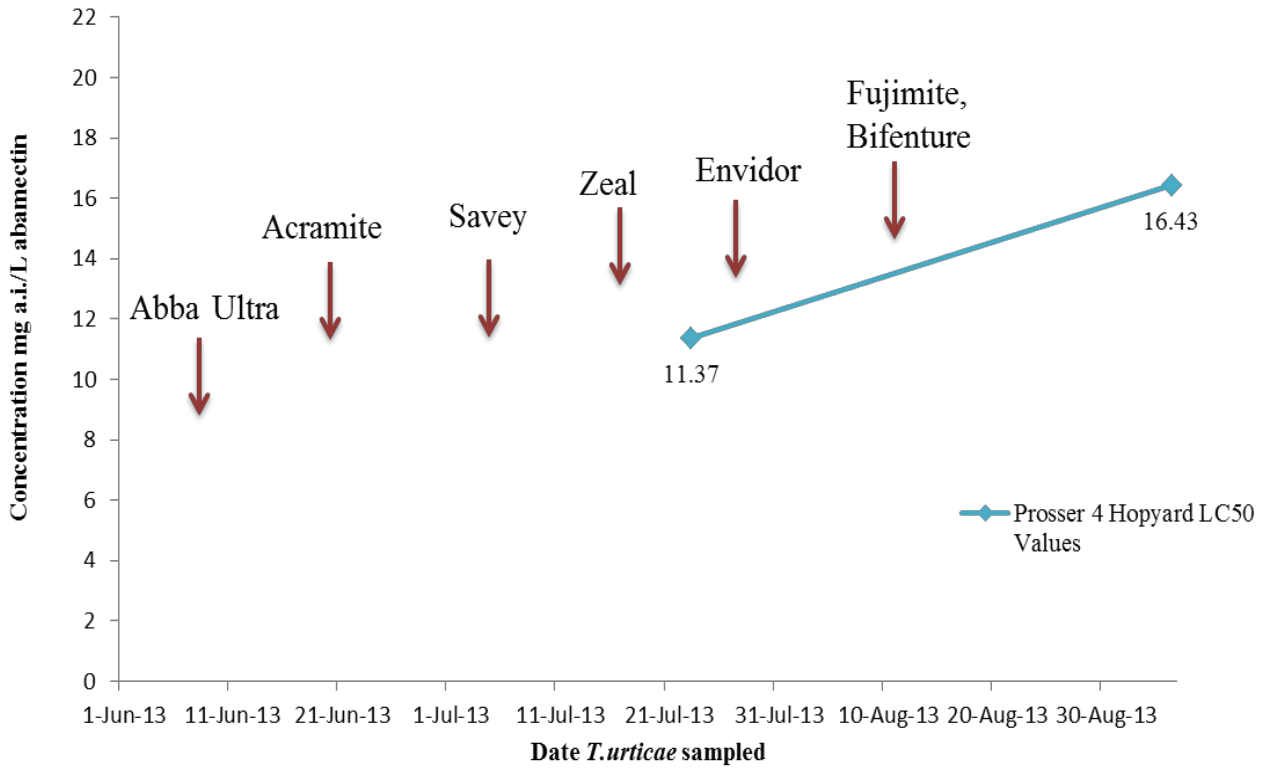

Figure 7. LC_{50} values of Prosser 4 *T. urticae* population in response to abamectin, and in relation to the acaricide applied, as depicted by the arrows above.

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