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Establishment of Tetranychid Mites in vitro

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Additional index words. *Tetranychus urticae*, somaclonal variation, surface disinfestation, tissue culture, axenic cultures

Summary. **Procedures were developed to determine if live, adult two-spotted spidermites (*Tetranychus urticae* Koch) could be surface disinfested before being introduced into in vitro cultures of torenia (*Torenia fournieri* L.). Three time periods (5, 10, and 15 minutes) and five levels of sodium hypochlorite (0.05% to 0.25%) were evaluated. Surface disinfestation was accomplished by agitating 2 × 3 cm pieces of infested bean leaves in sodium hypochlorite solutions and then drying in a mite drier apparatus. All sodium hypochlorite concentrations disinfested the mites completely, however high concentration levels were lethal to the mites. Exposure periods of 10 and 15 minutes also significantly increased mortality. For optimum disinfestation of two-spotted spidermites with minimum mortality, a concentration of 0.05% sodium hypochlorite and 0.05% Tween-20 for 5 minutes should be used.**

For either the study of plant processes or of a plant's relationship to its environment, the plant should be free of microorganisms. Axenic cultures are necessary because microorganisms can influence plant nutrition, physiology, and health. The need for asepsis while working with plant tissue culture requires that all culture vessels, instruments, and media be sterile. A variety of wet and dry heat treatments, such as radiation, filtration, gas, and chemical agents, are available for sterilization (Klein and Klein, 1970). In addition, simple precautions, such as maintaining a high level of cleanliness, will reduce the risk of widespread contamination (Kreider, 1968). The disinfestation of plant tissue culture media and apparati is usually accomplished by autoclaving.

Several chemical agents are used for to surface disinfest plant material, including sodium hypochlorite, calcium hypochlorite, mercuric chloride, hydrogen peroxide, silver nitrate, and bromine water. The choice of chemical and the time of exposure depends on the sensitivity of the material to be disinfested. Overzealous disinfestation may not only remove all microorganisms, but it may also be lethal to the plant tissue. Therefore, optimum conditions have to be determined for each tissue or situation.

The chemical agent should be easily removed after application because the retention of noxious chemicals seriously affects the establishment of cultures. Repeated rinses with distilled water will wash most chemical agents away, whereas others degrade to less-toxic chemicals that can be washed away. For example, sodium hypochlorite breaks down to chlorine, its active agent, and sodium hydroxide; the latter is removed during rinsing. Hydrogen peroxide decomposes and evaporates. Silver nitrate can be inactivated by the addition of sodium chloride (NaCl) to render the sterilizing agent harmless to the tissue. Dilute mercuric chloride is a satisfactory sterilizing agent, but difficult to remove.

Studies on the surface disinfestation of insects or arthropods for introduction into in vitro cultures have not been reported; however, techniques exist to evaluate the effects of acaricides on spidermites. Methods to evaluate the resistance of spidermite populations to acaricides should be simple, provide reproducible results, and simulate, as closely as possible, the conditions under which the acaricide will be used for mite control. The slip-dip method, as described by Voss (1961), fulfills the first two criteria; however, it measures only topical toxicity and is difficult to use with acaricides that are effective against adult female mites. Furthermore, it is

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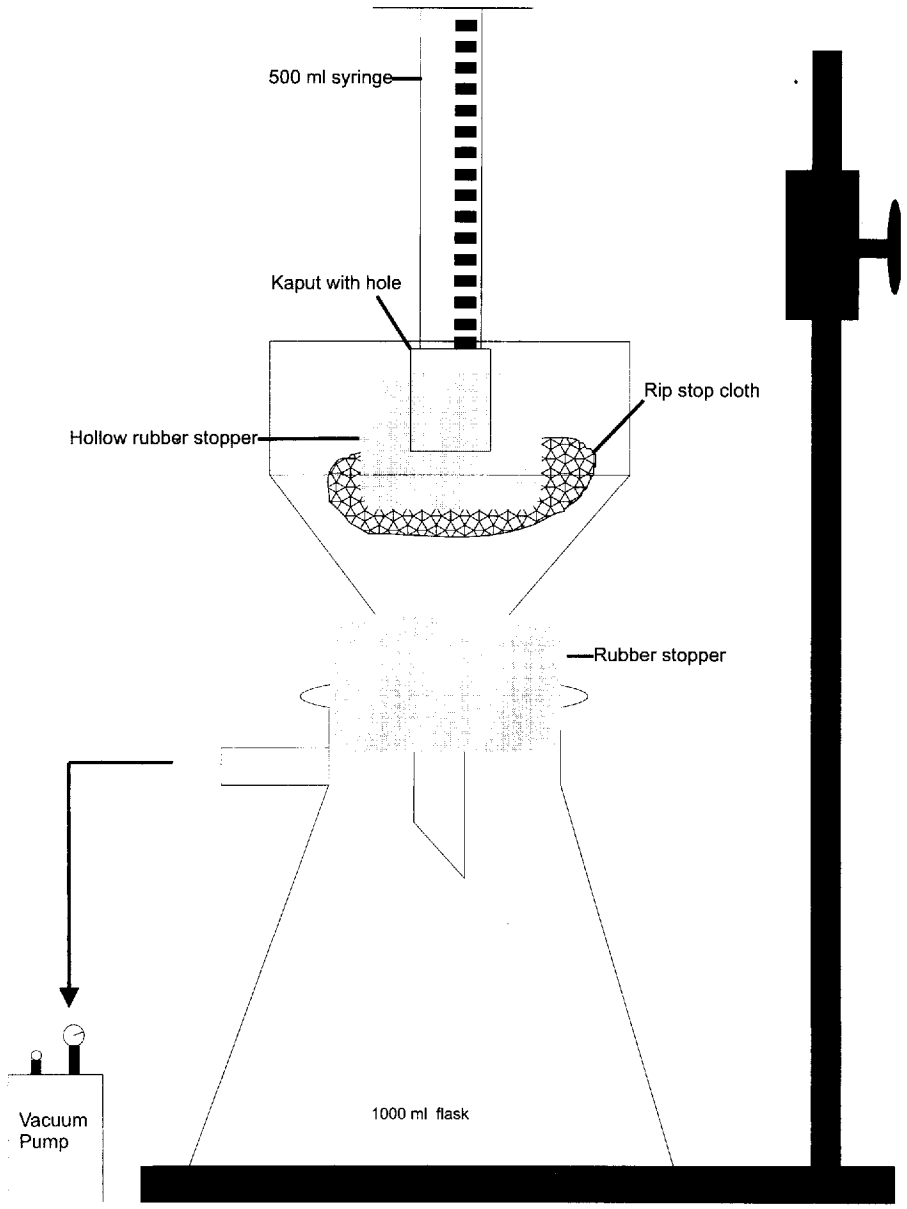

Fig. 1. Diagram of the mite removal and drier apparatus for the disinfestation of mites in vitro.

not suitable for use with slow-acting acaricides because control mortality becomes excessive after 3 d (Walker et al., 1973).

This study was undertaken to determine if two-spotted spidermites could be surface disinfested before introduction onto sterile shoot cultures of *Torenia fournieri* This research was the initial stage in a program whose objective was to examine the potential of developing insect-resistant plants through somaclonal variation (Hadi and Bridgen, 1996).

Materials and methods

MITE DISINFESTATION. Two-spotted spidermites (*Tetranychus urticae*) were obtained from John Sanderson, Dept. of Entomology, Cornell Univ., Ithaca, N.Y., and used to establish a colony on bean (*Phaeseulous vulgare* L.) in a growth chamber. Plants were grown in 1.7-L^3 pots at 23 °C with continuous cool-white light at 120 $\mu mol \cdot m^{-1} \cdot s^{-2}$. Host plants were replaced with 10 new plants 12 to 15 cm in height every 15 d. To begin new colonies, 20 mites were taken from the older plants with a camel-hair brush and placed on the top three leaves of each of the new plants.

Five levels of sodium hypochlorite were tested: 0.05%, 0.10%, 0.15%, 0.20%, and 0.25% (v/v). Each concentration was evaluated for three time periods: 5, 10, and 15 min. Replications were completed over time, with each experimental unit containing 15 samples. All experiments were replicated three times. A treatment of 0% sodium hypochlorite was not included in these evaluations because previous studies demonstrated that no treatment resulted in 100% contamination all the time.

Fifteen magnetic stir plates were set up in a laminar-flow hood for each block over time. Each sodium hypochlorite treatment was brought up to a final volume of 200 mL with deionized water and placed in a 300-mL beaker that contained a sterile stir bar and 100 µL of Tween-20. Treatments were randomly assigned to the magnetic stir plates and agitated at 120 rpm. Sodium hypochlorite concentrations were obtained from a commercial bleach solution with 5.25% sodium hypochlorite as the active agent.

Mites were gathered from the colonies by removing heavily infested leaves from host plants and cutting them into 2 × 3-cm sections. Ten pieces of leaf were placed in each beaker. After the completion of each treatment, sterile forceps were used to hold the leaf pieces while both sides were sprayed with sterile deionized water from a wash bottle. This process removed any mites still adhering to the surface of the leaf. After the pieces of leaves had been washed, the solution containing the mites was filtered through a mite removal and drier apparatus.

The mite removal and drier apparatus (Fig. 1) was a 500-mL polypropylene disposable syringe that had been attached to a two-pronged clamp on a support rod. The lower part of the syringe was resting on a no. 12 rubber stopper in which a hole had been drilled. The stopper was placed in the top of a Buchner funnel that was lined with a piece of ripstop nylon cloth. The funnel drained into a side-arm Erlenmeyer flask that was connected to a vacuum pump. After the solution was poured into the syringe, the vacuum pump was switched on. Mites were retained on the nylon cloth while the solution was sucked into the flask. The interwoven nature of the ripstop cloth allowed the bleach solution to be removed quickly without damaging the mites.

The pieces of ripstop clothes with mites were then placed in sterile petri dishes (100 × 15 mm) in the laminar flow hood for 15 min to dry. Dishes were sealed with Parafilm and placed in the growth chamber at 23 °C for 24 h at 120 $\mu mol \cdot m^{-1} \cdot s^{-2}$. Efficiency of the treatments were assessed using a modified Murashige and Skoog (MS) (1962) me-

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Table 1. Sums of squares (SS) for sources of variation in the response of mites to different sodium hypochlorite concentrations and time of exposure to these concentrations.[z]

Source	df	SS	F value	*P*
Model	23	19.8669	9.68	0.01
Block	9	5.1684	6.44	0.01
Concentration	4	11.5918	32.48	0.01
L	1	10.4682	117.32	0.01
Q	1	0.3757	4.21	NS
C	1	0.7408	8.30	0.05
Q	1	0.0069	0.08	NS
Time	2	2.8100	15.76	0.01
L	1	2.7773	31.13	0.01
Q	1	0.0326	0.37	NS
Concentration × Time	8	0.2966	0.42	NS
Error	126	11.2432		

[z]Analysis was done on transformed data (sqrt. +0.5); CV = 1.4223, R^2 = 0.638600
[NS]Nonsignificant; L = linear, C = cubic, Q = quadratic.

dium amended with 30 g·L^{-1} of sucrose and 6 g·L^{-1} of agar (Sigma Corp., St. Louis). The pH was then adjusted to 5.7 and autoclaved for 20 min under 1.05 kg·cm^{-2} at 120 °C. The sterile medium was poured into sterile 100 × 15-mm plastic petri plates.

After 24 h in the growth chamber, petri plates containing the mites were opened in the laminar flow hood. Thirteen mites were randomly picked from each plate and transferred to MS medium. These petri plates were sealed with Parafilm and placed in the growth chamber at 23 °C for 15 d at 120 µmol·m^{-1}·s^{-2}. The number of contaminated petri plates were recorded on day 16.

Mortality evaluation. In addition to evaluating the sodium hypochlorite effectiveness for mite disinfestation, a modified version of the slip-dip technique was used to determine mite mortality to the treatment (Voss, 1961). The technique has been proposed as the standard assay method for determining acaricide resistance in spidermites (Bussivine, 1980). Double sided tape (3M Co., St. Paul, Minn.) was used to prepare slides for the slip-dip technique. The tape was placed on a 7.5 × 2.5-cm microscope slide, leaving 1 cm on each side empty. Ten mites were randomly selected and placed with a fine camel-hair brush (No. 00) on their backs on the tape. Care was taken not to touch the brush to the adhesive of the tape.

Each slide was submerged in sodium hypochlorite solution for 5, 10, and 15 min and then left to air dry at room temperature (25 °C) on a slide rack. The slides were then placed in sterile disposable petri dishes (100 × 15 mm) lined with filter paper (Whatman no. 1, 7.0 cm size) and wetted with 3 mL of distilled water. The petri plates were sealed with Parafilm and placed in a growth chamber at 26 °C under constant cool-white light. Ten replications of each treatment were tested. Mites were counted as dead 48 h post treatment if no leg movement was observed when stroked with a camel-hair brush. All count data were transformed using square root plus 0.5 transformation. Linear regression and analysis of variance statistics were performed using SAS statistical package (SAS, 1988).

Results and discussion

Previous studies have demonstrated that mites can withstand mild concentrations of sodium hypochlorite (Scriven

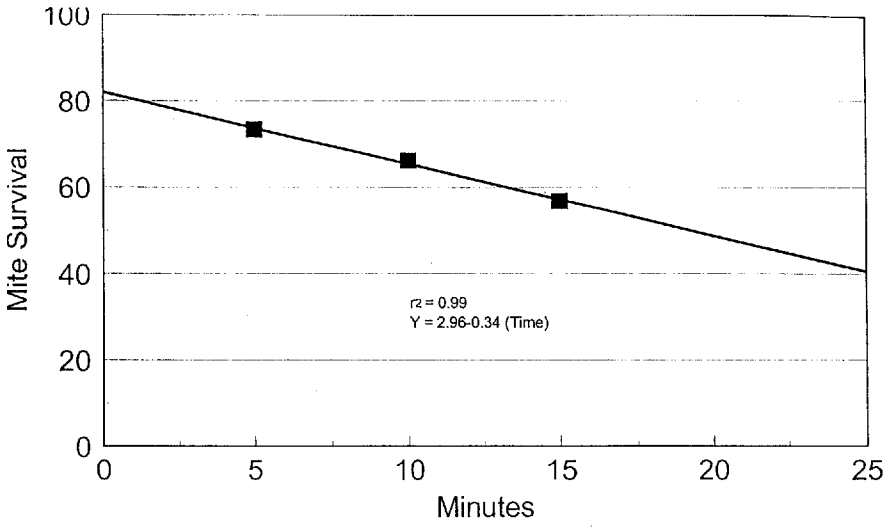

Fig. 2. Percentage of mite survival in sodium hypochlorite at different treatment times.

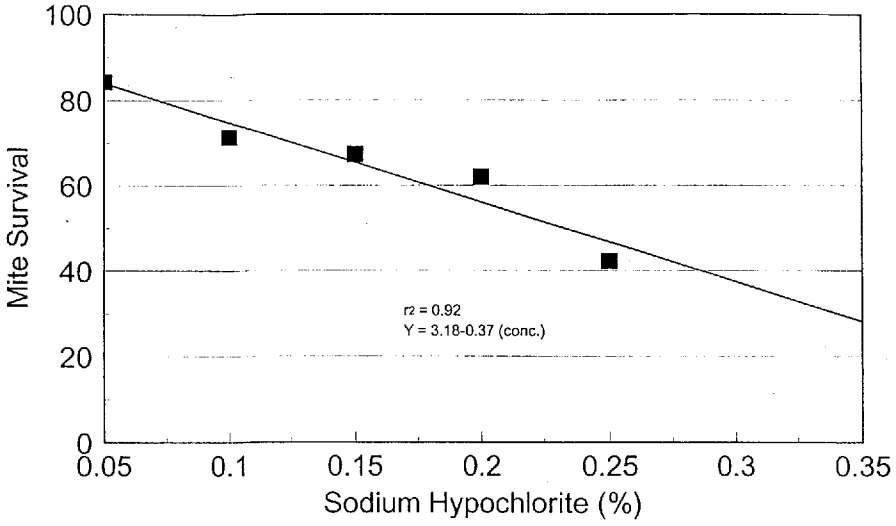

Fig. 3. Percentage of mite survival in different concentrations of sodium hypochlorite.

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and McMurtry, 1971). All five concentrations of sodium hypochlorite tested produced axenic mites at all three treatment durations. There were no significant differences between treatments with respect to surface disinfestation of mites. No differences in mite disinfestation were observed with the different time and concentration treatments. However, the higher concentrations of sodium hypochlorite and longer time periods were more lethal to the mites.

A modified version of the slip-dip technique was used to confirm mortality levels. Highly significant differences in mite mortality were observed between time and concentration treatments. Significant differences were not observed in the interaction response. All three time periods differed from each other significantly (Table 1). As the time in the sodium hypochlorite concentrations increased from 5 to 15 min, mite mortality increased. A linear response was observed for time; as time increased mite mortality increased (Fig. 2). The percentage of mite survival also decreased as the sodium hypochlorite concentration increased from 0.05 to 0.25%. The concentration having 0.05% sodium hypochlorite differed significantly from the rest of the concentrations having the highest survival rate. A linear response was observed with concentration and survival; the higher the concentration of sodium hypochlorite, the higher the mite mortality (Fig. 3). Croughan and Quisenberry (1989) reported of similar relationships when working with fall armyworm (*Spodoptera frugiperda* J.E. Smith).

From the results of this study, we conclude that the concentration of 0.05% sodium hypochlorite and 0.05% Tween-20 for a time period of 5 min is adequate for surface sterilization of mites without causing excessive mortality of mites before they are introduced in vitro. The technique allows surface-disinfested mites to be introduced into culture. Once this is accomplished, plant material can be evaluated for resistance, thereby offering the ability to evaluate plant material in vitro and possibly reducing considerable time, space, and expense as opposed to a field evaluation.

Most of the progress in the area of plant resistance to insects has been due to conventional insect evaluation and plant breeding techniques. In the future, accurate and economical techniques of plant and insect evaluations developed from biotechnology will prove useful as molecular plant technologies are developed for use in crop protection. However, problems inherent in the development of resistant cultivars will most likely follow the development of resistant cultivars, whether by conventional or transgenic means. New techniques to identify plant resistance to insects using all available technologies will be a main element of future crop insect pest management systems.

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Seeding Uniformity of Precision Seeders

Regina P. Bracy and Richard L. Parish[1]

ADDITIONAL INDEX WORDS. seeding, seed metering, planters, cabbage, carrot, cucumber, onion, spinach

SUMMARY. Stanhay, Carraro, and Gaspardo precision vegetable seeders were evaluated for seeding uniformity with seeds of five vegetable crops—cabbage (*Brassica oleracea* L. Capitata group), carrot (*Daucus carota* L.), cucumber (*Cucumis sativus* L.), onion (*Allium cepa* L. Cepa group), and spinach (*Spinacia oleracea* L.). Five measurements [mean, percentage of misses, percentage of multiples, quality of feed, and precision (defined as the coefficient of variation after misses and multiples were discarded)] were used to evaluate seeder uniformity. Using all five measurements provided a more complete determination of the metering uniformity of the seeders than was possible in prior work when only mean and coefficient of variation were used. The belt seeder (Stanhay) was effective at singulating spherical seeds (cabbage) and nearly spherical seeds (onion)as the most precise vacuum seeder (Carraro). Seeding uniformity of all seeders with elongated (carrot and cucumber) or angular (spinach) seeds was inadequate for precision seeding.

Seed spacing uniformity is very important when direct-seeding vegetable crops. Plant spacing can affect growth and yield (Thornley, 1983; Willey and Heath,

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