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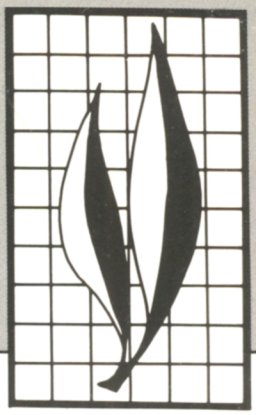

Evaluation of Genetically Improved Strains of *Metaseiulus occidentalis* (Nesbitt) (Acarina: Phytoseiidae) for Integrated Control of Spider Mites on Roses in Greenhouses

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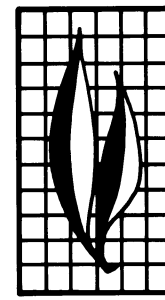

ABSTRACT

Biological control of spider mites is difficult in California greenhouses. Reliable supplies of high quality predators are lacking, available natural enemies are susceptible to pesticides, and predators are thought to be unable to regulate spider mites in sufficiently low densities to prevent economic loss, especially on ornamental plants. This paper evaluates the performance of genetically improved strains of a phytoseiid, *Metaseiulus occidentalis* (Nesbitt), that possess traits that could provide effective, permanent control of *Tetranychus urticae* Koch on roses.

Laboratory tests determined the relative toxicity of 11 rose house pesticides to a non-diapausing, carbaryl- and organophosphorus-resistant strain of *M. occidentalis* and *T. urticae*. At normal label rates, acephate was the only one rated highly toxic to this predator strain. Carbaryl, pirimicarb, endosulfan, hexakis, dienochlor, benomyl, triforine, parinol, piperalin, and oxycarboxin were rated as having low toxicity to *M. occidentalis*. Hexakis and benomyl were toxic to *T. urticae*, endosulfan was moderately toxic, and all others exhibited low toxicity.

The non-diapausing strain of *M. occidentalis* remained reproductive all year under simulated rose house conditions, whereas the diapausing strain did not. Biological attributes of the non-diapausing strain were sufficiently good to conclude this strain could be effective all year.

Metaseiulus occidentalis was evaluated for its ability to regulate *T. urticae* on small noncommercial rose house plots during 2 years. Carbaryl, pirimicarb, triforine, oxycarboxin, hexakis, and dienochlor were successfully used for pest and disease control without disrupting the predators, confirming the laboratory data. *Metaseiulus occidentalis* is unlikely to provide complete biological control of *T. urticae* on greenhouse roses, however. Acaricidal control may be needed (using reduced rates) while *M. occidentalis* establishes, and following annual pruning. Large-scale commercial trials should be considered with the non-diapausing pesticide-resistant strain.

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Evaluation of Genetically Improved Strains of *Metaseiulus occidentalis* (Nesbitt) (Acarina: Phytoseiidae) for Integrated Control of Spider Mites on Roses in Greenhouses[1]

INTRODUCTION

THERE ARE THREE BASIC biological control tactics: Classical or inoculative biological control, conservation, and augmentation (DeBach 1964). Virtually all biological control practiced in greenhouses is augmentative. This is because greenhouse production usually involves intensive short-term cropping with multiple pest/disease problems requiring regular use of pesticides. The short-term nature of greenhouse crops does not favor the permanent establishment of natural enemies. Regular pesticide applications in many crop systems greatly restrict the permanent retention of natural enemies, and small greenhouses may form ecological islands so that dispersal within and from outside the greenhouse of both pests and natural enemies is unable to contribute to population stability. Such dispersal is normally essential if natural enemies are to work effectively as classical biological control agents.

There have been several outstanding successes using augmentation in glasshouses, particularly with *Encarsia formosa* Gahan, a parasite of greenhouse whitefly (*Trialeurodes vaporariorum* (Westwood)), and with *Phytoseiulus persimilis* Athias-Henriot, a predator of the two spotted spider mite (*Tetranychus urticae* Koch) (Hussey and Bravenboer 1971; Woets and van Lenteren 1981). Augmentation has been most successful on short-term vegetable crops such as tomatoes and cucumbers in Europe where there are concentrated and intensive greenhouse industries. During the 1960s pesticide resistance in populations of *T. urticae* aided the acceptance of biological control by *P. persimilis* (French et al. 1976). In general, resistance to acaricides in spider mites has not become a problem outside Europe, and growers have not found it necessary to implement biological control.

Tauber and Helgesen (1978) listed two major reasons why implementation of biological control has been delayed in greenhouse crops in the U.S. The first reason encompassed research and development of requisite biological information that forms the basis for implementing specific biological control systems. The second included industry-related factors that influenced the acceptance of biological control in crop production practices. Many of the industry-related factors, such as a reliable supply of predators and parasites, could be overcome if classical biological control were shown to be successful.

The characteristics of the plant/pest/natural enemy system favoring classical biological control can be split into two components: (1) those characteristics associated with the plant and growth conditions of the crop; and (2) those related to the pest/natural enemy interaction and reaction to the physical environment.

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1. It would be desirable for the greenhouse crop to be semipermanent (several years) to avoid the problem of asynchrony between the natural enemy and the habitat. That is, long-term crops provide a continuous suitable environment for habitation by the natural enemy. Short-term crops could be suitable in circumstances where strip harvesting is practiced and where the natural enemies are sufficiently mobile so that synchrony with the habitat is always maintained somewhere in the greenhouse. Second, the crop type and variety may influence both the growth rate potential of the pest and the natural enemy (van Lenteren et al. 1980). Third, aesthetic injury to the crop may be important. For example, high densities of mites can be tolerated on cucumbers without reducing yield. However, low densities on ornamental plants, although not reducing yield, may downgrade the value of the crop. Fourth, most crops have a limited environmental range under which they ideally grow. Temperature, humidity, and day length can be fairly precisely controlled in modern glasshouses. The natural enemies that are introduced must be able to function effectively under the physical conditions utilized for crop production.

2. The characteristics of the pest/natural enemy system must be such that their interaction and reaction to the environment (both the physical environment and pesticides) result in stable pest regulation, or at least regulation that can maintain the pest below an economic or aesthetic injury level, with minimal assistance from pesticides.

First, for the natural enemy to survive and reproduce, the use of pesticides should be avoided, or if used, the natural enemy must be tolerant of, or resistant to them. Ideally, the natural enemy survives better than the pest. Second, the natural enemy must be well adapted to the physical conditions under which the crop is grown. Third, the searching capacity/prey requirements of the natural enemy must be such that the natural enemy and pest can coexist indefinitely at low levels in the greenhouse; that is, overexploitation of the pest by the natural enemy should not occur regularly. Patchiness may prevent such overexploitation. If patchiness does not exist because of rapid dispersal of both the pest and natural enemy into unoccupied resource areas, instability of pests by natural enemies may occur (Huffaker 1958; Takafuji 1977). Fourth, the life cycles of the pest and natural enemy must be synchronized. That is, any influence that greenhouse conditions have on the incidence of diapause or availability of suitable host/prey stages in the pest, must also influence the natural enemy so as to maintain seasonal synchrony.

Roses, carnations, and gardenias are grown in greenhouses for several years and are continuously cropped and thus are good candidates for classical biological control programs. These crops, however, have lower economic thresholds compared with vegetable crops, because of the importance of aesthetic injury. Of the seven major groups of greenhouse pests (aphids, whiteflies, spider mites, leafrollers, leafminers, mealybugs, and thrips), spider mites may be the best candidates for classical biological control. First, there are several natural enemies that are effective biological control agents in outdoor agricultural systems (McMurtry et al. 1970), including a rose garden in Canberra, Australia (Carmody et al. 1981). Second, a number of spider mite natural enemies, especially in the Phytoseiidae, have developed resistance to pesticides (Croft 1976; Hoy 1985) or have been artificially selected for resistance to several broad spectrum pesticides (Roush and Hoy 1981a, 1981b; Hoy and Knop 1981; Hoy 1985). This is particularly important in crops where the control of other pests and diseases is primarily chemical. Third, spider mites are, with perhaps the exception of mealybugs, the least mobile of the major pest groups. Their usual method of aerial dispersal is probably curtailed in a greenhouse because of reduced air movement which could result in patchiness in spider mite distribution.

A number of predators of tetranychids have been investigated as biological control agents in greenhouses (Hamlen 1980; Sabelis 1981; McMurtry 1982). *Phytoseiulus persimilis,* a phytoseiid, has been the most commonly and successfully used predator. It was first investigated by Dosse (1958) and subsequently by others in many countries (Begljarov 1967; Berendt 1980; Böhm 1966; Boys and Burbutis 1972; Bravenboer 1959; Bravenboer and Dosse 1962; Chant 1961; Gould 1970, 1971, 1980; Gould and Light 1971; Harris 1971; Hussey 1965; Hussey et al. 1965; Langenscheidt 1966; Legowski 1966; Lindquist et al. 1979; Markkula 1974; Markkula and Tiittanen 1976, 1980; Mori 1975; Simmonds 1972; Stenseth 1974; Svenson 1974; Tonks and Everson 1977; Woets 1974). Other phytoseiids such as *Typhlodromus athiasae* Porath and Swirski (Hessein 1978), *T. pyri* Scheuten (Herbert 1962), *Amblyseius fallacis* (Garman) (Burnett 1971), *T. longipilus* Nesbitt (=*Metaseiulus occidentalis*) (Bravenboer 1959; Rabbinge and Hoy 1980) and *Phytoseiulus macropilis* (Banks) (Hamlen 1978; Hamlen and Lindquist 1981; Lindquist et al. 1979) have also been studied in greenhouses but as yet are not used commercially.

Very few of the insect predators of spider mites (McMurtry et al. 1970) have been evaluated in greenhouses. Bravenboer (1959) compared the coccinellid *Stethorus punctillum* Weise with the phytoseiids *T. longipilus* (=*M. occidentalis*) and *P. riegeli* (=*P. persimilis*) and concluded that *S. punctillum* was only effective if *T. urticae* populations were high and at these densities there would be economic losses. Coville and Allen (1977) concluded from life table studies that the predatory thrips *Scolothrips sexmaculatus* (Pergande) could have potential in greenhouses. Other insect predators such as *Oligota* sp. (Staphylinidae), *Orius* sp. and *Geocoris* sp. (Hemiptera), and *Mycodiplosis* sp. (Cecidomymidae) have not been considered for use in greenhouses although they are at times numerous outdoors.

The monilaceous fungus *Hirsutella thompsonii* Fisher has been introduced into Israel for investigation as a possible biological control of the spider mite *T. cinnabarinus* (Boisduval) in greenhouses (Gerson et al. 1979), but could only be useful if humidities could be raised to saturation and temperatures kept below 37°C (Gerson et al. 1979). Gardner et al. (1982) in Georgia, U.S., have also found that it is difficult to infect *T. urticae* with *H. thompsonii* under normal greenhouse conditions.

Under specific circumstances, many predators of tetranychids have the potential to be effective biological control agents in greenhouses. Whether any particular species is effective will depend on the favorability of a number of factors.

Table 1 summarizes some of the characteristics of four predators of *T. urticae* that could be considered as classical biological control agents on long-term crops in greenhouses. Each predator serves as a representative of groups of predators having similar characteristics. *Phytoseiulus persimilis* is a large, active phytoseiid and thus represents species such as *P. macropilis; M. occidentalis* represents smaller, less active species. The coccinelid *S. punctillum,* the only *Stethorus* sp. investigated in greenhouses, possesses characteristics common to many other *Stethorus* sp. (long-lived, high fecundity, and highly mobile). *Scolothrips sexmaculatus,* although not used in greenhouses, is an important predator of *T. urticae* on strawberries in southern California (Oatman and Voth 1972) and may have certain advantages over *P. persimilis* if it were to be used in greenhouses (Coville and Allen 1977). However, biological control agents cannot be judged as groups. There are approximately one thousand described species of phytoseiids with a range of feeding habits (D. Chant, pers. commun.), and there are about forty species of *Stethorus.* Thus, no single species can adequately represent all other related species for greenhouse situations.

TABLE 1. ENVIRONMENTAL AND BIOLOGICAL FACTORS EXPECTED TO INFLUENCE PERMANENT ECONOMIC REGULATION* OF *TETRANYCHUS URTICAE* AND PERMANENT ESTABLISHMENT OF PREDATORS OF *T. URTICAE* IN GREENHOUSES

Factor	*Phytoseiulus persimilis*†	*Metaseiulus occidentalis*‡	*Stethorus punctillum*§	*Scolothrips sexmaculatus*¶
Temperature tolerance	—	A	A	A
Humidity tolerance	A	?(A)	?(A)	?(A)
Synchrony (diapause)	A	?(A)	?(A)	?(A)
Pesticide resistance	—	?(A)	?(—)	?(—)
Speed of control	++	+	+++	++
Searching capacity	++	+	+++	++
Prey requirements	++	+	+++	++
Dispersal	?(+)	?(+)	+++	++

*—not adequate; A adequate; + low; ++ moderate; +++ high; ? unknown; ( ) probably.

References used to make relative assessments of characteristics were:

†Mori and Chant 1966a, 1966b; Force 1967; Laing and Huffaker 1969; Pruszynski 1976; Schulten et al. 1976; Hussey and Scopes 1977; Stenseth 1979; van Zon and Wysoki 1978.

‡Laing and Huffaker 1969; Tanigoshi et al. 1975; Roush and Hoy 1981a; Hoy and Knop 1979.

§McMurtry et al. 1970; Huffaker et al. 1970.

¶Coville and Allen 1977; Gilstrap and Oatman 1976.

Table 1 compares factors, including pesticide resistance, that influence the ability of the four predators to serve as permanent biological control agents of *T. urticae* in greenhouses. *Phytoseiulus persimilis* has acquired resistance to some organophosphorus pesticides (Schulten et al. 1976), has no diapause (Hussey and Scopes 1977), and, therefore, is seasonally synchronized with its prey. It has been shown to tolerate a wide range of humidities (Mori and Chant 1966a, 1966b). Temperatures above 30°C were found to be detrimental to *P. persimilis* (Pruszyński 1976; Hussey and Scopes 1977), and predatory efficiency declined above 20°C (Force 1967). Vorishilov (1979) selected a strain of *P. persimilis* that tolerated high temperatures (39°C), thus increasing the probability of spider mite control under hot conditions, although this strain has not yet been tested in greenhouses.

Metaseiulus occidentalis is resistant to a range of pesticides (Croft 1976). It has an adult female diapause (Croft 1971; Hoy and Flaherty 1970; Lee and Davis 1968), but under greenhouse conditions this may not be induced, because a constant temperature of 22°C prevents diapause irrespective of photoperiod (Hoy 1975a). The effect of fluctuating temperatures has not been determined. *Metaseiulus occidentalis* also has been shown to tolerate a wide range of temperatures (12°-33°C) (Tanigoshi et al. 1975; Sabelis 1981) and to tolerate at least a moderate range of humidities (40%-70%) (Laing and Huffaker 1969; Sabelis 1981). The effect of very high humidities appears to be poor colony growth under laboratory conditions (Hoy, unpublished data).

The other two species are poorly adapted to at least one of the four environmental factors with both *Stethorus* sp. (Coccinellidae) (McMurtry et al. 1970) and probably *S. sexmaculatus* (Pergande) (Thripidae) susceptible to many pesticides. Their ability to tolerate a wide range of temperatures and relative humidities has not been documented. Diapause attributes of

these two species have not been adequately described, although some information for two species of *Stethorus* (Tanigoshi and McMurtry 1977; Richardson 1977) and *S. sexmaculatus* (Gilstrap and Oatman 1976; Coville and Allen 1977) has been published.

Table 1 also compares factors relating to prey regulation for the four predators. First, the rate of control of the prey is unimportant if the economic threshold is not exceeded. However, if disruptions to a balanced predator/prey interaction occur (via cultural practices or pesticide applications), then a rapid rate of control would be advantageous in preventing excessive injury. A pesticide-resistant predator is unlikely to experience disruptions as often as susceptible predators. Second, the greater food requirements of larger predator species often is not offset by a higher searching capacity, resulting in prey regulation at a higher level than for smaller species (Huffaker et al. 1970; Sabelis 1981). The same reasoning probably accounted for the ability of *M. occidentalis* to regulate populations of *T. urticae* on strawberry plants at lower levels than *P. persimilis* (Laing and Huffaker 1969). Third, if dispersal of a predator occurs readily, then the predator will tend to be in synchrony with its prey over a larger area than if mobility is low (Huffaker et al. 1970). A system with a highly mobile predator should be more prone to overexploitation than one involving a less mobile predator that permits patchiness. Systems utilizing the highly mobile insect predators could, therefore, be more prone to overexploitation than systems using the less mobile phytoseiids. Likewise, *P. persimilis* is a more active phytoseiid than *M. occidentalis* and has a higher prey consumption and intrinsic rate of increase, which may explain the greater tendency for overexploitation of prey by *P. persimilis* (Laing and Huffaker 1969). In summary, phytoseiid predators appear to be better candidates for classical biological control of *T. urticae* than the two insect predators. As pesticide resistance is of paramount importance, *M. occidentalis* should be a better choice than *P. persimilis.* In addition, *M. occidentalis* is less likely to overexploit its prey than *P. persimilis.* The same reasoning suggests that *P. persimilis* would be a better choice than *M. occidentalis* as a predator of *T. urticae* in augmentation programs involving regular releases of predators into short-term crops that are seldom treated with pesticides.

Hoy (1976 and 1979) discussed genetic improvement programs for biological control agents but did not specifically discuss greenhouse programs. Genetic selection of biological control agents for use in greenhouses is particularly logical because the natural enemy is being used under artificial conditions and there would be little interspecific competition between the released, improved natural enemies and unselected, wild natural enemies. Predators have already been genetically improved for use in greenhouses. Selections for a limited range of organophosphorus pesticide resistance (Schulten et al. 1976) and tolerance to higher temperatures (Voroshilov 1979) in *P. persimilis* were designed to improve this predator's performance in greenhouses. Resistance to carbaryl (Roush and Hoy 1981a, 1981b) and permethrin (Hoy and Knop 1981) has been selected for in *M. occidentalis* for use in deciduous orchards and vineyards. In addition, a non-diapausing pesticide-resistant strain of *M. occidentalis* has been selected for use in greenhouses (Hoy 1984).

To evaluate the potential efficiency of *M. occidentalis* as a natural enemy in greenhouses, a number of areas of uncertainty need to be resolved. Resolution of these uncertainties forms the basis of this paper, the experiments being designed to evaluate the potential of *M. occidentalis* as a predator of *T. urticae* in California rose houses. A laboratory evaluation of an array of rose-house pesticides against both *T. urticae* and a non-diapausing strain of *M. occidentalis* was conducted. The ability of *M. occidentalis* to disperse from bean plants

in response to low prey densities and its ability to tolerate high laboratory humidities also were evaluated. Different strains of *M. occidentalis* were used in small experimental, non-commercial greenhouse plots of roses to help evaluate the ability of this predator to regulate *T. urticae* populations. Three different colonies of *M. occidentalis* are referred to in this paper: An organophosphorus-resistant (OP) and carbaryl-susceptible strain with a normal diapause, which is referred to as the OP-resistant strain; an organophosphorus- and carbaryl-resistant strain with a normal diapause, which is referred to as the carbaryl-OP strain; and a non-diapausing and organophosphorus- and carbaryl-resistant strain derived from the normal strain, which is referred to as the carbaryl-OP-ND strain.

MATERIALS AND METHODS

Pesticide Screening

Colony sources and maintenance

The carbaryl-OP-ND strain (Hoy 1984) was maintained at 22° to 26°C under an 18 h photophase on waxed paper discs surrounded by moist cotton and fed all stages of *T. urticae,* which had been brushed from bean leaves. The colony of *T. urticae* was continuously maintained on bean plants (*Phaseolus vulgaris* (L.)) in a greenhouse at UC Berkeley and had been exposed to a variety of chemicals over the years.

Pesticides and test methods

Eleven formulated pesticides (table 2) were tested against both the carbaryl-OP-ND strain of *M. occidentalis* and *T. urticae,* each at three rates: half label, label, and five times label rates. Each pesticide was made up in distilled water containing 0.1 percent surfactant (Triton AG-98) no more than 2 h prior to use.

Experiments were conducted with 1.5 cm diameter rose leaflet discs (cv. Royalty) cut from young leaves and placed on moist cotton. Sets of 15 discs were sprayed for 1 to 2 sec. with a fine mist of the test pesticide using a pressurized atomizer (Crown-Sprā Tool) held ca. 35 cm from the discs. The treated discs were air dried for 1 to 3 h before mites were added. A mixture of all stages of *T. urticae* brushed from bean leaves was added to ten discs and five gravid *M. occidentalis* females were then placed on each. To each of the other five discs, five gravid *T. urticae* females were added. All discs were held at 27°C under an 18 h photophase for 48 h, when mortality and egg production were assessed. Mites that ran off the discs and drowned were considered to be dead. Drowning of *M. occidentalis* females was kept to a minimum (<4%) by assuring that their food was not placed on the edge of the disc. After 48 h all females were removed and the leaf discs were returned to the incubator for another 5 days, at which time the eggs of both species had hatched and the immatures had had time to develop to the protonymph stage. Counts were then made of egg and larval mortality. Ample food, especially eggs of *T. urticae,* was made available to *M. occidentalis* for the entire experiment.

Each day that a test was run, controls consisting of 10 discs containing a total of 50 gravid female *M. occidentalis* and 5 discs containing 25 gravid female *T. urticae* were sprayed with distilled water and surfactant. Most tests were replicated on 3 separate days.

TABLE 2. LABEL RATES AND USES FOR 11 COMMONLY USED ROSE HOUSE PESTICIDES

Trade name & formulation	Common name	Label rate (g AI/liter)	Species controlled*
Orthene 75% SP	Acephate	0.90	Thrips *(Frankliniella occidentalis)*, leaf-rollers *(Platynota stultana)*, aphids (several species)
Sevin 80% WP	Carbaryl	2.40	*P. stultana*
Pirimor 50% WP	Pirimicarb	0.30	Aphids
Thiodan 33% EC	Endosulfan	0.56	Aphids, *F. occidentalis*
Vendex 50% WP	Hexakis	0.65	Spider mites *(Tetranychus urticae)*
Pentac 50% WP	Dienochlor	0.33	*T. urticae*
Benlate 50% WP	Benomyl	0.30	Powdery mildew *(Sphaerotheca pannosa)*, *Botrytis* sp.
Triforine 18.2% EC	Triforine	0.18	*S. pannosa*
Parnon 4% EC	Parinol	0.025	*S. pannosa*
Pipron 82.4% EC	Piperalin	0.31	*S. pannosa*
Plantvax 75% WP	Oxycarboxin	1.29	Rust *(Phragmidium disciflorum)*

*Jefferson et al. 1971; Allen et al. 1980; McCain 1979.

A mean of 145 (range 100-150) female *M. occidentalis* were tested at each rate for each pesticide. The number of *M. occidentalis* eggs used to determine egg mortality averaged 557 (range 200-1,025), except for the acephate experiment, where only 98, 69, and 24 eggs were deposited at the ½, 1, and 5 times label rate, respectively. The number of *M. occidentalis* larvae hatching averaged 492 (range 129-952), except for the acephate experiment, which had 81, 66, and 21 larvae hatching at the three rates. A mean of 70 (range 50-125) female *T. urticae* were tested at each rate for each pesticide. The average number of *T. urticae* eggs used to determine egg mortality was 707 (range 211-1,814), except for the endosulfan experiment at the highest rate, where only 19 eggs were deposited. The number of *T. urticae* larvae used to estimate larval survival averaged 612 (range 130-1,654), except for the endosulfan residues at the highest rate where only 16 larvae hatched, and the two highest rates of benomyl, where 56 and 0 larvae hatched, respectively.

Adult female, egg, and larval mortalities were corrected for control mortality using Abbott's (1925) correction applied to each replicate. An average mortality for each stage was calculated over all replicates. Egg production was determined as a percentage of the control production and averaged over all replicates. To make an overall assessment of the toxicity of each pesticide to the two mite species, a toxicity index (TI) was devised which was calculated by adding three times the number of females surviving 48 h to the number

of progeny reaching protonymph stage, and dividing this sum by the equivalent sum obtained for the control test. A pesticide with a TI value of 0.5 or greater was considered to be of low toxicity; pesticides with TI values of 0.25 to 0.49 were judged to be moderately toxic; and pesticides with a TI of less than 0.25 were judged to be highly toxic. Survival of females was given greater weight in the index because of their importance in an immediate numerical response and because high prey mortality is caused by *M. occidentalis* females (Sabelis 1981). *Tetranychus urticae* females also are heavily weighted in the index because they cause most of the leaf damage.

To determine that OP and carbaryl resistance had been retained in the non-diapausing strain of *M. occidentalis,* experiments were conducted comparing this strain with the OP-carbaryl (normal) resistant strain developed by Roush and Hoy (1981a, 1981b) and a strain susceptible to both OP and carbaryl insecticides collected from blackberries in Berkeley (Hoy and Knop 1979). Carbaryl was applied at 2.4 g AI/liter (the label rate) and diazinon at 12.0 g AI/liter (10 times the label rate). However, female mortality was assessed only at 48 h and only one experiment was conducted using 50 females of each strain. Water controls were tested for each strain at the same time.

Life History Attributes

Fecundity and longevity

Fecundity and longevity of both the carbaryl-OP and the carbaryl-OP-ND strains were studied to determine if selection for "non-diapause" had altered these important attributes. All experiments were done under temperatures that followed a daily sine curve between 17.5°C and 24.5°C, averaging 21°C. The first experiment was conducted in January 1980 under a 10 h photophase. Both colonies were then maintained for 15 months under non-diapause inducing conditions prior to repeating the experiment under a 16 h photophase. Each experiment was conducted with 22 to 25 predators; each female was mated upon maturing and placed on an individual 1.5 cm diameter spider mite-infested bean leaf disc resting on moist cotton. Females were examined daily and eggs removed. Eggs were reared to adulthood and the sex ratio determined under the 16 h photophase.

Humidity tolerance

Egg hatch and the developmental success rate of larvae of the carbaryl-OP-ND strain were determined at low, medium, and high humidities, each at 20° and 30°C. Saturated salt solutions were used to produce desired humidities, which were confirmed using a lithium chloride probe on a dew point hygrometer (YSI model 91). Calcium chloride gave 32 percent and 24 percent relative humidity (R.H.); sodium nitrite gave 74 percent and 70 percent R.H.; and potassium phosphate gave 97 percent and 93 percent R.H. at 20° and 30°C, respectively. The salt solutions were prepared as described by Winston and Bates (1960) and placed in 23 cm by 15 cm plastic dishes, which were placed on the bottom of 30.75 liter stainless steel and glass desiccator cabinets. The three desiccators, each containing a different salt solution, were placed in a Percival environment chamber set at either 20° or 30°C. Humidities equilibrated within 6 h at both 20° and 30°C. Individual eggs (less than 6 h old) and larvae of *M. occidentalis* were placed on the top of 2.5 cm

diameter black plastic vial caps that were held on a tray 8 cm above the salt solution. The edges of the caps were ringed with Stickem-Special thinned with xylene to confine the predators to the caps. Twenty-five eggs and 25 larvae were tested per replicate with three replicates for each humidity. A mixture of all stages of *T. urticae* was added to each cap prior to adding the predator larvae. The number of eggs hatching and larvae maturing was determined after 5 days at 20° C and 3 days at 30° C. Desiccator cabinets were not opened during the course of the experiment.

Greenhouse Trials

Experimental design

The experiments were conducted in a greenhouse cubicle (6.2 m × 4.5 m) at the University of California, Berkeley, between June 1979 and May 1981. Seventy-six rose plants, cv. Royalty, were used, each growing in a 19-liter can in UC planting mix (50% Colma sand and 50% peat moss to which is added, per cubic meter, the following fertilizer: 4.1 kg oyster shell, 2.4 kg dolomite lime, 1.5 kg single superphosphate, and 0.6 kg potassium nitrate). The bushes were randomly assigned to three treatments: (1) 34 to a normal commercial treatment of regular pesticide applications, including acaricides when necessary; (2) 34 to a "predator" treatment for biological control of *T. urticae* by *M. occidentalis* (this treatment did not exclude the use of acaricides, as the aim was to achieve a reduction in the use of acaricides, but not necessarily to eliminate their use); (3) 8 to a control treatment which received applications of acaricides only when the plants were defoliating due to high mite densities. This control indicated that high spider mite populations could and would develop in this greenhouse. The treatments were arranged in blocks in the greenhouse to help account for patchiness in mite populations and temperature gradients (fig. 1). Blocks 1 and 3 each contained a bank of 12 (2 × 6) bushes of treatments (1) and (2) and the middle block contained a bank of 10 (2 × 5) bushes of treatments (1) and (2) separated by the 8 (2 × 4) control bushes. The control bushes were, therefore, situated in the middle of the cubicle and were surrounded by all three blocks. Each treatment was separated from neighboring treatments in the same and other blocks by at least one meter. No attempt was made to prevent the movement of *T. urticae* or *M. occidentalis* between blocks.

Predator releases

At the start of the experiment a moderately resistant colony of *M. occidentalis* was found on the control plants, both treatments in block 3, and the commercial treatment in block 1. *Tetranychus urticae* was found in all seven plots.

The carbaryl-OP resistant colony with a normal diapause developed by Roush and Hoy (1981a, 1981b) was released on June 21 and July 6, 1979. On both occasions five adult female predators were placed on each bush near areas showing spider mite damage. When this strain entered diapause during the first winter the carbaryl-OP-ND strain (5 females/bush) was released onto the same bushes on December 29, 1979 and on January 13, 1980.

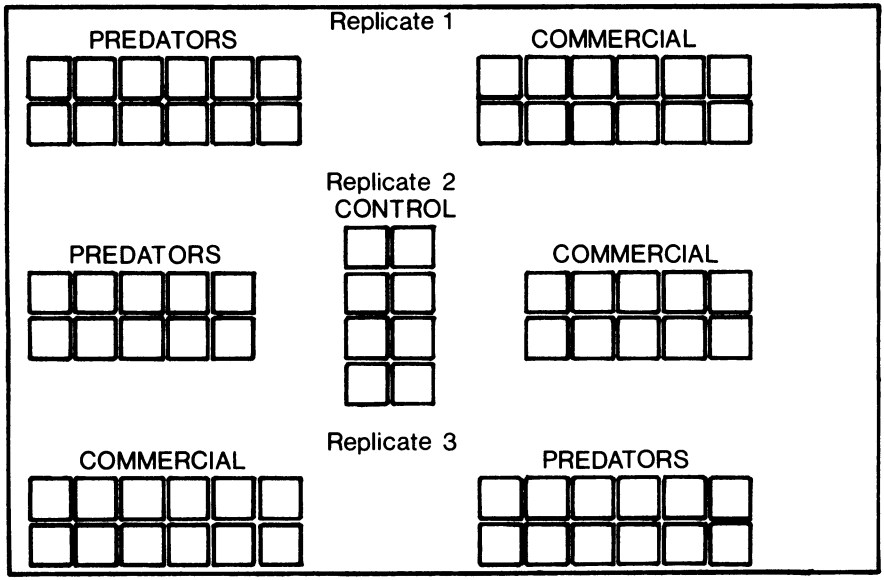

Fig. 1. Plan of the cubicle (6.2 m × 4.5 m) in a UC Berkeley greenhouse used. Each square represents one rosebush (cv. Royalty). Predator treatments had releases of *M. occidentalis* plus applications of acaricides as necessary. Commercial treatments had regular applications of acaricides and the control treatment received acaricide applications only when the bushes approached defoliation.

Pesticide treatments and fertilization

Six different pesticides were applied to the bushes during the 2 years. The pesticides used, the rate at which they were applied, and their frequency and reason for use were: Triforine (triforine), 0.18 g AI/liter, applied every 7 to 14 days for control of powdery mildew (*Sphaerotheca pannosa* var. *rosae* (Wallr.) Lev.); Vendex (hexakis), 0.65 g AI/liter, applied as required for control of *T. urticae* (see fig. 4, page 19); Pentac (dienochlor), 0.33 g AI/liter, applied October 19, October 31, 1979, and July 22, 1980, for control of *T. urticae* (on commercial treatments only); Sevin (carbaryl), 2.4 g AI/liter, applied August 8, 1979, March 6, and August 30, 1980, for control of omnivorous leafroller (*Platynota stultana* Walsingham); Plantvax (oxycarboxin), 1.29 g AI/liter, applied January 4 and January 17, 1980, for control of rust (*Phragmidium disciflorum* (Tode) James); Pirimor (pirimicarb), 0.30 g AI/liter applied February 16, 1980, for control of aphids (*Macrosiphum* sp.). A wetter (Triton AG 98) was added at a rate of 0.2 mL/liter to all sprays. The pesticides were applied using a hand compression Hudson sprayer. Approximately 8 liters of pesticide were used each time all 76 bushes were sprayed. When treatments were selectively sprayed with acaricides, a large sheet of plastic was used to protect surrounding treatments from spray drift.

Approximately every 2 weeks about 5 g of fertilizer (26% N, 3% P as P_2O_5, and 3% K as K_2O) were added to each rose plant container. Each container received about 350 mL of water every day through an automatic drip line.

Mite population estimates

Leaflets were sampled from two regions on each bush every 2 to 4 weeks: Two terminal leaflets were sampled from leaves at the hook (bud from which the next flower is produced) and two terminal leaflets from the third five-leaflet leaf above the hook (usually the bottom leaf of the harvested bloom). The leaflets were examined under a stereomicroscope and all stages of both *T. urticae* and *M. occidentalis* recorded.

All the bushes were pruned on June 12, 1980, and leaflet sampling recommenced on July 9, 1980.

Yield and damage measurements

Bushes were allowed to produce flowers continuously. No attempt was made to synchronize bushes so as to provide discrete cropping periods. Flowers were cut three times per week. In general, cuts were made just above the second five-leaflet leaf above the hook. Cuts were occasionally made above the first five-leaflet leaf below the hook during spring when the bushes were tall and growing vigorously. The following measurements were made on each flower or bush.

1. Stem length from below the base of the bud.
2. Number of leaflets on the stem.
3. Number of flowers per bush.
4. Percentage of leaflets showing injury by *T. urticae.*

These values were averaged for each week to produce four summary numbers for each treatment in each replicate for 45 consecutive weeks from August 6, 1979, and 43 consecutive weeks from July 14, 1980.

Monitoring for diapause in *M. occidentalis*

On November 21, 1979, five strips of black cloth were wrapped around the canes of replicate 3 of the biological control treatment, the bands being about 15 cm above the soil surface. The cloth provided an artificial shelter for diapausing *T. urticae* and *M. occidentalis.* This technique had been used in orchards to detect overwintering mite species, including *M. occidentalis* (Field 1978, 1979). During November and December 1979 when *M. occidentalis* was found in the bands, they were removed and placed on spider mite-infested bean leaf discs resting on moist cotton. The discs were held in the greenhouse, observed daily, and the time to egg deposition recorded.

These observations prompted more extensive diapause samples of *M. occidentalis* during the winter of 1980-1981, a year after the carbaryl-OP-ND strain had been released, as knowledge of the diapause status of *M. occidentalis* populations was considered critical for evaluating the potential of this predator. On September 17, 1980, six bands were placed on each treatment in each replicate, including the control block. The bands were removed weekly for 22 weeks and the predators transferred to leaf discs in the greenhouse for observations on oviposition. During December and January, corresponding to the sample dates from the bands, 20 carbaryl-OP-ND gravid females that had been reared in the laboratory under 16 h photophase and mean 21°C (daily temperatures fluctuated on a sine curve between 17.5° and 24.5°C) were removed from their colony, starved for 4 days on individual bean leaf discs in the greenhouse, and then transferred to leaf discs containing *T.*

urticae. Their time to initiating oviposition was recorded and served as a comparison for those removed from the bands.

Analyses

Each of the four yield and damage measurements for each year was subjected to separate analysis of variance. Measurements on stem length were made only until the end of November 1980, but all other measurements were made to the end of May 1981.

Persistence of non-diapause and carbaryl resistance

At the end of the greenhouse experiment, 20 *M. occidentalis* females were collected from each bank of plants, and reared through approximately two generations at 26° C and 18 h photophase. Fifty eggs were then taken from each of the seven colonies and used to establish new colonies that were reared at 19° C and 8 h photophase, conditions that would induce a high incidence of diapause in native *M. occidentalis* populations (Hoy 1975a, 1975b). Upon maturity, 20 mated females were removed from each colony and placed on individual 1.5 cm *T. urticae*-infested bean leaf discs and examined daily for egg deposition for 3 weeks. In addition to the seven colonies from the rose bushes, three other laboratory colonies were tested. These were: (1) a line of the carbaryl-OP-ND colony that had been reared continuously at 19° C and 8 h photophase for 2 years, (2) a line from the same carbaryl-OP-ND colony that, for the previous 1.5 years, had been reared under non-diapause-inducing conditions (26° C and 18 h photophase), and (3) a line from the original carbaryl-OP diapausing colony that had been used for the first releases of *M. occidentalis;* this colony had also been maintained under non-diapause-inducing conditions for the previous 2 years.

Levels of carbaryl resistance in the same 10 colonies also were evaluated along with a carbaryl-susceptible colony collected from Berkeley blackberries. For each colony, 50 gravid females were exposed to a dried residue of carbaryl on 1.5 cm rose (cv. Royalty) leaflet discs resting on moist cotton. The discs were sprayed with a 2.4 g AI/liter carbaryl suspension using a hand-held atomizer (Crown Sprā-Tool). A mixture of all stages of *T. urticae* was brushed onto the discs and then five *M. occidentalis* females were placed on each of 10 discs. Fifty females from each colony were placed on leaf discs sprayed with distilled water for controls. Mortality was assessed after 48 h at 27° C.

RESULTS AND DISCUSSION

Pesticide Screening

The carbaryl-OP-ND strain of *M. occidentalis* retained the same levels of OP and carbaryl resistance found in the diapausing strain from which it was selected. Survival rates of adult females of the carbaryl-OP-ND, the carbaryl-OP, and the carbaryl-OP-susceptible strain were 81.8 percent, 83.3 percent, and 0.0 percent, respectively, when treated with diazinon.

Female mortality and egg production are given in figure 2 for *M. occidentalis* and *T. urticae* exposed to 11 pesticides. Acephate was the most toxic chemical tested, causing very high mortality of the carbaryl-OP-ND strain of *M. occidentalis* at all three test rates and few

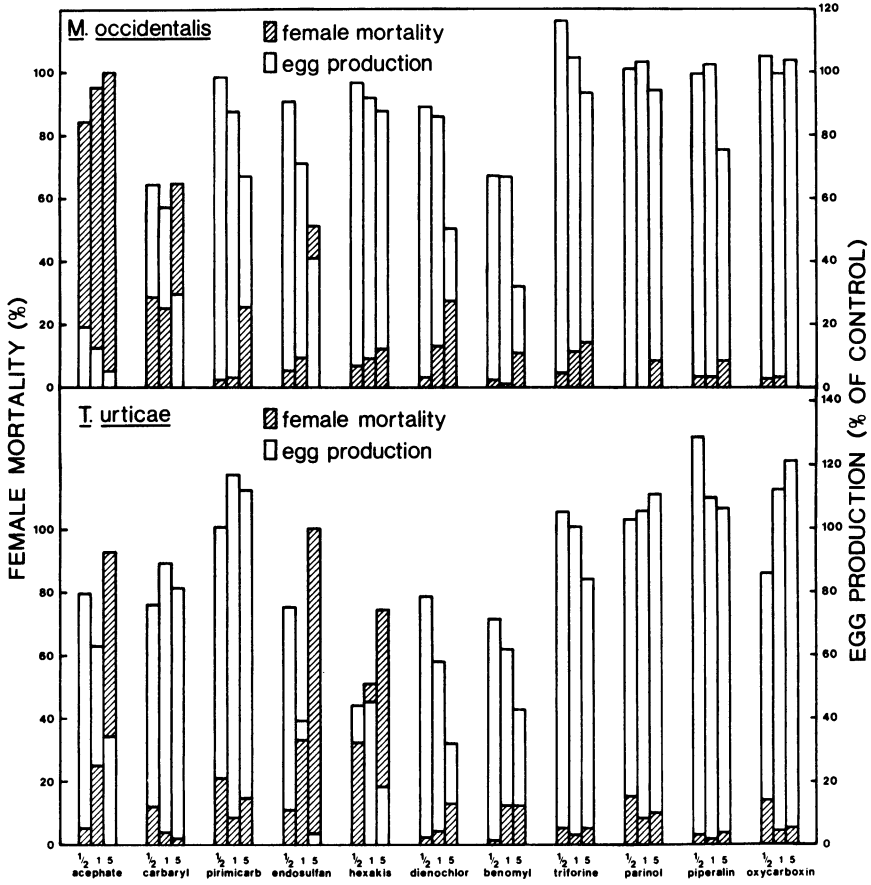

Fig. 2. Mortality of females and egg production on rose leaflet discs after 48 h exposure of the carbaryl-OP-ND strain of *M. occidentalis* and *T. urticae* to 11 commonly used rose house pesticides at rates ½, 1, and 5 times the label rate.

eggs were laid. Carbaryl and endosulfan at five times the label rate caused high mortality of female *M. occidentalis,* and consequently low egg production. Most other pesticides caused only low to moderate mortality of *M. occidentalis* females and low to moderate reductions in egg production. However, benomyl at its highest rate caused low egg production without appreciable mortality of the females. Colonies susceptible to carbaryl are also susceptible to benomyl and produce even fewer eggs at label rates (Hoy and Standow 1981).

For *T. urticae,* the insecticides acephate and endosulfan at five times the label rate caused high mortality as did the acaricide hexakis at label and five times label rates. Egg production was greatly reduced by acephate at five times label rate, by endosulfan at label and five times label rates, by hexakis at all rates, and by dienochlor and benomyl at five times label rate. All other pesticides and rates caused low to moderate mortality of female *T. urticae* and a low to moderate reduction in egg production.

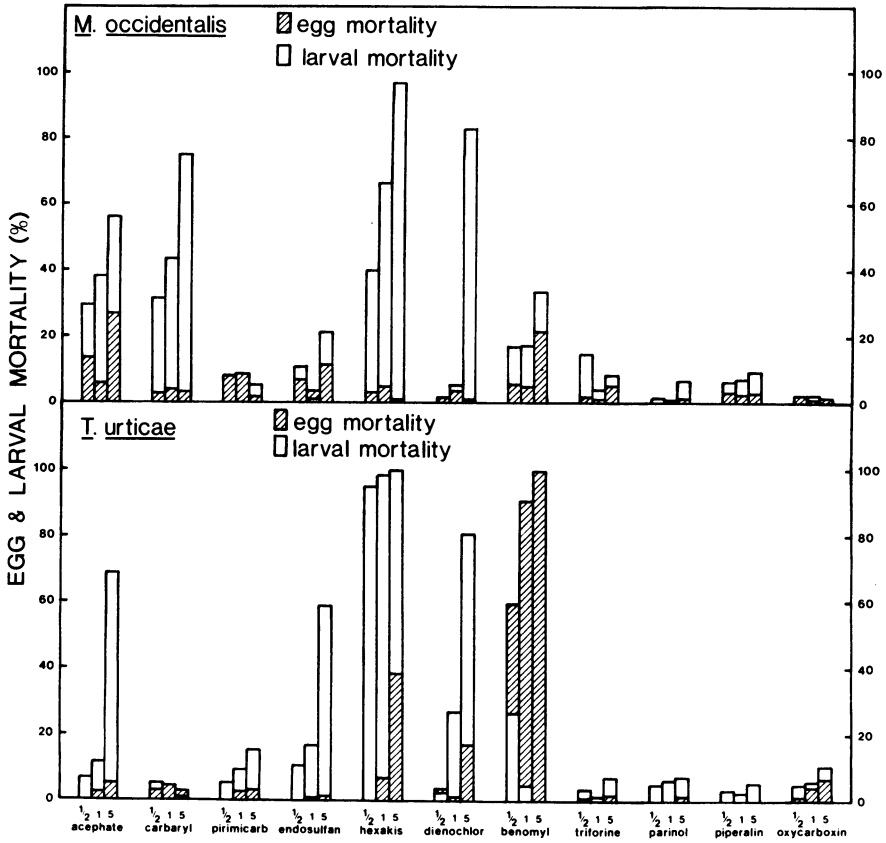

Fig. 3. Egg and larval mortality of the carbaryl-OP-ND strain of *M. occidentalis* and *T. urticae* on rose leaflet discs sprayed with 11 commonly used rose house pesticides at rates ½, 1, and 5 times the label rate.

Egg and larval mortality are presented in figure 3 for the carbaryl-OP-ND strain of *M. occidentalis* and *T. urticae* when exposed to foliage residues of 11 pesticides. The mortality values obtained for *M. occidentalis* when exposed to acephate at the highest rate and for *T. urticae* when exposed to endosulfan at five times the label rate are less reliable than the other values because of the low number of eggs (less than 24 eggs) laid. No estimate of the mortality of *T. urticae* larvae at five times label rates of benomyl could be obtained because no eggs hatched. Mortality of *M. occidentalis* eggs was low for all pesticides. However, mortality of larvae of *M. occidentalis* was moderate to high for all rates of acephate, carbaryl, and hexakis, and high for the highest rate of dienochlor. All five fungicides (benomyl, triforine, parinol, piperalin, and oxycarboxin) had little detrimental effect on eggs and larvae of *M. occidentalis.* Benomyl was slightly more toxic to eggs and larvae than were the other four fungicides. In contrast, strains of *M. occidentalis* susceptible to carbaryl exhibit high egg and larval mortality when exposed to benomyl (Hoy and Standow 1981).

For *T. urticae,* high egg mortality occurred at all rates of benomyl and at five times the label rate of hexakis; all other pesticides and rates caused only low to moderate egg mortality (fig. 3). Larval mortality was high at all rates of hexakis and at five times label rate of acephate, endosulfan, and dienochlor.

The effects of 11 pesticides on the two species are summarized using toxicity indices (TI) which combine adult, egg, and larval mortality (table 3). Acephate at all rates and carbaryl at five times the label rate were highly toxic to this carbaryl-OP-ND strain of *M. occidentalis.* Carbaryl is commonly highly toxic to unselected native populations of *M. occidentalis.* Thus, this carbaryl-OP-ND strain is substantially more resistant to carbaryl than native unselected populations. Hexakis at all rates, benomyl at the two highest rates, and acephate, endosulfan, and dienochlor at their highest rate were considered highly toxic to *T. urticae.* All other pesticides and rates were low to moderately toxic to both species.

The carbaryl-OP-ND strain of *M. occidentalis* showed high resistance or tolerance at label rates to all pesticides tested except acephate. Acephate is an OP insecticide commonly used for the control of many insect pests in rose houses in California (table 2). Acephate probably does not act on *M. occidentalis* in the same way other OPs act as there has been shown to be considerable general OP resistance in this and other strains of *M. occidentalis* (Hoy 1982). The strain originally selected for carbaryl resistance by Roush and Hoy (1981a) exhibits cross-resistance to benomyl, a fungicide known to sterilize females of other strains of *M. occidentalis* (Hoy and Standow 1981) and many phytoseiids (Croft 1976). Of the 11 pesticides tested, acephate (Lindquist et al. 1979), carbaryl (Shinkaji 1976), endosulfan (Smith et al. 1963), and benomyl (Parr and Binns 1971) are known to be toxic to *P. persimilis* and cannot be used successfully in integrated pest management programs involving this species.

The long-term effects of dienochlor on *M. occidentalis* are unknown. This highly effective acaricide acts slowly on *T. urticae,* but forms the basis of mite control in California rose houses. It may likewise have similar long-term effects on *M. occidentalis.* Hexakis, however, has been used successfully as a selective acaricide in conjunction with *P. persimilis* in greenhouses (Lindquist et al. 1979) and *M. occidentalis* in orchards (Jeppson et al. 1975; Downing and Moilliet 1975). This study also shows this acaricide to be highly selective at label-use rates.

Carbaryl, pirimicarb, endosulfan, hexakis, benomyl, triforine, parinol, piperalin, oxycarboxin, and perhaps dienochlor, could be safely used for the control of a variety of pests and diseases of glasshouse roses where the carbaryl-OP resistant or the carbaryl-OP-ND strain of *M. occidentalis* is used as a predator of *T. urticae.* However, care should be taken in extrapolating these laboratory results (table 3) to greenhouse situations. The laboratory toxicity indices frequently may indicate toxicities greater than are experienced in the greenhouse. In laboratory tests higher mortality could occur because the mites are unable to escape to unsprayed refuges or areas with poorer pesticide coverage. However, sometimes the laboratory results may show lower mortalities than will occur in the greenhouse because high prey mortality may result in higher predator mortality through starvation. The effects of some of these chemicals on *M. occidentalis* and *T. urticae* were, therefore, investigated under greenhouse conditions and are reported in the section on greenhouse trials.

TABLE 3. RELATIVE TOXICITY INDICES OF 11 COMMONLY USED ROSE HOUSE PESTICIDES FOR THE CARBARYL-OP-ND STRAIN OF *M. OCCIDENTALIS* AND *T. URTICAE*

Pesticide	Test dose (× label rate)	Toxicity indices (TI)* and rank† for *Metaseiulus occidentalis*	Toxicity indices (TI)* and rank† for *Tetranychus urticae*
Acephate	½	0.13 (•••)	0.81 (•)
	1	0.07 (•••)	0.59 (•)
	5	0.01 (•••)	0.07 (•••)
Carbaryl	½	0.60 (•)	0.75 (•)
	1	0.55 (•)	0.91 (•)
	5	0.24 (•••)	0.85 (•)
Pirimicarb	½	0.96 (•)	0.90 (•)
	1	0.92 (•)	0.99 (•)
	5	0.72 (•)	0.91 (•)
Endosulfan	½	0.87 (•)	0.76 (•)
	1	0.80 (•)	0.45 (••)
	5	0.38 (••)	0.01 (•••)
Hexakis	½	0.76 (•)	0.16 (•••)
	1	0.60 (•)	0.10 (•••)
	5	0.50 (•)	0.05 (•••)
Dienochlor	½	0.97 (•)	0.81 (•)
	1	0.86 (•)	0.58 (•)
	5	0.39 (••)	0.24 (•••)
Benomyl	½	0.77 (•)	0.40 (••)
	1	0.76 (•)	0.23 (•••)
	5	0.53 (•)	0.21 (•••)
Triforine	½	1.01 (•)	1.02 (•)
	1	0.96 (•)	1.02 (•)
	5	0.90 (•)	0.83 (•)
Parinol	½	1.04 (•)	0.99 (•)
	1	1.11 (•)	0.99 (•)
	5	0.91 (•)	0.97 (•)
Piperalin	½	0.95 (•)	1.18 (•)
	1	0.99 (•)	1.11 (•)
	5	0.83 (•)	1.06 (•)
Oxycarboxin	½	1.06 (•)	0.86 (•)
	1	1.00 (•)	1.02 (•)
	5	1.07 (•)	0.98 (•)

$$*TI = \frac{3 \times \text{no. females surviving} + \text{no. progeny developing to protonymph at test dose}}{3 \times \text{no. females surviving} + \text{no. progeny developing to protonymph in control}}$$

†• = low toxicity; •• = moderate toxicity; ••• = high toxicity.

Life History Attributes

Fecundity and longevity

Fecundity and longevity, on bean leaf discs, of the carbaryl-OP-ND and the carbaryl-OP strain from which it was derived, are compared in table 4. At a photophase of 10 h, all of the carbaryl-OP resistant females entered diapause, and of those that eventually oviposited, the mean pre-oviposition period was ca. 73 days. Egg production by these females was very low (only ca. 8 eggs per female). Hoy (1975b) recorded low egg production from diapausing *M. occidentalis* (12.6 eggs per female under an 8 h photophase at 19°C). In addition, only 61 percent of these diapausing females oviposited before they died. The longevity and pre-oviposition period of the carbaryl-OP strain were similar to those reported by Roush and Hoy (1981b) for the same strain. Only 18.2 percent of the carbaryl-OP-ND strain entered diapause under a 10 h photophase, and all females eventually oviposited. For those females in diapause, longevity was similar to the normal carbaryl-OP strain but they commenced egg production significantly earlier and laid more eggs than the normal strain (table 4). The females not in diapause had pre-oviposition periods, egg production, and longevities similar to predators reared under non-diapausing conditions (16 h photophase) (table 4). When the two strains were compared under non-diapause-inducing conditions (16 h photophase and mean 21°C), there were no significant differences between their pre-oviposition periods, fecundity, and longevity. The sex ratios for the two strains also were not significantly different, being 1.81:1 (female:male) and 1.65:1 for the carbaryl-OP and the carbaryl-OP-ND strains, respectively.

TABLE 4. PERCENTAGE OF NORMAL AND NON-DIAPAUSING *M. OCCIDENTALIS* FEMALES ENTERING DIAPAUSE, THEIR PRE-OVIPOSITION PERIOD, EGG PRODUCTION, AND LONGEVITY AT A MEAN 21°C AND 10 AND 16 H PHOTOPHASES

Colony	Photophase *h*	Percent in diapause	Mean pre-oviposition period (days) (SD)*		Mean total no. of eggs/female (SD)*		Mean longevity (days) (SD)*	
			D	ND	D	ND	D	ND
Carbaryl-OP	10	100	72.7 a (36.6)	—‡	8.1 a (5.1)	—	69.8 a (42.4)	—
Carbaryl-OP-ND	10	18.2	46.5 a (12.8)	4.1 b (2.8)	16.8 b (9.5)	18.4 b (7.7)	68.5 a (5.4)	23.0 b (10.7)
Carbaryl-OP	16	0	—	2.3 a (1.3)	—	23.7 a (9.6)	—	19.9 a (8.3)
Carbaryl-OP-ND	16	0	—	2.4 a (1.1)	—	25.6 a (6.1)	—	20.5 a (6.5)

*D = diapausing individuals; ND = non-diapausing individuals. Comparisons made within a photophase are not significantly different if followed by the same letter (Bonferroni inequality t-test $p \leq 0.01$). Comparisons between photophases are not valid.

‡Blanks signify no data because no females occurred in the category.

Humidity tolerance

The survival of eggs and larvae at low (24%-32%), medium (70%-74%), and high (93%-97%) relative humidities at each of two temperatures (20° and 30° C) are shown in table 5. The effects of the various relative humidities can only be compared across constant temperatures (Ferro and Chapman 1979). The lowest humidity at both temperatures significantly reduced egg hatch ($p \leq 0.05$). These results suggest that this strain of *M. occidentalis* can survive a wide range of environmental conditions, but that the eggs are susceptible to desiccation at low humidities, and the larvae do not mature well at high temperatures with high vapor pressures. These restrictions are unlikely to greatly reduce the effectiveness of *M. occidentalis* on greenhouse roses, as high humidities with condensed moisture are avoided to prevent rust developing on the leaves and *Botrytis* on the flowers. Humidities as low as 32 percent are unlikely to occur on rose leaf surfaces in greenhouses.

Some aspects of the influence of humidity on *P. persimilis* have been examined by Mori and Chant (1966a, 1966b) and Sabelis (1981). Although survival of immature stages was not reported, adult female *P. persimilis* lived longer when free water was available, but they were also less active at high humidities (Mori and Chant 1966b). However, at high humidities *P. persimilis* were less efficient predators than at low humidities because their searching activity was lower at the higher humidities (Mori and Chant 1966a).

TABLE 5. SURVIVAL OF EGGS AND LARVAE OF THE CARBARYL-OP-ND STRAIN OF *M. OCCIDENTALIS* AT LOW, MEDIUM, AND HIGH HUMIDITIES AT 20° AND 30° C

Stage	Temperature (° C)	Mean* survival (%) at three relative humidities (vapor pressure in mm Hg)		
		32% (5.6)	*74% (12.8)*	*97% (16.9)*
Eggs	20	22.8 a†	92.0 b	81.2 ab
Larvae	20	62.8 a	61.2 a	58.8 a
		24% (7.6)	*70% (22.2)*	*93% (29.8)*
Eggs	30	1.2 a	85.2 b	69.2 b
Larvae	30	72.0 a	81.2 a	53.2 b

*Means of 3 replicates, 25 individuals/replicate.

†Values in the same row followed by the same letter are not significantly different (Bonferroni inequality t-test $p \leq 0.05$).

Greenhouse Trials

Although mite population estimates were made at the hook and the third five-leaflet leaf above the hook, only those from the latter are presented here. Mite numbers at the hook reflected the same population trends as the populations on the higher sampled leaflets; however, mites (both *T. urticae* and *M. occidentalis*) were almost always more abundant at the hook.

Population studies for 1979-1980

The predators released on the rose bushes could be found up on the flower stems within a week (fig. 4). The first application of carbaryl resulted in a decline in predator densities on the plots that had populations of the endemic population of *M. occidentalis* that was moderately carbaryl-susceptible. In the two predator release plots where prerelease (endemic) populations of *M. occidentalis* were absent, *M. occidentalis* densities increased from the precarbaryl treatment sample to the postcarbaryl treatment sample, verifying the high carbaryl resistance of the released predators.

During the first 5 months of the experiment (until mid-November 1979), only one application of the acaricide hexakis was applied to replicates 1 and 2 of the predator treatment (on July 7, 1979), 16 days after the first predator release (fig. 4). The third predator treatment replicate required two applications of hexakis over the same period, the second being necessary following an increase of *T. urticae* that occurred after an August 8 application of carbaryl that reduced predator densities. During the same 5 months six applications of acaricide were made to the commercial treatments, and defoliation was imminent once on the control. Although damage estimates did not commence until August, during the subsequent 3.5 months to mid-November, mite damage in replicate 1 was lower in the predator treatment than in the commercial treatment; in replicate 2 both treatments were about equivalent and in replicate 3 the commercial treatment had lower damage. There was extremely high damage in the control block (fig. 5).

During November and December, spider mite densities increased rapidly in all plots (fig. 4). Predators could only be found on replicate 3 of the predator release treatment during these months. This was the only plot containing predators that had a continuous supply of prey during November and December. The failure of *M. occidentalis* to maintain spider mite control during late autumn and early winter was most likely exacerbated by *M. occidentalis* females entering diapause, as the black cloth bands contained adult female predators on each of the three dates they were examined. Fourteen of the predators recovered from the bands failed to oviposit within 16 days at greenhouse temperatures and day lengths with abundant prey, which strongly suggests they were in diapause. The other four predators collected on November 23 had a mean pre-oviposition period of 8.5 days, which is abnormally long for reproductive females (Tanigoshi et al. 1975). It is hypothesized (Field and Hoy 1985) that food shortages under the temperatures and photoperiods near the critical temperatures and photophases may have triggered an increased incidence of diapause induction. If the food supply is resumed soon enough, then the induction process may be reversed, but with the result of an increased pre-oviposition period. Thus, continued reproduction in *M. occidentalis* could occur into late autumn if food were plentiful, but earlier induction of diapause would ensue if food were to become scarce.

To prevent excessive damage to the flower crop on the predator-treated plots, applications of the acaricide hexakis were necessary during November and December (fig. 4). The carbaryl-OP-ND strain was released in December and again in January and presumably established on all three predator replicates. By mid-June 1980, *M. occidentalis* had controlled the spider mites on the release plots, although three applications of hexakis had to be made during the establishment phase. By June an influx of spider mites from outside the cubicle had resulted in replicate 3 becoming heavily infested near the tops of the bushes, causing extensive mite damage to harvested flowers (fig. 5).

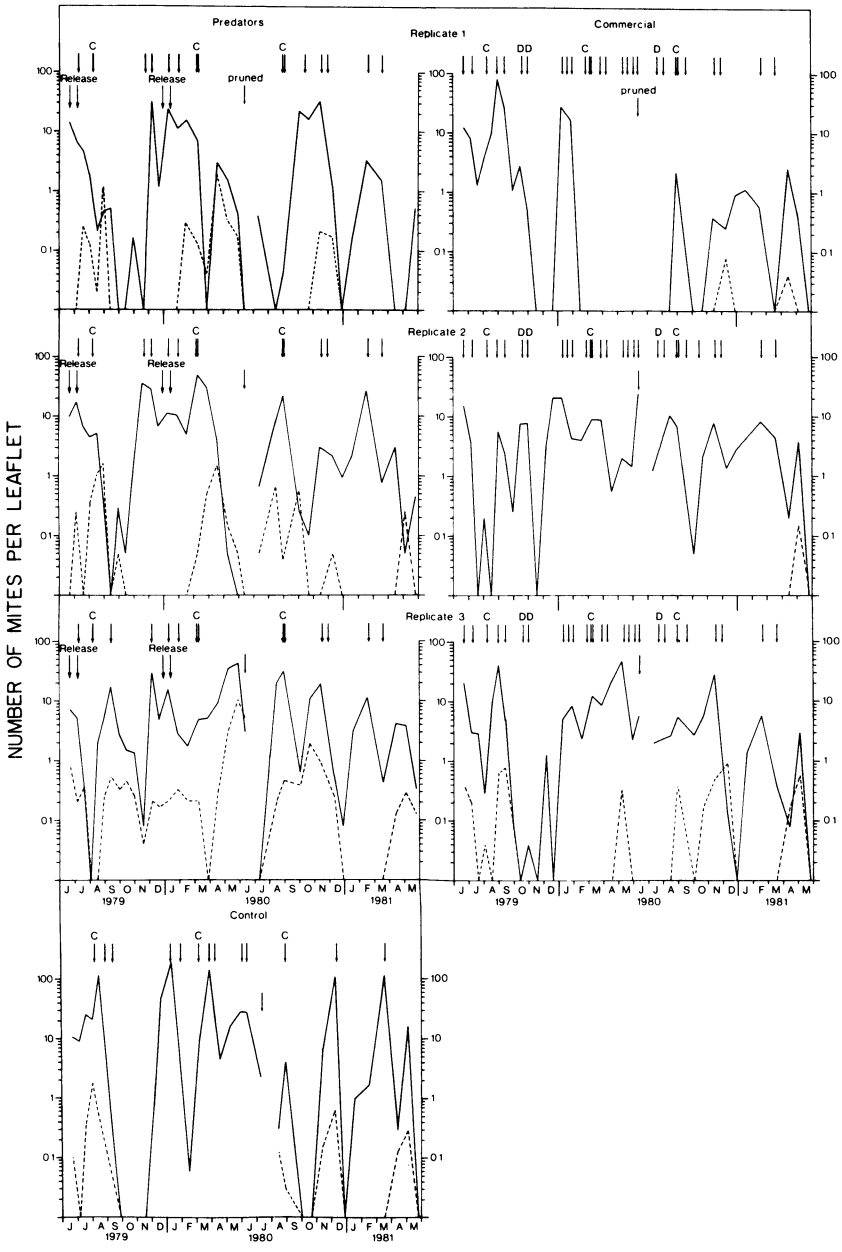

Fig. 4. Mean number (log scale) of all stages of *M. occidentalis* (— — —) and *T. urticae* (———) per leaflet at the third five-leaflet leaf above the hook on rosebushes. Arrows indicate: times of release for *M. occidentalis* on the predator treatments; time of pruning; *C*, application of carbaryl; *D*, application of the acaricide dienochlor; other arrows indicate application of the acaricide hexakis.

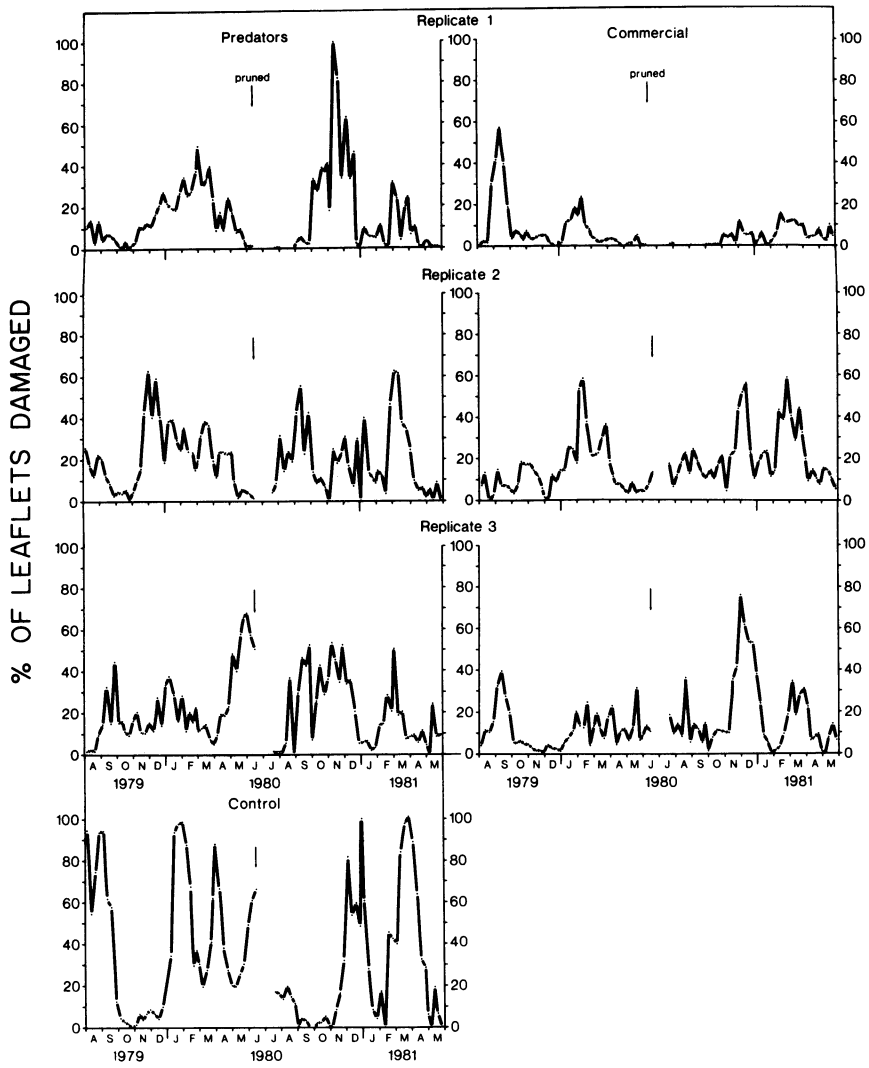

Fig. 5. Weekly average percentages of leaflets damaged by *T. urticae* on harvested greenhouse-grown roses (cv. Royalty). Predator treatments had releases of *M. occidentalis* and limited use of acaricides. Commercial treatments were sprayed regularly with acaricides. The control treatment was sprayed with an acaricide only when defoliation was imminent.

During the first year, the commercial treatment received 17 acaricide applications, the control plot eight applications, and the predator treatments six applications (table 6).

Damage and yield analyses for 1979-1980

Yield and damage measurements based on weekly averages of the various measurements are shown in table 6. The stem length, number of leaflets per flower stem, and number of flowers per bush did not significantly differ between the treatments. There was significantly more leaf damage on the predator treatments than on the commercial treatments. Both predator and commercial treatments had less damage than the control ($p \leq 0.01$). The influence of acaricides and predators cannot be completely separated when trying to explain the control of spider mites during this experiment. However, *M. occidentalis* contributed greatly to the control of *T. urticae* between March and June 1980, as no acaricides were applied to the predator treatments during this period, whereas high spider mite populations had to be controlled with acaricides on all other plots.

Population studies for 1980-1981

Pruning appeared to disrupt the control of *T. urticae* on all predator-treated plots, necessitating an application of hexakis on July 22 (fig. 4). Acaricide applications were also necessary on all commercial treatment plots at this time, although in the control plot, the ratio of predators to prey was large enough to control *T. urticae* without the need for acaricides.

As in the previous year, predator numbers declined in late autumn, and applications of hexakis had to be made to prevent excessive damage. Predators began reappearing in the leaflet samples during February and by the end of May spider mite densities were low on

TABLE 6. YIELD AND DAMAGE MEASUREMENT FROM GREENHOUSE ROSES (CV. ROYALTY) SUBJECTED TO THREE TREATMENTS FOR THE CONTROL OF SPIDER MITES, 1979-1980 AND 1980-1981

Treatment	Number of acaricides used	Mean stem length (cm)*	Mean no. leaflets/flower stem*	Mean no. flowers/bush/ week*	Mean percentage of leaflets damaged†
1979-1980					
Commercial	17	57.4 a	38.0 a	1.50 a	11.3 a
Predators	6	58.5 a	38.5 a	1.43 a	19.4 b
Control	8	57.5 a	38.3 a	1.56 a	40.4 c
1980-1981					
Commercial	8.3	42.1 a	29.9 a	1.19 a	14.8 a
Predators	5.3	41.4 a	29.4 a	1.18 a	19.9 b
Control	2	39.3 a	29.7 a	1.26 a	29.7 c

*Values in columns followed by the same letter are not significantly different ($p \leq 0.05$). Comparisons are only valid within each year.

†Values in column followed by a different letter are significantly different (Bonferroni inequality t-test, $p \leq 0.01$, based on an arcsin transformation for percentage of leaflets damaged). Comparisons are only valid within each year.

all plots, including the three commercial treatment replicates and the control, all of which had become inhabited by *M. occidentalis.*

It appears that the carbaryl-OP-ND strain had not maintained its ability to reproduce under the winter diapause-inducing conditions in the greenhouse. The number of female *M. occidentalis* in the bands and the percentage of these predators terminating diapause "rapidly" (within 3 weeks of being fed) and "slowly" (> 3 weeks) for 22 weekly samples (September 24, 1980, to February 20, 1981) are shown in figure 6. The highest numbers of predators were found during December, when there was the highest incidence of strong diapause (slow termination). No predators were found in the bands during the first and last 3 weeks of sampling (fig. 6), suggesting that they would not normally seek such shelter under long day lengths. No spider mites were found in the bands during the study.

All the *M. occidentalis* removed from the bands fit the description of diapausing females as given by Hoy and Flaherty (1970). In all experiments comparing the mean times to oviposition, except two where the sample sizes were small, the starved, laboratory-reared carbaryl-OP-ND predators produced eggs significantly sooner (1.4-2.8 days) than the predators from the bands (4.0-15.0 days) (Wilcoxon rank sum test $p \leq 0.05$), suggesting that a previous lack of food was not the only factor contributing to delayed oviposition. It appears that the conditions in the greenhouse resulted in behavior indicative of diapause in the putatively non-diapausing greenhouse strain. However, the diapause was weak as it was terminated shortly after the resumption of feeding.

Damage and yield analyses for 1980-1981

Yield and damage measurements for 1980-1981 based on weekly averages are shown in table 6. As in the previous year, stem length, number of leaflets per flower stem, and the number of flowers per bush did not significantly differ between the treatments. However,

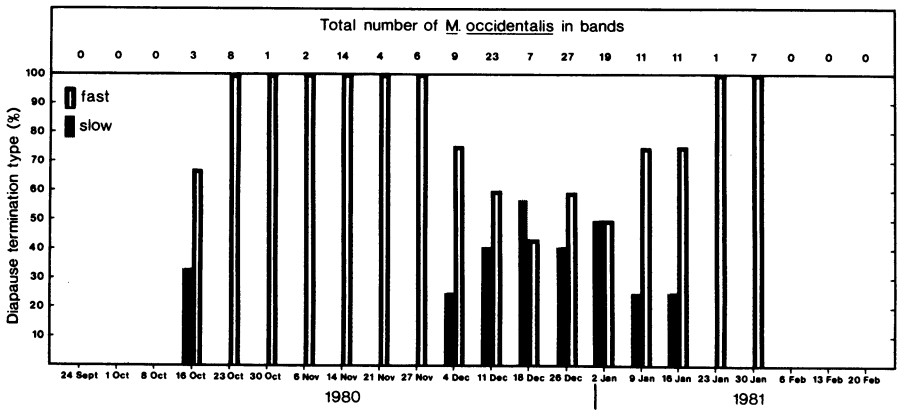

Fig. 6. Numbers of *M. occidentalis* found in black cloth bands on greenhouse-grown roses (cv. Royalty) during the fall and winter of 1980-1981, and the percentage of these predators terminating diapause rapidly (≤ 3 weeks) and slowly (> 3 weeks) on each sample date.

there was still significantly more damage on the predator treatments than on the commercial treatments. Both had less damage than the control ($p \leq 0.05$).

Fewer acaricides were applied to all plots in the second year than in the first (table 6, fig. 4). Considerable control of *T. urticae* could be attributed to *M. occidentalis* in the control treatment, particularly during late summer and the following spring. Thus, defoliation was approached only twice and leaflet damage was considerably lower than in the previous year (table 6). For the predator treatments, two replicates received one less acaricide spray and the third received the same number of applications as in the previous year. The resultant damage was similar to 1979-1980. About half the number of acaricide applications were made to the commercial treatments in the second year compared with the first, but the damage was a little higher in the second year (table 6). There also was more predator activity in the commercial treatments during the second year that reduced the need for acaricides (fig. 4).

Although no attempt was made to determine the commercial acceptability of the flowers produced, any noticeable mite damage on leaflets, particularly those close to the flower, would result in downgrading the bloom. However, damage almost invariably occurs first on the lower leaves, many of which (up to 25%) are removed during packing operations. Thus, about 85 percent of flowers from the "commercial" blocks, 70 percent from the "predator" blocks, and 50 percent from the control block could have been commercially acceptable blooms without downgrading.

Persistence of non-diapause and carbaryl resistance

Colonies obtained from the seven rose plots at the end of the experiments responded as if they had been derived from the original diapausing carbaryl-OP resistant colony released onto the bushes 2 years earlier. Diapause incidence was high (85%-100% diapause) as was the resistance to carbaryl (7%-27% mortality). The two laboratory carbaryl-OP-ND colonies also had high carbaryl resistance (18% mortality) and had a low incidence of diapause when reared under 8 h, 10°C. The non-diapausing laboratory strain that had been reared under non-diapause-inducing conditions for 18 months prior to testing had a lower incidence of diapause (0%) than the non-diapausing strain that had been reared continuously under diapause-inducing conditions (20%). The laboratory carbaryl-OP strain had 95 percent of the females in diapause when reared under an 8 h photophase and 19°C and had high carbaryl resistance (17% mortality).

It appears that while the carbaryl resistance was maintained throughout the experiment, the non-diapause characteristic failed to persist, despite persisting in the isolated base colony, which was continuously reared under non-diapause-inducing conditions in the laboratory. Preliminary laboratory results (Hoy, unpublished data), suggest that the non-diapausing characteristic is not governed by a single dominant or semidominant gene and is, therefore, lost if populations mate with normal individuals. As no attempt was made to remove the original diapausing predators from the bushes prior to releasing the non-diapausing strain, it is likely that the non-diapausing characteristic was lost when the two populations interbred.

CONCLUSIONS

Since these experiments were conducted with high spider mite populations on the nearly adjacent control plot and in the surrounding areas of the greenhouse, the test conditions were more severe than might be encountered with commercially grown greenhouse roses. Any future greenhouse experiments should be carried out with larger plots where the migration of *T. urticae* from surrounding treatments can be minimized. However, several conclusions and recommendations can be made from these greenhouse and laboratory studies.

1. The carbaryl-OP-ND or the carbaryl-OP strains of *M. occidentalis* can be integrated into a rose pest control system without being seriously affected by chemical controls being used for other pests and diseases. During the greenhouse studies carbaryl, pirimicarb, triforine, oxycarboxin, hexakis, and dienochlor were used for pest and disease control without apparent negative effects to either of these strains. This verifies results obtained in the laboratory for the carbaryl-OP-ND strain. Other strains of *M. occidentalis* have resistance to sulfur (Hoy and Standow 1981) and permethrin (Hoy and Knop 1981). If resistance to these chemicals could be added to the carbaryl-OP resistant strain, there would be few currently used California rose house pesticides toxic to *M. occidentalis.*

2. If *M. occidentalis* is to be successful as an effective, permanent, long-term predator of *T. urticae* on greenhouse roses, a non-diapausing strain of the predator should be established. Other strains of the predator with a normal diapause should be excluded or eliminated from the greenhouse to prevent loss of the non-diapausing characteristic through interbreeding. The non-diapausing strain has adequate reproductive potential and humidity tolerance to be an effective predator of *T. urticae* on roses throughout the year. It also has the ability to aerially disperse on wind currents in greenhouses when food is in short supply (Field and Hoy 1985).

3. *Metaseiulus occidentalis* is unlikely to accomplish complete biological control of *T. urticae* on greenhouse roses because of the low injury threshold. Applications of a selective acaricide would be necessary during the establishment phase of the predator, and, possibly, following the annual pruning.

4. The use of genetically improved natural enemies for greenhouses appears to be feasible in the near future. However, it is imperative that a genetic analysis of the improved traits be conducted *prior* to the release of the genetically improved strain. Had the genetic basis of the non-diapausing trait been known prior to conducting these greenhouse experiments, appropriate action could have been taken to eliminate interbreeding of the two strains. A series of steps, including a genetic analysis of the improved traits, has been delineated by Hoy (1979) as necessary components of genetic improvement projects, and if these had been adhered to, this release probably would have been successful.

5. Although *M. occidentalis* persisted in the greenhouse for the length of these experiments, under commercial conditions where lower densities of spider mites are maintained, long-term persistence of *M. occidentalis* might be more difficult. To test this, experiments are needed under a large-scale commercial situation to fully evaluate the efficiency of the carbaryl-OP-ND strain of *M. occidentalis* in regulating *T. urticae* while preventing excessive yield loss and aesthetic injury to glasshouse roses, or other long-term ornamental crops.

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