PESTICIDE SUSCEPTIBILITY OF *CYBOCEPHALUS NIPPONICUS* AND *RHYZOBIUS LOPHANTHAE* (COLEOPTERA: CYBOCEPHALIDAE, COCCINELLIDAE)

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ABSTRACT

The susceptibility of the predatory beetles *Cybocephalus nipponicus* Endrödy-Younga and *Rhyzobius lophanthae* Blaisdell to 6 pesticides commonly used for treating cycad aulacaspis scale, *Aulacaspis yasumatsui* Takagi, was tested. Three concentrations (half field rate, field rate, and twice field rate) of each pesticide were tested against both beetle species with a coated glass vial bioassay. Nearly 100% mortality in both beetle species occurred at all concentrations when treated with methidathion, dimethoate, and malathion. Insecticidal soap, fish oils, and imidacloprid were much less toxic. At one-half the field rate, *C. nipponicus* had 66% mortality with insecticidal soap, 76% mortality with imidacloprid, and 83% mortality with fish oil. At one-half the field rate, *R. lophanthae* had 43% mortality with insecticidal soap, 63% mortality with imidacloprid, and 46% mortality with fish oil. Mortality rate for each beetle species rose with increasing concentration of each pesticide and the soap and oil were the least toxic of all pesticides tested.

Key Words: biocontrol, coated glass vial bioassay, predatory beetle, toxicity test

RESUMEN

Se investigó la susceptibilidad de los escarabajos depredadores *Cybocephalus nipponicus* Endrödy-Younga y *Rhyzobius lophanthae* Blaisdell a seis pesticidas comunmente usados en el control de la escama de las cícadas, *Aulacaspis yasumatsui* Takagi. Se probaron tres concentraciones (mitad de la tasa recomendada en el campo, la tasa recomendada en el campo, y doble la tasa recomendada en el campo) de cada pesticida contra cada especies de escarabajo, usando frascos de vidrio aplicado para bioensayos. La mortalidad en ambas especies de escarbajos fue casi 100% a todas las concentraciones de metidatión, dimetoato, y malatión. Jabón insecticida, aceite de pescado, e imidacloprid fueron mucho menos tóxicos. A la mitad de la tasa recomendada en el campo, los niveles de mortalidad de *C. nipponicus* fueron 66% con jabón insecticida, 76% con imidacloprid, y 83% con aceite de pescado. A la mitad de la tasa recomendada en el campo, los niveles de mortalidad de *R. lophanthae* fueron 43% con jabón insecticida, 63% con imidacloprid, y 46% con aceite de pescado. La tasa de mortalidad por cada especie de escarabajo aumentó con mayores concentraciones de cada pesticida y el jabón y aceite de pescado fueron los menos tóxicos de todos los pesticidas probados.

Translation provided by the authors.

Beetles of the families Coccinellidae and Cybocephalidae are the most economically important groups of predators of diaspidid scales in the world (Blumberg & Swirski 1982). *Cybocephalus nipponicus* Endrödy-Younga (Cybocephalidae) and *Rhyzobius lophanthae* Blaisdell (Coccinellidae) are commonly used as biological control agents for many armored scale pests. *Rhyzobius lophanthae* has been established in Florida since the 1930s (according to specimen label data in the Florida State Collection of Arthropods). *Cybocephalus nipponicus*, misidentified as *Cybocephalus binotatus* Grouvelle, was recently released in south Florida in an effort to control the cycad aulacaspis scale (CAS), *Aulacaspis yasumatsui* Takagi (Homoptera: Diaspididae) (Anon. 1998; Howard et al. 1999; Howard & Weissling 1999). CAS is the most economically damaging scale to cycads that the state of Florida has ever seen (Hodges et al. 2003). Although *C. nipponicus* is present in Hawaii (Heu & Chun 2000), *R. lophanthae* is usually suggested as the better control agent of CAS (Heu et al. 2003; A. Hara, personal communication). In both places, CAS has continued to spread and multiply. A more promising approach to controlling CAS would be one using integrated pest management (IPM). In this manner, a combination of pesticides and biological control would be used to combat CAS.

There has been some success controlling CAS with various pesticides. Oils, either an ultra-fine horticultural oil or a product containing fish oils, seem to be the most effective chemical control method (Hodges et al. 2003). This is not surprising given that oils have long been used to control armored scale insects. The oil not only covers the insects and suffocates them but also covers the surface of the plant making it difficult for crawlers to settle onto the plant (Howard & Weissling 1999). Soaps are quite popular with homeowners; but they must be applied frequently, in some cases once a week (personal observation). The effective application of pesticides for control of CAS is difficult due to the scale's tendency to heavily infest the abaxial surface of leaves, a site difficult to spray (Howard & Weissling 1999). In the case of *Cycas revoluta* Thunberg (Cycadaceae), the architecture of the plant itself, with the margins of the leaflets curling down and inward to form an arch on the abaxial surface of the leaflet, makes foliar treatments inefficient (Hodges et al. 2003). Frequent or "as needed" applications of oils seems to be the most effective technique for controlling CAS, and by mixing oil with contact pesticides such as malathion, even greater scale mortality can be achieved (Hodges et al. 2003). Systemic pesticides such as dimethoate and contact pesticides like methidathion have yielded mixed results, being very effective in some instances and completely ineffective in other cases (Hodges et al. 2003). Imidacloprid used as a soil drench can be very effective, but Howard & Weissling (1999) found that this product had to be mixed at very high concentrations to be effective. This product can also be used as a foliar spray.

The reproductive biology of *C. nipponicus* makes it a good biological control agent. Alvarez & Van Driesche (1998) found that, at low scale densities, *C. nipponicus* was able to maintain its populations and maintain populations of euonymus scale, *Unaspis euonymi* (Comstock), and San Jose scale, *Quadraspidiotus perniciosus* (Comstock), in check. In the presence of greater scale densities, *C. nipponicus* will increase egg production accordingly. With a total life cycle from egg to adult only taking around 44 days (Smith & Cave 2006), it is conceivable that 5-6 generations could be produced every year in Florida. *Cybocephalus nipponicus* is available commercially in the U.S. market.

Rhyzobius lophanthae is an exceptional biological control agent because of its high fecundity, lack of parasitoids, absence of diapause, and resistance to low temperatures especially in the immature stages (Rubstov 1952; Smirnoff 1950; Stathas 2000). Female *R. lophanthae* are able to lay hundreds of eggs in a lifetime (Stathas 2000). *Rhyzobius lophanthae* also seems to be able to resist extreme heat, but Atkinson (1983) found that adult *R. lophanthae* could not survive for long at 42°C. *Rhyzobius lophanthae* is also available commercially in the U.S. market.

This study was conducted to determine the susceptibility of *C. nipponicus* and *R. lophanthae* to 6 pesticides commonly used in the control of CAS. Given the established presence of both predators on cycads in south Florida and their commercial availability, it is very important to learn what effects the commonly used pesticides against CAS will have on them. This information is vital for development of IPM programs aimed at controlling CAS.

Materials and Methods

Insects

Adult *R. lophanthae* were reared at, and purchased from, Rincon-Vitova Insectaries (Ventura, California). Adult *C. nipponicus* also were purchased from Rincon-Vitova but were reared by Philip Alampi Beneficial Insect Laboratory, New Jersey Department of Agriculture. Both beetle species were maintained in Plexiglas cages at 25°C and 80% relative humidity prior to testing. All life stages of CAS were provided as a food source.

Food was not provided during testing because of the very small size of the beetles (1 mm in width and 2.5 mm in length). The beetles could have conceivably perched on the food source for long periods of time, never coming into contact with the walls of the treated vial. Preliminary studies indicated that a 24-h period without food would not unduly stress the beetles. On average, untreated *C. nipponicus* survived for 8-9 d (n = 30) and untreated *R. lophanthae* lived for 5-6 d (n = 30) before dying of starvation. Cotton used to stopper the vials was soaked in water to prevent dehydration.

Bioassays with the Coated Glass Vial Method

A coated glass vial method (Plapp 1971; Amalin et al. 2000; Snodgrass 1996; Snodgrass et al. 2005) was used to determine the chemical susceptibility of adult *R. lophanthae* and *C. nipponicus* to 6 pesticides used to control CAS (Howard et al. 1997; Howard & Weissling 1999; Weissling et al. 1999; Hodges et al. 2003; Emshousen & Mannion 2004). This is a very effective method for testing the chemical susceptibility of small arthropods (Amalin et al. 2000) such as *R. lophanthae* and especially *C. nipponicus* because of its extremely small size. The 6 pesticides tested were fish oil emulsion (Organocide®), insecticidal soap (Garden Safe, Inc.), imidacloprid (Provado®), malathion (Spectracide, Inc.), methidathion (Supracide®), and dimethoate (Cygon®). The fish oil and insecticidal soap were purchased as commercial grade, while the imidacloprid (99% purity), malathion (98% purity), methidathion (98.6% purity), and dimethoate (98.7% purity) were purchased as the technical grade from Chem Service (West Chester, PA).

All pesticides were dissolved in acetone, except the insecticidal soap, which does not dissolve in acetone. Instead, the insecticidal soap was dissolved in 95% ethanol. The fish oil was shaken in a paint shaker after being placed in acetone in order to break the oil into fine globules. Each pesticide was separated into 3 dilutions: field rate, twice field rate, and one-half field rate. The field rate was taken from label data for each pesticide as directed for use against scale insects. A small amount (0.5 mL) of the pesticide working solution was dispensed into 20-mL scintillation vials. Concentrations of active ingredient for the working solution and the amount of active ingredient residue within the vials can be seen in Table 1. Vials were hand rotated until the acetone or ethanol completely evaporated leaving an insecticidal residue on the inner surface. Vials treated with only acetone or ethanol, as well as untreated vials, were used as controls. A single beetle was placed into a treated vial. All beetles had emerged from pupae within the previous 14 d. Vials were sealed with cotton soaked in water allowing the beetles to drink. Vials were placed upright in a ventilated cabinet with a fume hood and at a constant temperature of 25°C and 80% relative humidity for 24 h. For each treatment of 10 beetles, 5 females and 5 males were used. Each treatment of 10 beetles was replicated 3 times for each dosage. All trials were carried out the same day that the pesticide was applied to the vials.

Mortality of beetles was determined immediately after the 24-h period. A beetle was considered dead if it was not moving or could not right itself. Percent mortality was measured as the proportion of 30 beetles dead after a 24-h exposure to the pesticides.

Statistical Analyses

All descriptive statistics were generated in EXCEL (Microsoft 2000). The mortality rates for each pesticide were compared by the Student-Newman-Keuls mean separation test (SAS Institute 2001).

TABLE 1. FIELD RATES (1X) FOR EACH PESTICIDE USED.

Insecticide	Working solution (µg*AI/mL)	Insecticide residue (µg*AI/cm²)
Organocide®	47000	8.29
Insecticidal Soap®	512300	27.71
Imidacloprid	106	2.40
Methidathion	233	5.26
Dimethoate	305	6.91
Malathion	1990	45.07

*AI = Active Ingredient.

RESULTS

Of the 6 pesticides tested on adult *C. nipponicus* and *R. lophanthae*, 3 (methidathion, dimethoate, and malathion) caused >90% mortality at all concentrations, while the other 3 (fish oil, insecticidal soap, and imidacloprid) were less toxic but still caused very high mortality (Tables 2 and 3).

Effects of Pesticides on *C. nipponicus*

Cybocephalus nipponicus was extremely susceptible to all pesticides. The three least toxic pesticides were imidacloprid, insecticidal soap, and fish oil (Table 2). There were significant differences ($P < 0.05$; Table 4) in mortality between concentrations among these 3 pesticides. Fish oil was not only toxic to the beetle, but due to its very small size, *C. nipponicus* would often get trapped in small globules of oil, eventually dying from suffocation.

Effects of Pesticides on *R. lophanthae*

Rhyzobius lophanthae was more tolerant than *C. nipponicus* to the experimental pesticides, although mortality rates were high for this species, too. The 3 least toxic pesticides to *R. lophanthae* were imidacloprid, insecticidal soap, and fish oil (Table 3). There were significant differences in

TABLE 2. PERCENT MORTALITY OF *CYBOCEPHALUS NIPPONICUS* PER 30 INDIVIDUALS EXPOSED. X = FIELD RATE.

Pesticide	% Beetle mortality			
	at 0X	at 0.5X	at 1X	at 2X
Organocide®	—	83	100	96
Insecticidal Soap®	—	66	86	96
Imidacloprid	—	76	93	100
Methidathion	—	100	100	100
Dimethoate	—	100	96	100
Malathion	—	93	100	100
Control (Acetone)	0	—	—	—
Control (Ethanol)	0	—	—	—
Control (No coating)	0	—	—	—

TABLE 3. PERCENT MORTALITY OF *RHYZOBIUS LOPHANTHAE* PER 30 INDIVIDUALS EXPOSED. X = FIELD RATE.

	% Beetle mortality			
Pesticide	at 0X	at 0.5X	at 1X	at 2X
Organocide®	—	46	83	100
Insecticidal Soap®	—	43	76	96
Imidacloprid	—	63	80	100
Methidathion	—	100	100	100
Dimethoate	—	100	96	100
Malathion	—	93	90	96
Control (Acetone)	0	—	—	—
Control (Ethanol)	6	—	—	—
Control (No coating)	0	—	—	—

survivorship between concentrations of these 3 pesticides (Table 4). *Rhyzobius lophanthae*, about twice the size of *C. nipponicus*, had much less difficulty traversing oil globules on the surface of the vials.

DISCUSSION

In the present study, a significant difference ($P < 0.05$) was observed between mortality in the control and that of even the lowest pesticide concentration. This sensitivity to pesticides makes an IPM approach to the control of CAS quite difficult. Unfortunately, most of the success in chemically controlling CAS has involved very toxic pesticides often being used at higher than recommended doses (Howard & Weissling 1999; Weissling et al. 1999).

The high mortalities experienced by *C. nipponicus* and *R. lophanthae* are not unexpected. Nakao et al. (1985) found that all 18 species of Coccinellidae inhabiting Japanese citrus groves were severely affected by the application of pesticides, including methidathion and dimethoate. They also found that *Cybocephalus gibbulus* Erichson, one of the most common scale predators found in Japanese citrus groves, was virtually eliminated by long-term pesticide use. Oils have proven to be the most effective pesticides used against many plant-sucking pests, while maintaining the natural enemy populations. Erkiliç & Uygun (1997) found that oils were much less toxic to *Cybocephalus fodori minor* (Endrödy-Younga) and *Chilocorus bipustulatus* (Linnaeus) than was methidathion. In fact, they went as far as saying that methidathion should not be used in IPM programs.

In natural conditions, the predatory beetles may not be in contact with the pesticide for as long as the exposures in this experiment. However, *C. nipponicus* and *R. lophanthae* are uniquely suited for life in chemically-treated environments. Both beetle species place their eggs underneath the scale cover and at least part of larval development takes place beneath the armored scale, allowing the beetles some protection from both the elements and pesticides (Smirnoff 1950; Alvarez & Van Driesche 1998; Stathas 2001). In Greece, Katsoyannos (1984) found that *C. fodori* was able to survive in pesticide-treated

TABLE 4. STUDENT-NEWMAN-KEULS TEST SHOWING RANKED VALUES OF MORTALITY OF ADULT *CYBOCEPHALUS NIPPONICUS* AND *RHYZOBIUS LOPHANTHAE* BY 4 DOSES OF IMIDACLOPRID, INSECTICIDAL SOAP, AND ORGANOCIDE.[1]

	Dose	Imidacloprid	Organocide®	Insecticidal soap
C. nipponicus	0.0X	2.0 a	2.0 a	2.0 a
	0.5X	5.5 b	5.3 b	5.3 b
	1.0X	8.5 c	8.6 c	8.3 c
	2.0X	10.0 c	10.0 c	10.3 c
R. lophanthae	0.0X	2.0 a	2.0 a	2.0 a
	0.5X	5.6 b	5.0 b	5.3 b
	1.0X	7.3 b	8.0 c	7.8 c
	2.0X	11.0 c	11.0 d	10.8 d

[1]Means within columns with the same letter are not significantly different based on Student-Newman-Keuls mean separation test, $P = 0.05$.

fruit orchards. In date palm plantations in Israel, Kehat et al. (1974) found that, while all coccinellids in a chemically-treated plantation died, species of *Cybocephalus* survived.

For some pesticides, it is apparent that from these tests, the lower the concentration of the pesticide, the lower the mortality. However, these tests were conducted in a laboratory environment wherein the test subjects were in constant contact with the pesticide for 24 h. A whole host of factors, such as humidity, UV degradation, evaporation, and precipitation, will influence pesticide activity in the field. Nevertheless, whenever possible, insecticidal soaps and fish oils should be used. While many homeowners use various types of soaps to treat CAS, this method requires treatment every 7 to 10 d, thus increasing exposure of the beetles to the pesticide. If more toxic pesticides must be used, then applying them to "hot spots" rather than broadcast spraying may protect the scale predators from complete annihilation. This type of selective spraying may also protect other entomophagous insect populations from being decimated (Kuznetsov 1997). The results of these laboratory experiments yield some baseline data from which more research in the field can be conducted.

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