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UNIVERSITY OF CALIFORNIA
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Biological Studies on *Galendromus flumenis* (Acari: Phytoseiidae),
a Predator of Banks Grass Mite

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Entomology

by

Fatemeh Ganjisaffar

August 2016

Dissertation Committee:

Dr. Thomas M. Perring, Chairperson

Dr. Subir Ghosh

Dr. Timothy D. Paine

Dr. Erin W. Rankin

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The Dissertation of Fatemeh Ganjisaffar is approved:

Committee Chairperson

University of California, Riverside

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ABSTRACT OF THE DISSERTATION

Biological Studies on *Galendromus flumenis* (Acari: Phytoseiidae),
a Predator of Banks Grass Mite

by

Fatemeh Ganjisaffar

Doctor of Philosophy, Graduate Program in Entomology
University of California, Riverside, August 2016
Dr. Thomas M. Perring, Chairperson

The predatory mite, *Galendromus flumenis* (Chant) (Acari: Phytoseiidae), is the most abundant predator and the only phytoseiid species on date bunches infested with Banks grass mite, *Oligonychus pratensis* (Banks) (Acari: Tetranychidae). Biological studies were conducted on this predatory mite in order to develop and optimize biological control strategies against the Banks grass mite.

In the first chapter, studies showed that *G. flumenis* feeds on all immature stages of Banks grass mite, but it prefers eggs over the other stages. Functional response studies indicated that the predator displays a type II response on all immature stages of the prey. The mutual interference study in Chapter 2 revealed that total predation increased with increasing *G. flumenis* density in two arena sizes. However, there was not a proportional increase in per capita predation due to mutual interference. The interference effect was high in small

arenas due to higher encounter rates between predators. Also, higher predator density and smaller arena size caused significantly higher dispersal rate of predators.

In Chapter 3 data show that *G. flumenis* develops under a wide range of temperatures from 18°C to 42°C, but survival rates were highest between 26°C and 38°C. The optimal temperature for development (T_{opt}) was calculated to be 37.6°C. Further work on the population growth and reproduction of *G. flumenis* (Chapter 4) showed that predators cannot oviposit at 38°C (T_{opt}). At 34°C, the next temperature with the shortest developmental time for the predator, *G. flumenis* showed medium reproductive capacity (1.6 eggs/ day and 19.9 eggs/ ovipositional period), which is inferior to its prey. The net reproductive rate of prey can reach three times the maximum potential of *G. flumenis*, contributing to an intrinsic rate of natural increase of the prey that is more than double that of the predator.

In the final chapter, treatment with the miticide hexythiazox had little or no effect on immature development and reproduction of emerging adult predators. Hexythiazox did not have any adverse effect on the fecundity of treated females, hatch rate of eggs, progeny development, or sex ratio.

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Chapter 1

Introduction

Date Palm, *Phoenix dactylifera* L. (Arecaceae)

Production and Yield

Dates have been cultivated for some 6000 years in the Mesopotamian region in what is now the country of Iraq (Wrigley 1995; Zohary and Hopf 2000). Since then the date palm has been spread across the world by seed or offshoot propagation (Nixon and Carpenter 1978; Zaid and de Wet 2002a). Among the 100 million date palms of the world, 60% exist in North Africa and Middle East (Wakil et al. 2015) with Egypt, Iran and Saudi Arabia as the current top three date producing countries (FAOSTAT 2014) (Figure 1-1).

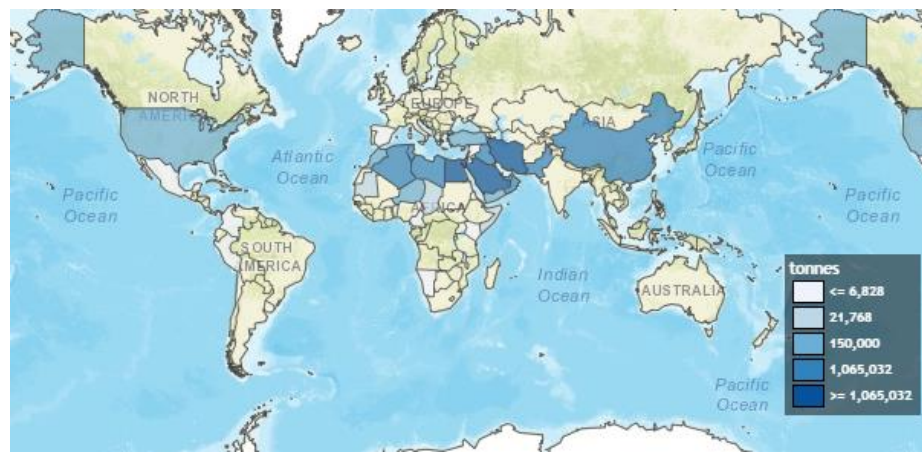


Figure 1-1 Average date production by country from 2013 through 2014 (FAOSTAT 2014).

In 2015, 43,600 tons of dates valued at \$68 million were harvested from 10,000 acres of date gardens in the United States (USDA 2016). Commercial date production in the United States is centered in the Coachella Valley (Riverside County, California), with recent expansion into the Bard/Winterhaven area (Imperial County, California) and Yuma, Arizona (Wakil et al. 2015). From more than 3000 date varieties worldwide (Zaid and Arias-Jimenez 2002), 196 varieties occur in the United States (Nixon 1950). The dominant variety in the Coachella Valley has been the Deglet Noor (85% of the acreage) (Warner

1988), but recent plantings of Medjool are quickly making this the dominant economic variety.

Biology and Cultivation

Date palm is a perennial and dioecious monocot with male and female flowers being produced in clusters (called inflorescence) on separate palm trees. Both female and male inflorescences are enclosed in a hard, fibrous cover known as the spathe which splits open as the flowers mature exposing them for pollination purposes. The spathe protects the delicate flowers from intense heat and sunlight during the early stage of flower development. The flowers on female trees are borne on a flat, tapering peduncle or rachis, commonly known as the “fruit stalk” with many unbranched strands arranged in spirals on the rachis (Chao and Krueger 2007). As the inflorescence bearing the fruit develops it is commonly referred to as a “bunch.” The date fruit goes through four distinct ripening stages derived from Iraqi Arabic as “Kimri,” “Khalal,” “Rutab,” and “Tamar” (Reuveni 1986). During the Kimri stage, the green fruit rapidly increases in size and weight. The dates change color from green to a color characteristic of the variety during the Khalal stage. The fruit remains turgid and astringent during Khalal, and reaches its full size and weight. During the Rutab stage the fruit skin darkens to amber, brown, or nearly black, accompanied by softening and decreasing astringency. During the Tamar stage, the fruit loses much of its water and the sugar-to-water ratio is high enough to prevent fermentation, similar to raisins (Chao and Krueger 2007). There are also periods of natural fruit abscission to which the Deglet Noor variety is especially prone to insect infestation (Nay 2006). In the Coachella Valley, the most significant fruit abscission occurs during the

Kimri stage in which 20-40% of the fruit abscise from the bunch (Kehat et al. 1976; Nixon and Carpenter 1978; Warner 1988). This period of fruit abscission is commonly referred to as “June drop,” which begins in early to mid-June and lasts 3-4 weeks. A second, less extensive period of fruit abscission (5-15% fruit drop) begins in late July and early August during the Khalal stage, and may last 2-3 weeks (Nay 2006).

Because of the biology of the date palm, its cultivation is very unique. There are a number of cultural practices such as pollination, bunch tie-down, covering, harvesting, and pruning that require access to the crown of the tree (a height which can be in excess of 25 m in old trees). Although the practice of climbing the trees for access to the crown is still found in all date-producing areas, the use of mechanical lifts is common in more advanced or industrialized production areas, such as the United States (Nixon and Carpenter 1978). Artificial pollination is critical for the success of production, therefore pollen is collected from a few male trees grown in date gardens in January and February, and it is used to pollinate female inflorescences during March and April. In June and July, bunches are tied to the leaf stalks to support the weight of the fruit. Fruit thinning often is practiced to decrease alternate bearing, increase fruit size, improve fruit quality, advance fruit ripening, and facilitate bunch management. Fruit thinning can be carried out in three ways: removal of entire bunches, reduction in the number of strands per bunch, and reduction in the number of fruits per strand. From July through September, bunches of dates usually are covered (bagged) with brown craft paper or mesh bags. Bagging can protect fruit bunches from high humidity and rain, minimize damage from sunburn, and decrease losses from birds and insects (Nixon and Carpenter 1978; Zaid and de Wet 2002b). In the United States,

soft varieties like Medjool are harvested from mid-August through September and for drier varieties like Deglet Noor, fruit is harvested in October and November.

Flood or furrow irrigation is the oldest form of irrigation and is still used in many areas including the Coachella Valley. Sprinkler, micro sprinkler, and drip irrigation often are used in newer plantations (Abdul-Baki et al. 2002). In date gardens of the Coachella Valley, trees are planted at a density of 49 trees per acre, although some growers have experimented with higher densities, even up to 150 trees per acre. The average economic life of a date palm is 40 to 50 years, but some are still productive up to 150 years (Chao and Krueger 2007).

Arthropod Pests of Date Palm

El-Shafie (2012) reviewed pest and disease of date palm and listed 112 species worldwide including 22 species attacking stored dates. The major pests currently attacking dates in the Coachella Valley are the Banks grass mite, *Oligonychus pratensis* Banks (Acari: Tetranychidae), four species of nitidulid beetles, *Carpophilus dimidiatus* (Fabricius), *C. hemipterus* (L.), *Urophorus humeralis* (Fabricius), *Haptoncus luteolus* (Erichson) (Coleoptera: Nitidulidae), the raisin moth, *Cadra figulilella* (Gregson) (Lepidoptera: Pyralidae) and the carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) (Carpenter and Elmer 1978; Warner 1988).

Banks Grass Mite, *Oligonychus pratensis* (Banks)

Taxonomy and Distribution

The first description of Banks grass mite was made by Nathan Banks (1912) from mites collected on timothy grass (*Phleum pratense* L) near the town of Pullman,

Washington. He named the mite *Tetranychus pratensis* Banks. Two years later, he collected what he thought was a new species from date palms near El Centro (Imperial County, California) and described it as *Tetranychus simplex* Banks (Banks 1914). The name underwent several changes until Pritchard and Baker (1955) gave it its current binomial name, *Oligonychus pratensis* (Banks). Although this mite was known previously as the timothy mite and date mite, the committee on common names of the Entomological Society of America in 1960 officially selected the Banks grass mite as the common name for *O. pratensis* (Elmer 1965).

Banks grass mite is believed to be native to the United States since it was found on native host plants and is a different species from those collected from dates in other countries (Stickney et al. 1950). According to the comprehensive review by Negm et al. (2015), Banks grass mite has been reported as an agricultural pest of grasses and dates in 22 states of the United States, Mexico, Costa Rica, El Salvador, Honduras, Colombia, Brazil, and Puerto Rico. In the old world, Banks grass mite has been identified in many parts of Africa and Asia (Negm et al. 2015).

Host Plants

Banks grass mite is reported from a wide range of host plants in 10 plant families (Negm et al. 2015), but economic losses occur in just two families; the Arecaceae and Poaceae. Pest management is required on date palm (Elmer 1965), timothy grass (Negm et al. 2015), grain sorghum (Pate and Neeb 1971; Owens et al. 1976), corn (Walter and Wene 1956; Schweissing 1973; Ehler 1974), Bermuda grass (Tuttle and Baker 1964; Jeppson et al. 1975) and sometimes wheat (Harvey 1954; DePew 1960).

Biology

Similar to other species of mites in the Tetranychidae, Banks grass mite has arrhenotokous parthenogenesis producing haploid males from non-fertilized eggs (Malcolm 1955). Males have faster developmental rates than females, and guard quiescent female deutonymphs (Malcolm 1955). Newly emerged females are immediately mated by the tending male. Females typically mate multiple times (Malcolm 1955). The life stages of Banks grass mite consist of egg, larva, larval chrysalis, protonymph, protochrysalis, deutonymph, deutochrysalis, and adult (Figure 1-2).



Figure 1-2 Different life stages of Banks grass mite, *Oligonychus pratensis*. A: egg, B: six-legged larva, C: protonymph, D: deutonymph, E: adult female.

All life stages of the mite are present throughout the year on the fronds and grasses in date gardens, suggesting that feeding and reproduction occurs year-round and there is no true diapause. Numbers of mites on date fronds are low from October through February and they peak from March through September (Gispert et al. 2001). In April, Banks grass mite females infest green immature date fruits in the early Kimri stage (Figure 1-3). They move to date bunches by crawling from infested fronds or by aerial dispersal from fronds and grasses.



Figure 1-3 Early infestation of the Kimri dates by Banks grass mite.

During the months of April through June, when the normal day-time temperatures are above 39°C and humidity is low, colony development is very rapid as Perring et al. (1984) determined that the population doubling time is 48 hours under such condition. Mite colonies reach their maximum abundance in mid-July when the entire bunch of fruit is covered with their heavy webbing (Figure 1-4).



Figure 1-4 Heavy webbing produced by Bank grass mite on date bunches.

Banks grass mite numbers begin to decline with the onset of the khalal stage as a result of thickening epidermis and an increase in soluble carbohydrates in the ripening date. Perring et al. (1983) found that increased soluble carbohydrates in the leaves of maturing

grain sorghum negatively affected Banks grass mite densities. The Banks grass mites leaving the date fruits move to date fronds and grasses in the date garden and continue to grow to re-infest date bunches the following April (Gispert et al. 2001).

Damage and Economic Loss

Banks grass mite feeds on the epidermis of the Kimri dates with its stylet-like chelicerae (Malcolm 1955; Krantz 1978). The mites rasp the fruit surface causing fruits that harden prematurely, shrivel, and crack (Elmer 1965; Carpenter and Elmer 1978) (Figure 1-5). Scarred fruits are considered low quality at packing houses, being unmarketable for fresh market consumption. Up until 1998, when the miticide Savey® (Hexythiazox) was registered for use in the date industry in California, Banks grass mite caused annual losses plus control costs between \$1 million and \$2.5 million (Negm et al. 2015). Nearly all of the damage by Banks grass mite is on Deglet Noor dates.



Figure 1-5 Rasping and bronzing of the fruit surface due to the feeding damage by Banks grass mite.

Management

Chemical control of Banks grass mite originated in the mid-1950s when sulfur dust was introduced into the industry (Vincent and Lindgren 1958; Carpenter and Elmer 1978;

Nixon and Carpenter 1978). As recent as 1997, up to 8 applications of sulfur dust were blown into the date bunches from June through September costing the industry \$0.5 million each year. However, due to the high cost of application, environmental concerns of dusting, complaints of neighboring communities, and field failures with sulfur, the California Date Commission in 1997 started supporting research to understand the biology and ecology of Banks grass mite and develop a more integrated management program. Studies by Gispert et al. (2001) indicated the development of resistance to sulfur in Banks grass mite. In addition, a negative impact of sulfur on the predatory mite, *Galendromus mcgregori* (probably misidentified *Galendromus flumenis* – see below) was demonstrated (Gispert et al. 2001). At the same time, studies on the growth regulator, Hexythiazox (Savey®) was shown to be effective for controlling mites with a one-time, early season application. Given the environmental concerns, non-target effects, and developed resistance to sulfur, a Section 18 Emergency Use registration from the United States Environmental Protection Agency was issued in 1998 (Environmental Protection Agency 1998), and the product received full registration in 2003 (Environmental Protection Agency 2003). Savey® is the current industry standard for controlling Banks grass mite in California (Mauk et al. 2005), and resistance to Savey® has not been documented in the United States (Perring, unpublished data).

Since it is not practical to sample individual mites on date bunches, there is no established economic threshold for Banks grass mite on dates, and Savey® is applied on a calendar basis. However, promising results have been gained from mite web rating as a reliable estimate of mite density and its subsequent damage to the dates. In addition, some

growers are relying on webbing assessment to identify specific bunches that require spot treatments with Savey® saving significant chemical and application costs.

With a growing interest in sustainable practices for managing arthropod pests including Banks grass mite, growers realize that reliance on Savey®, even if it is an effective miticide, is placing too much pressure on a single strategy. Evaluation of alternative miticides and studies on the biology of natural enemies will provide additional tools for managing Banks grass mite on dates.

Biological Control

Surveys in date gardens in the United States have identified only a few predator species for Banks grass mite. Based on abundance, mites in the Phytoseiidae are the most important. Other predators found in date bunches, only after the spider mite colonies are well established, include *Stethorus* sp. (Coleoptera: Coccinellidae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), and *Scolothrips sexmaculatus* (Pergande) (Thysanoptera: Thripidae) (Negm et al. 2015).

Gispert et al. (2001) found two phytoseiid species, *Galendromus mcgregori* (Chant) and *Neoseiulus comitatus* (DeLeon). *G. mcgregori* was found on date fronds and bunches, while *N. comitatus* was collected from Bermuda grass, *Cynodon dactylon* (L.) Persoon, on the floor of the date garden. Both species are reported to be Type II phytoseiids which are selective predators of mites in the Tetranychidae (McMurtry and Croft 1997), and laboratory studies showed that they prey readily on Banks grass mite (Gispert et al. 2001). In 2012, Banks grass mite infested date bunches were sampled, and the predatory mite *Galendromus flumenis* (Chant) (Figure 1-6) was collected. Muma (1963) noted that “the

dorsal scutal setation of *G. flumenis* is very similar to that of *G. mcgregori* except the setae are somewhat shorter on this species, especially the dorsal setae.” Therefore, it is possible that *G. flumenis* collected recently and *G. mcgregori* collected in 2001 are the same species.



Figure 1-6 Predatory mite *Galendromus flumenis* collected from date bunches infested with Banks grass mite.

Predatory mite, *Galendromus flumenis* (Chant)

Taxonomy

Galendromus flumenis belongs to the subfamily Typhlodrominae, and Tribe Metaseiulini. The species first was described by Chant (1957) from mites collected on soapollie, *Shepherdia canadensis* Nutt (Family: Elaeagnaceae), in British Columbia, Canada. He named the mite *Typhlodromus flumenis* Chant. In 1961, Muma determined that this mite belonged in the new genus *Galendromus*, thus giving rise to *Galendromus flumenis* (Chant) (Muma 1961). A year later, both Wainstein (1962) and Hirschmann (1962) moved the mite back to the genus *Typhlodromus*. Schuster and Pritchard (1963) placed the mite in the genus *Metaseiulus*. In the same year, Muma (1963) moved the mite back to the genus *Galendromus*, giving its current binomial name, *Galendromus flumenis*

(Chant). According to Prasad (2012), there is still a controversy about the genus of this species, and both *Metaseiulus* and *Galendromus* genera names are used for the species.

Distribution

Galendromus flumenis has been found in the states of British Columbia and Ontario in Canada (Chant 1957; Anderson et al. 1958; Chant et al. 1974; Chant and Yoshida-Shaul 1984). It also has been reported from El Salvador (Denmark and Andrews 1981) and Mexico (Denmark and Evans 2011). In the United States, *G. flumenis* has been identified on various deciduous and evergreen trees, shrubs and herbs in the states of Arizona, California, Florida, Missouri, New Jersey, New Mexico, Ohio, Oregon, Pennsylvania, Washington and Wyoming (Schuster and Pritchard 1963; Specht 1968; Tutte and Muma 1973; Charlet and McMurtry 1977; Lehman 1982; Prischmann and James 2003; Croft and Luh 2004; Prischmann and James 2005; James and Prischmann 2010; Denmark and Evans 2011). It is a dominant species in non-sprayed apple orchards (Croft and Luh 2004) and vineyards of Oregon and Washington States (Prischmann and James 2003).

Life-Style Type

Galendromus flumenis can prey on the mites from the families of Tetranychidae, Tenuipalpidae, Eriophyiidae, and Winterschmidtidae, as well as small insects such as thrips, whiteflies, and pollen (Croft and Jorgensen 1969; Blackwood et al. 2004). Based on the life-style classification by McMurtry and Croft (1997), *G. flumenis* is placed on the specialist side of Type III approaching Type II (Blackwood et al. 2004). This classification is due to the fact that *G. flumenis* performs relatively poorly on pollens compared to Type

III species, and is not well adapted for predation on *Tetranychus sp.* compared to Type II species.

Objectives of the Present Study

According to the findings of Gispert et al. (2001) and personal observations, *G. flumenis* is found in low numbers on date bunches that are insufficient for the economic suppression of Banks grass mites. Therefore, the main objective of this dissertation research was to understand why *G. flumenis* is not effective at regulating Banks grass mite populations on dates in California. For this purpose, a series of laboratory experiments were designed to elucidate the biological properties of *G. flumenis*.

In the first two research chapters (Chapters 2 and 3) behavioral traits that contribute to the success of the predator in a biological control program were studied. Prey-stage preference of *G. flumenis* to constant densities of different stages of Banks grass mite and the functional response of the predator to the varying densities of each life stage of Banks grass mite were investigated in Chapter 2. The possibility of any mutual interference effect between *G. flumenis* females was evaluated in Chapter 3. In addition, the effects of predator density and arena size on the foraging efficiency and dispersal rate of *G. flumenis* were studied.

The next two chapters dealt with the biology of *G. flumenis*. The relationship between temperature and development of *G. flumenis* was studied in Chapter 4 aiming to understand the thermal requirements of *G. flumenis* and to determine the optimal temperature for the development of this predator. In Chapter 5, the reproductive potential and the population growth of *G. flumenis* was assessed.

The focus of Chapter 6 was on the compatibility of the miticide hexythiazox (Savey[®]) with *G. flumenis*. The effect of field rate applications of Savey[®] on different life stages of the predator was evaluated.

The last chapter (Chapter 7) summarizes all pertinent information from the preceding chapters in an effort to provide insight into the potential efficiency of *G. flumenis* for managing Banks grass mite on dates.

References

- Abdul-Baki, A., Aslan, S., Linderman, R., Cobb, S., Davis, A. 2002. Soil, water, and nutritional management of date orchards in the Coachella Valley and Bard. 2nd edition. California Date Commission, Indio.
- Anderson, N.H., Morgan, C.V.G., Chant, D.A. 1958. Notes on occurrence of *Typhlodromus* and *Phytoseius* spp. in southern British Columbia (Acarina: Phytoseiidae). *The Canadian Entomologist* 90, 275-279.
- Banks, N. 1912. New American mites. *Proceedings of the Entomological Society of Washington* 14, 96-99.
- Banks, N. 1914. New mites. *Journal of Entomology and Zoology* 6, 1-57.
- Blackwood, J.S., Luh, H.K., Croft, B.A. 2004. Evaluation of prey-stage preference as an indicator of life-style type in phytoseiid mites. *Experimental and Applied Acarology* 33, 261-280.
- Carpenter, J.B., Elmer, H.S. 1978. Pests and diseases of the date palm. United States Department of Agriculture Agricultural Handbook 527, 1-42.
- Chant, D.A. 1957. Description of some phytoseiidae mites (Acarina: Phytoseiidae). Part I. Nine new species from British Columbia with key to the species of British Columbia. Part II. Redescription of eight species described by Berlese. *The Canadian Entomologist* 89, 289-308.
- Chant, D.A., Hansell, R.I.C., Yoshida-Shaul, E. 1974. The genus *Typhlodromus* Scheuten (Acarina: Phytoseiidae) in Canada and Alaska. *Canadian Journal of Zoology* 52, 1265-1291.
- Chant, D.A., Yoshida-Shaul, E. 1984. A world review of four species in the genus *Typhlodromus* Scheuten (Acari: Phytoseiidae): *brevicollis*, *luculentis*, *carinulatus*, and *pinnatus*. *Canadian Journal of Zoology* 62, 2631-2642.
- Chao, C.T., Krueger, R.R. 2007. The date palm (*Phoenix dactylifera* L.): overview of biology, uses, and cultivation. *Hortscience* 42, 1077-1082.
- Charlet, L.D., McMurtry, J.A. 1977. Systematics and bionomics of predaceous and phytophagous mites associated with pine foliage in California. *Hilgardia* 45, 173-236.
- Croft, B.A., Jorgensen, C.D. 1969. Life history of *Typhlodromus mcgregori* (Acarina: Phytoseiidae). *Annals of the Entomological Society of America* 62, 1261-1267.

Croft, B.A., Luh, H.-K. 2004. Phytoseiid mites on unsprayed apple trees in Oregon, and other western states (USA): distributions, life-style types and relevance to commercial orchards. *Experimental and Applied Acarology* 33, 281-326.

Denmark, H.A., Andrews, K.L. 1981. Plant associated phytoseiidae of El Salvador, Central America (Acarina: Mesostigmata). *The Florida Entomologist* 64, 147-158.

Denmark, H.A., Evans, G.A. 2011. *Phytoseiidae of North America and Hawaii (Acari: Mesostigmata)*. Indira Publishing House, West Bloomfield.

DePew, L.J. 1960. Control of *Oligonychus pratensis* attacking winter wheat in western Kansas. *Journal of Economic Entomology* 53, 1061-1063.

Ehler, L.E. 1974. A review of the spider mite problem on corn and grain sorghum in west Texas. *Texas Agricultural Experiment Station Bulletin* 1149, 1-15.

Elmer, H.S. 1965. Banks grass mite, *Oligonychus pratensis*, on dates in California. *Journal of Economic Entomology* 58, 531-534.

El-Shafie, H.A.F. 2012. Review: list of arthropod pests and their natural enemies identified worldwide on date palm, *Phoenix dactylifera* L. *Agriculture and Biology Journal of North America* 3, 516-524.

Environmental Protection Agency. 1998. Hexythiazox, pesticide tolerances for emergency exemption. *Federal Register* 63, 54595-54599. Available at: <http://www.gpo.gov/fdsys/granule/FR-1998-10-13/98-27397>

Environmental Protection Agency. 2003. Hexythiazox, pesticide tolerance. *Federal Register* 68, 10370-10377. Available at: <http://www.federalregister.com/Browse/Document/usa/na/fr/2003/3/5/03-5194>

FAOSTAT. 2014. Food and Agricultural commodities production. Available at: <http://faostat3.fao.org/browse/Q/QC/E>

Gispert, C., Farrar, C., Perring, T.M. 2001. Seasonal abundance and the Banks grass mite *Oligonychus pratensis* (Banks) (Prostigmata: Tetranychidae) and a predatory mite, and their response to sulfur treatment on commercial date palms *Phoenix dactylifera* L. in Southern California. In: Halladay, R.B., Walter, D.E., Proctor, H.C., Norton, R.A., Colloff, M.J. (Eds.), *Proceedings of the 10th International Congress of Acarology*. CSIRO Publishing, Melbourne, pp. 403-408.

Harvey, T.L. 1954. Chemical control of a spider mite on wheat in eastern New Mexico. *Journal of Economic Entomology* 47, 593-597.

- James, D.G., Prischmann, D. 2010. The impact of sulfur on biological control of spider mites in Washington State vineyards and hop yards. In: Sabelis, M.W., Bruin, J. (Eds.). Trends in acarology. Proceedings of the 12th International Congress, Springer, Dordrecht, pp. 477-482.
- Jeppson, L.R., Keifer, H.H., Baker, E.W. 1975. Mites injurious to economic plants. University of California Press, Berkeley.
- Kehat, M., Blumberg, D. Greenburg, S. 1976. Fruit drop and damage in dates (*Myelois ceratoniae* Zeller-Pyralidae). Annales du Service des Epiphyties 4, 93-99.
- Krantz, G.W. 1978. A manual of acarology, 2nd edition. Oregon State University Bookstores, Inc., Oregon.
- Lehman, R.D. 1982. Mites (Acari) of Pennsylvania conifers. American Entomological Society 108, 181-286.
- Malcolm, D.R. 1955. Biology and control of the timothy mite, *Paratetranychus pratensis* (Banks). Washington Agricultural Experiment Station Bulletin 17, 1- 35.
- Mauk, P.A., Perring, T.M., Shea, T. 2005. Management of Banks grass mite in dates. University of California Cooperative Extension, Topics in Subtropics Newsletter 3, 1-2.
- McMurtry, J.A., Croft, B.A. 1997. Life-styles of phytoseiid mites and their roles in biological control. Annual Review of Entomology 42, 291-321.
- Muma, M.H. 1961. Subfamilies, genera and species of Phytoseiidae (Acarina: Mesostigmata). Bulletin of the Florida State Museum, Biological Science 5, 267-302.
- Muma, M.H. 1963. The genus *Galendromus* Muma, 1961 (Acarina: Phytoseiidae). The Florida Entomologist 1, 15-41.
- Nay, J.E. 2006. Biology, ecology, and management of the carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae), a pest of dates, *Phoenix dactylifera* L., in Southern California. Ph.D. Dissertation, University of California, Riverside.
- Negm, M.W., de Moraes, G.J., Perring, T.M. 2015. Mite pests of date palms. In: Wakil, W., Faleiro, J.R., Miller, T.A. (Eds.). Sustainable pest management in date palm: current status and emerging challenges. Springer International Publishing, Switzerland, pp. 347-389.
- Nixon, R.W. 1950. Imported cultivars of dates in the United States. United States Department of Agriculture Circular #834, Washington, DC.

- Nixon, R.W., Carpenter, J.B., 1978. Growing dates in the United States. United States Department of Agriculture Information Bulletin 207, 1-63.
- Owens, J.C., Ward, C.R., Teetes, G.L. 1976. Current status of spider mites in corn and sorghum. Proceedings of the 31st Annual Corn and Sorghum Research Conference 31, 38-64.
- Pate, T.L., Neeb, C.W. 1971. The Banks grass mite problem in the Trans-Pecos area of Texas. Texas Agricultural Experiment Station progress report #2871, Texas.
- Perring, T.M., Holtzer, T.O., Toole, J.L. 1984. Influence of temperature and humidity on pre-adult development of the Banks grass mite (Acari: Tetranychidae). Environmental Entomology 13, 338-343.
- Perring, T.M. 1983. Influences of temperature, humidity, and corn canopy microenvironment on population dynamics of the grass mite, *Oligonychus pratensis* (Banks). Ph.D. Dissertation, University of Nebraska.
- Perring, T.M., Archer, T.L., Krieg, D.L., Johnson, J.W. 1983. Relationships between the Banks grass mite (Acariformes: Tetranychidae) and physiological changes of maturing grain sorghum. Environmental Entomology 12, 1094-1098.
- Parasad, V. 2012. Checklist of the world (Acari: Mesostigmata). Indira Publishing House, West Bloomfield, Michigan.
- Prischmann, D.A., James, D.G. 2003. Phytoseiidae (Acari) on unsprayed vegetation in southcentral Washington: implications for biological control of spider mites on wine grapes. International Journal of Acarology 29, 279-287.
- Prischmann, D.A., James, D.G. 2005. Impact of management identity on mites (Acari: Tetranychidae, Phytoseiidae) in southcentral Washington wine grapes. International Journal of Acarology 31, 277-288.
- Pritchard, A.E., Baker, E.W. 1955. A revision of the spider mite family Tetranychidae. Memoirs of the Pacific Coast Entomological Society 2, 1-472.
- Reuveni, O. 1986. Date. In: Monselise, S.P. (Ed.). CRC handbook of fruit set and development. CRC Press, Boca Raton, pp. 119-144.
- Schuster, R.O., Pritchard, A.E. 1963. Pytoseiid mites of California. Hilgardia 34, 191-285.
- Schweissing, F.C. 1969. Cultural practice for possible use in reducing spider mite damage in corn. Colorado State University Experiment Station Progress Report, Colorado.

- Specht, H.B. 1968. Phytoseiidae (Acarina: Mesostigmata) in the New Jersey apple orchard environment with descriptions of spermathecae and three new species. *The Canadian Entomologist* 100, 673-692.
- Stickney, F.S., Barnes, D.F., Simmons, P. 1950. Date palm insects in the United States. United States Department of Agriculture Circular 846, 15-22.
- Tuttle, D.M., Baker, E.W. 1964. The spider mites of Arizona (Acarina: Tetranychidae). Arizona Agricultural Experiment Station Technical Bulletin 158, 1-38.
- Tuttle, D.M., Muma, M.H. 1973. Phytoseiidae (Acarina: Mesostigmata) inhabiting agricultural and other plants in Arizona. Arizona Agricultural Experiment Station Technical Bulletin 208, 1-55.
- USDA. 2016. United States Department of Agriculture noncitrus fruits and nuts, 2015 summary. Available at:
<http://usda.mannlib.cornell.edu/usda/current/NoncFruiNu/NoncFruiNu-07-06-2016.pdf>.
- Vincent, L.E., Lindgren, D.L. 1958. Control of the date mite, *Oligonychus pratensis* (Banks) in California. *Date Growers' Institute Annual Report* 35, 15-17.
- Wainstein, B.A. 1962 Révision du genre *Typhlodromus* Scheuten, 1857 et systématique de la famille des Phtoseiidae (Berlese, 1916). (Acarina: Parasitiformes). *Acarologia* 4, 5-30.
- Wakil, W., Faleiro, J.R., Miller, T.A., Bedford, G.O., Krueger, R.R. 2015. Date palm production and pest management challenges. In: Wakil, W., Faleiro, J.R., Miller, T.A. (Eds.). *Sustainable pest management in date palm: current status and emerging challenges*. Springer International Publishing, Switzerland, pp. 1-11.
- Walter, V.E., Wene, G.P. 1956. *Oligonychus pratensis*, a new mite on corn. *Journal of Economic Entomology* 49, 255-265.
- Warner, R.L. 1988. Contributions to the biology and the management of the carob moth, *Ectomyelois ceratoniae* (Zeller) in 'Deglet noor' date gardens in the Coachella Valley of California. Ph.D. Dissertation, University of California, Riverside.
- Wrigley, G. 1995. Date palm. In: Smartt, J., Simmonds, N.W. (Eds.). *Evolution of crop plants*. 2nd edition. Longman Group, Essex, pp. 399-403.
- Zaid, A., Arias-Jimenez, E.J. 2002. Date palm cultivation. Food and Agriculture Organization Plant Production and Protection Paper #156, Rome.

Zaid, A., de Wet, P.F. 2002a. Date palm propagation. In: Zaid, A. (Ed.). Date palm cultivation. Food and Agriculture Organization Plant Production and Protection Paper #156, Rome, pp. 73-105.

Zaid, A., de Wet, P.F. 2002b. Pollination and bunch management. In: Zaid, A. (Ed.). Date palm cultivation. Food and Agriculture Organization Plant Production and Protection Paper #156, Rome, pp. 145-175.

Zohary, D., Hopf, M. 2000. Domestication of plants in the old world: the origin and spread of cultivated plants in west Asia, Europe, and the Nile Valley. Oxford University Press, Oxon.

Chapter 2

Prey Stage Preference and Functional Response of *G. flumenis*

Abstract

The Banks grass mite, *Oligonychus pratensis* (Banks) (Acari: Tetranychidae), is a serious pest in dates (*Phoenix dactylifera* L.) in the New World. Currently Banks grass mite is managed using the miticide, Savey[®], and alternative strategies are necessary to remove pressure from a single control method due to the risk of resistance evolution. For this purpose, studies are underway to develop biological control strategies using the predatory mite, *Galendromus flumenis* (Chant) (Acari: Phytoseiidae). The current study determined the consumption rate of *G. flumenis* at constant densities of Banks grass mite eggs, larvae, protonymphs and deutonymphs, and defined the functional response of predator females. The predator consumed significantly more eggs than other prey stages, and displayed a Type II functional response on all prey stages. The highest attack rate and shortest handling time were obtained for predators feeding on prey larvae and eggs, respectively. The proportions of prey consumed by *G. flumenis* were higher at lower densities for all stages of Banks grass mite, implying that *G. flumenis* should be more effective at suppressing Banks grass mite populations at lower densities. Therefore, in an augmentative release program, *G. flumenis* would need to be released early in the infestation.

Introduction

The Banks grass mite, *Oligonychus pratensis* (Banks) is native to the United States (Stickney et al. 1950) and widely distributed throughout the country (Malcolm 1955; Pritchard and Baker 1955; Elmer 1965; McGregor and Stickney 1965; USDA 1972; Jeppson et al. 1975). Banks grass mite is reported from a wide range of host plants in 10

plant families, but economic losses occur in just two host families; the Arecaceae and Poaceae (Perring unpublished data). Damaged crops include dates (Stickney et al. 1950; Elmer 1965), corn (Walter and Wene 1956; Ehler 1974; Owens et al. 1976), Bermuda grass (Tuttle and Baker 1964; Carpenter and Elmer 1978), grain sorghum (Flechtmann and Hunter 1971; Ehler 1974; Owens et al. 1976), sugar cane (Jeppson et al. 1975), and wheat (Harvey 1954; DePew 1960). Banks grass mite first was reported on date palms, *Phoenix dactylifera* L., in California by Banks (1914), and has become a serious pest for date producers. The mites feed on date fruit in the Kimri stage (green fruits) where their rasping of the fruit surface causes cracked skin (Elmer 1965) and bronzing scars (Carpenter and Elmer 1978), resulting in unmarketable fruit.

Oligonychus pratensis management depends mainly on chemical applications. Currently there are two materials registered for this use, sulfur dust and Savey® (Hexythiazox). However, sulfur dust suppresses natural enemies, leading to Banks grass mite outbreaks (Gispert et al. 2001), and resistance to sulfur has been demonstrated (Perring unpublished data). In addition, dusting is unpopular with surrounding communities, thus only a handful of organic growers still use sulfur dust. This leaves the date industry with Savey®, a material effective with a single early-season application (Mauk et al. 2005), but not a sound practice as a single method of mite control due to the risk of resistance evolution.

An alternative and ecologically friendly control measure against Banks grass mite is biological control using predatory mites in the family Phytoseiidae. *Galendromus flumenis* (Chant) is naturally present on date bunches in the Coachella Valley (Riverside County,

California). It first was described as *Typhlodromus flumenis* by Chant (1957) from mites collected on soapberry, *Shepherdia canadensis* Nutt (Family: Elaeagnaceae), in British Columbia, Canada. In the United States, *G. flumenis* has been identified on deciduous and evergreen trees, shrubs and herbs (Schuster and Pritchard 1963; Specht 1968; Tuttle and Muma 1973; Lehman 1982; Prischmann and James 2003), and it has been reported as a dominant species in non-sprayed apple orchards (Croft and Luh 2004) and vineyards (Prischmann and James 2003). Reported prey include Tetranychidae, Tenuipalpidae, Eriophyiidae, Winterschmidtidae, and small insects such as thrips, whiteflies, and Pollen (Croft and Jorgensen 1969; Blackwood et al. 2004). Based on the life-style classification by McMurtry and Croft (1997), *G. flumenis* is placed on the specialist side of Type III approaching Type II (Blackwood et al. 2004). This classification is due to the fact that *G. flumenis* performs relatively poorly on pollens compared to Type III species, and is not well adapted for predation on *Tetranychus sp.* compared to Type II species.

Currently there is no information on predator-prey interactions between *G. flumenis* and Banks grass mite on dates. Therefore, a set of experiments were designed to provide insight into the potential efficiency of this predatory mite for managing Banks grass mite on dates, as part of an integrated pest management program. The objectives of this study were to determine the consumption rate and prey stage preference of *G. flumenis* when offered different stages of Banks grass mite at constant densities, and to evaluate the functional response of *G. flumenis* to each prey stage.

Materials and Methods

Mite Culture Maintenance

Initial populations of both Banks grass mite and *G. flumenis*, were collected from date bunches in the Coachella Valley and a colony of Banks grass mite was established on field corn (*Zea mays* variety 31G71). Corn plants were grown in 1-gallon pots in a greenhouse, and those with 7-8 fully developed leaves were moved to an environmentally controlled room (30°C, 50±10% RH and 16L: 8D photoperiod) and infested with Banks grass mite (Figure 2-1). Predatory mites were reared on a 10×10 cm black ceramic tile resting on a water-saturated foam in a stainless steel pan (20×20 cm). Strips of tissue paper were placed around the edges of the tile to prevent mites from escaping. Water was added to the pans daily to keep the tissue papers wet. A microscope cover slip with a few cotton strands underneath was placed on the tile to provide ovipositional sites for the predatory mites (McMurtry and Scriven 1965) (Figure 2-2). Predators were fed three times a week with mixed life stages of Banks grass mite brushed from infested corn leaves using a mite brushing machine (Bioquip Products, Rancho Dominguez, California). The rearing arenas were maintained in a growth chamber (Conviron, model EF7-H) at 30±1°C, 50±10% RH, and 16L: 8D photoperiod.



Figure 2-1 Field corn plants (*Zea mays*) grown in 1-gallon pots in the greenhouse (Left), corn plants infested with Banks grass mite in an environmentally controlled room (Middle), and an infested corn leaf with higher magnification (Right).

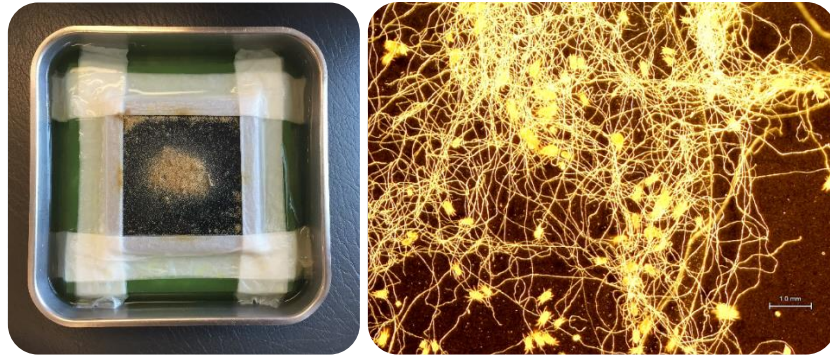


Figure 2-2 Rearing arena for *Galendromus flumenis* made according to McMurtry and Scriven (1965) (Left), and higher magnification of cotton strands with mites using them as shelter and ovipositional sites (Right).

Experimental Procedure

Newly-emerged *G. flumenis* females were transferred individually from the stock colony to experimental arenas, into which was added a male from the colony. Experimental arenas consisted of a corn leaf abaxial side up on a wet cotton layer in a Petri dish (3 cm diameter). This Petri dish had a 5 mm hole drilled in its bottom, and was placed in the middle of a larger 5 cm diameter Petri dish containing water. The leaf margin was surrounded with a water-saturated cotton strip to confine mites and to maintain freshness in the leaf. Each 5-cm dish had a lid ventilated with a 1-cm hole covered with fine mesh (Figure 2-3). The *G. flumenis* mite pairs were provided with a mix of all prey stages, and allowed to feed and mate for 24 h, and then females were transferred individually to new arenas and were starved for the next 24 h. In the prey stage preference test, each 24 h starved female predator was provided with 50 Banks grass mite eggs, newly emerged larvae, protonymphs and deutonymphs for a total of 200 prey items. In preliminary experiments, *G. flumenis* females did not feed on adult prey in the presence of immature stages (0/15), thus adults were excluded from the experiment. Predators were allowed to

feed on prey items for 24 h after which the number of prey individuals consumed per predator was counted. Fifteen *G. flumenis* females were used as replicates.

The same type of experimental arenas were used for the functional response experiments. Each 24 h starved female predator was exposed to densities of 2, 4, 8, 16, 32, and 64 Banks grass mite eggs or newly emerged larvae, protonymphs and deutonymphs. An additional density of 128 larvae and densities of 128, 160, 192, and 224 eggs were tested in order to ensure that the predators were satiated. Fifteen *G. flumenis* females were used as replicates for each density level. After 24 h, the number of prey individuals consumed was recorded.

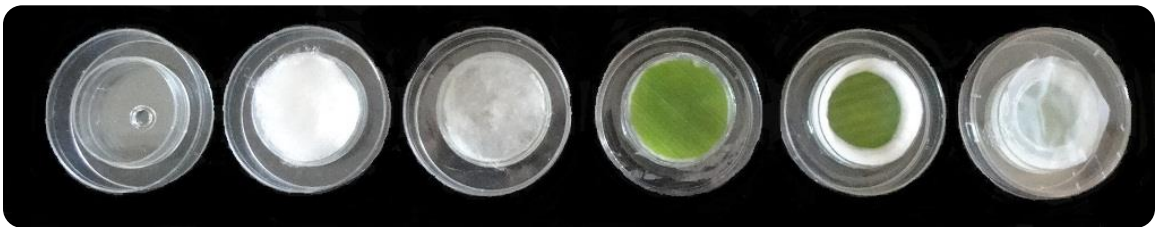


Figure 2-3 Experimental arena made with corn leaf (Left to right). A 3 cm diameter Petri dish with a 5 mm hole drilled in its bottom was placed in the middle of a larger 5 cm diameter Petri dish containing water. A cotton layer was placed in the small Petri dish and wetted with water on top of which a corn leaf abaxial side up was put. The leaf margin was surrounded with a water-saturated cotton strip to confine mites and to maintain freshness in the leaf. Each 5-cm dish had a lid ventilated with a 1-cm hole covered with fine mesh.

Statistical analysis

The numbers of prey consumed by *G. flumenis* were tested for normality (Shapiro-Wilk test (Shapiro and Wilk 1965)) and homogeneity of variances (Bartlett test (Snedecor and Cochran 1989)) using a significance level of 0.05. Data then were analyzed by one-way ANOVA followed by Games-Howell test ($P < 0.01$) using R version 3.0.3 for windows (R Core Team 2014).

A logistic regression of the proportion of prey consumed as a function of initial prey density was used to determine the shape of the functional response curve of *G. flumenis* to different stages of Banks grass mite:

$$\frac{N_e}{N_0} = \frac{\exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}{1 + \exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}$$

where N_e is the number of prey consumed, N_0 is the initial prey density, (N_e/N_0) is the probability of prey consumption, and P_0 , P_1 , P_2 , and P_3 are the maximum likelihood estimates of the intercept, linear, quadratic, and cubic coefficients, respectively (Juliano 2001). The sign of P_1 and P_2 are used to determine the type of functional response. When the linear coefficient is significantly negative ($P_1 < 0$), the predator displays a type II functional response which indicates the proportion of prey consumed declines monotonically with the initial prey density. When the linear coefficient is positive ($P_1 > 0$), and the quadratic coefficient is negative ($P_2 < 0$), the predator displays a type III functional response (Juliano 2001). The logistic regression analysis indicated that our data fit a type II functional response for all stages of Banks grass mite, therefore, further analyses were restricted to a type II response. Since the experiment was carried out without prey replacement during the course of the experiment, the appropriate model to estimate the handling times (T_h) and the attack rates (a) for a type II functional response is the Roger's random predator equation (Rogers 1972):

$$N_e = N_0[1 - \exp(\alpha(T_h N_e - T))]$$

in which N_e is the number of prey consumed, N_0 is the initial prey density, T is the searching time (24 h), a is the attack rate, and T_h is the handling time. Nonlinear least square

regression (NLIN procedure, SAS Institute 2012) was used to estimate parameters of the Roger's equation. After a and T_h were determined for the original data (m_i), the differences among a values, as well as T_h values were tested for significance by estimating the variance using the jackknife technique (Meyer et al. 1986). The jackknife pseudo-values (m_j) were calculated for the n samples using the following equations:

$$m_{j\alpha} = n \cdot m_{t\alpha} - (n - 1)m_{i\alpha}$$

$$m_{jT_h} = n \cdot m_{tT_h} - (n - 1)m_{iT_h}$$

The mean values of $(n-1)$ jackknife pseudo-values for a and T_h for each prey stage were subjected to Kruskal-Wallis test (nonparametric ANOVA), followed by Dunn's multiple comparison test ($P < 0.01$) (R Core Team 2014).

Results

The mean consumption rate of *G. flumenis* significantly differed across four Banks grass mite life stages. The highest number of prey consumed was observed for eggs, followed by larvae, protonymphs and deutonymphs, respectively (Table 2-1). The predator consumed 46, 25, 14 and 3% of the initial density of eggs, larvae, protonymphs and deutonymphs, respectively (Table 2-1).

Logistic regression yielded a significant negative linear parameter ($P_1 < 0$) for all prey stages, suggesting that the predator displayed a Type II functional response on all life stages (Table 2-2). Functional response curves showed a higher proportion of prey consumed at lower densities of all prey stages (Figure 2-4). The proportion of prey consumed for each prey stage declined with increasing prey density.

The attack rate (α) was highest for predators feeding on larvae ($1.7974 \pm 0.0748 \text{ h}^{-1}$), followed by eggs, protonymphs and deutonymphs (Table 2-3). The shortest handling time (T_h) was observed on eggs ($0.1531 \pm 0.0012 \text{ h}$), followed by larvae, protonymphs, and deutonymphs. However, there was no significant difference between the handling times estimated for protonymphs and deutonymphs (Table 2-3). The coefficient of determinations, R^2 [1– (residual sum of squares/ total sum of squares)], and standard errors of the estimated parameters indicated that the random predator equation described the functional response of *G. flumenis* appropriately.

Table 2-1 Mean consumption rate and proportion of prey consumed (\pm SE) for *Galendromus flumenis* feeding on different stages of Banks grass mite

Prey Stage	Mean prey consumption	N_e/N_0
Egg	$22.80 \pm 1.76 \text{ a}$	$0.46 \pm 0.04 \text{ a}$
Larva	$12.53 \pm 1.20 \text{ b}$	$0.25 \pm 0.02 \text{ b}$
Protonymph	$6.87 \pm 1.02 \text{ c}$	$0.14 \pm 0.02 \text{ c}$
Deutonymph	$1.47 \pm 0.26 \text{ d}$	$0.03 \pm 0.01 \text{ d}$

Means within the same column followed by the same letter are not significantly different (Games-Howell Test, $P < 0.01$); N_e : the number of prey consumed; N_0 : initial prey density

Table 2-2 Maximum likelihood estimates from logistic regressions of the proportion of prey consumed as a function of initial Banks grass mite prey density by adult female *Galendromus flumenis*

Prey Stage	Parameters	Estimates	SE	χ^2	P value
Egg	Intercept (P_0)	9.3146	1.3263	49.33	<0.0001
	Linear (P_1)	-0.0870	0.0255	11.60	0.0007
	Quadratic (P_2)	0.000317	0.000161	3.86	0.0494
	Cubic (P_3)	-4.54E-7	3.31E-7	1.88	0.1698
Larva	Intercept (P_0)	12.4511	3.0056	17.16	<0.0001
	Linear (P_1)	-0.5720	0.1669	11.75	0.0006
	Quadratic (P_2)	0.00944	0.00264	12.78	0.0003
	Cubic (P_3)	-0.00004	0.000012	14.20	0.0002
Protonymph	Intercept (P_0)	6.9330	1.6326	18.03	<0.0001
	Linear (P_1)	-0.4426	0.1887	5.50	0.0190
	Quadratic (P_2)	0.00914	0.00613	2.23	0.1357
	Cubic (P_3)	-0.00006	0.000056	1.32	0.2507
Deutonymph	Intercept (P_0)	3.4997	0.4852	52.03	<0.0001
	Linear (P_1)	-0.3458	0.0647	28.53	<0.0001
	Quadratic (P_2)	0.00974	0.00230	17.96	<0.0001
	Cubic (P_3)	-0.00009	0.000022	14.97	0.0001

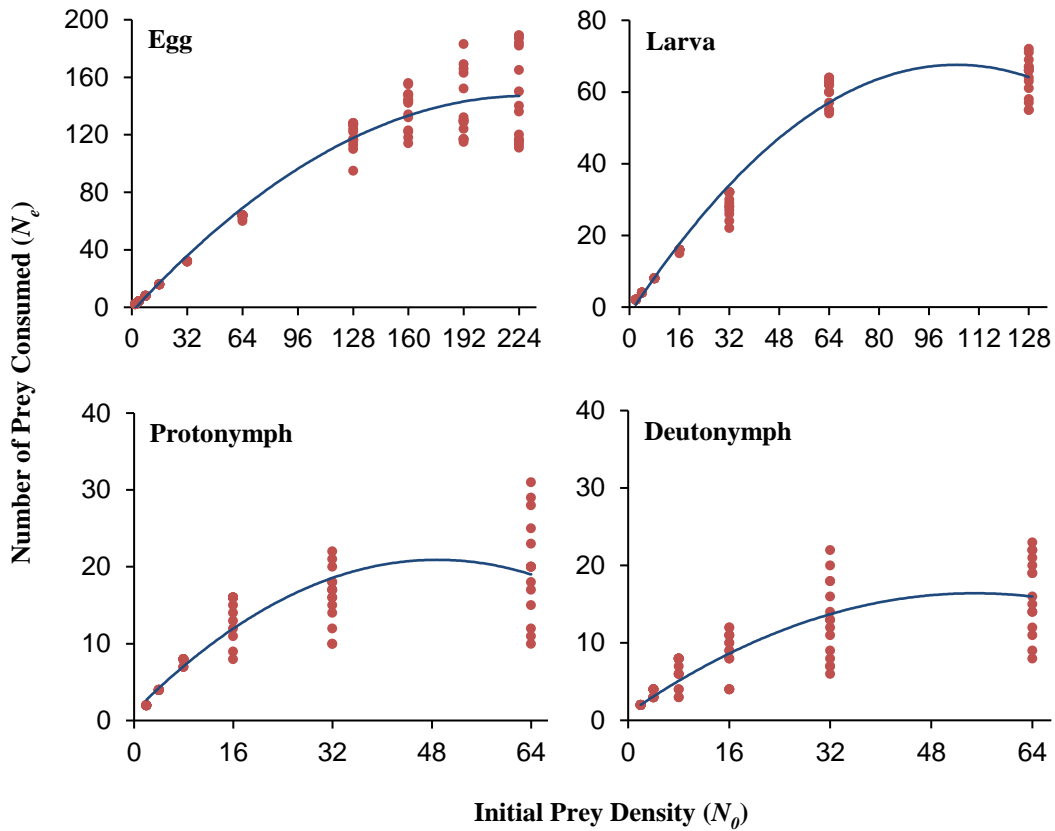


Figure 2-4 Functional responses of adult female *Galendromus flumenis* to different stages of Banks grass mite. Points represent the observed numbers of prey consumed at each initial prey density, and lines were predicted by the random predator equation (Rogers 1972).

Table 2-3 Mean values \pm SE of attack rate and handling time for *Galendromus flumenis* feeding on different life stages of Banks grass mite

Prey Stage	Attack rate (a)	Asymptotic 95% CI		Handling time (T_h)	Asymptotic 95% CI		R^2
		Lower	Upper		Lower	Upper	
Egg	0.6083 ± 0.0183 b	0.1657	0.9792	0.1531 ± 0.0012 a	0.1387	0.1660	0.98
Larva	1.7974 ± 0.0748 a	0.4812	2.7277	0.3692 ± 0.0016 b	0.3574	0.3815	0.99
Protonymph	0.2291 ± 0.0093 c	0.0806	0.3405	1.1619 ± 0.0201 c	1.0313	1.2939	0.94
Deutonymph	0.0664 ± 0.0023 d	0.0403	0.0946	1.2259 ± 0.0239 c	1.0134	1.4394	0.90

Means within the same column followed by the same letter are not significantly different (Dunn's Multiple Comparison Test, $P < 0.01$).

Discussion

Galendromus flumenis prefers eggs of Banks grass mite over other stages when offered the choice. However, in a prey-stage preference study using *T. urticae* eggs and larvae as prey, 8 h starved *G. flumenis* females showed no preference (Blackwood et al. 2004). This suggests that prey-stage preference of *G. flumenis* may change by the prey species, predator hunger level, prey defense mechanisms, and/or the nutritional value of prey individuals (Sandness and McMurtry 1970; Blackwood et al. 2001; Badii et al. 2004; Carrillo and Pena 2012).

The consumption rate of *G. flumenis* is inversely related to the life stage, and thus size, of Banks grass mite. The greater consumption of eggs compared to the other stages could be explained by the following reasons: 1) *G. flumenis* has an innate tendency to feed on smaller prey; 2) eggs have lower biomass compared to other stages and predators need to feed on a greater number of eggs to get the same amount of nutrients; 3) eggs are immobile and defenseless, therefore, they are easier to handle and subdue; 4) it may be easier for predators to penetrate the egg chorion compared to the sclerotized cuticle of nymphs; 5) According to Perring et al. (1984), the egg stage is the longest immature stage of Banks grass mite (approximately 3 days at 30°C), therefore, eggs are available to predators for more time than the other immature prey stages. A preference for eggs is an important factor from a pest management point of view as this predator will kill a large number of eggs before they hatch and begin to feed on the dates.

The negative values estimated for the linear parameters, as well as the inverse density-dependent relationship between the proportion of prey consumed and the initial prey

density on all life stages of the prey, are compatible with a Type II functional response. Type II functional responses are common in many phytoseiid species, including *Typhlodromus bagdasarjani* Wainstein and Arutunjan (Farazmand et al. 2012), *Neoseiulus californicus* (McGregor) (Farazmand et al. 2012), *Neoseiulus womersleyi* (Schicha) (Ali et al. 2011), *Kampimodromus aberrans* (Oudemans) (Kasap and Atlihan 2011), *Euseius hibisci* (Chant) (Badii et al. 2004), *Phytoseiulus persimilis* Athias-Henriot (Skirvin and Fenlon 2003), and *Amblyseius cucumeris* Oudemans (Shipp and Whitfield 1991). Holling (1965) stated that only predators displaying a Type III functional response contribute to regulating their prey populations because only this type of response will allow for long-term persistence. However, while functional response is an important aspect of natural enemy behavior, it is not the sole factor determining the success or failure of a biological control agent. Other aspects, such as predator numerical response, high intrinsic rate of population increase, high searching efficiency (Hassell 1978), prey patchiness, intraguild predation, competition, host plant characteristics, and abiotic environmental factors can influence the efficiency of predators (Cédola et al. 2001; Pervez and Omkar 2005; Ahn et al. 2010; Gontijo et al. 2012). In addition, the functional response of a predator may shift from Type II to Type III in response to environmental conditions, such as host plant (Skirvin and Fenlon 2001), or the size of the search areas (Takafuji and Deguchi 1980). Higher proportions of prey were consumed by *G. flumenis* at lower densities of all stages of Banks grass mite, suggesting a high searching ability of *G. flumenis*, and implying that the predator should be more effective at suppressing Banks grass mite populations at lower

densities. Therefore, in an augmentative release program, *G. flumenis* would need to be released early in the infestation.

The attack rate and handling time are parameters used to determine the magnitude of functional responses (Pervez and Omkar 2005). The handling time is a good indicator of the consumption rate and predator efficacy since it determines the cumulative time spent on capturing, killing, and digesting the prey (Veeravel and Baskaran 1997). Handling time may be affected by various factors such as the speed of the predator, prey movement, and the time spent subduing individual prey (Hassell 1978), which may be related to behavioral and physical prey defense mechanisms (Ali et al. 2011). In the present study, the handling time recorded for Banks grass mite eggs was less than other stages, which was expected due to the immobility of eggs and their inability to defend themselves. However, the highest attack rate was observed on Banks grass mite larvae. Since larvae are small, but motile, and eggs are stationary, encounter rates between *G. flumenis* and larvae will be greater than with eggs. Table 4 summarizes estimated attack rates and handling times for some phytoseiid species and compares them with those obtained for *G. flumenis*. Compared to other species, *G. flumenis* has the highest attack rate on larvae, and a relatively good attack rate on eggs. However, the attack rate on nymphal stages is much lower than other species, implying that *G. flumenis* will not be an effective control agent late in the infestation when the population is comprised of more nymphal stages. The handling time was also longer for nymphal stages (Table 4).

Functional response studies conducted in small laboratory arenas have been criticized for ignoring the environmental complexities and multispecies prey and/or predator systems

that occur in the field (O'Neil 1989; Wiedenmann and O'Neil 1991; Messina and Hanks 1998). In the current study, for example, the experiments were done in arenas where no or very little spider mite webbing was produced. In the field, all prey stages are surrounded by webbing which may limit the predation efficiency of the predator. Thus, future studies will evaluate the impact of *G. flumenis* on Banks grass mite in field trials, guided by the results of the current study which suggest that, because *G. flumenis* shows a Type II functional response, maximum impact will be obtained if it is released early in the season, when prey densities are low.

References

- Ahn, J.J., Kim, K.W., Lee, J.-H. 2010. Functional response of *Neoseiulus californicus* (Acari: Phytoseiidae) to *Tetranychus urticae* (Acari: Tetranychidae) on strawberry leaves. *Journal of Applied Entomology* 134, 98-104.
- Ali, M.P., Naif, A.A., Huang, D. 2011. Prey consumption and functional response of a phytoseiid predator, *Neoseiulus womersleyi*, feeding on spider mite, *Tetranychus macfarlanei*. *Journal of Insect Science* 11, 1-11.
- Badii, M.H., Hernández-Ortiz, E., Flores, A.E., Landeros, J. 2004. Prey stage preference and functional response of *Euseius hibisci* to *Tetranychus urticae* (Acari: Phytoseiidae, Tetranychidae). *Experimental and Applied Acarology* 34, 263-273.
- Banks, N. 1914. New mites. *Journal of Entomology and Zoology* 6, 1-57.
- Blackwood, J.S., Luh, H.K., Croft, B.A. 2004. Evaluation of prey-stage preference as an indicator of life-style type in phytoseiid mites. *Experimental and Applied Acarology* 33, 261-280.
- Blackwood, J.S., Schausberger, P., Croft, B.A. 2001. Prey-stage preference in generalist and specialist phytoseiid mites (Acari: Phytoseiidae) when offered *Tetranychus urticae* (Acari: Tetranychidae) eggs and larvae. *Environmental Entomology* 30, 1103-1111.
- Carpenter, J.B., Elmer, H.S. 1978. Pests and diseases of the date palm. United States Department of Agriculture Agricultural Handbook 527, 1-42.
- Carrillo, D., Pena, J.E. 2012. Prey-stage preferences and functional and numerical responses of *Amblyseius largoensis* (Acari: Phytoseiidae) to *Raoiella indica* (Acari: Tenuipalpidae). *Experimental and Applied Acarology* 57, 361-372.
- Cédola, C.V., Sánchez, N.E., Liljesthröm, G.G. 2001. Effect of tomato leaf hairiness on functional and numerical response of *Neoseiulus californicus* (Acari: Phytoseiidae). *Experimental and Applied Acarology* 25, 819-831.
- Chant, D.A. 1957. Description of some phytoseiidae mites (Acarina: Phytoseiidae). Part I. Nine new species from British Columbia with key to the species of British Columbia. Part II. Redescription of eight species described by Berlese. *The Canadian Entomologist* 89, 289-308.
- Croft, B.A., Jorgensen, C.D. 1969. Life history of *Typhlodromus mcgregori* (Acarina: Phytoseiidae). *Annals of the Entomological Society of America* 62, 1261-1267.

- Croft, B.A., Luh, H.-K. 2004. Phytoseiid mites on unsprayed apple trees in Oregon, and other western states (USA): distributions, life-style types and relevance to commercial orchards. *Experimental and Applied Acarology* 33, 281-326.
- DePew, L.J. 1960. Control of *Oligonychus pratensis* attacking winter wheat in western Kansas. *Journal of Economic Entomology* 53, 1061-1063.
- Ehler, L.E. 1974. A review of the spider mite problem on corn and grain sorghum in west Texas. *Texas Agricultural Experiment Station Bulletin* 1149, 1-15.
- Elmer, H.S. 1965. Banks grass mite, *Oligonychus pratensis*, on dates in California. *Journal of Economic Entomology* 58, 531-534.
- Farazmand, A., Fathipour, Y., Kamali, K. 2012. Functional response and mutual interference of *Neoseiulus californicus* and *Typhlodromus bagdasarjani* (Acari: Phytoseiidae) on *Tetranychus urticae* (Acari: Tetranychidae). *International Journal of Acarology* 38, 369-376.
- Flechtmann, C.H.W., Hunter, P.E. 1971. The spider mites (Prostigmata: Tetranychidae) of Georgia. *Journal of the Georgia Entomological Society* 6, 16-30.
- Gispert, C., Farrar, C., Perring, T.M. 2001. Seasonal abundance and the Banks grass mite *Oligonychus pratensis* (Banks) (Prostigmata: Tetranychidae) and a predatory mite, and their response to sulfur treatment on commercial date palms *Phoenix dactylifera* L. in Southern California. In: Halladay, R.B., Walter, D.E., Proctor, H.C., Norton, R.A., Colloff, M.J. (Eds.). *Proceedings of the 10th international congress of acarology*, CSIRO Publishing, Melbourne, pp. 403-408.
- Gontijo, L.M., Nechols, J.R., Margolies, D.C., Cloyd, R.A. 2012. Plant architecture and prey distribution influence foraging behavior of the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae). *Experimental and Applied Acarology* 56, 23-32.
- Harvey, T.L. 1954. Chemical control of a spider mite on wheat in eastern New Mexico. *Journal of Economic Entomology* 47, 593-597.
- Hassell, M. 1978. *The dynamics of arthropod predator-prey systems*. Princeton University Press, Princeton.
- Holling, C.S. 1965. The functional response of predators to prey density and its role in mimicry and population regulation. *Memoirs of the Entomological Society of Canada* 48, 3-60.
- Jeppson, L.R., Keifer, H.H., Baker, E.W. 1975. *Mites injurious to economic plants*. University of California Press, Berkeley.

- Juliano, S.A. 2001. Nonlinear curve fitting: predation and functional response curves. In: Scheiner, S.M., Gurevitch, J. (Eds.). Design and analysis of ecological experiments 2. Oxford University Press, New York, pp. 178-196.
- Kasap, I., Atlihan, R. 2011. Consumption rate and functional response of the predaceous mite *Kampimodromus aberrans* to two-spotted spider mite *Tetranychus urticae* in the laboratory. *Experimental and Applied Acarology* 53, 253-261.
- Kuşutan, O., Çakmak, İ. 2009. Development, fecundity, and prey consumption of *Neoseiulus californicus* (McGregor) fed *Tetranychus cinnabarinus* Boisduval. *Turkish Journal of Agriculture and Forestry* 33, 19-28.
- Landeros, J., Cerda, P., Badii, M.H., Aguirre, L.A., Cerna, E., Ochoa, Y.M. 2013. Functional response of *Neoseiulus californicus* on *Tetranychus urticae* on apple leaves. *Southwestern Entomologist* 38, 79-84.
- Lehman, R.D. 1982. Mites (Acari) of Pennsylvania conifers. *American Entomological Society* 108, 181-286.
- Malcolm, D.R. 1955. Biology and control of the timothy mite, *Paratetranychus pratensis* (Banks). *Washington Agricultural Experiment Station Bulletin* 17, 1- 35.
- Mauk, P.A., Perring, T.M., Shea, T. 2005. Management of Banks grass mite in dates. *University of California Cooperative Extension, Topics in Subtropics Newsletter* 3, 1-2.
- McGregor, E.A., Stickney, F. 1965. Distribution and host plants of *Oligonychus pratensis* (Banks). *Proceedings of the Entomological Society of Washington* 67, 28.
- McMurtry, J.A., Croft, B.A. 1997. Life-styles of phytoseiid mites and their roles in biological control. *Annual Review of Entomology* 42, 291-321.
- McMurtry, J.A., Scriven, G.T. 1965. Insectary production of phytoseiid mites. *Journal of Economic Entomology* 58, 282-284.
- Messina, F.J., Hanks, J.B. 1998. Host plant alters the shape of the functional response of an aphid predator (Coleoptera: Coccinellidae). *Environmental Entomology* 27, 1196-1202.
- Meyer, J.S., Ingersoll, C.G., McDonald, L.L., Boyce, M.S. 1986. Estimating uncertainty in population growth rates: jackknife vs. bootstrap techniques. *Ecology* 67, 1156-1166.
- O'Neil, R.J. 1989. Comparison of laboratory and field measurements of the functional response of *Podisus maculiventris* (Heteroptera: Pentatomidae). *Journal of the Kansas Entomological Society* 62, 148-155.

- Owens, J.C., Ward, C.R., Teetes, G.L. 1976. Current status of spider mites in corn and sorghum. Proceedings of the 31st Annual Corn and Sorghum Research Conference 31, 38-64.
- Perring, T.M., Holtzer, T.O., Toole, J.L. 1984. Influence of temperature and humidity on pre-adult development of the Banks grass mite (Acari: Tetranychidae). Environmental Entomology 13, 338-343.
- Pervez, A., Omkar. 2005. Functional responses of coccinellid predators: an illustration of a logistic approach. Journal of Insect Science 5, 1-6.
- Prischmann, D.A., James, D.G. 2003. Phytoseiidae (Acari) on unsprayed vegetation in south central Washington: implications for biological control of spider mites on wine grapes. International Journal of Acarology 29, 279-787.
- Pritchard, A.E., Baker, E.W. 1955. A revision of the spider mite family Tetranychidae. Memoirs of the Pacific Coast Entomological Society 2, 1-472.
- Rasmy, A.H., Abou-Elella, G.M., Osman, M.A. 2014. Functional response of the phytoseiid mite *Typhlodromus negevi* Swirski and Amitai to the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae). Archives of Phytopathology and Plant Protection 47, 1327-1334.
- R Core Team, 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Rogers, D. 1972. Random search and insect population models. Journal of Animal Ecology 41, 353-360.
- Sandness, J.N., McMurtry, J.A. 1970. Functional response of three species of Phytoseiidae (Acarina) to prey density. The Canadian Entomologist 102, 692-704.
- SAS Institute. 2012. SAS enterprise guide 5.1. SAS Institute Inc., Cary, North Carolina.
- Schuster, R.O., Pritchard, A.E. 1963. Pytoseiid mites of California. Hilgardia 34, 191-285.
- Shapiro, S.S., Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). Biometrika 52, 591-611.
- Shipp, J.L., Whitfield, G.H. 1991. Functional response of the predatory mite, *Amblyseius cucumeris* (Acari: Phytoseiida), on western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). Environmental Entomology 20, 694-699.

- Skirvin, D.J., Fenlon, J.S. 2001. Plant species modifies the functional response of *Phytoseiulus persimilis* (Acari: Phytoseiidae) to *Tetranychus urticae* (Acari: Tetranychidae): implications for biological control. *Bulletin of Entomological Research* 91, 61-67.
- Skirvin, D.J., Fenlon, J.S. 2003. The effect of temperature on the functional response of *Phytoseiulus persimilis* (Acari: Phytoseiidae). *Experimental and Applied Acarology* 31, 37-49.
- Snedecor, G.W., Cochran, W.G. 1989. *Statistical methods*. Iowa State University Press, Iowa.
- Specht, H.B. 1968. Phytoseiidae (Acarina: Mesostigmata) in the New Jersey apple orchard environment with descriptions of spermathecae and three new species. *The Canadian Entomologist* 100, 673-692.
- Stickney, F.S., Barnes, D.F., Simmons, P. 1950. Date palm insects in the United States. United States Department of Agriculture Circular 846, 15-22.
- Takafuji, A., Deguchi, K. 1980. Functional responses of a predacious phytoseiid mite in different sizes of experimental universe. *Applied and Experimental Zoology* 15, 355-357.
- Tuttle, D.M., Baker, E.W. 1964. The spider mites of Arizona (Acarina: Tetranychidae). *Arizona Agricultural Experiment Station Technical Bulletin* 158, 1-38.
- Tuttle, D.M., Muma, M.H. 1973. Phytoseiidae (Acarina: Mesostigmata) inhabiting agricultural and other plants in Arizona. *Arizona Agricultural Experiment Station Technical Bulletin* 208, 1-55.
- USDA. 1972. Banks grass mite situation in the U.S.-1971. United States Department of Agriculture Cooperative Economic Insect Report 22, 218-223.
- Veeravel, R., Baskaran, P. 1997. Searching behaviour of two Coccinellid Predators, *Coccinella transversalis* Fab. and *Cheilomenes sexmaculatus* Fab., on eggplant infested with *Aphis gossypii* Glov. *International Journal of Tropical Insect Science* 17, 363-368.
- Walter, V.E., Wene, G.P. 1956. *Oligonychus pratensis*, a new mite on corn. *Journal of Economic Entomology* 49, 255-265.
- Wiedenmann, R.N., O'Neil, R.J. 1991. Searching behavior and time budgets of the predator *Podisus maculiventris*. *Entomologia Experimentalis et Applicata* 60, 83-93.

Chapter 3

Effects of Predator Density and Size of Searching Area on the Mutual Interference and Dispersal Rate of *G. flumenis*

Abstract

The Banks grass mite, *Oligonychus pratensis* (Banks) (Acari: Tetranychidae), causes significant damage to California dates if not controlled. Studies are underway to develop biological control strategies against the Banks grass mite in dates using the predatory mite *Galendromus flumenis* (Chant) (Acari: Phytoseiidae). This predator is found in low numbers that are insufficient for the economic suppression of Banks grass mites, and our research aims to understand why it fails to keep up with prey densities. In the current study, the hypothesis that mutual interference among *G. flumenis* females leads to reduced predation rate was tested. In addition, the effect of arena size on the foraging efficiency and dispersal rate of the predators was investigated. With increasing *G. flumenis* density, the searching efficiency of the predators decreased significantly from 0.280 to 0.123 in small arenas. Consequently, the per capita predation decreased significantly from 241.0 eggs to 89.8 eggs as predator densities increased from 1 to 5 females. The interference coefficient (m) was negative and estimated to be -0.524. In the large arenas, no significant difference was observed in searching efficiency at different predator densities, suggesting that no mutual interference existed at these levels. The arena size had a significant effect on the predation rate and searching efficiency of *G. flumenis* at low predator numbers. Also, the effects of predator density and arena size on the dispersal rate of the predators were significant. For both arenas, higher numbers of predators contributed to more dispersal. This effect was more profound in the small arena. These results provide a better understanding of the predator-prey interactions, and help in designing strategies for more efficient augmentative releases of *G. flumenis*.

Introduction

The Banks grass mite, *Oligonychus pratensis* (Banks) (Acari: Tetranychidae) first was reported on date palms, *Phoenix dactylifera* L., in California by Banks (1914), and currently is widely distributed throughout the world damaging dates, grain crops, and grasses (Negm et al. 2015). In California, Banks grass mite has been a serious pest of dates since the early 1900s. Every year, the date growers in the Coachella Valley (Riverside County, California) suffer from severe losses due to Banks grass mite infestations. A single early-season application of Savey[®] (Hexythiazox) is the main strategy that date growers use for Banks grass mite management. However, their concerns about the development of resistance to Savey[®] encourages a move towards studies on alternative control measures such as biological control. Mites in the family Phytoseiidae that have short life cycles, and the ability to use alternative foods and search efficiently for prey at low densities, are considered to be important predators of several phytophagous mites and small insects on various crops (McMurtry et al. 1970; Helle and Sabelis 1985; McMurtry and Croft 1997; McMurtry et al. 2013). *Galendromus flumenis* (Chant) is the only phytoseiid species and the key predator of Banks grass mite on date bunches in the Coachella Valley (Ganjisaffar and Perring 2015a). Here, we aim to understand the foraging behavior of *G. flumenis* and factors influencing the predator-prey interactions in an effort to predict the efficacy of *G. flumenis* after release and the biological control outcomes in the field. In addition, our work is informing potential release strategies for *G. flumenis* against Banks grass mite in dates (Ganjisaffar and Perring 2015a, b).

One of the simplest models to describe the density-dependent predator-prey dynamics

is the Nicholson-Bailey (1935) model. This model defines the area of discovery (a) as the average area searched by a predator during the search time. Therefore, the area of discovery is a measure of a predator's searching efficiency that remains constant and independent of prey and predator densities. However, Hassell and Varley (1969) suggested an inverse relationship between the predator's searching efficiency and its density, and incorporated the effect of predator density into the Nicholson-Bailey model to provide stability. This stabilizing effect was named mutual interference (m). They showed that the greater the interference constant, the greater the tendency for the interaction to become stable since at high predator densities, mutual interference reduces both the prey consumption and the predator's rate of increase. In fact, as predator density increases, individuals will waste a great portion of their time with encounters of other conspecifics rather than searching for and handling prey (Henne and Johnson 2010). Furthermore, females that are already feeding may stop when disturbed (Evans 1976). As a result, the attack rate and the predation rate will decline even though food is in excess (Evans 1976; Eveleigh and Chant 1982; Zhang and Croft 1995; Reis et al. 2003). Increasing densities of predators also may lead to higher dispersal rates from experimental arenas (Khuchlein 1966) and lower egg production per female (Evans 1976). In addition, high predator density can affect survival of some phytoseiid species such as *Amblyseius fallacis* (Garman), *Amblyseius andersoni* Chant, *Typhlodromus occidentalis* (Nesbitt), *Typhlodromus pyri* Scheuten, and *Phytoseiulus persimilis* Athias-Henriot (Zhang and Croft 1995; Nachman 2006). The objective of this study was to determine the potential effects of increasing densities of *G. flumenis* on its foraging efficiency and dispersal.

Materials and Methods

Mite Colony Maintenance

Colonies of *G. flumenis* and Banks grass mite have been maintained in our laboratory at $30\pm 1^\circ\text{C}$, $60\pm 10\%$ RH, and 16L: 8D photoperiod since they were collected from date bunches in the Coachella Valley in 2012. Every year, the colonies have been supplemented with field-collected mites to ensure genetic variability. Banks grass mite was reared on field corn plants (*Zea mays* variety 31G71) with 7-8 fully developed leaves. *G. flumenis* were reared on a 10×10 cm black ceramic tile resting on a water-saturated foam in a stainless steel pan (20×20 cm). The edges of the tile were covered with strips of tissue paper immersed in water in the pan to prevent mites from escaping. A microscope cover slip with a few cotton threads underneath was placed in the center of the tile to provide ovipositional site and shelter for the predatory mites (McMurtry and Scriven 1965; Ganjisaffar and Perring 2015a). Three times per week, predators were provided with mixed life stages of Banks grass mite brushed from infested corn leaves using a mite brushing machine (Bioquip Products, Rancho Dominguez, CA, USA).

Experimental Procedure

Newly-emerged *G. flumenis* females and males (the latter of unknown age) were moved from the stock colony to excised corn leaves (4-5 cm in width and 10 cm in length). The excised leaves were placed abaxial side up on a wet cotton layer in a Petri dish (14 cm diameter). This Petri dish had a 1 cm hole drilled in its bottom, and was placed in a pan containing water (Figure 3-1).



Figure 3-1 Arena with excised corn leaf for *Galendromus flumenis* females to feed and mate before being starved for the experiment.

Galendromus flumenis females were allowed to mate and feed on a mix of all prey stages for 48 h. Then, these gravid females were moved individually to arenas and were starved for the next 24 h. The arenas consisted of two Petri dishes, a 3 cm Petri dish with a 5 mm hole in its bottom was placed in a 5 cm Petri dish containing water. A cotton layer was placed in the small Petri dish on top of which a corn leaf cut to fit the 3 cm dish was placed abaxial side up. The leaf margins were covered with a water-saturated cotton strip to prevent mites from escaping and to maintain freshness of the leaf. A 1-cm hole was made in the lids of the large Petri dish, and covered with fine mesh for ventilation. Starved females at densities of 1, 2, 3, 4, and 5 were moved to experimental arenas with 1000 Banks grass mite eggs. To obtain these eggs, 150 Banks grass mite females were transferred to experimental arenas, and allowed to oviposit for 36-48 h. After this time, the females were removed and the eggs were counted and adjusted so that 1000 eggs were available in each arena. Only Banks grass mite eggs were used as prey since our previous experiments revealed that egg is the most preferred prey stage for *G. flumenis* females and 1000 Banks grass mite eggs would be in excess (Ganjisaffar and Perring 2015a). Predators were

allowed to feed 24 h, after which the number of Banks grass mite eggs consumed and the number of predators leaving the arenas were counted. *G. flumenis* females found alive on the wet cotton surrounding the experimental arenas were considered as leaving individuals. The experiments were conducted in small (5 cm diameter) and large (8 cm diameter) corn leaf arenas to test if the size of the arena (Figure 3-2), which determined the functional search area, had any effect on the predation capacity and dispersal of *G. flumenis*. Each *G. flumenis* density was replicated at least 10 times since the arenas with leaving predators were excluded from the predation analyses. The experimental arenas were maintained in a growth chamber (Percival model 130BLL) at $30\pm 1^{\circ}\text{C}$, a photoperiod of 16L: 8D and a relative humidity of $60\pm 10\%$ RH.

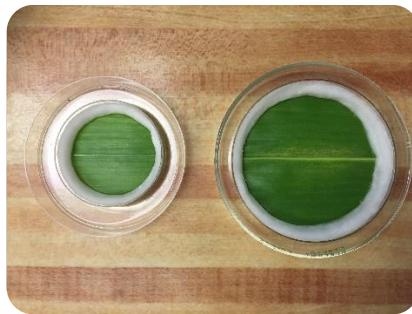


Figure 3-2 Two different arena sizes, small (5 cm diameter) and large (8 cm diameter) used in the study.

Statistical Analysis

Data of total predation, per capita predation and searching efficiency (a) of *G. flumenis* for each arena size (small and large) were tested for normality (Shapiro-Wilk Test (Shapiro and Wilk 1965)) and homogeneity of variances (Bartlett Test (Snedecor and Cochran 1989)) using a significance level of 0.05. Normal data then were analyzed for testing the differences in the mean values by one-way ANOVA followed by Tukey's HSD test ($P < 0.01$). For data that did not meet the homogeneity of variances assumption, Games-Howell

test ($P < 0.01$) was used for mean comparison. In addition, a comparison of the total predation, per capita predation, and searching efficiency (a) of *G. flumenis* at each predator density was done between the two different arena sizes. Normal data were tested for the homogeneity of variances (Variance Ratio Test) using a significance level of 0.05, and then were analyzed using either Two Sample t-test or Welch's t-test.

Searching efficiency (a) was estimated according to the following equation by Hassell (1978):

$$a = \left(\frac{1}{PT} \right) \ln \left(\frac{N_t}{N_t - N_a} \right)$$

where a is the searching efficiency, P is the predator density, T is the duration of the interaction in days (1 day in this experiment), N_t is the initial number of prey, and N_a is the total number of consumed prey. Mean searching efficiency values were fitted against predator densities on a logarithmic scale. The points were fitted to a linear regression using the Least Square Method according to the equation proposed by Hassell and Varley (1969):

$$\log a = \log Q - m \log P$$

where a is the searching efficiency, P is the predator density, Q is the quest constant (the searching efficiency for an individual predator), and m is the mutual interference constant (slope of the regression line of $\log a$ on $\log P$). Dispersal data for two arena sizes, five predator densities and their interactions were analyzed using a negative binomial generalized linear model ($P < 0.01$). Kruskal-Wallis rank sum test was used for mean comparison of *G. flumenis* dispersal rates at different predator densities ($P < 0.01$). All statistical analyses were performed using R version 3.3.0 for windows (R Core Team 2016).

Results

Total predation by *G. flumenis* females increased with predator density within both small ($F_{4,45} = 5.48$; $P < 0.01$) and large (Games-Howell test, $P < 0.01$) arenas. The highest number of Banks grass mite eggs were consumed at the predator density of 5 (448.9 ± 33.9 eggs in the small and 515.2 ± 22.6 eggs in the large arenas), however there was no significant difference in total predation among predator densities of 2, 3, 4, and 5 in the small arenas (Table 3-1). The per capita predation rates decreased significantly from 241.0 ± 23.1 eggs for 1 predator to 89.6 ± 6.8 eggs for 5 predators in small (Games-Howell test, $P < 0.01$) and from 134.1 ± 4.6 eggs for 1 predator to 103.0 ± 4.5 eggs for 5 predators in large arenas ($F_{4,45} = 4.41$; $P < 0.01$). There was no significant difference in the per capita predation among predator densities of 1, 2, and 3 in the small and among densities 1-4 in the large arenas (Table 3-1). The searching efficiency decreased significantly from 0.280 to 0.123 in small arena with increasing predator densities ($F_{4,45} = 7.28$; $P < 0.01$). However, in the large arena, no significant effect of predator density on searching efficiency of *G. flumenis* was observed ($F_{4,45} = 0.71$; $P = 0.59$) (Table 3-1).

Table 3-1 Predation and searching efficiency (mean \pm SE) of *Galendromus flumenis* at different predator densities on Banks grass mite eggs in two different size of arenas

Arena size	Predator density (<i>P</i>)	Total predation n=10	Per capita predation n=10	Searching efficiency (<i>a</i>) n=10
Small arena (5 cm Diameter)	1	241.0 \pm 23.1 b	241.0 \pm 23.1 a	0.280 \pm 0.032 a
	2	355.2 \pm 33.8 ab	177.6 \pm 16.9 ab	0.226 \pm 0.027 ab
	3	408.7 \pm 44.2 ab	136.2 \pm 14.7 abc	0.184 \pm 0.027 ab
	4	414.3 \pm 35.5 a	103.6 \pm 8.9 bc	0.138 \pm 0.015 b
	5	448.9 \pm 33.9 a	89.8 \pm 6.8 c	0.123 \pm 0.013 b
Large arena (8 cm Diameter)	1	134.1 \pm 4.6 d	134.1 \pm 4.6 a	0.144 \pm 0.005 a
	2	227.5 \pm 16.4 d	113.8 \pm 8.2 ab	0.130 \pm 0.010 a
	3	329.2 \pm 18.7 c	109.7 \pm 6.2 ab	0.134 \pm 0.010 a
	4	434.7 \pm 15.1 b	108.7 \pm 3.8 ab	0.144 \pm 0.007 a
	5	515.2 \pm 22.6 a	103.0 \pm 4.5 b	0.147 \pm 0.010 a

The effect of arena size on foraging behavior of *G. flumenis* was significant only at predator numbers of 1 (Total predation: $t(9.67)= 4.55$; Per capita predation: $t(9.67)= 4.55$; Searching efficiency: $t(9.49)= 4.22$, $P < 0.01$) and 2 (Total predation: $t(13.01)= 3.40$; Per capita predation: $t(13.01)= 3.40$; Searching efficiency: $t(11.58)= 3.29$, $P < 0.01$) (Figure 3-3). The effect of arena size was not significant for predator numbers of 3 (Total predation: $t(12.12)= 1.66$, $P= 0.12$; Per capita predation: $t(12.12)= 1.66$, $P= 0.12$; Searching efficiency: $t(11.36)= 1.77$, $P= 0.10$), 4 (Total predation: $t(12.18)= -0.53$, $P= 0.61$; Per capita predation: $t(12.17)= -0.53$; Searching efficiency: $t(12.45)= -0.33$, $P= 0.74$) and 5 (Total predation: $t(18)= -1.63$, $P= 0.94$; Per capita predation: $t(18)= -1.63$, $P= 0.94$; Searching efficiency: $t(18)= -1.49$, $P= 0.92$) (Figure 3-3).

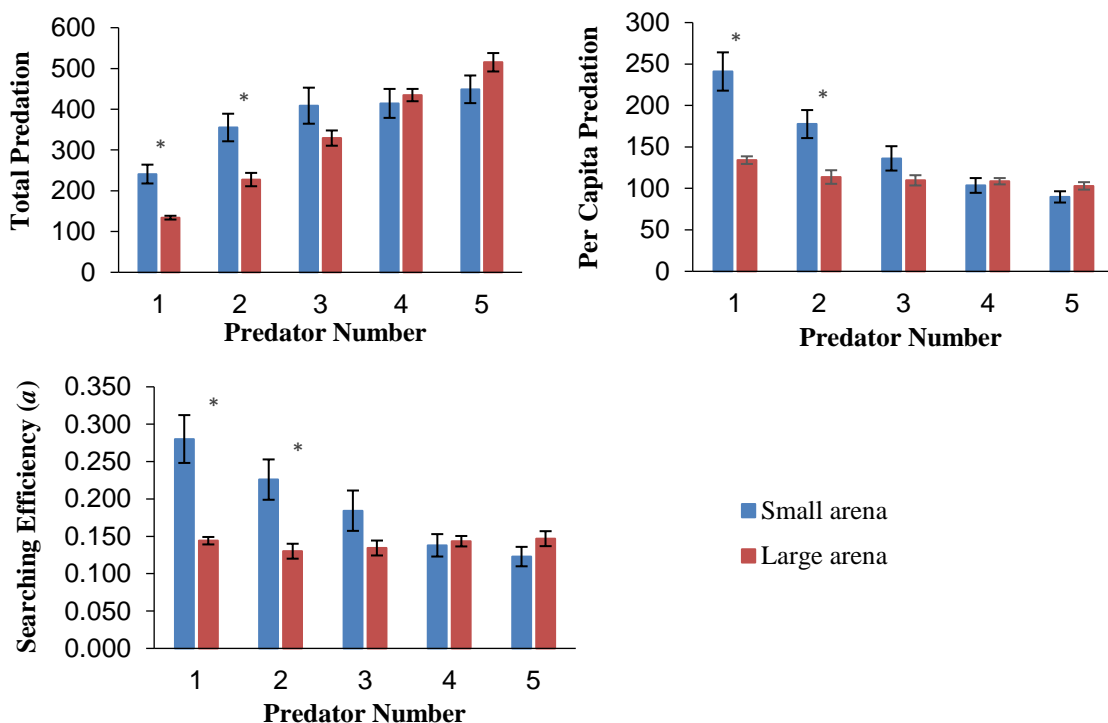


Figure 3-3 The effect of arena size on the foraging efficiency of *Galendromus flumenis* on Banks grass mite eggs. The asterisk symbol (*) was used to show that means are significantly different (Two Sample t-test or Welch's t-test, $P < 0.01$).

The linear regressions between the logarithm of searching efficiency and the logarithm of the predator density for small and large arenas are represented by the following equations:

Small arena: $\log a = -0.55 - 0.524 \log P$

Large arena: $\log a = -0.87 + 0.014 \log P$

The statistics shows that there was a linear relationship between the logarithm of searching efficiency and the logarithm of the predator density for the small arena ($F_{1,3}= 83.12, P < 0.01, R^2 = 0.965$). The slope of the regression line, i.e. the mutual interference constant (m), for the small arena was negative and estimated to be -0.524 (Figure 3-4). For the large arena, no significant relationship was observed between the logarithm of searching efficiency and the logarithm of the predator density ($F_{1,3}= 0.07, P = 0.81, R^2 = 0.022$) (Figure 3-4), suggesting that the mutual interference among *G. flumenis* females was negligible in this larger space.

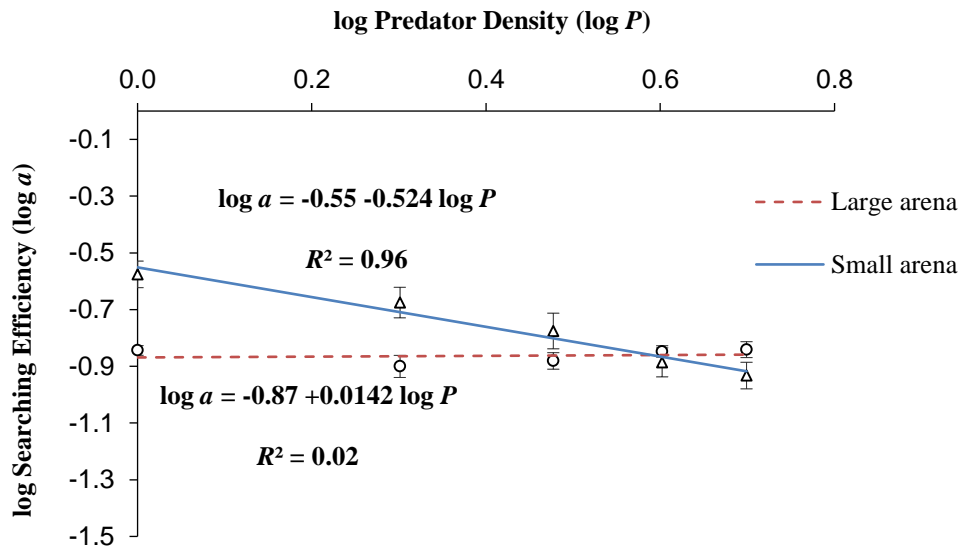


Figure 3-4 Regression line of mutual interference of *Galendromus flumenis* on Banks grass mite eggs.

The arena size and the predator density had a significant effect on the number of predators leaving the experimental arenas (dispersal rate) ($P < 0.01$). The interaction effect between predator density and the arena size was not significant ($P = 0.20$). In both small and large arenas, as density of *G. flumenis* females increased, the number of predators attempting to leave the arenas increased significantly (Figure 3-5). No dispersal was observed at predator density of 1 in the small arenas, and also at densities of 1-3 predators in the large arenas. However, at higher predator densities there were higher rates of dispersal, with more predators leaving in the small arenas (Figure 3-5).

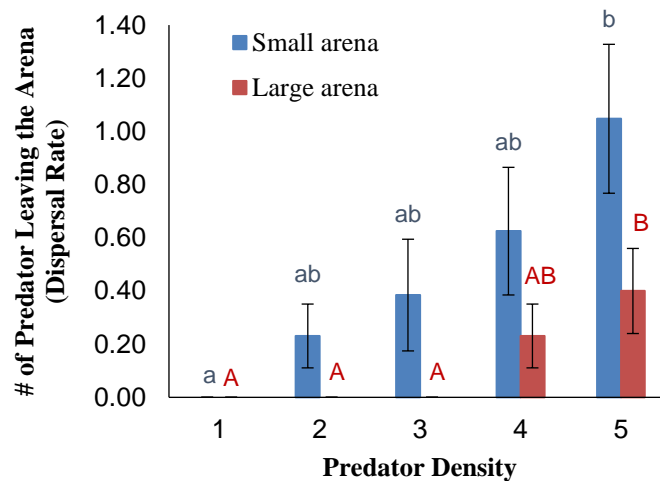


Figure 3-5 Effect of arena size and predator density on the dispersal rate of *Galendromus flumenis*. Bars with different lowercase letters (small arena) or uppercase letters (large arena) are significantly different (Kruskal-Wallis rank sum test, $P < 0.01$).

Discussion

Total predation increased with increasing density of *Galendromus flumenis*, however, it did not result in a proportional increase in the per capita predation due to the mutual interference effect which reduced the searching efficiency of the predatory mites. This interference effect has been demonstrated in other phytoseiid species, including *A. fallacis*,

A. andersoni, *T. occidentalis*, *T. pyri* (Zhang and Croft 1995), *P. persimilis* (Fernando and Hassel 1980; Nachman 2006), *Neoseiulus californicus* (McGregor), *Typhlodromus bagdasarjani* Wainstein & Arutunjan (Farazmand et al. 2012), and *Phytoseiulus plumifer* (Canestrini & Fanzago) (Khodayari et al. 2016). The interference effect is, in part, due to confinement in small experimental arenas which likely resulted in higher encounter rates between predators; our results indicated that in large arenas such mutual interference was insignificant due to reduced predator densities, and lower number of predators per square centimeters of the arena.

At low predator densities, when only 1 or 2 individuals were available in the experimental arenas, predation rate and searching efficiency of *G. flumenis* was higher in small arenas than in large arenas. This can be explained by the fact that in the small arenas, 1000 Banks grass mite eggs were laid close to each other, and predators required little searching time to find the prey. However, the same number of eggs in the large arena were dispersed at further distances from each other, thus requiring a greater amount of prey searching time by the predator. When predator density was increased to 3 or more individuals, there were no significant differences between small and large arenas in prey consumption and searching efficiency. This suggests that at the higher densities, while frequent encounters in the small arenas reduced consumption and searching efficiency due to predator interactions, these interactions did not occur in the large arenas, and each predator consumed the same number of prey and had the same searching efficiency.

It has been shown that mutual interference among predators affects the stability of predator-prey interactions; the greater the value of the mutual interference constant, the

greater the stability of the interaction (Beddington 1975). In fact, there is a trade-off between the benefits and costs of aggregation for predators (Van Der Meer and Ens 1997). Well-fed predators show area-concentrated searching behavior (Kaiser 1983) which results in aggregation of individuals in patches with abundant prey (Murdie and Hassell 1973). In this situation, they have high searching efficiency and consume large numbers of prey. The cost is that as they become more aggregated, mutual interference reduces predator foraging efficiency and thus their rate of increase (Eveleigh and Chant 1982). The present study shows that concentrating mites in a small area has the same impact of increasing mutual interference, and thereby reducing searching efficiency, and per capita predation. Another response to aggregation is cannibalism which leads to the elimination of potential intraspecific competitors for food, oviposition sites and shelters (Elgar and Crespi 1992; Schausberger 2003). However, we found no cannibalism in the present study. In addition, when predators aggregate in patches of high prey density, they likely will encounter each other, which may result in individuals attempting to leave the area. Kuchlein (1966) showed that increasing densities of the predatory mite, *Thyphlodromus longipes* Nesbitt, led to increased dispersal rates from experimental leaf arenas containing prey mites. The same trend was also observed in the present study.

Our laboratory experiments provided predators with only one stage of the prey (eggs) confined in small arenas. This may lead to biased and inaccurate predictions of the predator performance in the field when all life stages of prey are distributed in patches over larger spatial scales. Still, the findings from this study are important as they provide fundamental information regarding *G. flumenis-O. pratensis* interactions, information that is

challenging to obtain from field studies. For example, measuring the predation rate of *G. flumenis* at the scale of an entire date palm tree is not feasible because; 1) date palms are large trees with many fronds and bunches of fruits, 2) mites are very small and occur in large numbers, and 3) the remains of a consumed prey, especially eggs, are impossible to find and record. One possibility would be to assume that a single date fruit is the smallest homogenous spatial unit in the date palm, and that prey and predators on that fruit behave as in experimental leaf arenas. In this manner, parameters obtained from our arena experiment can be used to estimate predation rate of Y predators inhabiting a date fruit with X prey. Future field studies will estimate predator-prey ratios on date fruits which will enable us to draw more robust conclusions about the effectiveness of *G. flumenis* for Banks grass mite control.

This study also contributes to our growing knowledge in designing strategies for efficient augmentative releases of *G. flumenis*. Studies on parasitoids have shown that due to the mutual interference effect, higher release rates are less effective or even ineffective compared to lower release rates (Wen and Brower 1994; Hoddle et al. 1997). Since our results suggest that predator aggregation and mutual interference among predators will increase negative interactions in small spaces, we need to manage release rates to most effectively cover the search area, while at the same time, not adversely impacting the foraging efficiency of *G. flumenis*.

References

- Banks, N. 1914. New mites. *Journal of Entomology and Zoology* 6, 1-57.
- Beddington, J.R. 1975. Mutual interference between parasites or predators and its effect on searching efficiency. *Journal of Animal Ecology* 44, 331-340.
- Elgar, M.A., Crespi, B.J. 1992. *Cannibalism: ecology and evolution among diverse taxa*. Oxford University Press, United Kingdom.
- Evans, H.F. 1976. Mutual interference between predatory anthocorids. *Ecological Entomology* 1, 283-286.
- Eveleigh, E.S., Chant, D.A. 1982. Experimental studies on acarine predator-prey interactions: the effects of predator density on prey consumption, predator searching efficiency, and the functional response to prey density (Acarina: Phytoseiidae). *Canadian Journal of Zoology* 60, 611-629.
- Farazmand, A., Fathipour, Y., Kamali, K. 2012. Functional response and mutual interference of *Neoseiulus californicus* and *Typhlodromus bagdasarjani* (Acari: Phytoseiidae) on *Tetranychus urticae* (Acari: Tetranychidae). *International Journal of Acarology* 38, 369-376.
- Fernando, M.H.J.P., Hassell, M.P. 1980. Predator-prey responses in an acarine system. *Reseraches on Population Ecology* 22, 301-322.
- Ganjisaffar, F., Perring, T.M. 2015a. Prey stage preference and functional response of the predatory mite *Galendromus flumenis* to *Oligonychus pratensis*. *Biological Control* 82, 40-45.
- Ganjisaffar, F., Perring, T.M. 2015b. Relationship between temperature and development of *Galendromus flumenis* (Acari: Phytoseiidae), a predator of Banks grass mite (Acari: Tetranychidae). *Experimental and Applied Acarology* 67, 535-546.
- Hassell, M.P. 1978. *The dynamics of arthropod predator-prey systems*. Princeton, New Jersey.
- Hassell, M.P., Varley, G.C. 1969. New inductive population model for insect parasites and its bearing on biological control. *Nature* 223, 1113-1137.
- Helle, W., Sabelis, M.W. 1985. *Spider mites, their biology, natural enemies and control*. Vol. 1B. Elsevier, The Netherlands.

- Henne, D.C., Johnson, S.J. 2010. Laboratory evaluation of aggregation, direct mutual interference, and functional response characteristics of *Pseudacteon tricuspis* Borgmeier (Diptera: Phoridae). *Biological Control* 55, 63-71.
- Hoddle, M., Van Driesche, R., Sanderson, J. 1997. Biological control of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on poinsettia with inundative releases of *Encarsia formosa* (Hymenoptera: Aphelinidae): are higher release rates necessarily better? *Biological Control* 10, 166-179.
- Jeppson, L.R., Keifer, H.H., Baker, E.W. 1975. Mites injurious to economic plants. University of California Press, Berkeley.
- Kaiser, H. 1983. Small scale spatial heterogeneity influences predation success in an unexpected way: model experiments on the functional response of predatory mites (Acarina). *Oecologia* 56, 249-256.
- Khodayari, S., Fathipour, Y., Sedaratian, A. 2016. Prey stage preference, switching and mutual interference of *Phytoseius plumifer* (Acari: Phytoseiidae) on *Tetranychus urticae* (Acari: Tetranychidae). *Systematic and Applied Acarology* 21, 347-355.
- Khuchlein, J.H. 1966. Mutual interference among the predaceous mite *Typhlodromus longipes* Nesbitt (Acari: Phytoseiidae). 1. Effects of predator density on oviposition rate and migration tendency. *Mededelingen Rijksfaculteit Landbouwwetenschappen Gent* 31, 740-745.
- McMurtry, J.A., Croft, B.A. 1997. Life-styles of phytoseiid mites and their roles in biological control. *Annual Review of Entomology* 42, 291-321.
- McMurtry, J.A., de Moraes, G.J., Sourassou, N.F. 2013. Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. *Systematic and applied acarology* 18, 297-320.
- McMurtry, J.A., Huffaker, C.B., van de Vrie, M. 1970. Ecology of tetranychid mites and their natural enemies: a review. I. Tetranychid enemies: their biological characters and the impact of spray practices. *Hilgardia* 40, 331-390.
- McMurtry, J.A., Scriven, G.T. 1965. Insectary production of phytoseiid mites. *Journal of Economic Entomology* 58, 282-284.
- Murdie, G., Hassell, M.P. 1973. Food distribution, searching success and predator-prey models. In: Hiorns, R.W. (Ed.). *The mathematical theory of the dynamics of biological populations*. Academic Press, London, pp. 87-101.

- Nachman, G. 2006. The effects of prey patchiness, predator aggregation, and mutual interference on the functional response of *Phytoseiulus persimilis* feeding on *Tetranychus urticae* (Acari: Phytoseiidae, Tetranychidae). *Experimental and Applied Acarology* 38, 87-111.
- Negm, M.W., de Moraes, G.J., Perring, T.M. 2015. Mite pests of date palms. In: Wakil, W., Faleiro, J.R., Miller, T.A. (Eds.). *Sustainable pest management in date palm: current status and emerging challenges*. Springer International Publishing, Switzerland, pp. 347-389.
- Nicholson, A.J., Bailey, V.A. 1935. The balance of animal populations. *Proceedings of the Zoological Society of London* 105, 551-598.
- R Core Team. 2016. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna.
- Reis, P.R., Sousa, E.O., Teodoro, A.V., Neto, M.P. 2003. Effect of prey densities on the functional and numerical response of two species of predaceous mites (Acari: Phytoseiidae). *Neotropical Entomology* 32, 461-467.
- Shapiro, S.S., Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52, 591-611.
- Snedecor, G.W., Cochran, W.G. 1989. *Statistical methods*. Iowa State University Press, Iowa.
- Zhang, Z-Q., Croft, B.A. 1995. Intraspecific competition in immature *Amblyseius fallacis*, *Amblyseius andersoni*, *Tvohlodromus occidentalis* and *Tvohlodromus pyri* (Acari: Phytoseiidae). *Experimental and applied Acarology* 19, 65-77.
- Van Der Meer, J., Ens, B.J. 1997. Models of interference and their consequences for the spatial distribution of ideal and free predators. *Journal of Animal Ecology* 66, 846-858.
- Wen, B., Brower, J.H. 1994. Suppression of Maize Weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae), Populations in Drums of Corn by Single and Multiple Releases of the Parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae). *Journal of the Kansas Entomological Society* 67, 331-339.

Chapter 4
Relationship Between Temperature and Development of
G. flumenis

Abstract

The Banks grass mite, *Oligonychus pratensis* (Banks) (Acari: Tetranychidae), is a serious pest of grains, grasses and dates. In order to develop and optimize biological control strategies against the Banks grass mite, the survival and development of the predator, *Galendromus flumenis* (Chant) (Acari: Phytoseiidae), at eleven constant temperatures between 12°C and 44°C were determined. The survival rates of *G. flumenis* (67.4- 89.5%) were highest between 26°C and 38°C although it developed successfully from egg to adult at temperatures ranging from 18°C to 42°C. The lower temperature threshold (T_0) and thermal constant (K) for total immature development were 13.3°C and 145.3 degree-days, respectively. The upper temperature threshold was 44.3°C, and the optimal temperature for development was calculated to be 37.5°C. These results indicate that *G. flumenis* is better adapted to high temperatures than most predators in the Phytoseiidae. In addition, the thermal requirements for total development of *G. flumenis* was found to be very close to those of *O. pratensis* which indicates that there should be synchrony between the occurrence of the prey and the predator. Therefore, the lack of predation observed in the field is related to other factors, such as the developmental time between *O. pratensis* and *G. flumenis*, or the inability of the predator to establish at the same time, and in the same location, as the prey.

Introduction

The Banks grass mite, *Oligonychus pratensis* (Banks) (Acari: Tetranychidae) is a serious pest of dates, grain crops, and grasses in many parts of North America, Central America, South America, Africa, the Middle East, and Asia (Jepson et al. 1975). In the

United States where it is native, it has been a persistent pest of California dates since the early 1900s. Savey® (Hexythiazox) and sulfur dust are the only registered materials for Banks grass mite control on dates. Sulfur is rarely used due to environmental concerns (Gispert et al. 2001), safety issues (Ganjisaffar and Perring 2015a), and resistance of Banks grass mites to this chemical (Perring unpublished data). Savey® is an ovicide with an unknown mode of action which is used for the control of mite growth through activity on eggs or early developmental stages. It performs effectively with a single early-season application (Mauk et al. 2005), however, growers are concerned about development of resistance to Savey®. Therefore, to enhance biological control as an alternative control method, surveys for biological control agents of Banks grass mite in date gardens of the Coachella Valley (Riverside County, California) were conducted and revealed that *Galendromus flumenis* (Chant) (Acari: Phytoseiidae) was the only phytoseiid species on date bunches (Ganjisaffar and Perring 2015a).

The first description of *G. flumenis* was made by Chant (1957) from mites collected on soopolallie, *Shepherdia canadensis* Nutt (Family: Elaeagnaceae), in British Columbia, Canada. He named the mite *Typhlodromus flumenis* Chant. In 1961, Muma determined that this mite belonged in the new genus *Galendromus*, thus giving rise to *Galendromus flumenis* (Chant) (Muma 1961). A year later, both Wainstein (1962) and Hirschmann (1962) moved the mite back to the genus *Typhlodromus*. Schuster and Pritchard (1963) placed the mite in the genus *Metaseiulus*. In the same year, Muma (1963) moved the mite back to the genus *Galendromus*, giving its current binomial name, *Galendromus flumenis* (Chant). According to Prasad (2012), there is still a controversy about the genus of this

species, and both *Metaseiulus* and *Galendromus* genera names are used for the species. In the United States, *G. flumenis* has been found as a dominant predatory mite in non-sprayed apple orchards (Croft and Luh 2004) and vineyards (Prischmann and James 2003).

Previous research indicated that *G. flumenis* feeds on all stages of Banks grass mite but it prefers eggs over the other stages (Ganjisaffar and Perring 2015). In the current study, we investigated the effects of temperature on the developmental rate of immature stages and their survival, to improve our knowledge of the thermal requirements (temperature thresholds, thermal constant, and optimal temperature) for *G. flumenis*. From these studies, we developed a temperature-dependent developmental rate model for this species. Thermal models are useful for predicting the seasonal occurrence and population dynamics in the field (Logan et al. 1976; Frazer and McGregor 1992; Briere and Pracros 1998; Huffaker et al. 1999; Rodriguez- Saona and Miller 1999) and the greenhouse (Lee and Ahn 2000, Lee and Gillespie 2011). In addition, they can be helpful in optimizing mass rearing of natural enemies under laboratory conditions (Rodriguez-Saona and Miller 1999; Broufas et al. 2007), and determining the appropriate release timing and geographic regions in which a particular natural enemy can be used (Lee and Gillespie 2011).

Materials and Methods

Mite Culture Maintenance

Both species of mites, *G. flumenis* and Banks grass mite, were collected from date bunches in the Coachella Valley. The stock colony of Banks grass mite was initiated on field corn plants (*Zea mays* variety 31G71) with 7-8 fully developed leaves in an environmentally controlled room (30°C, 50±10% RH and 16L: 8D photoperiod). Black

ceramic tiles (10×10 cm) were used to rear the predatory mites as described by McMurtry and Scriven (1965). Briefly, the tile was placed on a water-saturated foam in a stainless steel pan (20×20 cm). The edges of the tile were covered with strips of tissue paper immersed in water in the pan; this provided moisture and prevented mites from escaping. Ovipositional sites and shelters were provided by placing a microscope cover slip with a few cotton threads underneath. Corn leaves infested with Banks grass mite were brushed three times a week to provide mixed stages of prey for the predatory mite culture. A mite brushing machine (Bioquip Products, Rancho Dominguez, California) was used to brush the corn leaves. The *G. flumenis* rearing arenas were maintained in a growth chamber (Conviron, model EF7-H) at $30\pm 1^\circ\text{C}$, $50\pm 10\%$ RH, and 16L: 8D photoperiod.

Experimental Procedure

To obtain a cohort of eggs for the experiment, a large number of adult females and males of *G. flumenis* were transferred from the stock colony to excised corn leaves (4-5 cm in width and 10 cm in length) which contained mixed stages of Banks grass mite. The excised leaves were placed abaxial side up on a wet cotton layer in a Petri dish (14 cm diameter). This Petri dish had a 1 cm hole drilled in its bottom, and was placed in a stainless steel pan containing water. These adult mites were maintained in a growth chamber at $30\pm 1^\circ\text{C}$, $50\pm 10\%$ RH, and 16L: 8D photoperiod for 12 h. Eggs laid by the predators within the 12 h were transferred individually to the experimental units; 60 eggs were transferred for each temperature. Experimental units consisted of two Petri dishes. The smaller Petri dish (3 cm diameter) with a 5 mm hole in its bottom was placed in a larger one (5 cm diameter) which contained water. A cotton layer was placed in the small Petri dish on top

of which a corn leaf cut to fit the 3 cm dish was placed abaxial side up. The margin of the leaf was covered with water-saturated cotton strips to prevent mites from escaping and to prolong leaf freshness. Lids of the large Petri dishes had a 1-cm hole covered with fine mesh for ventilation. These experimental units were maintained in growth chambers (Percival model 130BLL) at eleven constant temperatures (12, 14, 16, 18, 22, 26, 30, 34, 38, 42 and 44°C), a photoperiod of 16L: 8D and a relative humidity of 50±10% RH. This temperature range covered the expected field conditions during a typical date growing season in the Coachella Valley. The temperature in each growth chamber was recorded continuously with a data logger (HOBO® UX100-001).

After the *G. flumenis* eggs hatched, approximately 150 Banks grass mite eggs were supplied to each predator daily. To obtain these eggs, 50 Banks grass mite females were transferred to a new experimental unit, and allowed to oviposit for 24 h. After this time, the females were removed and the eggs were counted and adjusted so that 150 eggs were available in each unit. The predators then were moved to these new units containing the eggs.

The predators were observed every 12 h to determine the duration of each developmental stage, and the corresponding survivorship. The presence of an exuvium was used as the indication of a successful molting (Figure 4-1). For calculation purposes, we established the time of molting or death to have occurred at the midpoint between two successive observations (Perring et al. 1984). Individuals trapped in the wet cotton surrounding the leaf disk arena were excluded from data analysis.

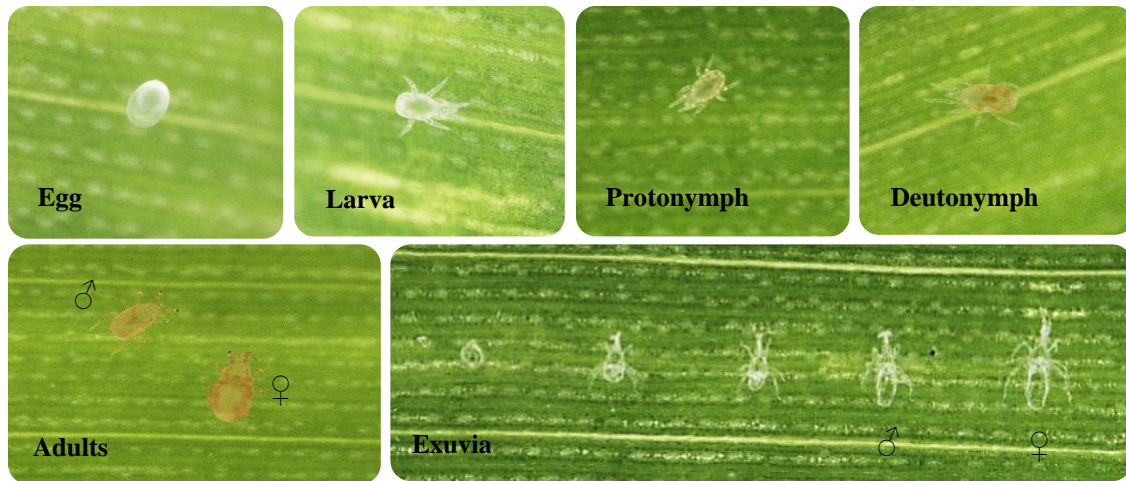


Figure 4-1 Different life stages of *Galendromus flumenis* and their exuvia.

Statistical Analysis

Percentages of immature survival at different temperatures were compared using the chi-square test ($P < 0.01$), after correction with the Bonferroni method followed by Marascuilo's post hoc multiple proportion comparisons ($P < 0.05$). Data of developmental times were tested for normality by Minitab 17 software (Minitab 2014). All data were normally distributed and analyzed using one-way ANOVA followed by post hoc Tukey's HSD test ($P < 0.01$) (SAS Institute 2012).

The relationship between developmental rate (D_r), which is the reciprocal of developmental time (D_t) in days, and temperature (T) for each developmental stage and total immature development was described by one linear and four nonlinear models using SPSS 22 (IBM SPSS Statistics 2013). We used the most common models that allow estimation of parameters with biological significance for development, i.e. the lower and upper temperature thresholds, and optimal temperature (Table 4-1). In the linear model, the lower temperature threshold (T_0) and the thermal constant (K) (cumulative degree-days)

were calculated as $T_0 = -a/b$ and $K = 1/b$, respectively (Campbell et al. 1974). The goodness of fit for each model was assessed by the adjusted R^2 (R^2_{adj}) (Kvalseth 1985), and Akaike Information Criterion (AIC) (Akaike 1974):

$$R^2_{adj} = 1 - \left(\frac{n-1}{n-p} \right) (1 - R^2)$$

$$AIC = n \ln \left(\frac{RSS}{n} \right) + 2p$$

where n = the number of observations, p = the number of model parameters, including the intercept, and RSS = the residual sum of squares. The model with the smallest value of AIC and the highest value of R^2_{adj} was considered the best.

Table 4-1 Models used to describe the relationship between developmental rate (D_r) and temperature in *Galendromus flumenis*

Equation	Model	Reference
$D_r = a + bT$	Linear	Simpson (1903)
$D_r = \Delta \times \left[e^{\rho \cdot T} - e^{\left(\rho \cdot T_{max} - \frac{T_{max}-T}{\Delta T} \right)} \right]$	Logan 6	Logan et al. (1976)
$D_r = e^{\rho \cdot T} - e^{\left(\rho \cdot T_M - \frac{T_M-T}{\Delta T} \right)} + \lambda$	Lactin 2	Lactin et al. (1995)
$D_r = a \times T(T - T_0) \times (T_{max} - T)^{1/2}$	Briere 1	Briere et al. (1999)
$D_r = a \times T(T - T_0) \times (T_{max} - T)^{1/m}$	Briere 2	Briere et al. (1999)

Linear model: a and b are equation constants and T is the rearing temperature ($^{\circ}C$).

Logan 6: T is the rearing temperature ($^{\circ}C$), Δ is the maximum developmental rate, ρ is a constant defining the rate of increase to optimal temperature, T_{max} is the upper temperature threshold, and ΔT is the temperature range over which physiological breakdown becomes the overriding influence.

Lactin 2: T , ρ , T_{max} and ΔT are as in Logan 6, λ forces the curve to intercept the Y -axis at a value below zero, and thus allows estimation of the lower temperature threshold, and T_M is the supraoptimal temperature at which $D_r = \lambda$.

Briere models: T is the rearing temperature ($^{\circ}C$), a is an empirical constant, T_0 is the lower temperature threshold, T_{max} is the upper temperature threshold, and m in Briere 2 is an empirical constant.

Results

Survival during the development of all immature stages except the deutonymphs significantly varied among the different temperatures tested (Table 4-2, Egg: $df= 8$, $\chi^2= 238.00$, $P< 0.01$; Larva: $df= 7$, $\chi^2= 61.06$, $P< 0.01$; Protonymph: $df= 6$, $\chi^2= 39.50$, $P< 0.01$; Deutonymph: $df= 6$, $\chi^2= 3.36$ $P= 0.76$; Total development: $df= 6$, $\chi^2= 50.40$, $P< 0.01$). Survival rates for the total immature development were higher in the temperature range of 26-38°C (Table 4-2). However, no significant differences were found among these temperatures. The most vulnerable stage to temperature was the protonymph, as the highest mortality occurred in this stage across the temperature range. While mites did not survive to the adult stage at 14°C and 16°C, the survivorship from egg to adult was between 37.5% at 42°C and 89.5% at 30°C.

Galendromus flumenis developed successfully from egg to adult at temperatures that ranged from 18 to 42°C (Table 4-2); no eggs hatched at 12°C and 44°C. At 14°C, 23.3% of the eggs hatched, but the larvae that emerged did not survive. At 16°C, the emerged protonymphs did not develop to the deutonymph stage. Mean developmental time of each immature stage was significantly influenced by temperature (Table 4-2, Egg: $F_{8,438}= 1455.62$; Larva: $F_{7,405}= 409.33$; Protonymph: $F_{6,269}= 291.55$; Deutonymph: $F_{6,237}= 248.07$; Total development: $F_{6,237}= 522.48$, all $P< 0.01$). The mean incubation period for eggs ranged from 14.04 days at 14°C to 1.77 days at 38°C. At all temperatures tested, the larval stage was the shortest, ranging from 3.06 days at 18°C to 0.30 day at 38°C. Similar trends were observed in the nymphal developmental times. At all temperatures except 42°C, the protonymph stage was longer than the deutonymph stage. The total developmental time

from egg to adult ranged from 26.98 days at 18°C to 5.93 days at 38°C. Each time was significantly different ($P < 0.01$) from the other temperatures, with the exception of 30°C and 42°C which were statistically similar (Table 4-2).

Table 4-2 Mean developmental times (D_t) \pm SE (days) and stage survival (%) of *Galendromus flumenis* immature at different constant temperatures

Temp (°C)		Egg	Larva	Protonymph	Deutonymph	Egg-Adult
14	D_t	14.04 \pm 0.42 a				
	Survival	23.3% C (14)				
16	D_t	8.42 \pm 0.10 b	3.06 \pm 0.09 a			
	Survival	93.3% A (56)	79.2% B (42) (3)			
18	D_t	4.03 \pm 0.09 c	1.50 \pm 0.06 b	11.11 \pm 0.34 a	10.44 \pm 0.42 a	26.98 \pm 0.67 a
	Survival	93.3% A (56)	100% A (55) (1)	65.4% B (34) (3)	91.2% A (31)	55.4% BC (31) (4)
22	D_t	3.60 \pm 0.08 d	1.20 \pm 0.04 c	9.39 \pm 0.40 b	4.77 \pm 0.19 b	18.80 \pm 0.50 b
	Survival	91.7% AB (55)	100% A (55)	64.0% B (32) (5)	93.8% A (30)	54.6% BC (30) (5)
26	D_t	2.49 \pm 0.04 e	0.57 \pm 0.03 d	5.90 \pm 0.29 c	2.96 \pm 0.11 c	12.02 \pm 0.35 c
	Survival	95.5% A (57)	100% A (57)	78.3% AB (36) (11)	91.7% A (33)	67.4% ABC (33) (11)
30	D_t	2.13 \pm 0.04 fh	0.43 \pm 0.03 de	3.05 \pm 0.06 d	2.70 \pm 0.15 c	8.35 \pm 0.16 d
	Survival	100% A (60)	100% A (60)	94.8% A (55) (2)	94.4% A (51) (1)	89.5% A (51) (3)
34	D_t	1.83 \pm 0.03 fg	0.34 \pm 0.03 e	2.99 \pm 0.08 d	1.98 \pm 0.06 d	7.00 \pm 0.14 e
	Survival	100% A (60)	100% A (60)	87.5% AB (49) (4)	89.4% A (42) (2)	77.8% AB (42) (6)
38	D_t	1.77 \pm 0.02 g	0.30 \pm 0.02 e	2.00 \pm 0.07 e	1.85 \pm 0.06 d	5.93 \pm 0.09 f
	Survival	100% A (60)	98.3% AB (59)	98.3% A (57) (1)	85.2% A (46) (3)	82.1% AB (46) (4)
42	D_t	2.24 \pm 0.02 eh	0.39 \pm 0.04 de	3.25 \pm 0.20 d	3.35 \pm 0.07 c	9.24 \pm 0.25 d
	Survival	65.5% B (39)	89.7% AB (35)	74.2% AB (23) (4)	91.3% A (21)	37.5% C (21) (4)

Numbers in the first parentheses are numbers of live individuals for each developmental stage used to calculate the mean. Numbers in the second parentheses are missing or drowned individuals that were excluded from analysis.

Means within the same column followed by the same lower case letter (Tukey's HSD test, $P < 0.01$) or capital letter (Chi-square test, $P < 0.05$) were not significantly different.

The developmental rate increased linearly with increasing temperature up to 38°C, therefore, linear regressions were conducted in this range of temperatures. The lower temperature thresholds (T_{min}) were estimated to be 8.7, 14.0, 15.7, 12.7 and 13.3°C for egg, larva, protonymph, deutonymph and total development, respectively (Figure 4-2). The thermal constants (K) for the development of egg, larva, protonymph, deutonymph and total development were 47.6, 7.1, 48.5, 44.5 and 145.3 degree-days, respectively (Table 4-3). The R^2_{adj} coefficient which was used to fit the linear regression of developmental rate on temperature was high in all developmental stages (0.92-0.99), therefore, the linear model adequately describes the lower temperature threshold (T_{min}) and the cumulative degree-days (K) required for development of *G. flumenis*.

The nonlinear relationship between temperature and developmental rate was best described by the Logan 6 ($R^2_{adj} = 0.90-0.99$) and Lactin 2 models ($R^2_{adj} = 0.89-0.99$). The Briere 2 model did not provide good parameters estimations ($R^2_{adj} = 0.65-0.82$). The Briere 1 model was the best in describing the egg development. Larva and deutonymph development was best described by the Lactin 2 model, while the protonymph and total development was best described by the Logan 6 model (Table 4-3, Figure 4-2).

Table 4-3 Estimated parameters of six temperature-dependent models with values of their evaluation criteria

Model	Parameter	Egg	Larva	Protonymph	Deutonymph	Total Development
Linear	<i>a</i>	-0.18319	-1.98755	-0.32260	-0.28627	-0.09178
	<i>b</i>	0.02103	0.14154	0.02061	0.02249	0.00688
	<i>T_{min}</i>	8.7	14.0	15.7	12.7	13.3
	<i>K (DD)</i>	47.6	7.1	48.5	44.5	145.3
	<i>RSS</i>	0.01021	0.11786	0.00871	0.00388	0.00010860
	<i>R²</i>	0.96	0.99	0.94	0.97	0.99
	<i>R²_{adj}</i>	0.95	0.98	0.92	0.97	0.99
Briere 1	<i>a</i>	0.000182	0.001298	0.000197	0.000243	0.000069
	<i>T_{min}</i>	7.0	14.0	16.0	13.8	14.0
	<i>T_{max}</i>	44.8	44.9	44.4	43.1	43.9
	<i>T_{opt}</i>	36.6	37.6	37.6	36.2	36.8
	<i>RSS</i>	0.005551	0.168771	0.016290	0.005034	0.000508
	<i>R²</i>	0.98	0.98	0.88	0.97	0.96
	<i>R²_{adj}</i>	0.97	0.97	0.81	0.95	0.94
	<i>AIC</i>	-60.52	-24.87	-36.44	-44.66	-59.79
Briere 2	<i>a</i>	0.000012	0.000017	0.000000168	0.000004	0.00000067
	<i>m</i>	0.314	0.070	0.005	0.067	0.047
	<i>T_{min}</i>	9.8	14.7	16.2	15.6	15.2
	<i>T_{max}</i>	50.8	52.1	51.5	47.2	49.5
	<i>T_{opt}</i>	35.8	37.8	37.8	34.8	36.2
	<i>RSS</i>	0.006363	0.352410	0.022646	0.013408	0.001205
	<i>R²</i>	0.98	0.96	0.83	0.91	0.91
	<i>R²_{adj}</i>	0.96	0.93	0.65	0.73	0.82
	<i>AIC</i>	-57.29	-16.98	-32.14	-35.80	-52.67
Logan 6	ρ	0.119	0.150	0.089	0.142	0.116
	Δ	0.481	0.664	0.018	0.105	0.005
	ΔT	8.296	6.549	1.644	6.829	5.160
	<i>T_{max}</i>	45.5	44.7	43.0	43.8	44.2
	<i>T_{opt}</i>	37.2	38.1	39.3	36.9	37.6
	<i>RSS</i>	0.011732	0.126670	0.006426	0.004675	0.000083
	<i>R²</i>	0.95	0.99	0.95	0.97	0.99
	<i>R²_{adj}</i>	0.93	0.98	0.90	0.94	0.99
	<i>AIC</i>	-51.78	-25.16	-40.95	-43.18	-71.40
Lactin 2	ρ	0.020	0.048	0.014	0.017	0.006
	ΔT	6.837	3.743	0.162	2.620	1.348
	λ	-1.221	-1.824	-1.232	-1.238	-1.081
	<i>T_M</i>	52.2	46.0	42.3	46.0	45.5
	<i>T_{min}</i>	10.3	12.6	15.0	12.6	13.0
	<i>T_{max}</i>	47.0	44.9	42.1	43.5	43.0
	<i>T_{opt}</i>	36.4	38.2	41.3	37.5	39.0
	<i>RSS</i>	0.004827	0.070750	0.007330	0.002729	0.000094
	<i>R²</i>	0.98	0.99	0.94	0.98	0.99
	<i>R²_{adj}</i>	0.97	0.99	0.89	0.96	0.99
	<i>AIC</i>	-59.78	-29.82	-40.03	-46.95	-70.53

Model parameters were described in Table 1.

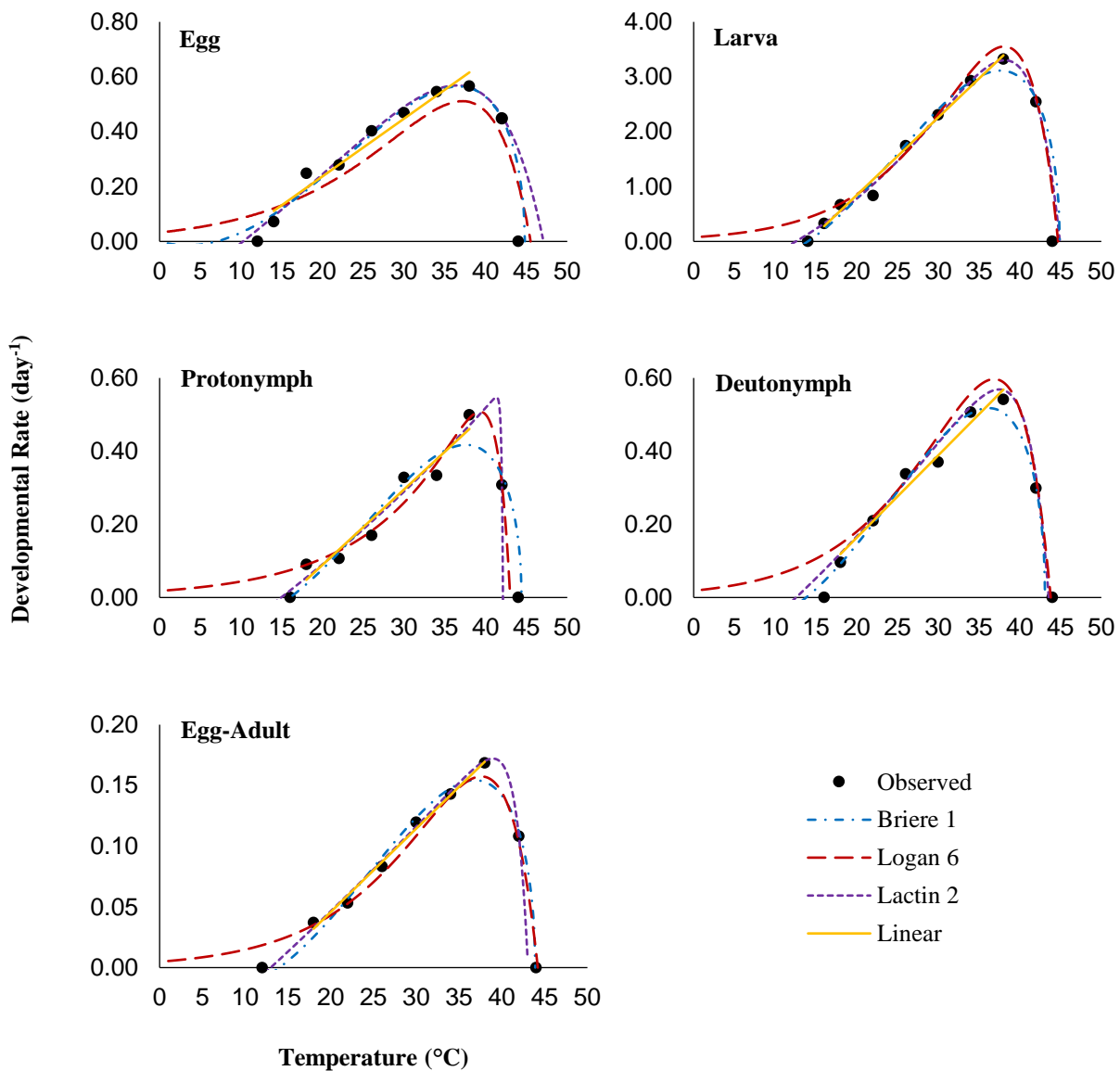


Figure 4-2 Linear (orange solid line), Briere 1 (blue dash-dot line), Logan 6 (red dashed line) and Lactin 2 (purple dotted line) models fitted to the observed developmental rates (black points) for immature stages of *Galendromus flumenis*.

Discussion

Immature survival of phytoseiids typically is high when plenty of food is available, however abiotic factors such as temperature can significantly affect immature survival (Sabelis 1985). The survival rates of *G. flumenis* were highest between temperatures of 26°C and 38°C (67.4%-89.5%, respectively), thus the fastest population growth may be expected to occur within this temperature range. However, a significant reduction in survival rate was observed at temperatures below 26°C and above 38°C suggesting lower population growth of *G. flumenis* at those temperatures. *Galendromus flumenis* develops under a wide range of temperatures from 18°C to 42°C. The larval stage was the shortest life stage at all temperatures which may be an adaptation to the vulnerability of this life stage. Larvae hardly move, and are easy prey for other predators and conspecifics. Also, they do not feed and have restricted growth, therefore a short developmental time for the larval stage promotes mite survival.

The linear model predicted a lower T_{min} for the egg stage (8.7°C, Table 4-3) than that observed in the experiment (no egg hatch at 12°C). This discrepancy may be due to intrinsic model weaknesses in fitting data across such broad temperature ranges (Roy et al. 2002). However, it also could be due to our experimental procedure. In the current study, experiments were initiated with eggs laid at 30°C and held at this temperature for an average of 12 h prior to placing them in the different experimental temperatures. This 12 h interval was included in our analysis. Therefore, our results may overestimate developmental time for eggs at temperatures below 30°C and underestimate developmental

time at temperatures above 30°C. An overestimation of development at low temperatures results in the T_{min} being artificially low.

Galendromus flumenis has higher thermal constants, T_{min} , T_{opt} , and T_{max} than other phytoseiid species (Table 4-4). This is reasonable, given the hot desert environment from which the *G. flumenis* evaluated in this study was collected. Since no other studies to date have looked at temperature-dependent development of *G. flumenis* collected from cooler climatic conditions, we cannot compare our results to other data. Interestingly, the lower and upper temperature thresholds, and the optimal temperature for the total development of Banks grass mite are 13.5°C, 44.0°C, and 37°C, respectively (calculated from Perring et al. (1984)), which are very close to *G. flumenis* (13.3°C, 44.3°C, and 37.6°C based on the predictions of the linear and Logan 6 model). This suggests that *G. flumenis* should be active at the same time that Banks grass mite is active, and there should be a synchrony between the prey and predator such that overwintering adults of both will emerge in early spring and start reproducing at the same time.

Table 4-4 Summary of temperature thresholds and thermal constants for the total development of different phytoseiid species

Species	Food/Prey	K	T_{min}	T_{opt}	T_{max}	References
<i>Galendromus flumenis</i>	<i>O. pratensis</i>	145.3	13.3	37.6	44.3	Present study
<i>Typhlodromus bagdasarjani</i>	<i>T. urticae</i>	162.0	9.2	34	41.8	Ganjisaffar et al. (2011)
<i>Amblyseius swirskii</i>	Pollen		11.3	31.5	37.4	Lee and Gillespie (2011)
<i>Phytoseius plumifer</i>	<i>T. urticae</i>	125.3	10.7	33.6	37.4	Kouhjani Gorji et al. (2008)
<i>Phytoseiulus longipes</i>	<i>T. evansi</i>	28.7 ^c	12.0			Ferrero et al. (2007)
<i>Kampimodromus aberrans</i>			10.5	27.6	32.4	Broufas et al. (2007)
<i>Iphiseius degenerans</i>	<i>T. urticae</i>	76.9	9.5	29.9	35.9	Tsoukanas et al. (2006)
<i>Amblyseius californicus</i>	<i>T. urticae</i>	59.2	10.9	≈30		Gotoh et al. (2004)
<i>Euseius finlandicus</i>	Pollen	93.5	8.9	≈30	36.5	Broufas and Koveos (2001)
<i>Amblyseius womersleyi</i>	<i>T. urticae</i>	52.1 ^c	11.6	33		Lee and Ahn (2000)

K = Thermal constant (degree-days), T_{min} = Lower temperature threshold (°C), T_{opt} = Optimal temperature (°C), T_{max} = Upper temperature threshold (°C). T refers to *Tetranychus* and O refers to *Oligonychus*. Superscript c refers to calculated from the original data.

However, this is not what is observed in the field, as dates are heavily damaged by Banks grass mite early in the season. One critical difference is the time between prey infestation of date bunches and the occurrence of predators. Gispert et al. (2001) found that *G. flumenis* (previously as *Galendromus mcgregori*) is present in date bunches from June through September, peaking in mid-July, while Banks grass mites start infesting fruits two months earlier in April. Another distinction is the relative difference in development between the prey and predator at the time they move from overwintering sites onto date bunches. According to the meteorological data from the California Irrigation Management Information System (CIMIS) station in the Coachella Valley (OASIS.A (#136), for ten consecutive years (2005-2014), the average daily temperature during April and May was 23°C. The Logan 6 model predicts that the total developmental time for *G. flumenis* at 23°C is 17.2 days. However, Banks grass mite can develop in 12.6 days at the same temperature (Perring et al. 1984). Therefore, the shorter developmental time of Banks grass mite may help it to establish quicker than the predator early in the season. In addition, the developmental time of these species at their respective optimal temperatures is different. Banks grass mite optimal development from egg to adult is 4.87 days at 36°C (Perring et al. 1984) while *G. flumenis* optimal immature development is 6.4 days at 37.6°C (based on the predictions of the Logan 6 model). Furthermore, Banks grass mite females invade date gardens in larger numbers than the predator, being blown by the wind from overwintering sites in grasses in and around the date garden. Banks grass mites raise their posture on declining food sources which contributes to them being picked up by the wind (Margolies 1987) and they are known to balloon on webbing which facilitates their dispersal (Perring,

personal observation). The predators are not as numerous immigrating into the garden, and they must crawl from overwintering sites in search for patches of Banks grass mite. This gives Banks grass mite a much greater advantage in establishing, and puts the predator at a disadvantage for infesting date bunches. Finally, another reason for low numbers of *G. flumenis* early in the season could be a disparity between the impact of Savey® on the predator and the prey mites. Further research is needed to explicitly test the above possibilities. In addition, work must be done to determine the reproductive potential and the intrinsic rate of natural increase (r_m) of *G. flumenis* before we can fully understand *G. flumenis*-Banks grass mite interactions in the field. The present study contributes to our understanding of the relationship between *G. flumenis* development and temperature. The fact that *G. flumenis* can survive and develop at high temperatures makes it a good candidate for biological control of spider mites on crops grown in hot weather conditions. In addition, our results suggest that augmentative release of *G. flumenis* on dates during the months of April and May may help to compensate for the low numbers of the predator and will improve the effectiveness of this predatory mite in the biological control of Banks grass mite.

References

- Akaike, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19, 716-723.
- Briere, J.F., Pracros, P. 1998. Comparison of temperature-dependent growth models with the development of *Lobesia botrana* (Lepidoptera: Tortricidae). *Environmental Entomology* 27, 94-101.
- Briere, J.F., Pracros, P., Le Roux, A.Y., Pierre, J.S.A. 1999. A novel rate model of temperature-dependent development for arthropods. *Environmental Entomology* 28, 22-29.
- Broufas, G.D., Koveos, D.S. 2001. Development, survival and reproduction of *Euseius finlandicus* (Acari: Phytoseiidae) at different constant temperatures. *Experimental and Applied Acarology* 25, 441-460.
- Broufas, G.D., Pappas, M.L., Koveos, D.S. 2007. Development, survival, and reproduction of the predatory mite *Kampimodromus aberrans* (Acari: Phytoseiidae) at different constant temperatures. *Environmental Entomology* 36, 657-665.
- Campbell, A., Frazer, B.D., Gilbert, N., Gutierrez, A.P., Mackauer, M. 1974. Temperature requirements of some aphids and their parasites. *Journal of Applied Ecology* 11, 431-438.
- Chant, D.A. 1957. Description of some phytoseiidae mites (Acarina: Phytoseiidae). Part I. Nine new species from British Columbia with key to the species of British Columbia. Part II. Redescription of eight species described by Berlese. *The Canadian Entomologist* 89, 289-308.
- Croft, B.A., Luh, H.-K. 2004. Phytoseiid mites on unsprayed apple trees in Oregon, and other western states (USA): distributions, life-style types and relevance to commercial orchards. *Experimental and Applied Acarology* 33, 281-326.
- Ferrero, M., de Moraes, G.J. 2007. Life tables of the predatory mite *Phytoseiulus longipes* feeding on *Tetranychus evansi* at four temperatures (Acari: Phytoseiidae, Tetranychidae). *Experimental and Applied Acarology* 41, 45-53.
- Frazer, B.D., McGregor, R.R. 1992. Temperature-dependent survival and hatching rate of seven species of Coccinellidae. *The Canadian Entomologist* 124, 305-312.
- Ganjisaffar, F., Perring, T.M. 2015. Prey stage preference and functional response of the predatory mite *Galendromus flumenis* to *Oligonychus pratensis*. *Biological Control* 82, 40-45.

- Gispert, C., Farrar, C., Perring, T.M. 2001. Seasonal abundance and the Banks grass mite *Oligonychus pratensis* (Banks) (Prostigmata: Tetranychidae) and a predatory mite, and their response to sulfur treatment on commercial date palms *Phoenix dactylifera* L. in Southern California. In: Halladay, R.B., Walter, D.E., Proctor, H.C., Norton, R.A., Colloff, M.J. (Eds.). Proceedings of the 10th international congress of acarology. CSIRO Publishing, Melbourne, pp. 403-408.
- Gotoh, T., Yamaguchi, K., Mori, K. 2004. Effect of temperature on life history of the predatory mite *Amblyseius (Neoseiulus) californicus* (Acari: Phytoseiidae). *Experimental and Applied Acarology* 32, 15-30.
- Hirschmann, W. 1962. Gangsystematik der Parasitiformes, Teil 5. *Acarologie Schriftenreihe für Vergleichende Milbenkunde*, 1-56.
- Huffaker, C., Berryman, A., Turchin, P. 1999. Dynamics and regulation of insect populations. In: Huffaker, C.B., Gutierrez, A.P. (Eds.). *Ecological entomology*, 2nd edition. Wiley, New York, pp. 269-312.
- IBM SPSS Statistic 2013. SPSS 22.0 for windows, Chicago, Illinois.
- Jeppson, L.R., Keifer, H.H., Baker, E.W. 1975. *Mites injurious to economic plants*. University of California Press, Berkeley.
- Kouhjani Gorji, M., Kamali, K., Fathipour, Y., Ranjbar Aghdam, H. 2008. Temperature-dependent development of *Phytoseius plumifer* (Acari: Phytoseiidae) on *Tetranychus urticae* (Acari: Tetranychidae). *Systematic and Applied Acarology* 13, 172-181.
- Kvalseth, T.O. 1985. Cautionary note about R^2 . *American Statistician* 39, 279-285.
- Lactin, D.J., Holliday, N.J., Johnson, D.L., Craigen, R. 1995 Improved rate model of temperature-dependent development by arthropods. *Environmental Entomology* 24, 68-75.
- Lee, H.S., Gillespie, D.R. 2011. Life tables and development of *Amblyseius swirskii* (Acari: Phytoseiidae) at different temperatures. *Experimental and Applied Acarology* 53, 17-27.
- Lee, J.H., Ahn, J.J. 2000. Temperature effects on development, fecundity, and life table parameters of *Amblyseius womersleyi* (Acari: Phytoseiidae). *Environmental Entomology* 29, 265-271.
- Logan, J.A., Wolkind, D.T., Hoty, J.C., Tanigoshi, L.K. 1976. An analytic model for description of temperature dependent rate phenomena in arthropods. *Environmental Entomology* 5, 1133-1140.

- Margolies, D.C. 1987. Conditions eliciting aerial dispersal behavior in Banks grass mite *Oligonychus pratensis* (Acari: Tetranychidae). *Environmental Entomology* 16, 928-932.
- McGregor, E.A., Stickney, F. 1965. Distribution and host plants of *Oligonychus pratensis* (Banks). *Proceedings of the Entomological Society of Washington* 67, 28.
- Minitab Inc 2014. MINITAB release 17: statistical software for windows. Minitab Inc, USA.
- Muma, M.H. 1961. Subfamilies, genera and species of Phytoseiidae (Acarina: Mesostigmata). *Bulletin of the Florida State Museum, Biological Science* 5, 267-302.
- Muma, M.H. 1963. The genus *Galendromus* Muma, 1961 (Acarina: Phytoseiidae). *The Florida Entomologist* 1, 15-41.
- Parasad, V. 2012. Checklist of the world (Acari: Mesostigmata). Indira Publishing House, West Bloomfield, Michigan.
- Perring, T.M., Holtzer, T.O., Toole, J.L. 1984. Influence of temperature and humidity on pre-adult development of the Banks grass mite (Acari: Tetranychidae). *Environmental Entomology* 13, 338-343.
- Prischmann, D.A., James, D.G. 2003. Phytoseiidae (Acari) on unsprayed vegetation in south central Washington: implications for biological control of spider mites on wine grapes. *International Journal of Acarology* 29, 279-787.
- Rodriguez-Saona, C., Miller, J.C. 1999. Temperature dependent effects on development, mortality, and growth of *Hippodamia convergens* (Coleoptera: Coccinellidae). *Environmental Entomology* 28, 518-522.
- Roy, M., Brodeur, J., Cloutier, C. 2002. Relationship between temperature and developmental rate of *Stethorus punctillum* (Coleoptera: Coccinellidae) and its prey *Tetranychus mcdanieli* (Acari: Tetranychidae). *Environmental Entomology* 31, 177-187.
- Sabelis, M.W. 1985. Capacity for population increase. In: Helle, W., Sabelis, M.W. (Eds.). *Spider mites: their Biology, natural enemies and control*. vol. 1B. Elsevier, Amsterdam, The Netherlands, pp. 35-41.
- SAS Institute. 2012. SAS enterprise guide 5.1. SAS Institute Inc., Cary, North Carolina.
- Schuster, R.O., Pritchard, A.E. 1963. Pytoseiid mites of California. *Hilgardia* 34, 191-285.
- Simpson, C.B. 1903. The codling moth. United States Department of Agriculture Division of Entomology Bulletin 41, 1-105.

Tsoukanas, V.I., Papadopoulos, G.D., Fantinou, A.A., Papadoulis, G.T.H. 2006. Temperature-dependent development and life table of *Iphiseius degenerans* (Acari: Phytoseiidae). *Environmental Entomology* 35, 212-218.

Wainstein, B.A. 1962. Révision du genre *Typhlodromus* Scheuten, 1857 et systématique de la famille des Phytoseiidae (Berlese, 1916). (Acarina: Parasitiformes). *Acarologia* 4, 5-30.

Chapter 5
Population Growth and Reproduction Parameters of
G. flumenis

Abstract

The predatory mite, *Galendromus flumenis* (Chant) (Acari: Phytoseiidae), has shown promising traits for biological control of Banks grass mite, the major pest of date palms in California. In the present study, reproduction and population growth parameters of *G. flumenis* on Banks grass mite eggs were studied at 34°C, 50±10% RH and a photoperiod of 16L: 8D hours. 100 percent of eggs hatched and 63.5 percent of the emerged larvae survived to adulthood. The total immature developmental time was 5.7 and 5.5 days for females and males, respectively. The sex ratio of *G. flumenis* was 2.3: 1 (females: males). Mated females laid on average 1.6 eggs per day and 19.9 eggs during their mean ovipositional period of 12.5 days. The net reproductive rate (R_0) was 11.5 females/ female/ generation, the intrinsic rate of increase (r_m) was 0.200 females/ female/ day, the finite rate of increase (λ) was 1.222 population multiplication/ day, the mean generation time (T) was 12.2 days, and the doubling time (DT) was 3.5 days. The lower r_m value of *G. flumenis* than that of its prey (0.24-0.48) explains why Banks grass mite escapes control by *G. flumenis* in field. These results suggest that augmentative release of this predator would offset the lower r_m of the predator, thereby contributing to the control of Banks grass mite. Combined with the benefit of early releases determined in companion studies, future field studies with *G. flumenis* are being planned.

Introduction

California leads the nation in date production, with 99% of the production. In 2015, 43,600 tons of dates valued at \$68,016,000 were harvested from 10,000 hectares of date gardens in the Coachella Valley (Riverside County, California) (USDA 2016). The Banks

grass mite, *Oligonychus pratensis* (Banks) (Acari: Tetranychidae) has been a problematic pest of California dates since the early 1900s (Banks 1914). The mites' feeding damage leaves scarred and cracked fruits that are unmarketable (Elmer 1965; Carpenter and Elmer 1978). Chemical control of Banks grass mite is limited to Savey[®] (Hexythiazox), a growth regulator which is recommended to be sprayed on mite eggs or early developmental stages. However, growers' concerns about the development of resistance to Savey[®] and reliance on a single management strategy has resulted in support of research to find additional management tools. Among these efforts has been the use of biological control agents.

Mites in the family Phytoseiidae are considered as important predators of several phytophagous mites and small insects on various crops (Helle and Sabelis 1985; McMurtry and Croft 1997; McMurtry et al. 2013). Surveys for predatory mites in date gardens of the Coachella Valley revealed that *Galendromus flumenis* (Chant) was the only phytoseiid species in association with Banks grass mite on date bunches (Ganjisaffar and Perring 2015a). Therefore, a series of laboratory experiments were initiated to elucidate the biological properties of *G. flumenis* in an effort to predict the performance of the predator in the field. Previous research on the prey stage preference of *G. flumenis* indicated that *G. flumenis* feeds on all life stages of Banks grass mite, but it prefers eggs over the other stages, and displays a type II functional response on all Banks grass mite immature stages (Ganjisaffar and Perring 2015a).

Temperature-dependent studies showed that *G. flumenis* develops successfully from egg to adult under a wide range of temperatures from 18°C to 42°C, and the optimal temperature for development was calculated to be 37.6°C (Ganjisaffar and Perring 2015b).

Not only is this a high optimal temperature among phytoseiids, but also the thermal requirements of *G. flumenis* are very close to the Banks grass mite (Perring et al. 1984a). This suggests that the prey and predator should be active at the same time in date garden, with overwintering adults of both species emerging and reproducing at the same time. However, field observations do not agree with this prediction, and dates are heavily damaged by Banks grass mites early in the season (Gispert et al. 2001). Therefore, additional population parameters of *G. flumenis* were studied to complete a life table analysis. For this purpose, survivorship, reproduction and population growth of *G. flumenis* at its optimal temperature were studied. Our aim was to further understand why *G. flumenis* fails to keep up with Banks grass mite densities in field. In addition, information gained from life table studies can help understand release timing, release numbers, and geographic regions in which the predator can be used, thereby enhancing the use of *G. flumenis* in the management of Banks grass mite.

Materials and Methods

Mite Culture Maintenance

Colonies of *G. flumenis* and Banks grass mite were collected from date gardens in the Coachella Valley in 2012, and have been maintained in growth chambers at $30\pm 1^\circ\text{C}$, $50\pm 10\%$ RH, and 16L: 8D photoperiod since then. Every year, the colonies were supplemented with field collected mites to ensure genetic variability. Banks grass mite were reared on field corn plants, *Zea mays* variety 31G71, with 7-8 fully developed leaves. *G. flumenis* were reared on black ceramic tiles (10×10 cm) as described by McMurtry and Scriven (1965). The tile was resting on a water-saturated foam in a stainless steel pan

(20×20 cm) filled with water. To prevent mites from escaping, strips of tissue paper were placed around the edges of the tile which were immersed in water in the pan. A microscope cover slip with a few cotton threads underneath was placed in the center of the tile to provide ovipositional site and shelter for *G. flumenis*. Using a mite brushing machine (Bioquip Products, Rancho Dominguez, CA), Banks grass mite infested corn leaves were brushed three times a week to provide mixed stages of prey for *G. flumenis* culture.

Experimental Procedure

Studies to determine survival, reproduction and population growth parameters of *G. flumenis* were initiated at the optimal temperature of 37.6°C (\approx 38°C) with 50±10% RH, and 16L: 8D photoperiod. The newly emerged females at this temperature did not lay eggs; therefore, the study was conducted at 34°C which was the temperature with the next shortest developmental time (Ganjisaffar and Perring 2015b).

G. flumenis was reared for one generation on Banks grass mite eggs at 34°C. Then, 63 deposited eggs of newly emerged females were transferred individually to the experimental arenas which consisted of two Petri dishes. A 3 cm Petri dish with a 5 mm hole in the bottom was placed in a 5 cm Petri dish containing water. A cotton layer was placed in the small Petri dish on top of which a corn leaf cut to fit the 3 cm dish was placed abaxial side up. The leaf margin was covered with a water-saturated cotton strip to prevent mites from escaping and to maintain freshness in the leaf. A 1-cm hole was made in the lid of the large Petri dish, and this hole was covered with fine mesh for ventilation. Eggs of Banks grass mite were supplied as food since previous study indicated that *G. flumenis* prefers eggs over the other stages of Banks grass mite (Ganjisaffar and Perring 2015a). Development

from egg to adult was recorded every 24 h. Second generation newly emerged females were paired with a male obtained in the experiment or taken from the colony. The pair was kept together until the end of the study and males that escaped or died were replaced by new ones. Approximately 250 Banks grass mite eggs were supplied as food daily to each predator mite pair. To obtain these eggs, 50 Banks grass mite females were transferred to a new experimental arena, and allowed to oviposit for 24 h. After this time, the females were removed and the eggs were counted and adjusted so that 250 eggs were available in each arena. The predators were moved to these new arenas containing the eggs each day until the female died. Daily observations were conducted under a stereomicroscope (10X) to determine female reproduction and survivorship.

Statistical Analysis

Life and fertility tables were constructed using the survival and reproduction data according to Carey (1993). Then, according to the method described by Meyer et al. (1986), the Jackknife procedure was used to calculate the following population growth parameters and their mean and standard errors (SAS Institute 2013):

Net reproductive rate (R_0)	$\sum l_x m_x$
Intrinsic rate of population increase (r_m)	$\sum_{x=0}^n e^{-rx} l_x m_x = 1$
Mean generation time (T)	$\ln R_0 / r_m$
Doubling time (DT)	$\ln 2 / r_m$
Finite rate for increase (λ)	e^{r_m}

Briefly, the Jackknife procedure is based on recombining the original data, calculating pseudo-values for each recombination, and estimating the mean and standard error of the

parameters from the resulting frequency distribution of pseudo-values. The steps for the application of this method are as follow:

Step 1) true calculation of r_m , R_0 , λ , T and DT is done considering the survival and reproduction data for all the n females. The estimates obtained are denoted as $r_{m(all)}$, $R_{0(all)}$, $\lambda_{(all)}$, $T_{(all)}$ and $DT_{(all)}$.

Step 2) the procedure described is repeated n times, each time excluding one of the n females. Therefore, the remaining $n-1$ females are used to re-compute parameters, now named $r_{m(i)}$, $R_{0(i)}$, $\lambda_{(i)}$, $T_{(i)}$ and $DT_{(i)}$.

Step 3) pseudo-values are calculated for each parameter, subtracting the estimate in step 1 from the estimate in step 2, for example, the pseudo-values of r_m ($r_{m(j)}$) were calculated for n samples using the following equation:

$$r_{m(j)} = n \times r_{m(all)} - (n-1) \times r_{m(i)}$$

Step 4) after calculating all the n pseudo-values for r_m , the mean ($r_{m(mean)}$), variance ($VAR r_{m(mean)}$) and standard error ($SE r_{m(mean)}$) was calculated by the following equations:

$$r_{m(mean)} = \frac{\sum_1^n r_{m(j)}}{n}$$

$$VAR r_{m(mean)} = \frac{\sum_1^n (r_{m(j)} - r_{m(all)})^2}{n-1}$$

$$SE r_{m(mean)} = \sqrt{\frac{VAR r_{m(mean)}}{n}}$$

Results

100 percent of the eggs hatched and all of the newly emerged larvae developed to the protonymph stage. A sharp decline in the survival curve occurred during the protonymph stage (days 3-5) (Figure 5-1). Forty individuals of the original 63 eggs survived to adult (63.49% immature survival). The age specific survival rate (l_x) of *G. flumenis* was recorded as 0.83 at the time of first adult emergence (day 5) (Figure 5-1). The egg to adult developmental time was 5.6 and 5.5 days for females and males, respectively (Table 5-1). The pre-oviposition period was on average 2.7 days (Table 5-1), and the first oviposition occurred on day 6 (Figure 5-1). Females laid an average of 19.9 eggs during their mean oviposition period of 12.5 days, and the number of eggs laid daily by a female ranged from 0-3 eggs with the average being 1.6 eggs per day. The mean duration of the post-oviposition period was 2.5 days.

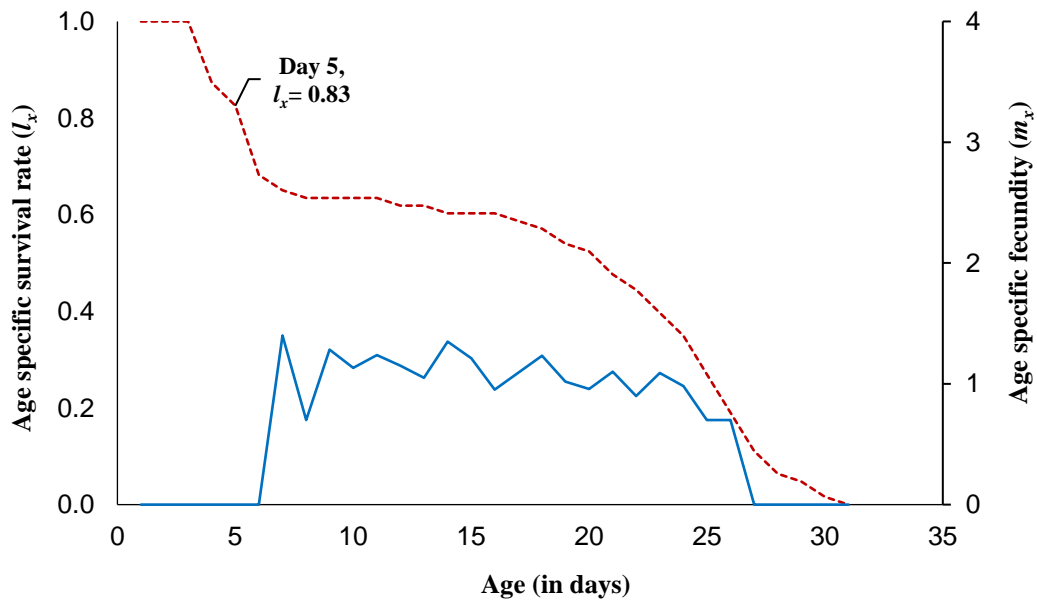


Figure 5-1 Age specific survival rate (dotted line) and fecundity (solid line) of *Galendromus flumenis* on Banks grass mite eggs.

Table 5-1 Mean (\pm SE) duration of different life stages and reproduction parameters of *Galendromus flumenis* on Banks grass mite eggs

Parameters	Female (n=28)	Male (n=12)	Combined sexes (n=40)
Egg to adult development	5.6 \pm 0.2	5.5 \pm 0.1	5.6 \pm 0.1
Adult longevity	17.7 \pm 0.6	16.8 \pm 1.8	17.4 \pm 0.7
Life span	23.3 \pm 0.6	22.3 \pm 1.8	23.0 \pm 0.7
Pre-oviposition period	2.7 \pm 0.1		
Oviposition period	12.5 \pm 0.7		
Post-oviposition period	2.5 \pm 0.1		
Total fecundity (eggs/female)	19.9 \pm 1.1		
Fecundity rate (eggs/female/day)	1.6 \pm 0.0		
Sex ratio (females: males)	2.33: 1		

n is the number of individuals tested (replicates).

The sex ratio of *G. flumenis* was biased towards females (2.3 females: 1 male). The population growth parameters were calculated as: net reproductive rate (R_0) = 11.5 females/ female/ generation, intrinsic rate of increase (r_m) = 0.200 female/ female/ day, the finite rate of increase (λ) = 1.222 population multiplication/ day, the mean generation time (T) = 12.2 days, and the doubling time (DT) = 3.5 days (Table 5-2).

Table 5-2 Population growth parameters of *Galendromus flumenis* on Banks grass mite eggs

Parameter	Mean \pm SE
Net reproductive rate (R_0)	11.5 \pm 1.3
Intrinsic rate of increase (r_m)	0.20 \pm 0.01
Finite rate of increase (λ)	1.22 \pm 0.01
Mean generation time (T)	12.2 \pm 0.5
Doubling time (DT)	3.5 \pm 0.2

Discussion

Egg to adult developmental time for *G. flumenis* in the present study at 34°C was 5.6 days. However, in another study in which *G. flumenis* eggs had been collected from the stock colony at 30°C and reared at 34°C, the immature developmental time was 7.0 days

(Ganjisaffar and Perring 2015b). This difference could be due to the shorter incubation period of eggs collected at 34°C compared to 30°C.

The fecundity rate of *G. flumenis* was 1.6 eggs/ day which is similar to other species in the genus *Galendromus* which are classified as phytoseiids with medium reproductive capacities (McMurtry and Croft 1997). In addition, McMurtry and Croft (1997) stated that Type II phytoseiids (selective predators of tetranychid mites, most frequently associated with dense-web-producing species) have r_m values of up to 0.4. However, they note that the r_m values for Type III species (generalist predators) are sometimes under 0.1, but can be as high as 0.25 when fed on spider mites or pollen. Based on this life-style classification, *G. flumenis* with a $r_m= 0.20$ would be placed on the specialist side of a type III predator approaching type II which also is in agreement with the classification made by Blackwood et al. (2004) based on the prey type.

Most of the *G. flumenis* females that had been reared at 38°C (optimal temperature for their development) died without laying eggs. It has been shown that high temperatures can result in mortality or reduced mobility of sperm thereby resulting in a failure in egg fertilization of phytoseiids (Ferragut et al. 1987; Broufas and Koveos 2001). According to the meteorological data from the California Irrigation Management Information System (CIMIS) station in the Coachella Valley (OASIS.A (#136)), the daily maximum temperatures exceed 38°C (up to 45°C) for a few hours during the summer months of June through September, which may adversely affect the population dynamics of *G. flumenis* and consequently, biological control of Banks grass mites. This finding is of great importance since it helps explain our field observations of Banks grass mites not being

controlled by *G. flumenis* at warm desert temperatures. In addition, a comparison among life table parameters of Banks grass mite and *G. flumenis* shows distinct differences between the prey and the predator. For example, at comparable temperatures the net reproductive rate of Banks grass mite can reach three times the maximum potential of *G. flumenis* (Table 5-3). This contributes to an intrinsic rate of increase of the prey mite that is more than double that of the predator mite, and much longer mean generation times and doubling times for the predator (Table 5-3). Taken together, these data explain why this predator is barely able to keep up with Banks grass mite densities, especially when densities of *G. flumenis* are low in the early season (Gispert et al. 2001) at the time when Banks grass mite populations begin to increase. However, while *G. flumenis* has inferior population growth parameters to its prey, it has other traits that can be exploited in a biological control strategy. These traits may help compensate for the lower population growth parameters of *G. flumenis*.

Table 5-3 A comparison between population growth parameters of *Galendromus flumenis* and its prey, Banks grass mite

Parameter	Banks grass mite 33-39°C	<i>G. flumenis</i> 34°C
Egg to adult development	4.9-5.3	5.6
Oviposition period	7-16	12.5
Net reproductive rate	6.45-33.36	11.5
Intrinsic rate of increase	0.24-0.48	0.20
Finite rate of increase	1.27-1.62	1.22
Mean generation time	5.93-8.99	12.2
Doubling time	1.6-2.9	3.5

Increasing the numbers of *G. flumenis* through augmentative releases should improve the management of Banks grass mite if releases are made early in the season when there is a higher proportion of prey eggs which are preferred life stage by the predator (Ganjisaffar

and Perring 2015a). Based on the negative impact of high temperatures determined in this study, we also recommend that releases be made during the cool morning hours of day and in the inner canopy of the trees or inside the date bunches with minimum exposure to the sun and heat. Future field studies will be conducted to evaluate these release strategies.

References

- Banks, N. 1914. New mites. *Journal of Entomology and Zoology* 6, 1-57.
- Blackwood, J.S., Luh, H.K., Croft, B.A. 2004. Evaluation of prey-stage preference as an indicator of life-style type in phytoseiid mites. *Experimental and Applied Acarology* 33, 261-280.
- Broufas, G.D., Koveos, D.S. 2001. Development, survival and reproduction of *Euseius finlandicus* (Acari: Phytoseiidae) at different constant temperatures. *Experimental and Applied Acarology* 25, 441-460.
- Carey, J.R. 1993. *Applied demography for biologists with special emphasis on insects*. Oxford University Press, New York.
- Carpenter, J.B., Elmer, H.S. 1978. Pests and diseases of the date palm. United States Department of Agriculture Agricultural Handbook 527, 1-42.
- Elmer, H.S. 1965. Banks grass mite, *Oligonychus pratensis*, on dates in California. *Journal of Economic Entomology* 58, 531-534.
- Ferragut, F., Garcia-Mari, F., Costa-Comelles, J., Laborda, R. 1987. Influence of food and temperature on development and oviposition of *Euseius stipulatus* and *Typhlodromus phialatus* (Acari: Phytoseiidae). *Experimental and Applied Acarology* 3, 317-329.
- Ganjisaffar, F., Perring, T.M. 2015a. Prey stage preference and functional response of the predatory mite *Galendromus flumenis* to *Oligonychus pratensis*. *Biological Control* 82, 40-45.
- Ganjisaffar, F., Perring, T.M. 2015b. Relationship between temperature and development of *Galendromus flumenis* (Acari: Phytoseiidae), a predator of Banks grass mite (Acari: Tetranychidae). *Experimental and Applied Acarology* 67, 535-546.
- Gispert, C., Farrar, C., Perring, T.M. 2001. Seasonal abundance and the Banks grass mite *Oligonychus pratensis* (Banks) (Prostigmata: Tetranychidae) and a predatory mite, and their response to sulfur treatment on commercial date palms *Phoenix dactylifera* L. in Southern California. In: Halladay, R.B., Walter, D.E., Proctor, H.C., Norton, R.A., Colloff, M.J. (Eds.). *Proceedings of the 10th international congress of acarology*. CSIRO Publishing, Melbourne, pp. 403-408.
- Helle, W., Sabelis, M.W. 1985. *Spider mites, their biology, natural enemies and control*. Vol. 1B. Elsevier, The Netherlands.

Janssen, A., Sabelis, M.W. 1992. Phytoseiid life-histories, local predator-prey dynamics, and strategies for control of tetranychid mites. *Experimental and Applied Acarology* 14, 233-250.

Maia A., Luiz, A.J.B., Campanhola, C. 2000. Statistical inference on associated fertility life table parameters using Jackknife technique: computational aspects. *Journal of Economic Entomology* 93, 511-518.

McMurtry, J.A., Croft, B.A. 1997. Life-styles of phytoseiid mites and their roles in biological control. *Annual Review of Entomology* 42, 291-321.

McMurtry, J.A., Scriven, G.T. 1965. Insectary production of phytoseiid mites. *Journal of Economic Entomology* 58, 282-284.

McMurtry, J.A., de Moraes, G.J., Sourassou, N.F. 2013. Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. *Systematic and Applied Acarology* 18, 297-320.

Perring, T.M., Holtzer, T.O., Toole, J.L. 1984a. Influence of temperature and humidity on pre-adult development of the Banks grass mite (Acari: Tetranychidae). *Environmental Entomology* 13, 338-343.

Perring, T.M., Holtzer, T.O., Kalisch, J.A., Norman, J.M. 1984b. Temperature and humidity effects on ovipositional rates, fecundity, and longevity of adult female Banks grass mite (Acari: Tetranychidae). *Annals of the Entomological Society of America* 77, 581-586.

SAS Institute. 2013. PROC user's manual, version 9.4. SAS Institute, Cary, North Carolina.

USDA. 2016. California agricultural statistics, 2013 crop year. Beltsville, USDA.

Chapter 6
Effects of the Miticide Hexythiazox on the Biology of
G. flumenis

Abstract

The Banks grass mite, *Oligonychus pratensis* (Banks) (Acari: Tetranychidae) is a major pest of dates. The miticide hexythiazox (Savey® 50 DF) is the current industry standard for Banks grass mite management on dates. However, sole reliance on hexythiazox as a single control strategy may lead to resistance in the mite population. Incorporating biological control into the Banks grass mite management on dates is a step toward a more integrated program. The predatory mite, *Galendromus flumenis* (Chant), is the most abundant predator and the only phytoseiid species on date bunches. Conservation of *G. flumenis* is essential for a successful integrated mite management program, and it is important to understand the impact of hexythiazox on the biological control of Banks grass mite by *G. flumenis*. For this purpose, the toxic effects of the recommended field rate of hexythiazox on the development of different immature stages and the fecundity of adult females of *G. flumenis* were evaluated. Laboratory bioassays were conducted in which predators were both treated topically by hexythiazox sprays, and exposed to residues on the arena surface and on the sprayed prey. Mortality due to hexythiazox was 0.09% in eggs, 2.63% in larvae, 0.00% in protonymphs, and 2.08% in adult females of *G. flumenis*. Hexythiazox did not have any significant effect on the fecundity of the treated females, hatch rate of the laid eggs, progeny development or sex ratio. Results suggest that hexythiazox would not disrupt the biological control of Banks grass mite by *G. flumenis* in the field.

Introduction

The Banks grass mite, *Oligonychus pratensis* (Banks) (Acari: Tetranychidae) has been a persistent pest of California dates since the early 1900s (Banks 1914). This mite feeds on the epidermis of the green developing dates (Kimri stage), disrupts the epidermal cell walls with its stylet-like chelicerae, and removes the plant sap and chlorophyll (Malcolm 1955; Krantz 1978). The mites rasp the fruit surface causing the skin to harden, shrivel, and crack (Elmer 1965; Carpenter and Elmer 1978). These scarred fruit are considered low quality at packing houses, being unmarketable for fresh market consumption.

Sulfur dust was introduced into the California date industry in mid-1950s for Banks grass mite control, and was the only control measure until 1997; up to 8 applications of sulfur were dusted into the date bunches (Negm et al. 2015). However, due to the danger of using sulfur dust to applicators, environmental concerns of dusting, complaints of neighboring communities, and field failures with sulfur (Negm et al. 2015), studies on the effectiveness of hexythiazox (Savey® 50 DF, Gowan Company, Yuma, Arizona, USA), an ovicide with an unknown mode of action, were initiated. Hexythiazox proved to be effective for the control of mite population growth through activity on eggs or early developmental stages with a one-time, early season application. Therefore, it received a Section 18 Emergency Use permit from the United States Environmental Protection Agency in 1998 and full registration in 2003 (EPA 2003). While sulfur is still used in organic date gardens, hexythiazox is the current industry standard for Banks grass mite management in California (Mauk et al. 2005).

The growing interest in sustainable pest management practices has encouraged biological control as an additional tool in Banks grass mite management program on dates to remove pressure from hexythiazox as a single tactic. Compared to other agroecosystems (Dean 1957; Pickett and Gilstrap 1986), only a few predator species are found in date gardens, likely due to the extreme high temperatures and low humidities in the desert environment. In the United States, mites in the Phytoseiidae are the most numerous and important predators associated with Banks grass mite. Our surveys in date gardens of the Coachella Valley (Riverside County, California) revealed that *Galendromus flumenis* (Chant) (Acari: Phytoseiidae) was the only phytoseiid species on date bunches (Ganjisaffar and Perring 2015).

Successful conservation of *G. flumenis* depends on its ability to survive exposure to the currently used miticide. Therefore, to enhance the biological control of Banks grass mite using this predator, it is essential to acquire information on the compatibility of hexythiazox with *G. flumenis*. For this purpose, a series of experiments were designed to assess the effect of field rate applications of hexythiazox on *G. flumenis* biology. We evaluated the survival of different life stages of *G. flumenis* to contact exposure, and the survival of *G. flumenis* to miticide residue on the foliage and prey treated with hexythiazox simulating field conditions. In addition, the effects of hexythiazox on fecundity, egg viability, developmental time and sex ratio of F₁ progeny of treated female predators were determined.

Materials and Methods

Mite Colony Maintenance

Colonies of *G. flumenis* and Banks grass mite were established with mites collected from date bunches in the commercial date gardens of the Coachella Valley in 2012. These colonies have been supplemented annually with field collected mites to ensure genetic variability. Banks grass mite was reared on field corn plants (*Zea mays* variety 31G71). *G. flumenis* was reared on a 10×10 cm black ceramic tile resting on a water-saturated foam in a stainless steel pan (20×20 cm). The edges of the tile were covered with strips of tissue paper immersed in water in the pan to prevent mites from escaping. A microscope cover slip with a few cotton threads underneath was placed in the center of the tile to provide ovipositional sites and shelter for the predatory mites (McMurtry and Scriven 1965; Ganjisaffar and Perring 2015). Predators were provided with mixed life stages of Banks grass mite brushed from infested corn leaves three times a week. Both colonies were maintained in environmentally controlled rooms at 30±1°C, 60±10% RH, and 16L: 8D photoperiod.

Experimental Arenas

Arenas consisted of two Petri dishes. A 3 cm Petri dish with a 5 mm hole in its bottom was placed in a 5 cm Petri dish containing water. A cotton layer was placed in the small Petri dish on top of which a corn leaf, cut to fit the 3 cm dish was placed abaxial side up. The leaf margin was covered with a water-saturated cotton strip to prevent mites from escaping and to maintain freshness in the leaf. A 1-cm hole was made in the lids of the large Petri dish, and covered with fine mesh for ventilation.

Chemical Tested

The recommended field concentration of hexythiazox (0.449g AI l^{-1}) was tested in a bioassay and distilled water was used as a non-treated control. This concentration was based on the maximum label rate applied at 935 L/ha of water. Treatments was applied using a deluxe airbrush (Central Pneumatic #69492, Camarillo, CA, USA) set at 5 psi held perpendicular at 15 cm away from the experimental arenas inhabited by both *G. flumenis* and Banks grass mite (Figure 6-1). All bioassays were held at $30\pm 1^\circ\text{C}$, $60\pm 10\%$ RH, and 16L: 8D photoperiod.

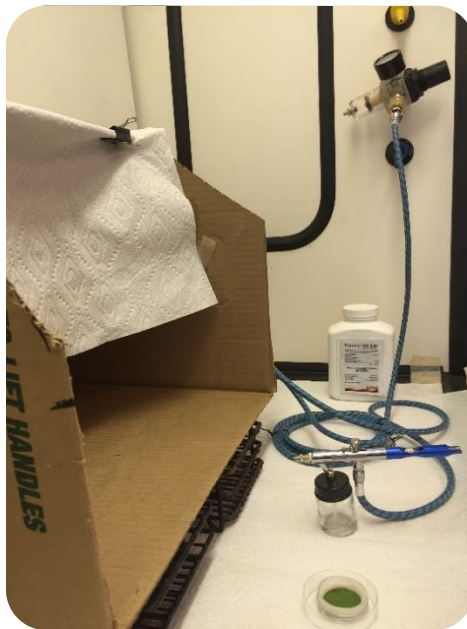


Figure 6-1 Deluxe airbrush and its setup for the chemical bioassay.

Treatment of Immature Stages

For the egg bioassay, approximately 150 *G. flumenis* gravid females were transferred from the stock colony to excised corn leaves infested with a mix of Banks grass mite stages. The excised leaves (4-5 cm in width and 10 cm in length) were placed abaxial side up on

a wet cotton layer in a Petri dish (14 cm diameter). This Petri dish had a 1 cm hole drilled in its bottom, and was placed in a pan containing water. After 24 h, 100 eggs were collected and moved individually to experimental arenas. Thus each treatment (hexythiazox and water control) had 50 replicates of a single *G. flumenis* egg. For the larvae, approximately 200 *G. flumenis* eggs were transferred from the stock colony to a clean excised corn leaf as described before. After 24 h, emerged larvae were moved individually to experimental arenas until 50 replicates for each treatment were obtained. For protonymphs, approximately 150 *G. flumenis* larvae were transferred from the stock colony to an excised corn leaf infested with Banks grass mite. After 24 h, 100 newly emerged protonymphs were collected and moved individually to experimental arenas, creating 50 replicates per treatment of a single *G. flumenis* protonymph. Before spraying the arenas, a surplus of all Banks grass mite stages was added to each arena for predator food. Mortality for each stage evaluated (egg, larvae, protonymphs) was recorded every 24 h until adulthood after which females were paired with a male and the number of eggs laid per female per day were counted after 24, 48, and 72h. The position of the eggs in each arena were marked with a felt-tip pen, and the marked eggs were allowed to hatch. In this way, the percentage of hatched eggs was determined. Therefore, *G. flumenis* had both direct exposure to the hexythiazox or water sprays and continued exposure to the residue from that sprays on the corn leaf and on the prey.

Treatment of Adult Females

One hundred newly emerged *G. flumenis* females were collected from the stock colony, transferred individually to experimental arenas, and paired with a male to ensure

mating. The pair was provided with a surplus of all Banks grass mite stages and then were sprayed with hexythiazox or water as described above. Each treatment had 50 replicates. Mortality of *G. flumenis* females was recorded at 24 h intervals for 5 days. Females were considered as dead if no movement was observed under the stereomicroscope when gently probed with a fine paint brush. The positions of the eggs laid on the first day of oviposition in each arena were marked with a felt-tip pen. The eggs laid on the next days were removed from the arena after the number was recorded. The egg hatchability, progeny development, survival and sex ratio were measured for the marked eggs.

Statistical analysis

All statistical analyses were performed using R version 3.3.0 for windows (R Core Team 2016). Mortality was corrected for the control mortality using Abbott's formula (Abbott 1925):

$$M = (M_t - M_c)/(100 - M_c) \times 100$$

where M is the corrected mortality, M_t is mortality in the hexythiazox treatment, and M_c is mortality in the control.

Means for the number of eggs per female per day, egg hatch, and developmental time of F_1 progeny of the treated females were tested for normality (Shapiro-Wilk Test) and homogeneity of variances (Levene Test) using a significance level of 0.05. Data then were analyzed by the Wilcoxon-Mann-Whitney Test ($P < 0.05$).

Results

Corrected mortality due to hexythiazox treatment was 0.09% in eggs, 2.63% in larvae and 0.00% in protonymphs (Table 6-1). Mortality remained very low in all immature stages

of *G. flumenis* during their development to the adult stage (Table 6-1). The mean number of eggs laid per female per day by *G. flumenis* females that were treated as eggs, larvae or protonymphs were not significantly different from the mean number of eggs in the control (Eggs: $P= 0.99$; Larvae: $P= 0.56$; Protonymphs: $P= 0.37$) (Table 6-2). In addition, there was no significant difference in the hatch rate of these eggs between two treatments ($P= 1$) (Table 6-2).

Table 6-1 Effects of hexythiazox on mortality of different immature stages of *Galendromus flumenis*

Treated Life Stage	% Corrected Mortality			
	Egg-Larva	Larva-Protonymph	Protonymph- Deutonymph	Deutonymph- Adult
Eggs	0.09 (46)	0.00 (45)	2.22 (45)	0.00 (44)
Larvae		2.63 (48)	3.21 (43)	3.29 (38)
Protonymphs			0.00 (49)	0.00 (49)

Number of replicates are given in the parentheses.

Table 6-2 Fecundity in females emerging from immature stages of *Galendromus flumenis* treated with hexythiazox or control

Treated Life Stage	Treatment	Replicates	Eggs/ female/ day	P	% Egg hatch	P
Eggs	Hexythiazox	34	2.3 ± 0.1 a	0.9898	99.41 ± 0.59 a	1
	Control	29	2.3 ± 0.1 a		100.0 ± 0.00 a	
Larvae	Hexythiazox	28	2.4 ± 0.1 a	0.5646	99.40 ± 0.60 a	1
	Control	31	2.5 ± 0.1 a		100.0 ± 0.00 a	
Protonymphs	Hexythiazox	31	1.9 ± 0.1 a	0.3682	99.19 ± 0.81 a	1
	Control	29	1.8 ± 0.1 a		99.31 ± 0.69 a	

Means within a row followed by the same letter are not significantly different ($P < 0.05$; Wilcoxon-Mann-Whitney Test).

Mortality caused by the field rate of hexythiazox was very low (2.08%) (Table 6-3), therefore, according to the IOBC standard (Hassan et al. 1994), this miticide is harmless to *G. flumenis*. The IOBC standard includes four categories: 1= harmless (mortality < 30 %), 2= slightly harmful (30% < mortality < 79 %), 3= moderately harmful (80% < mortality <

99 %), and 4= harmful (mortality >99 %). Females laid an average of 2.2 eggs per female per day in both treatments ($P= 0.42$). The hatching rate was high (98.83%) in the eggs laid by hexythiazox-treated females, and did not differ significantly ($P= 0.76$) from the egg hatch in the control (97.17%). The mean egg to adult developmental times were 5.8 and 5.5 days for female and male progeny of the hexythiazox treated females, respectively, which were not significantly different from those estimated for the Control ($P= 0.62$) (Table 6-3). There was no significant difference in the sex ratio (proportion of females and males) between the two treatments ($P= 0.33$) (Table 6-3).

Table 6-3 Effects of hexythiazox on survival, fecundity and progeny of *Galendromus flumenis* females

Treatment	% Corrected Mortality	Eggs/ female/ day	% Egg hatch	Developmental Time (F ₁)		Sex ratio females: males
				Female	Male	
Hexythiazox	2.08 (48)	2.2 ± 0.1 a (43)	98.83 ± 0.84 a (71)	5.8 ± 0.1 a (16)	5.5 ± 0.1 a (51)	0.29: 1 a
Control	-	2.2 ± 0.1 a (45)	97.17 ± 2.09 a (53)	5.9 ± 0.1 a (10)	5.6 ± 0.1 a (40)	0.25: 1 a
<i>P</i>		0.4185	0.7567	0.6169	0.8328	0.3321

Number of replicates are given in the parentheses.

Means within a column followed by the same letter were not significantly different ($P < 0.05$; Wilcoxon-Mann-Whitney Test).

Sex ratio (Proportion of females to males) between two treatments followed by the same letter were not significantly different ($P < 0.05$; Chi-square test).

Discussion

In the present study, *G. flumenis* was treated both topically by hexythiazox sprays, and exposed to residues on the arena surface and on the sprayed prey. Results indicated that hexythiazox is non-toxic to *G. flumenis*. Other studies have demonstrated that recommended field rates of hexythiazox are harmless (< 30% mortality) to some phytoseiid species such as, *Phytoseiulus persimilis* Athias-Henriot (Hassan et al. 1991; Oomen et al. 1991; Blumel and Gross 2001; Cote et al. 2002; Alzoubi and Cobanoglu 2008), *Neoseiulus*

womersleyi (Schicha) (Amano et al. 2004), *Neoseiulus californicus* McGregor (Amano et al. 2004; Alzoubi and Cobanoglu 2008), *Amblyseius potentillae* (Garman) (Hassan et al. 1991), *Amblyseius finlandicus* (Oudemans) (Hassan et al. 1991), *Amblyseius andersoni* (Chant) (Hassan et al. 1991), *Phytoseius plumifer* (Canestrini and Fanzago) (Nadimi et al. 2008; Nadimi et al. 2009), and *Typhlodromus pyri* Scheuten (Hassan et al. 1991).

The stage of exposure to a chemical can make a large difference in terms of susceptibility, particularly in the case of growth regulators (Stark and Banken 1999; Blumel et al. 2000). In addition, testing multiple life stages using longer evaluation periods provides much greater consistency and resolution to detrimental effects of a chemical (Angeli and Ioriatti 1994; Lefebvre et al. 2012). Therefore, the effects of hexythiazox were assessed on immature stages of *G. flumenis*, and little or no adverse effect on immature development and reproduction of the emerging adults was observed. Thus we confirmed the compatibility of hexythiazox with eggs, immatures and adults of the predator.

Our previous research indicated that the sex ratio for *G. flumenis* was female-biased, with a sex ratio (females: males) of 2.3: 1 (unpublished data). However, the sex ratio in the progeny of the *G. flumenis* females in the present study was male-biased in both treated and control populations. This varied sex ratio in the present study may be due to the fact that it was based on the sex of the adults emerging from the eggs laid on the first day of oviposition. It has been documented that the sex ratio in most phytoseiids is male biased on the first 2 days of oviposition and then becomes female-biased on the subsequent days (Nagelkerke and Sabelis 1998; Rahman et al. 2013).

This study shows that hexythiazox has no potential impact on the disruption of the biological control provided by *G. flumenis*, and supports the application of this miticide for Banks grass mite management in date gardens. In addition, hexythiazox can be incorporated in other integrated pest management programs in which *G. flumenis* serves as the key biological control agent.

References

- Alzoubi, S., Cobanoglu, S. 2008. Toxicity of some pesticides against *Tetranychus urticae* and its predatory mites under laboratory conditions. *American-Eurasian Journal of Agricultural and Environmental Sciences* 3, 30-37.
- Amano, H., Ishii, Y., Kobori, Y. 2004. Pesticide susceptibility of two dominant phytoseiid mites, *Neoseiulus californicus* and *N. womersleyi*, in conventional Japanese fruit orchards (Gamasina: Phytoseiidae). *Journal of Acarological Society of Japan* 13, 65-70.
- Angeli, G., Ioriatti, C. 1994. Susceptibility of two strains of *Amblyseius andersoni* Chant (Acari: Phytoseiidae) to dithiocarbamate fungicides. *Experimental and Applied Acarology* 8, 669-679.
- Banks, N. 1914. New mites. *Journal of Entomology and Zoology* 6, 1-57.
- Blümel, S., Pertl, C., Bakker, F.M. 2000. Comparative trials on the effects of two fungicides on a predatory mite in the laboratory and in the field. *Entomologia Experimentalis et Applicata* 97, 321-330.
- Carpenter, J.B., Elmer, H.S. 1978. Pests and diseases of the date palm. United States Department of Agriculture Agricultural Handbook 527, 1-42.
- Cote, K.W., Lewis, E.E., Schultz, P.B. 2002. Compatibility of acaricide residues with *Phytoseiulus persimilis* and their effects on *Tetranychus urticae*. *HortScience* 37, 906-909.
- Dean, H.A. 1957. Predators of *Oligonychus pratensis* (Banks), Tetranychidae. *Annals of the Entomological Society of America* 50, 164-165.
- Elmer, H.S. 1965. Banks grass mite, *Oligonychus pratensis*, on dates in California. *Journal of Economic Entomology* 58, 531-534.
- Environmental Protection Agency. 2003. Hexythiazox, pesticide tolerance. *Federal Register* 68, 10370-10377.
- Ganjisaffar, F., Perring, T.M. 2015. Prey stage preference and functional response of the predatory mite *Galendromus flumenis* to *Oligonychus pratensis*. *Biological Control* 82, 40-45.
- Hassan, S.A., Bigler, F., Bogenschutz, H., Boller, E., Brun, J., Calis, J.N.M., Chiverton, J., Coremans-Pelseneer, J., Duso, C., Lewis, G.B. 1991. Results of the fifth joint pesticide testing programme of the IOBC/WPRS-working group "pesticides and beneficial organisms". *Entomophaga* 36, 55-67.

- Hassan, S.A., Bigler, F., Boller, H.B., Brun, J., Calis, J.N.M. 1994. Results of the sixth joint pesticide testing programme of the IOBC/WPRS-working group “pesticides and beneficial organisms”. *Entomophaga* 39, 107-119.
- Krantz, G.W. 1978. A Manual of acarology, 2nd edition. Oregon State University Book stores, Inc., Oregon.
- Lefebvre, M., Bostanian, N.J., Mauffette, Y., Racette, G., Thistlewood, H.A., Hardman, J.M. 2012. Laboratory-based toxicological assessments of new insecticides on mortality and fecundity of *Neoseiulus fallacis* (Acari: Phytoseiidae). *Journal of Economic Entomology* 105, 866-871.
- Malcolm, D.R. 1955. Biology and control of the timothy mite, *Paratetranychus pratensis* (Banks). *Washington Agricultural Experiment Station Bulletin* 17, 1- 35.
- Mauk, P.A., Perring, T.M., Shea, T. 2005. Management of Banks grass mite in dates. *University of California Cooperative Extension, Topics in Subtropics Newsletter* 3, 1-2.
- McMurtry, J.A., Scriven, G.T. 1965. Insectary production of phytoseiid mites. *Journal of Economic Entomology* 58, 282-284.
- Nadimi, A., Kamali, K., Arbabi, M., Abdoli, F. 2008. Side-effects of three acaricides on the predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) under laboratory conditions. *Munis Entomology and Zoology* 3, 556-567.
- Nadimi, A., Kamali, K., Arbabi, M., Abdoli, F. 2009. Selectivity of three miticides to spider mite predator, *Phytoseius plumifer* (Acari: Phytoseiidae) under laboratory conditions. *Agricultural Sciences in China* 8, 326-331.
- Nagelkerke, C.J., Sabelis, M.W. 1998. Precise control of sex allocation in pseudo-arrhenotokous phytoseiid mites. *Journal of Evolutionary Biology* 11, 649-684.
- Negm, M.W., de Moraes, G.J., Perring, T.M. 2015. Mite pests of date palms. In: *Sustainable pest management in date palm: current status and emerging challenges*. Wakil, W., Faleiro, J.R., Miller, T.A. (Eds.). Springer International Publishing, Switzerland, pp. 347-389.
- Oomen, P.A., Romeijn, G., Wiegers, G.L. 1991. Side-effects of 100 pesticides on the predatory mite *Phytoseiulus persimilis* collected and evaluated according to the EPPO Guideline. *OEPP/EPPO Bulletin* 21, 701-712.
- Pickett, C.H., Gilstrap, F.E. 1986. Natural enemies associated with spider mites (Acari: Tetranychidae) infesting corn in the high plains region of Texas. *Journal of the Kansas Entomological Society* 59, 524-536.

Rahman, V.J., Babu, A., Roobakkumar, A., Perumalsamy, K. 2013. Life table and predation of *Neoseiulus longispinosus* (Acari: Phytoseiidae) on *Oligonychus coffeae* (Acari: Tetranychidae) infesting tea. *Experimental and Applied Acarology* 60, 229-240.

R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Stark, J.D., Banken, J.O. 1999. Importance of population structure at the time of toxicant exposure. *Ecotoxicology and Environmental Safety* 42, 282-287.

Chapter 7

Conclusion

We determined that *G. flumenis* has high consumption rates on early life stages of the Banks grass mite. It also displays a type II functional response indicating that a higher proportion of prey is consumed by *G. flumenis* at lower prey densities. These results suggest that the predator will be more effective at the beginning of the spider mite infestation, when the Banks grass mite densities are low and the population is comprised of a high proportion of eggs and larvae. Early releases also will increase the number of predators, providing better balance with the higher densities of prey mites that occur in the early season. Furthermore, temperatures in the early season are more favorable to ovipositional success of the predator compared to later in the season, and this will support better establishment of the predator. Augmentative release of *G. flumenis* on dates at this time should achieve maximum biological control of Banks grass mite.

Our studies also showed that when *G. flumenis* is aggregated, a mutual interference effect reduces its searching efficiency and per capita predation rates. This interference effect may be one of the factors that reduces the overall effectiveness of the predator in the field. However, since predators are not confined in the field and can disperse easily, such mutual interference effect may not be significant. Field trials are needed to study natural predator-prey ratios on date fruits to better understand their interactions. If such interference effect exists between individual predators in the field, then high release rates may have an adverse effect on the foraging efficiency of *G. flumenis*, and there may be a maximum release rate for the predator that needs to be considered.

Results of our temperature-dependent developmental study revealed that the optimal temperature for the development of *G. flumenis* is 38°C, suggesting that this predator is

adapted to high desert temperatures. The optimal temperature for *G. flumenis* development was determined to be very close to those of Banks grass mite. However, the developmental time of *G. flumenis* at lower temperatures early in the season (during the months of April and May) is longer than that of Banks grass mite, thus it cannot keep up with the prey population. This provides additional support for early season augmentation of *G. flumenis*.

The study on the population growth and reproduction of *G. flumenis* indicated that predators cannot produce eggs at 38°C, the optimal temperature for their development. Therefore, despite the rapid development of *G. flumenis* at high temperatures, their overall population growth is adversely affected at high temperatures. Based on these results, we recommend that releases be made during the cool morning hours of day and in the inner canopy of the trees or inside the date bunches with minimum exposure to the sun and heat. In addition, we determined that the population growth parameters of *G. flumenis*, which contribute to its intrinsic rate of natural increase (r_m), are inferior to the prey. This is probably the main reason for the low performance of *G. flumenis* in the field. Increasing the numbers of *G. flumenis* through augmentative releases will help compensate for the low r_m of *G. flumenis* and should improve the management of Banks grass mite.

To conclude our work, we evaluated the impact of hexythiazox (Savey® 50 DF), the current industry standard for Banks grass mite control on dates, on *G. flumenis*. All of our data support the augmentation of predators early in the season, and the timing of these releases precede the time when date producers apply hexythiazox. Therefore, it is critical to understand the impact of the miticide treatments on the predator. We found that hexythiazox had no impact on the predator, regardless of whether the predators were

sprayed or they were fed prey mites that had been sprayed. Not only was there no impact on any of the predator life stages, but sprayed mites continued to develop and lay eggs that developed normally. This finding ensures the compatibility of the miticide with early season augmentative releases of the predator.